Original Article

Value of circulating tumor cells in the diagnosis and treatment of solitary pulmonary nodules

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Background: Lung cancer has become the most common malignant tumor worldwide, with the highest rates of morbidity and mortality. The detection of circulating tumor cells (CTCs) can be simple, rapid, and minimally invasive, thus endowing them with a high value in the diagnosis of malignant tumors. We aimed to explore the correlation between CTCs in peripheral blood and benign or malignant solitary pulmonary nodules (SPNs).

Methods: A total of 223 patients with SPNs from January 2018 to May 2020 were recruited. During the same period, 20 healthy volunteers were recruited as controls. Venous blood samples were collected from participants for detecting CTCs using a folate receptor (FR)-positive cell detection kit, as well as tumor biomarkers.

Results: A significant difference in the level of CTCs were observed between the malignant SPNs group, the benign SPNs group, and the control group, which was markedly higher in the malignant SPNs group (10.48±3.49 FU/3 mL) than both the benign SPNs and control groups (6.38±0.53 and 4.45±1.21 FU/3 mL, respectively) (P<0.001). In addition, the level of CTCs was significantly higher in the benign SPNs group than in the control group (P=0.023). In particular, in the malignant SPNs group, patients older than 60 years (11.45±3.92 FU/3 mL) presented a notably higher level of CTCs than other patients (9.55±2.74 FU/3 mL). The patients were then classified according to the pathological subtypes of lung cancer. There was a significant difference in level of CTCs among patients with squamous cell carcinoma (9.10±1.94 FU/3 mL), adenocarcinoma (10.77±3.71 FU/3 mL), and adenosquamous cell carcinoma (11.78±2.61 FU/3 mL). Binary logistic regression analysis suggested that CTCs were an independent risk factor of malignant SPN (OR =3.698, 95% CI: 1.136–11.035, P=0.030). The sensitivity and specificity of CTCs in diagnosing malignant SPNs was significantly higher than tumor biomarkers (single or combined) [sensitivity =89.1%; specificity =92.3%; area under curve (AUC) (95% CI) =0.907 (0.861–0.942)].

Conclusions: Peripheral blood CTCs can be used in the diagnosis of malignant SPNs and are recommended for clinical application.

Keywords: Circulating tumor cells; solitary pulmonary nodules; lung cancer

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Introduction

Lung cancer has become the most common malignant tumor worldwide, with the highest rates of morbidity and mortality. In 2018, there were approximately 2.1 million new cases and 1.8 million deaths (1). In China, the morbidity and mortality rates of lung cancer rank are higher than all others. More than 80% of lung cancer cases are non-small cell lung cancers (NSCLCs). With the extensive application of low-dose spiral CT (LDCT) in China, and the popularity of physical examination, the rate of detection of solitary pulmonary nodules (SPNs) has increased. SPNs are either benign or malignant, and malignant SPNs are an early manifestation of lung cancer. Clinical evidence has shown that early screening and treatment of malignant SPNs can significantly decrease the mortality of lung cancer (2,3). Therefore, sensitive biomarkers should be identified to develop efficient tools for the diagnosis of SPNs.

As a subtype of tumor cells, circulating tumor cells (CTCs) shed into the vasculature from primary or metastatic solid tumors naturally or as a result of therapeutic procedures, and give rise to tumor development, metastasis, or recurrence (4-6). The detection of CTCs can be simple, rapid, and minimally invasive, thus endowing them with a high value in the diagnosis of malignant tumors, assessment of tumor staging, prediction of prognosis, evaluation of chemotherapy efficacy, and guidance of targeted medication. In the present study, we aimed to explore the correlation between peripheral blood CTCs with benign or malignant SPNs, thereby determining the potential of CTCs as a specific biomarker of lung cancer in early-stage screening of SPNs. We present the following article in accordance with the STARD reporting checklist (available at http://dx.doi.org/10.21037/atm-21-889).

Methods

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. All participants signed the informed consent. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013) .

