ORIGINAL ARTICLE

Assessment of a Combined Panel of Six Serum Tumor Markers for Lung Cancer

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Abstract

Rationale: We have previously identified six serum tumor markers (TMs) (carcinoembryonic antigen, carbohydrate antigen 15.3, squamous cell carcinoma–associated antigen, cytokeratin-19 fragment, neuron-specific enolase, and pro–gastrin-releasing peptide) related to the presence of lung cancer (LC).

Objectives: To validate their individual performance in an independent cohort, and to explore if their combined assessment (≥1 abnormal TM value) is a more accurate marker for LC presence.

Methods: We determined these six TMs in 3,144 consecutive individuals referred to our institution by their primary care physician because of the clinical suspicion of LC.

Measurements and Main Results: LC was excluded in 1,316 individuals and confirmed in 1,828 patients (1,563 with non–small cell LC and 265 with small cell LC). This study validated the previously reported performance of each individual TM. We also showed that their combined assessment (≥1 abnormal TM) had a better sensitivity, specificity, negative predictive value, and positive predictive value (88.5, 82, 83.7, and 87.3%, respectively) than each TM considered individually and that it increased the diagnostic performance (area under the curve) of a clinical model that included tumor size, age, and smoking status. In patients with radiographic nodules less than 3 cm, the negative predictive value of the TM panel was 71.8%, hence providing some support for a more conservative diagnostic approach. Finally we identified two TMs (neuron-specific enolase and pro–gastrin-releasing peptide) that differentiate the risk of non–small cell LC from that of small cell LC.

Conclusions: The combined assessment of a panel of six serum TMs is a more accurate marker for LC presence than these same TMs considered individually. The potential of these TMs in the diagnostic and screening settings deserves further research.

Keywords: CA15.3; CEA; CYFRA 21-1; NSE; ProGRP

Lung cancer (LC) is the most frequent and fatal human cancer. With a worldwide prevalence of 12.3% and a global incidence of 1.2 million new cases per year, LC causes 1.1 million deaths annually in the world and is responsible for 17.8% of the total number of cancer deaths per year (1–3). The diagnosis of LC might be relatively straightforward in some patients but cumbersome in others (2, 4). At variance with other cancer types (5, 6), the assessment of circulating tumor markers (TMs) in the clinical management of patients with suspected LC is not currently recommended because of the lack of solid scientific evidence (3, 7). Likewise, the potential role of TM quantification in LC screening programs is unknown (1, 8–10).

We have previously shown that, in a cohort of 647 patients with LC and 155 subjects without it, six specific serum TMs (carcinoembryonic antigen [CEA], carbohydrate antigen 15.3 [CA15.3], squamous cell carcinoma–associated antigen [SCC], cytokeratin-19 fragment [CYFRA 21-1], neuron-specific enolase [NSE], and pro–gastrin-releasing peptide

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At a Glance Commentary

Scientific Knowledge on the Subject: The role of serum tumor markers (TMs) for the diagnosis or screening of lung cancer (LC) is unclear. In previous studies our group identified six serum TMs (carcinoembryonic antigen, carbohydrate antigen 15.3, squamous cell carcinoma–associated antigen, cytokeratin-19 fragment, neuron-specific enolase, and pro–gastrin-releasing peptide) that were related with the presence of LC and its pathological subtypes, non–small cell and small cell LC.

What This Study Adds to the Field: This study validates prospectively the individual performance of these six TMs in a larger (n = 3,144) independent cohort of individuals with clinically suspected LC and shows that their combined assessment (i.e., ⩾1 abnormal TM value) is a more accurate marker for LC presence than the serum TM considered individually.

[ProGRP]) were individually associated with LC presence and LC type (non–small cell [NSCLC] vs. small cell LC [SCLC]) (11, 12). In this study we sought to validate the individual performance of these six TMs in an independent and larger cohort of individuals (n = 3,144) referred to our center because of the clinical suspicion of LC and because in other cancer types the assessment of several (vs. single) TMs improves diagnosis performance (7), we tested prospectively the hypothesis that the combined assessment of these six TMs (i.e., the presence of ⩾1 abnormal TM values) will also improve it in patients with suspected LC. Some of the results of this study have been previously reported in abstract form (13).

Methods

Study Design and Participants

This observational study was performed in a real-life clinical practice setting. Figure 1 presents the consort diagram of the study. We prospectively included in the study 3,144 individuals consecutively referred, mostly by their primary care physician, to the Lung Cancer Diagnostic Unit of our institution, a tertiary university hospital, to exclude the presence of LC. Figure 1 presents a detailed list of the presenting symptoms prompting referral. Of note, none of these 3,144 individuals had been included in any of our previous analysis (11, 12). The diagnosis of LC was established (or excluded) using standard clinical workup procedures, which included fiberoptic bronchoscopy, computed tomography and positron emission tomography scanning, fine-needle transthoracic aspiration, endobronchial or esophageal ultrasound, and/or resectional surgery, among others, as indicated by experienced clinicians following international guidelines (2).

LC Histological Typing and Staging

LC histological types were classified according to the 1999 World Health Organization recommendations (14). The differential diagnosis between SCLC and NSCLC was based on the morphological characteristics plus a positive CD56 and/or synaptophysin immunohistochemistry of the tumor (15). LC staging (TNM) was established according to international recommendations (16).

TM Measurements

Blood samples were obtained by peripheral venipuncture in all participants before the final diagnosis had been established and any anticancer therapy had been initiated. Yet, because this was a real-life clinical investigation, some patients were receiving treatment for other common chronic conditions, including chronic obstructive pulmonary disease, cardiovascular diseases, and/or diabetes, among others. After centrifugation, serum TMs were quantified in less than 5 hours from sampling, except for ProGRP and SCC, which were quantified in less than 2 days (discussed later). The serum levels of CEA, CYFRA 21-1, CA15.3, and NSE were measured with a commercially available electrochemiluminiscent assay (Elecsys; Roche Diagnostics, Penzberg, Germany), and those of SCC and ProGRP with an Architect automated assay (Abbott Laboratories, Chicago, IL). According to our previously published results (11, 12), the following thresholds were considered as the upper limit of normality: CEA, 5 ng/ml; CYFRA 21-1, 3.3 ng/ml; SCC, 2 ng/ml; CA15.3, 35 U/ml; NSE, 25 ng/ml; and ProGRP, 50 pg/ml. Accordingly, any individual TM value above these values was considered abnormal. When these six TMs were assessed in combination, we considered abnormal the presence of greater than or equal to one abnormal TM values.

Statistical Analysis

Results are presented as number, proportion, median, and interquartile range as appropriate. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas. TM levels were compared using nonparametric (Wilcoxon, Mann-Whitney *U*, Kruskal-Wallis) or parametric tests (Student's *t* test), as appropriate. Proportions between groups were compared using the chi-square test. Receiver operating characteristic (ROC) curves were constructed using the DeLong model (17). A *P* value less than 0.05 was considered statistically significant.