Baseline characteristics

In total, 223 patients with SPNs treated in the inpatient and outpatient departments of thoracic surgery at the First Affiliated Hospital of Soochow University from January 2018 to May 2020 were recruited into this retrospective analysis. During the same period, 20 healthy volunteers were recruited as controls. Venous blood samples were collected from participants for detecting CTCs using a folate receptor (FR)-positive cell detection kit [negative screening of immunomagnetic beads + ligand-targeted polymerase chain reaction (LT-PCR); National Machinery Registration Standard: 20163400061; Genosaber, Nantong, China]. Relative levels of carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 724 (CA724), cytokeratin fragment 21-1 (CYFRA21-1), and neuron-specific enolase (NSE) were detected using an automatic electrochemiluminescence immunoassay analyzer (Roche Cobas e601). Recruited patients with SPNs were surgically treated and pathologically diagnosed. Lung cancer was assessed according to the TNM Classification of Malignant Tumors (the 8th edition, 2018) published by the Union for International Cancer Control (UICC).

The inclusion criteria were as follows: (I) patients with chest CT images showing SPNs and a definite indication of surgery determined by experienced chief or associate chief physicians; (II) patients that had not undergone preoperative treatments, including anti-infection, anti-tuberculosis, chemotherapy, targeted medication, or combination treatment; (III) patients that did not have other types of malignant tumors; (IV) patients that showed normal functions in other vital organs; (V) patients who were willing to cooperate and provide informed consent; and (VI) healthy volunteers.

The exclusion criteria were as follows: (I) patients with clotted, hemolyzed, or contaminated blood samples; (II) the volume of the blood sample was less than 3 mL; (III) patients that had undergone nonstandard procedures in the collection, preservation, and analysis of blood samples; (IV) patients or their families were poorly compliant, did not cooperate with treatment, or refused to sign the informed consent; (V) clinical data were incomplete; (VI) patients diagnosed as small cell lung cancer; and (VII) patients with stage IV tumor.

Reference values

The reference value of CTCs was 8.70 FU according to the recommendation of the FR-positive cell detection kit. The reference values of serum tumor biomarkers were as follows: CEA <5 ng/mL, CA125 <35 U/mL, CA724 <6 U/mL, CYFRA21-1 <3.07 ng/mL, and NSE <7 ng/mL. A higher level than the reference value was considered as positive, and a lower level as negative. Any serum tumor

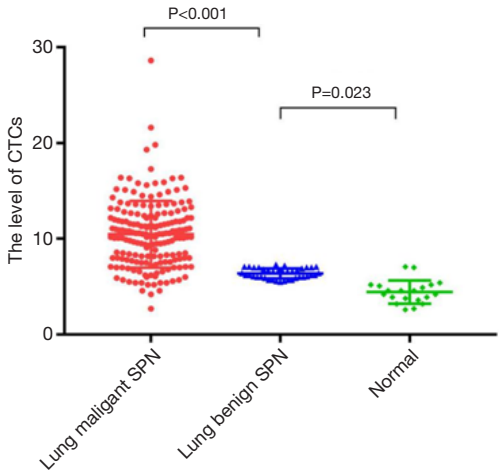

Figure 1 Comparison of peripheral CTC levels in the malignant SPNs group, benign SPNs group, and control group. CTC, circulating tumor cell; SPN, solitary pulmonary nodule.

biomarker higher than the reference value in combination treatment was considered as a positive result.

Indicators

The observed indicators included: (I) peripheral blood CTC level; (II) receiver operating characteristic (ROC) curves, sensitivity, and specificity of CTCs and serum tumor biomarkers in diagnosing malignant or benign SPNs.