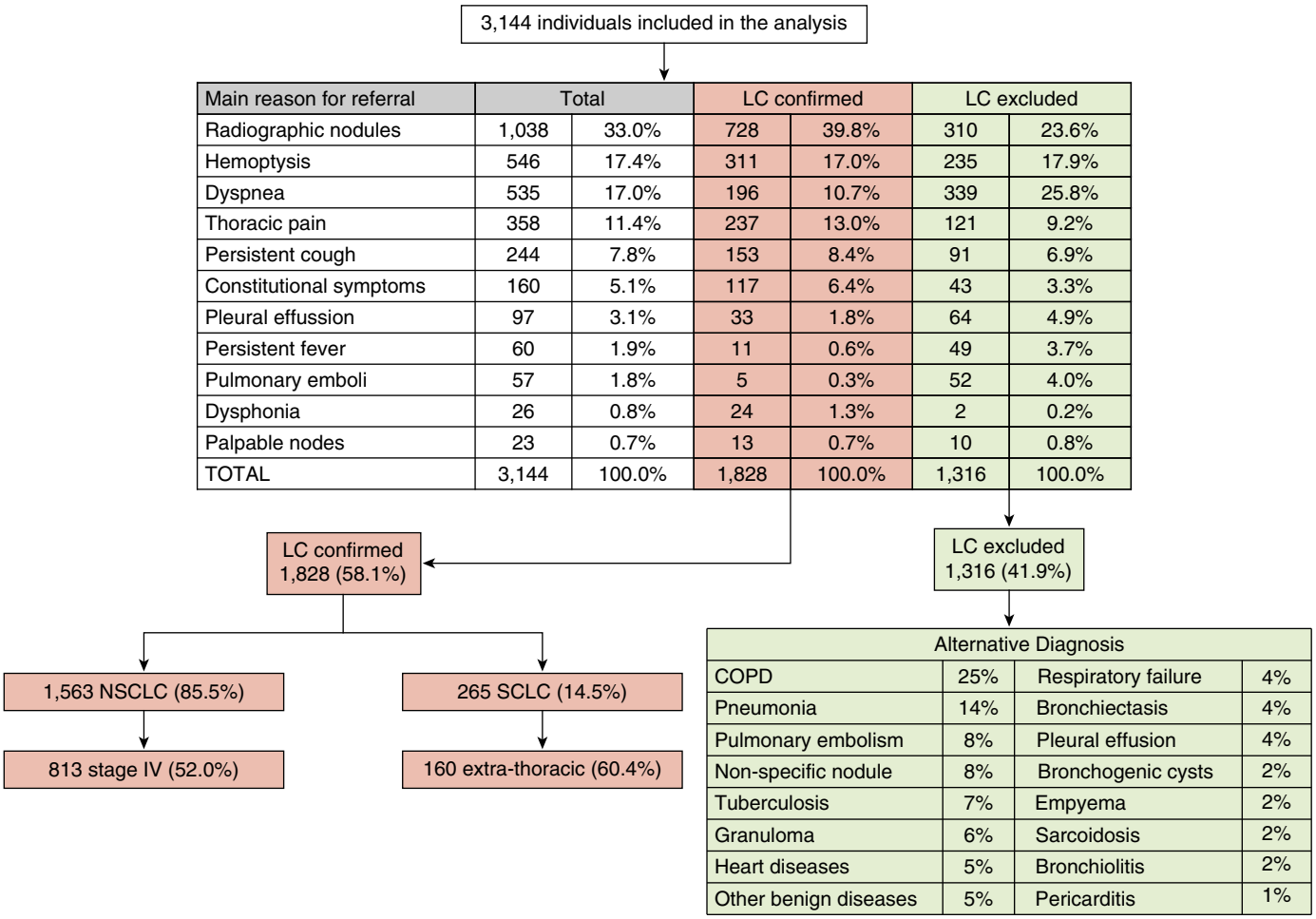
Results

Characterization of Participants

A diagnosis of LC was confirmed in 1,828 of the 3,144 individuals included in the study (58.1%) and excluded in 1,316 individuals (41.9%). Among the former, 1,563 had NSCLC (85.5%) and 265 SCLC (14.5%). Table 1 presents the main clinical characteristics of all participants, and Figure 1 the list of alternative diagnosis in patients without LC. Of note, patients with LC had a significantly higher smoking exposure history than those without it (Table 1).

LC versus No Cancer

Diagnostic performance of individual TM. Compared with patients without LC, the individual serum TM concentrations were significantly higher in patients with LC (Table 1) and the proportion of participants with abnormal individual TM values was remarkably low among those without LC but significantly higher in those with LC (Figure 2). Table 2 shows the proportion of false-positive results in patients without LC for each TM, stratified by the different final diagnosis categories. Finally, Table 3 presents the sensitivity, specificity, NPV,



Main reason for referral	Total		LC confirmed		LC excluded	
Radiographic nodules	1,038	33.0%	728	39.8%	310	23.6%
Hemoptysis	546	17.4%	311	17.0%	235	17.9%
Dyspnea	535	17.0%	196	10.7%	339	25.8%
Thoracic pain	358	11.4%	237	13.0%	121	9.2%
Persistent cough	244	7.8%	153	8.4%	91	6.9%
Constitutional symptoms	160	5.1%	117	6.4%	43	3.3%
Pleural effussion	97	3.1%	33	1.8%	64	4.9%
Persistent fever	60	1.9%	11	0.6%	49	3.7%
Pulmonary emboli	57	1.8%	5	0.3%	52	4.0%
Dysphonia	26	0.8%	24	1.3%	2	0.2%
Palpable nodes	23	0.7%	13	0.7%	10	0.8%
TOTAL	3,144	100.0%	1,828	100.0%	1,316	100.0%

Alternative Diagnosis			
COPD	25%	Respiratory failure	4%
Pneumonia	14%	Bronchiectasis	4%
Pulmonary embolism	8%	Pleural effusion	4%
Non-specific nodule	8%	Bronchogenic cysts	2%
Tuberculosis	7%	Empyema	2%
Granuloma	6%	Sarcoidosis	2%
Heart diseases	5%	Bronchiolitis	2%
Other benign diseases	5%	Pericarditis	1%

Figure 1. Clinical reasons that prompted referral and alternative diagnosis in patients in whom lung cancer was finally excluded. For further explanations, *see* text. COPD = chronic obstructive pulmonary disease; LC = lung cancer; NSCLC = non–small cell lung cancer; SCLC = small cell lung cancer.

and PPV of each individual TM. These values are very similar to those previously published by our group in other independent cohorts (11, 12), also included in Table 3 for easy comparison (numbers in parentheses).

Combined assessment of TM. When assessed in combination, 18% (235 of 1,316) of participants without LC had greater than or equal to one abnormal TM value. This proportion was significantly higher (88.5%) in patients with LC (87% in NSCLC and 97% in SCLC). The sensitivity, specificity, NPV, and PPV for LC of the combined TM assessment were 88.5, 82, 83.7, and 87.3%, respectively (Table 3).

False-positive results: sequential measurements. In a random subset of participants (n = 211) with greater than or equal to one abnormal TM value before a final diagnosis had been established, a second TM quantification was obtained 3–4 weeks later. The presence of LC was eventually confirmed in 89 of them and excluded in 122. In this second quantification, TM levels increased (by about 25%) in all patients with LC. By contrast, in participants without LC, TM values had returned to normal values in 87%, remained stable (±10% change) in 8%, and increased in the remaining 5%.

Relationship with nodule size. The prevalence and size of lung nodules were significantly higher in patients with LC than in those without it (Table 1). Table 4 shows that, within each nodule size category (<1, 1–3, and >3 cm) TM serum levels were most often significantly higher in patients with LC, and Figure 3 shows that, also within each nodule size category (<1, 1–3, and >3 cm), the proportion of participants with abnormal TM levels was higher in those with LC. Table 5 presents the sensitivity, specificity, PPV, and NPV of each individual TM stratified by nodule size. Of note, the sensitivity and NPV increased markedly when the six TM were assessed together (i.e., ⩾1 abnormal TM), as the proportion of participants with abnormal TM values did within each nodule size category (Figure 4). Finally, as shown in Table 5, the NPV of the combined TM assessment in patients with small nodules (<1 cm) was 91.4%, and in those with intermediate size nodules (1–3 cm) 60.8%. As shown in Tables E1 and E2 and Figures E1 and E2 in the online supplement, results were very similar when the population studied was dichotomized in two groups (⩽3 and >3 cm) rather than three.