Statistical analysis

Data were first subjected to the normality test. Normally distributed data were expressed as mean ± standard derivation; otherwise, they were expressed as medians. Differences of normally distributed data between groups were analyzed by the independent Student's *t*-test, and those among groups were analyzed by one-way analysis of variance (ANOVA). In addition, non-normally distributed data between groups were compared using the Mann-Whitney U test, and those among groups were compared by Kruskal-Wallis H test. Enumeration data were expressed as n (%), and were compared using the Chi-square test. Independent risk factors of malignant SPNs were assessed by binary logistic regression analysis. ROC curves were constructed for assessing diagnostic potentials, with sensitivity (%) as the Y-axis and 100-specificity (%) as the X-axis. The area under curve (AUC), sensitivity [sensitivity = true positive rate/(true positive rate + false negative rate) ×100%], and specificity [specificity = true negative rate/(true negative rate + false positive rate) ×100%] were calculated. SPSS 24.0, MedCalc_v12.3 and GraphPadPrism7 were used for statistical analyses. P<0.05 considered statistically significant.

Results

A total of 223 patients with SPNs were recruited. During the same period, 20 healthy volunteers were recruited as controls. Among the 184 (82.51%) patients with malignant SPNs were 79 males and 105 females, with an average age of 57.69±11.96 years. Of these, 34 (18.48%) were squamous cell carcinoma cases, 147 (79.89%) were adenocarcinoma cases, and three (1.63%) were adenosquamous cell carcinoma cases. Among the 39 (17.49%) patients with benign SPNs were 11 males and 28 females, with an average age of 60.12±9.75 years, including 10 (25.64%) sclerosing hemangioma cases, 15 (38.46%) hamartoma cases, 13 (33.33%) intrapulmonary lymph node cases, and one (2.56%) tuberculosis case.

CTC levels were subjected to the normality test. After the Kolmogorov-Smirnov test, Z values in the benign SPNs group, malignant SPNs group, and control group were 0.771 (P=0.593), 0.525 (P=0.946), and 0.833 (P=0.491), respectively, suggesting that CTC levels among the three groups were normally distributed, and were therefore expressed as mean ± standard derivation.

Level of CTCs in peripheral blood

Peripheral blood CTC levels in the malignant SPNs group, benign SPNs group, and control group were 10.48±3.49, 6.38±0.53, and 4.45±1.21 FU/3 mL, respectively. One-way ANOVA analysis showed that the CTC level was significantly different among the three groups (F=55.965, P<0.001). The subsequent *post-hoc* test suggested that peripheral blood CTC levels in the malignant SPNs group were significantly higher than those in both the benign SPNs group and the control group (both P<0.001). Moreover, a higher peripheral blood CTC level was detected in the benign SPNs group compared to the control group (P=0.023) (*Figure 1*).

As shown in *Table 1*, we recorded clinical data of patients in the malignant or benign SPNs groups, including CTCs (malignant/benign), age, pathological subtype, differentiation grade, location of SPNs (left/right lobe, upper/middle/lower lobe), largest diameter of SPNs, T stage, N stage, TNM stage, smoking history, and tumor

Table 1 Clinical data of patients in the malignant or benign SPNs groups

Clinical data	Malignant SPNs (n=184)	Benign SPNs (n=39)
CTCs level (FU/3 mL)	10.48±3.49	6.38±0.53
CTCs (malignant/benign)	164/20	3/36
Age (years)		
≤60	94	21
>60	90	18
Sex		
Male	79	11
Female	105	28
Pathological subtypes		
Squamous cell carcinoma	34	–
Adenocarcinoma	147	–
Adenosquamous cell carcinoma	3	–
Differentiation level		
G1	99	–
G2	67	–
G3	18	–
Location of SPNs		
Left lobe	91	19
Right lobe	93	20
Location of SPNs		
Upper lobe	121	20
Middle lobe	8	4
Lower lobe	55	15
Largest diameter of SPNs (cm)		
≤1	66	23
>1	118	16
T stage		
T1	126	–
T2	42	–
T3	16	–
N stage		
N0	153	–
N1	25	–
N2	6	–
TNM stage		
Stage I	137	–
Stage II	41	–
Stage III	6	–
Smoking history		
N/A	160	30
I/A	24	9
Tumor biomarkers (positive/negative)		
CEA	51/133	8/31
CA125	55/129	6/33
CA724	53/131	8/31
CYFRA21-1	56/128	7/32
NSE	76/108	5/34
CEA + CA125 + CA724 + CYFRA21-1 + NSE	109/75	9/30

SPN, solitary pulmonary nodule; CTCs, circulating tumor cell; N/A, not available; I/A, is available; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA724, carbohydrate antigen 724; CYFRA21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase.

biomarkers (negative/positive).

We subsequently performed subgroup analyses on peripheral blood CTC levels in malignant SPN patients classified by age, sex, pathological subtype, differentiation grade, location of SPN, largest diameter of SPN, T stage, N stage, TNM stage, and smoking history. The levels of CTCs were significantly higher in malignant SPN patients older than 60 years than other patients (11.45±3.92 *vs.* 9.55±2.74 FU/3 mL) (P<0.001). Classified by pathological subtypes, there was a notable difference in the level of CTCs among patients with squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma (9.10±1.94 *vs.* 10.77±3.71 *vs.* 11.78±2.61 FU/3 mL) (F=3.436, P=0.034). Peripheral blood CTC levels in patients with malignant SPNs classified by sex, differentiation grade, location of SPN, largest diameter of SPN, T stage, N stage, TNM stage, and smoking history were comparable (*Table 2*).

To assess the value of CTCs and other serum tumor biomarkers in diagnosing benign or malignant SPNs, binary logistic regression analysis was performed. Patients

Table 2 Clinical data of patients with malignant SPNs

Clinical data	Case number (n=184)	CTCs level (FU/3 mL)	F/χ^2	P
Age (years)				
≤60	94	9.55±2.74	4.671	<0.001
>60	90	11.45±3.92		
Sex				
Male	79	10.45±3.94	0.984	0.923
Female	105	10.50±3.13		
Pathological subtypes				
Squamous cell carcinoma	34	9.10±1.94	3.436	0.034
Adenocarcinoma	147	10.77±3.71		
Adenosquamous cell carcinoma	3	11.78±2.61		
Differentiation grade				
G1	99	10.39±3.74	0.899	0.409
G2	57	10.25±3.37		
G3	18	11.29±2.75		
Location of SPN				
Left lobe	91	10.49±4.02	3.731	0.968
Right lobe	93	10.47±2.91		
Location of SPN				
Upper lobe	121	10.51±3.38	0.943	0.391
Middle lobe	8	8.85±1.84		
Lower lobe	55	10.65±3.89		
Largest diameter of SPN (cm)				
≤1	66	10.22±3.53	0.512	0.475
>1	118	10.63±3.48		
T staging				
T1	126	10.57±3.71	0.964	0.383
T2	42	9.92±3.11		
T3	16	11.25±2.43		
N staging				
N0	153	10.44±3.40	0.344	0.709
N1	25	10.43±4.11		
N2	6	11.65±3.38		
TNM staging				
Stage I	137	10.35±3.50	0.553	0.576
Stage II	41	10.75±3.53		
Stage III	6	11.65±3.38		
Smoking history				
N/A	160	10.43±3.25	1.914	0.168
I/A	24	10.82±4.89		

SPN, solitary pulmonary nodule; CTC, circulating tumor cell; N/A, not available; I/A, is available.

Table 3 Logistic regression analysis of independent risk factors for malignant SPNs

Variables	B	SE	Wald	P	OR	95% CI
CTCs	1.318	0.702	4.817	0.030	3.698	1.136–11.035
CEA	0.518	0.626	0.725	0.415	1.661	0.526–5.336
CA125	0.221	0.359	0.624	0.234	1.567	0.367–4.267
CA724	0.456	0.547	0.636	0.365	1.778	0.412–3.612
CYFRA21-1	0.697	0.627	0.784	0.657	3.364	0.524–6.147
NSE	0.239	0.412	0.125	0.362	2.263	0.127–5.236

SPN, solitary pulmonary nodule; CTC, circulating tumor cell; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA724, carbohydrate antigen 724; CYFRA21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase.