Added diagnostic value of TM assessment. Several clinical characteristics including nodule size, age, and smoking status help clinicians to establish the risk of LC in a given patient (18). To investigate the added diagnostic value of the combined TM consideration on top of this clinical assessment, we compared the ROC curves

Table 1. Clinical Characteristics and Tumor Markers Values in All Participants

	No Cancer (*n* = 1,316)	*P* Values	Lung Cancer (*n* = 1,828)	NSCLC (*n* = 1,563)	*P* Values	SCLC (*n* = 265)
Females, %	37.5	<0.0001	21.4	22.3	<0.0001	16.6
Age, yr	68 (57–77)	NS	66 (58–75)	67 (58–75)	NS	65 (58–74)
Current smokers, %	34	<0.0001	53	51	<0.0001	64
Former smokers, %	31		35	36		31
Never smokers, %	35		12	13		5
Pack-years	12 (0–45)	<0.0001	40 (0–60)	40 (0–60)	<0.001	47 (15–70)
Presence of nodule	306 (23.2)	<0.0001	1,828 (100)	1,563 (100)	NS	265 (100)
Nodule size, mm	1.35 (0.8–2.5)	<0.0001	4.2 (2.9–5.4)	4.2 (2.8–5.5)	NS	4.3 (3.1–5.1)
CEA, ng/ml	2 (1.3–3.1)	<0.0001	6.2 (2.9 –23)	6.5 (3–23)*	0.018	4.6 (2.5–22.5)†
CYFRA 21-1, ng/ml	1.5 (1–2.1)	<0.0001	4 (2–9.7)	4.3 (2.1–10)*	0.0001	2.9 (1.9–7.3)*
SCC, ng/ml	0.6 (0.3–0.9)	NS	0.9 (0.5–1.7)	0.9 (0.5–1.9)*	0.0001	0.6 (0.4–1)
CA15.3, U/ml	15 (9–21)	<0.0001	21 (12–36)	21 (12.5–41)*	0.0001	17.5 (10–26)
NSE, ng/ml	11 (9–14)	<0.0001	14 (11–22)	13.7 (11–18)*	0.0001	67 (26.5–149)*
ProGRP, 50 pg/ml	21 (12–31)	<0.0001	24 (14–39)	22 (13–32)*	0.0001	333 (52–1,255)*

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; IQR = interquartile range; NS = nonsignificant; NSCLC = non–small cell lung cancer; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; SCLC = small cell lung cancer.
Data are given as n (%) or median (IQR) unless otherwise noted.
*$P < 0.0001$ versus no cancer.
†$P = 0.01$ versus no cancer.

derived from three different logistic regression models. The first one (Figure 5, *green curve*) included as predictors of LC presence nodule size (centimeters), age (years), and smoking status (current, former, ever smoker), as recommended (18). The second one (Figure 5, *blue curve*) was derived from considering the actual TM levels quantified in each participant (Table 1). The third one (Figure 5, *red curve*) included in the model all four predictors (nodule size, age, smoking status, and TM levels). The area under the curve (AUC) for the clinical model (*green curve*) was 0.85 (95% confidence interval, 0.83–0.88), for the TM model 0.89 (0.88–0.91; $P = 0.009$), and from the combination of both 0.93 (0.91–0.94; $P < 0.001$ vs. both previous models) (Figure 5).

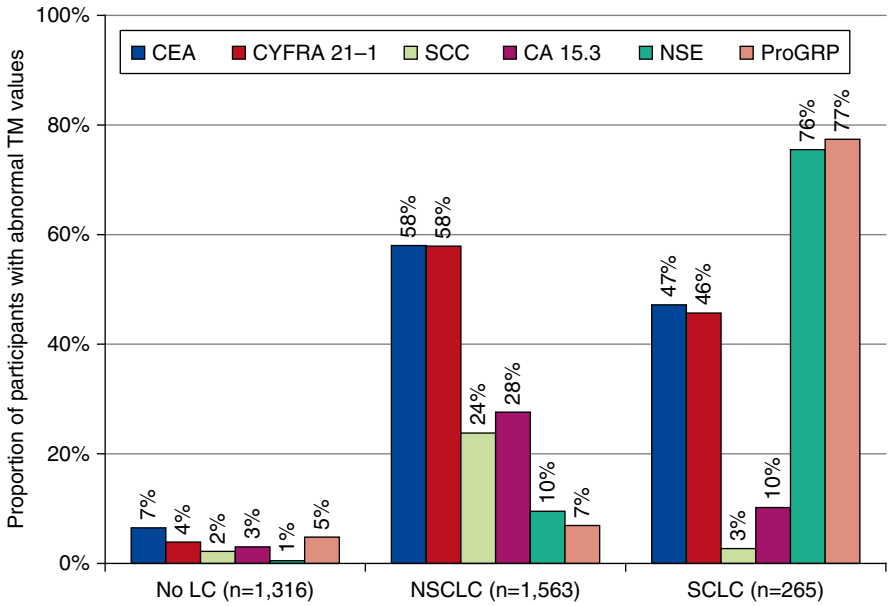


Figure 2. Proportion of participants with abnormal tumor marker values. For further explanations, *see* text. CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; LC = lung cancer; NSCLC = non–small cell lung cancer; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; SCLC = small cell lung cancer; TM = tumor marker.