Table 4 Diagnostic values for malignant SPNs

Indicators	Cut-off value	Sensitivity%	Specificity %	AUC (95% CI)
CTCs	8.7	89.1	92.3	0.907 (0.861–0.942)
CEA	5	27.7	78.9	0.533 (0.435–0.632)
CA125	35	29.9	84.2	0.571 (0.476–0.665)
CA724	6	28.8	78.9	0.539 (0.441–0.637)
CYFRA21-1	3.07	30.4	81.6	0.560 (0.464–0.656)
NSE	7	41.3	86.8	0.641 (0.553–0.728)
CEA + CA125 + CA724 + CYFRA21-1 + NSE	–	59.2	76.3	0.678 (0.588–0.768)

SPN, solitary pulmonary nodule; CTC, circulating tumor cell; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA724, carbohydrate antigen 724; CYFRA21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase.

with benign or malignant SPNs were enrolled as dependent variables (patients with benign SPNs =0; patients with malignant SPNs =1), and CTCs, CEA, CA125, CA724, CYFRA21-1, and NSE were independent variables (negative result =0, positive result =1).

The omnibus tests of model coefficients (χ^2=39.127, P<0.001), likelihood ratio test (maximum likelihood estimation =64.128), Cox & Snell R^2 (0.201), Nagelkerke R^2 (0.471), and Hosmer and Lemeshow test (χ^2=8.027, P=0.117) all suggested that the current model was well fitted with a significant difference. They suggested that peripheral blood CTC level was an independent risk factor of diagnosing malignant SPNs (OR =3.698, 95% CI: 1.136–11.035, P=0.030) (*Table 3*).

Sensitivity and specificity of CTCs and serum tumor biomarkers in diagnosing benign or malignant SPNs

To assess the value of CTCs in diagnosing benign or malignant SPNs, patients with benign or malignant SPNs were recruited as a control and experimental group, respectively. ROC curves were constructed, with sensitivity (%) as the Y-axis, and 100-specificity (%) as the X-axis, and the AUC was calculated. For comparison, ROC curves were constructed based on serum tumor biomarkers in diagnosing benign or malignant SPNs.

It was shown that the sensitivity and specificity of CTCs in diagnosing malignant SPNs were 89.1% and 92.3%, respectively [AUC (95% CI) =0.907 (0.861–0.942)], which were both superior to those of a single serum tumor biomarker or their combination (*Table 4* and *Figures 2,3*).

Discussion

At present, lung cancer can be screened mainly through cexfoliative cell examination of sputum (low sensitivity), chest X-ray examination (high false negative rate), LDCT (high false positive rate and high radiation exposure), and

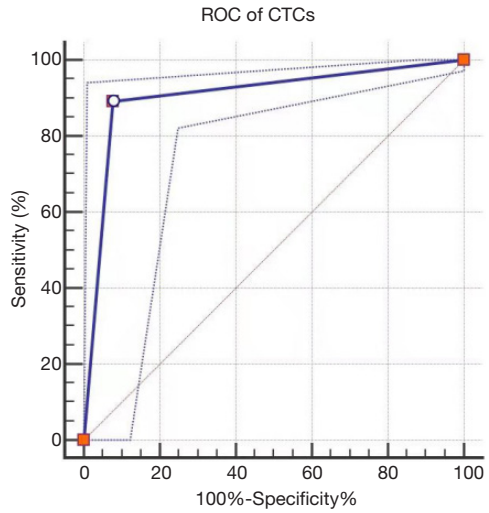

Figure 2 ROC curves of CTCs in diagnosing benign or malignant SPNs. ROC, receiver operating characteristic; CTC, circulating tumor cell; SPN, solitary pulmonary nodule.