TM Pattern in NSCLC and SCLC

NSCLC was characterized by statistically higher serum values (Table 1) and increased proportion of patients with abnormal values (Figure 2) of CEA, CYFRA 21-1, SCC, and CA15.3. The sensitivity of the combined TM assessment for the diagnosis of NSCLC was 87.1%, its specificity 82%, its NPV 84.3%, and its PPV 85.3%. Of the 1,563 with NSCLC, 758 (48.5%) had adenocarcinoma, 513 (33%) squamous cell carcinoma, 238 (15%) unspecific NSCLC, and 54 (3.5%) large cell LC. As shown in Table 6, adenocarcinomas were associated with significantly lower SCC and CYFRA 21-1 and higher CEA and CA15.3 serum levels than squamous carcinoma, whereas large cell LC had a similar TM pattern than adenocarcinoma albeit lower CEA serum levels ($P < 0.05$).

By contrast, SCLC (n = 265) was associated with significantly higher median serum values of NSE and ProGRP (Table 1) and a higher proportion of patients with abnormal values of these two specific TMs (Figure 2). Of note, abnormal SCC values were very unusual in SCLC. The sensitivity, specificity, NPV, and PPV of this combined TM assessment (i.e., ⩾1 abnormal TM value) for the diagnosis of SCLC were

Table 2. Rate of False-Positive Tumor Marker Values for Each Diagnostic Group in Participants in Whom Lung Cancer Was Excluded*

	Number of Patients	Percentage	False Positives (%)					
			CEA	CYFRA 21-1	SCC	CA15.3	NSE	ProGRP
COPD	334	25	10.8	1.8	1.2	2.4	0.6	4.2
Pneumonia	190	14	8.4	6.3	1.6	3.7	2.1	6.3
Pulmonary embolism	106	8	1.8	2.8	0.9	2.8	0	3.7
Nonspecific nodule	107	8	2.8	4.7	2.8	5.6	0	1.8
Tuberculosis	96	7	10.6	5.2	3.1	6.2	0	0
Granuloma	74	6	5.5	4.1	0	0	0	4.1
Heart diseases	70	5	1.4	5.7	4.3	1.4	0	11.4
Other benign diseases	70	5	2.8	0	4.3	0	0	2.8
Respiratory failure	54	4	5.6	7.4	5.6	1.9	0	11.2
Bronchiectasis	55	4	5.4	3.6	1.8	3.6	0	1.8
Pleural effusion	49	4	8.2	8.2	4	6.1	2	12.2
Bronchogenic cysts	30	2	0	3.3	0	0	0	0
Empyema	25	2	4	4	8	0	0	16
Sarcoidosis	22	2	0	0	4.5	9	0	0
Bronchiolitis	20	2	0	5	0	0	0	5
Pericarditis	14	1	0	0	7.1	0	0	0
Total	1,316	100	6.5	3.9	2.2	3.0	0.5	4.8

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; COPD = chronic obstructive pulmonary disease; CYFRA 21-1 = cytokeratin-19 fragment; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen.
*For further explanations, *see* text.

96.6, 82, 99.1, and 52.1%, respectively. ROC analysis (Figure 6) showed that NSE and ProGRP had the highest AUC (0.894 and 0.861, respectively) to discriminate between NSCLC and SCLC.

Relationship with LC Staging

Figure E3 presents the staging of LC at the time of diagnosis, both for NSCLC (Figure E3A) and SCLC (Figure E3C). The median serum concentration of TM increased ($P = 0.001$) in proportion to tumor stage in NSCLC for CEA, SCC, CYFRA 21-1, and CA15.3, and in SCLC for NSE and ProGRP (*see* Table E3). Similarly, the proportion of patients with at least one abnormal TM (i.e., combined assessment) increased in proportion to LC stage in NSCLC (*see* Figure E3B) but not in SCLC, which remained very high irrespectively of tumor stage (*see* Figure E3D).

In patients with early stages (I-II) of NSCLS, where surgery is expected to be of the highest therapeutic value, the combined TM assessment investigated here (i.e., ⩾1 abnormal TM value) had a sensitivity of 70.4%, specificity of 82%, NPV of 90.7%, and PPV of 52.9%. These values were similar to those associated with intrathoracic SCLC (94.3, 82, 99.4, and 29.6%, respectively).

Discussion

Our study explores the diagnostic performance of six serum TMs (CEA, CA15.3, SCC, CYFRA 21-1, NSE, and ProGRP), alone and in combination, in the largest cohort studied to date (n = 3,144) of individuals referred to exclude the

Table 3. Sensitivity, Specificity, NPV, and PPV of Each Individual TM Investigated and of Their Combined Assessment

	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Individual assessment				
CEA (<5 ng/ml)	56.5 (52.6)	93.5 (94.8)	60.7	92.3
CYFRA 21-1 (<3.3 ng/ml)	56.1 (55.3)	96.1 (93.9)	61.2	95.2
SCC (<2 ng/ml)	20.7 (15.6)	97.8 (95.9)	47	92.6
CA15.3 (<35 U/ml)	25.1 (33.9)	97 (96.6)	48.2	92.1
NSE (<25 ng/ml)	19.1 (25.1)	99.5 (98)	53.6	98
ProGRP (<50 pg/ml)	17.1 (32.1)	95.2 (98)	45.3	84
Combined assessment				
⩾1 abnormal TM value	88.5	82	83.7	87.3

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NPV = negative predictive value; NSE = neuron-specific enolase; PPV = positive predictive value; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; TM = tumor marker.
Proportions in parentheses in the sensitivity and specificity columns correspond to our previously published results in smaller independent cohorts (11, 12). Differences between the sensitivity and specificity of each individual TM and their combined assessment were statistically significant ($P < 0.001$).