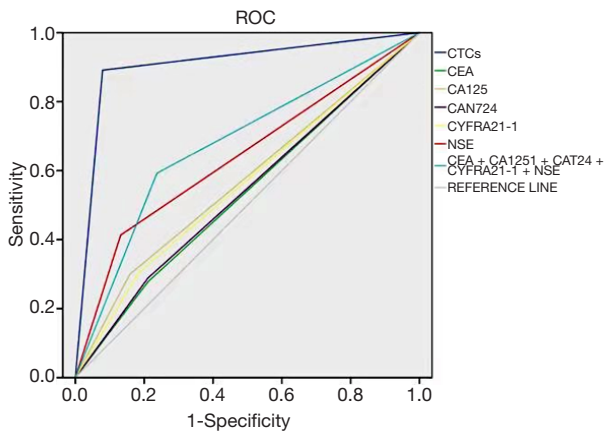

Figure 3 ROC curves of serum tumor biomarkers in diagnosing benign or malignant SPNs. ROC, receiver operating characteristic; CTC, circulating tumor cell; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA724, carbohydrate antigen 724; CYFRA21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase.

lung biopsy (invasive procedure, and the accuracy may be influenced by sample insufficiency) (7-12). In 2013, liquid biopsy was defined as a non-invasive procedure for early-stage cancer screening. It is also an auxiliary approach to develop therapeutic decisions for advanced cancer, featuring high accuracy, low sampling-related risk, less restriction by sample size, and simple preparation of samples (13-15). Blood samples are most commonly used in liquid biopsies. A wide range of biomarkers can be detected by liquid biopsy, including CTCs, circulating tumor DNAs (ctDNAs), exosomes, circulating tumor RNAs (ctRNAs), etc. Currently, only CTCs and ctDNAs detected by liquid biopsy are approved for clinical application (16).

The migration of CTCs into the bloodstream is affected by various factors, including anoikis, shear stress of blood flow, and immune elimination. As a result, CTCs remain at a low level (about 1 cell/mL of peripheral blood), which makes their detection challenging (17).

FR is a glycoprotein with a strong specificity to tissue and tumor, which is mainly distributed on the surface of cell membrane. It is highly expressed on tumor cell surfaces, but is barely or lowly expressed on normal cell surfaces (18,19). In 2013, Yue *et al.* (20) proposed LT-PCR, which is a novel method for detecting CTCs by specifically recognizing FR on the cell membrane. LT-PCR is being widely applied in clinical practice.

Some studies have pointed out the clinical value of CTCS in the treatment of lung cancer (21,22). In the present study, we detected significantly different CTC levels in patients with benign or malignant SPNs and healthy volunteers. The level of CTCs was markedly higher in the malignant SPNs group (10.48±3.49 FU/3 mL) than in the benign SPNs group (6.38±0.53 FU/3 mL) and the control group (4.45±1.21 FU/3 mL) (P<0.001). Moreover, the level of CTCs was significantly higher in the benign SPNs group than in the control group (P=0.023). In particular, in the malignant SPNs group, patients older than 60 years (11.45±3.92 FU/3 mL) presented a significantly higher CTC level than other patients (9.55±2.74 FU/3 mL). Classified by pathological subtypes of lung cancer, there was a significant difference in the CTC level among patients with squamous cell carcinoma (9.10±1.94 FU/3 mL), adenocarcinoma (10.77±3.71 FU/3 mL), and adenosquamous cell carcinoma (11.78±2.61 FU/3 mL). Binary logistic regression analysis suggested that CTCs were an independent risk factor of malignant SPNs (OR =3.698, 95% CI: 1.136–11.035, P=0.030). Compared with single or combined serum tumor biomarkers, the sensitivity and specificity of CTCs in the diagnosis of malignant SPNs were significantly higher [sensitivity =89.1%, specificity =92.3%, AUC (95% CI) =0.907 (0.861–0.942)]. Taken together, our results were consistent with previously reported findings (23,24).

There were several limitations in this study that should be noted. Firstly, this was a single-center study with a small

sample size. Secondly, the bias in selecting participants may have influenced the results. A multi-center study with a large sample size is required in the future to validate our findings. Finally, this study is relatively limited. In the future, we will carry out researches on the prognosis evaluation, surgical effect evaluation, chemotherapy efficacy evaluation and targeted drug use guidance of CTCs in lung cancer to prove the clinical application value of CTCs.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at http://dx.doi.org/10.21037/atm-21-889

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-21-889

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-21-889). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. All participants signed the informed consent. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

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