Table 4. Tumor Marker Values Stratified by Nodule Size and Type (Benign vs. Cancer)

	Nodule Size <1 cm			Nodule Size 1–3 cm			Nodule Size >3 cm		
	Benign (*n* = 113)	*P* Value	Cancer (*n* = 16)	Benign (*n* = 140)	*P* Value	Cancer (*n* = 256)	Benign (*n* = 53)	*P* Value	Cancer (*n* = 583)
CEA, ng/ml	2.2 (1.4–3.4)	0.01	3.5 (2.3–8.8)	2.4 (1.5–3.4)	0.001	4.3 (2.5–8.7)	2 (1.2–3.1)	0.001	4.6 (2.4–10.9)
CYFRA 21-1, ng/ml	1.4 (1–2.1)	NS	1.6 (1.2–2.0)	1.5 (1–2.1)	0.001	2 (1.4–3)	1.2 (0.9–1.9)	0.01	3.8 (2–7.7)
SCC, ng/ml	0.6 (0.3–1)	NS	0.6 (0.5–0,7)	0.7 (0.4–1)	0.009	0.9 (0.5–1.4)	0.5 (0.3–0.9)	0.001	1 (0.6–2.2)
CA15.3, U/ml	15 (10–19)	0.044	15.5 (11.8–25)	17 (9–23)	0.001	17 (10–25)	15 (10–19)	0.001	19 (11–28.8)
NSE, ng/ml	12 (10–14)	NS	10 (9–11)	11 (9–13)	0.001	12 (10–15)	11 (8.5–14)	0.001	13 (11–19)
ProGRP, pg/ml	24 (13–31.5)	NS	30 (16.5–38.8)	23 (15–32)	0.001	25 (18–37)	19 (12–26)	0.01	23 (14–37)

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NS = nonsignificant; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen.
Data are median (interquartile range). To avoid the potential bias caused by the presence of metastasis, 973 patients (53.2%) with disseminated lung cancer (813 with stage IV non–small cell and 160 with extrathoracic small cell lung cancer [Figure 1]) were excluded from this specific analysis. For further explanations, *see* text.

presence of LC. Results validate our previous observations (11, 12) on the individual performance of these TM in a larger and independent cohort of patients (Table 3); show that their combined assessment (i.e., the presence of ⩾1 abnormal TM value) improves this performance significantly; indicate that the addition of this combined TM assessment to a traditional clinical model that includes tumor size, age, and cumulative smoking exposure increases the AUC for LC diagnosis; and identified two TMs (NSE and ProGRP) that differentiate NSCLC from SCLC.

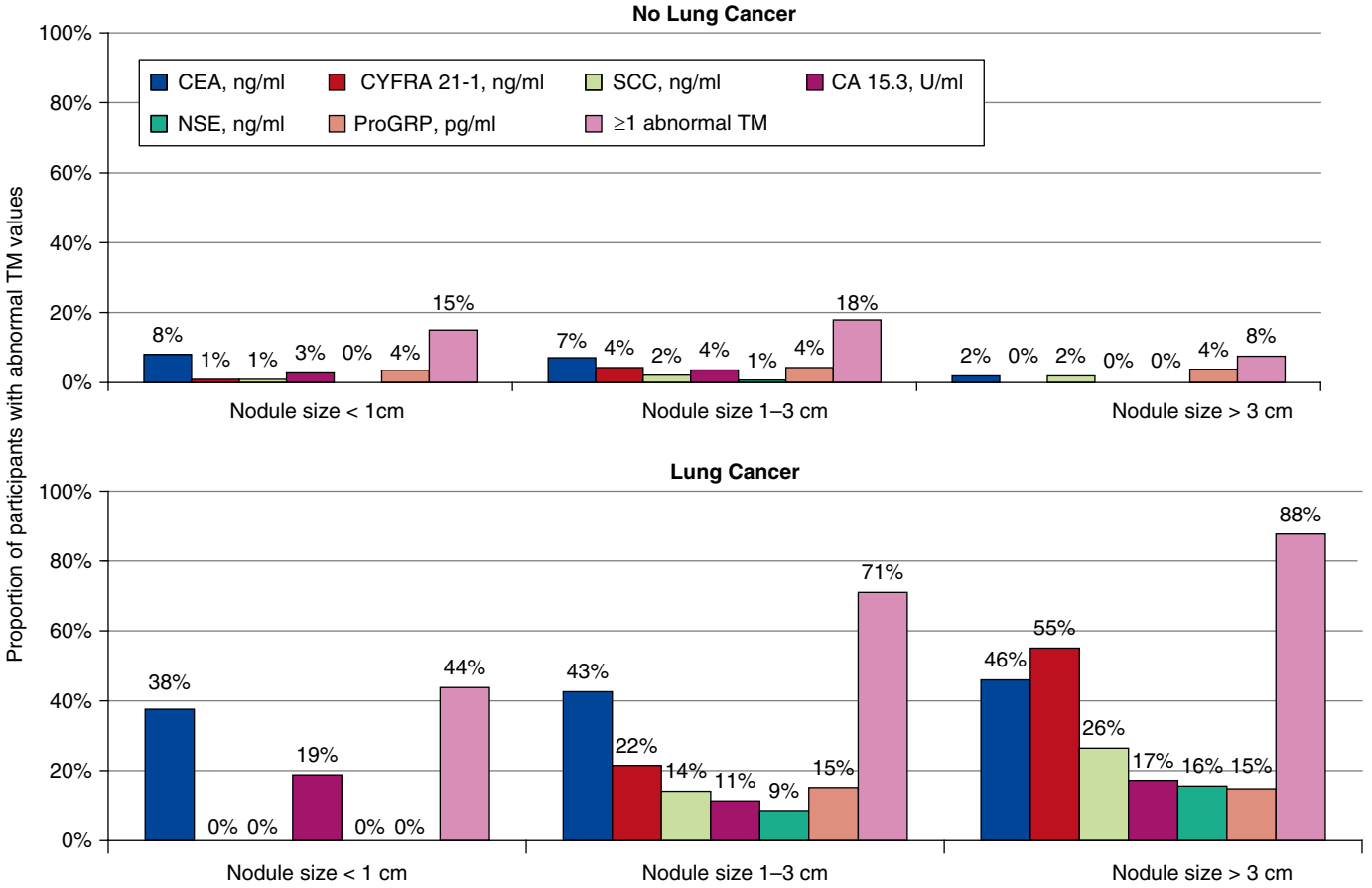


Figure 3. Proportion of participants (with and without lung cancer) with abnormal tumor marker values stratified by nodule size. For further explanations, *see* text. CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; TM = tumor marker.

Table 5. Sensitivity, Specificity, PPV, and NPV for Each Individual TM and of their Combined Assessment (⩾1 Abnormal TM), Stratified by Nodule Size

	Nodule Size <1 cm				Nodule Size 1–3 cm				Nodule Size >3 cm			
	SN	SP	PPV	NPV	SN	SP	PPV	NPV	SN	SP	PPV	NPV
Individual assessment												
CEA (<5 ng/ml)	37.6	92	40	91.2	42.6	92.9	91.6	47	46	98.1	99.6	14.2
CYFRA 21-1 (<3.3 ng/ml)	0	99.1	0	87.6	21.5	95.7	90.2	40	55.1	100	100	16.8
SCC (<2 ng/ml)	0	99.1	0	87.6	14.1	97.9	92.3	38.4	26.4	98.1	99.3	10.8
CA15.3 (<35 U/ml)	18.8	97.3	50	89.4	11.4	96.4	85.3	37.3	17.2	100	100	9.9
NSE (<25 ng/ml)	0	100	0	87.6	8.6	99.37	95.6	37.3	15.6	100	100	9.5
ProGRP (<50 pg/ml)	0	96.5	0	87.6	15.2	95.7	86.7	38.2	14.8	96.2	97.7	9.3
Combined assessment												
⩾1 abnormal TM	43.8	84.9	29.2	91.4	71.1	82.1	87.9	60.8	87.7	92.5	99.2	40.5

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NPV = negative predictive value; NSE = neuron-specific enolase; PPV = positive predictive value; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; SN = sensitivity; SP = specificity; TM = tumor marker.
Data are percentages. To avoid the potential bias caused by the presence of metastasis, patients with stage IV lung cancer were excluded from this analysis (813 non–small cell + 160 small cell lung cancer). For further explanations, *see* text.

Previous Studies

Many previous studies have investigated the utility of cancer antigens (CEA, CA15.3, SCC), proinflammatory cytokines (IL-6, tumor necrosis factor-α), growth factors (epidermal growth factor, vascular endothelial growth factor), hormones, proteases, and adhesion molecules, among others, in the diagnosis of LC (19–28). Most of them, however, included a relatively small number of patients with LC and yielded inconclusive results in patients with NSCLC, albeit NSE and ProGRP seemed more specific in patients with SCLC (7, 11, 12, 29–31). We previously showed in 647 patients with LC and 155 subjects without it, none of them included in the current study, that six TMs (CEA, CA15.3, SCC, CYFRA 21-1, NSE, and ProGRP) were significantly associated with the presence of LC (11, 12). Our current results confirm and, therefore, validate these previous observations.

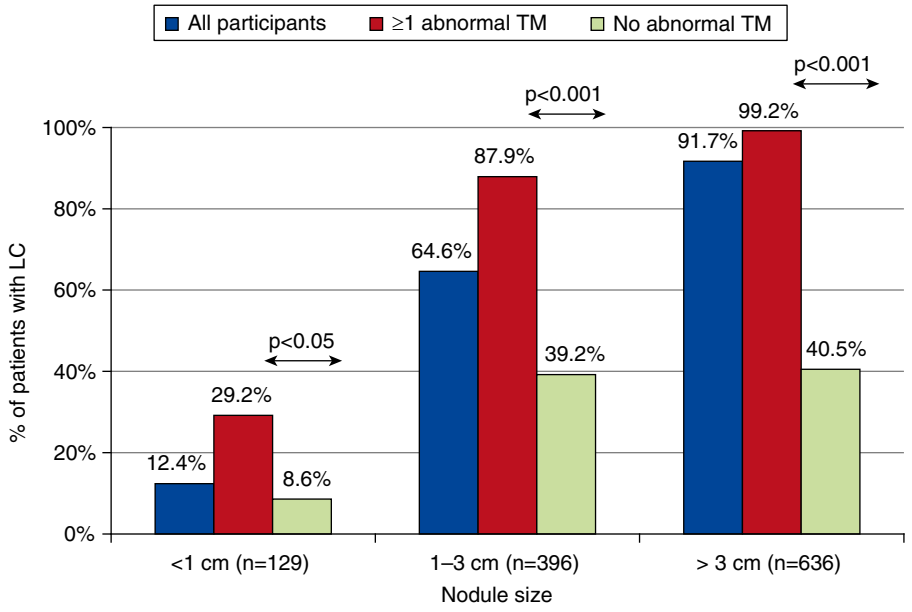


Figure 4. Proportion of participants with lung cancer (LC) by nodule size and presence/absence of abnormal tumor marker (TM) values. *P* values (chi-square test) correspond to the comparison between the *red* (⩾1 abnormal TM value) and *green* (no abnormal TM values) columns. For further explanations, *see* text.

However, in other cancer types it is well established that the assessment of a panel of several TMs, rather than a single one, improves diagnostic performance (7). In LC, Patz and coworkers (28) recently showed that the consideration of three TMs (CEA, α_1-antitrypsin, and SCC) combined with tumor size offered a sensitivity, specificity, PPV, and NPV of 80, 89, 89, and 81%, respectively, suggesting that this diagnostic strategy offered potential for the management of patients with pulmonary nodules. Our study develops this possibility further and investigates prospectively the added value of the combined assessment (i.e., ⩾1 abnormal value) of these six TMs (11, 12) on top of that of three variables (nodule size, cumulative smoking exposure, and age) often used in clinical practice to estimate the risk of LC in a given individual.

Interpretation of Findings

Participants without LC (true negatives and false positives). The circulating levels of the six TMs studied here were within the normal range in most participants without LC (Table 1, Figure 2). This indicates that the specificity of a normal TM panel (i.e., not a single abnormal TM value) to exclude the diagnosis of LC (i.e., true negatives) is quite high (82%). This is particularly relevant in patients with small (<1 cm) nodules, where the NPV of the combined TM panel was 91.4% (Table 5).

Conversely, 18% of participants without LC had greater than or equal to one

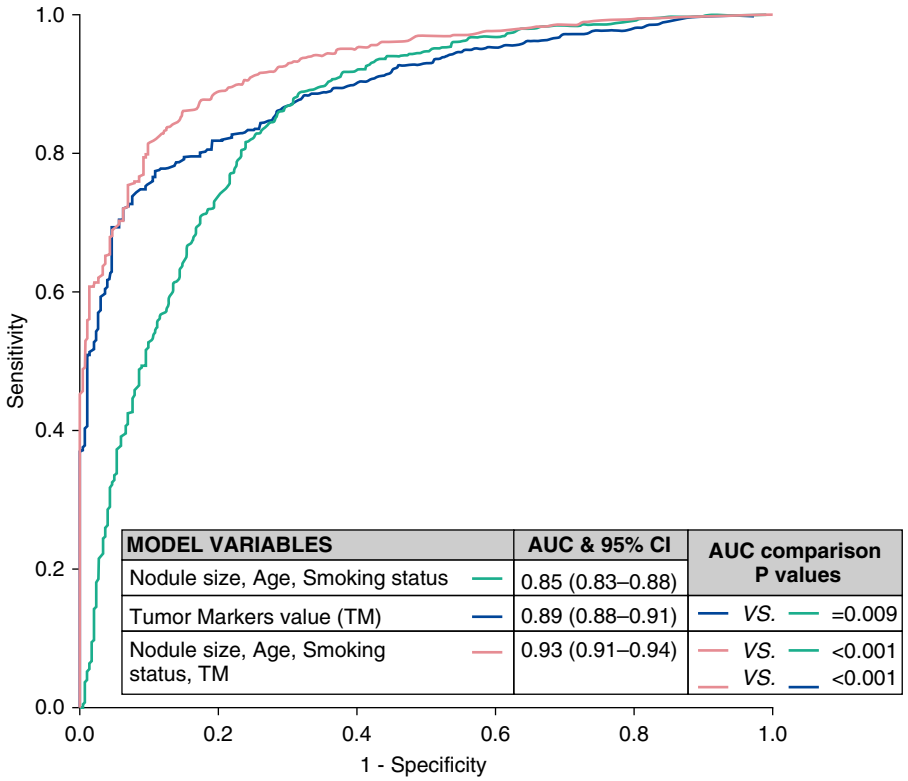


Figure 5. Receiver operating characteristic curves of a clinical model (tumor size, age, and cumulative smoking exposure), the tumor marker panel examined here, and the combination of both for the diagnosis of lung cancer. For further explanations, *see* text. AUC = area under the curve; CI = confidence interval; TM = tumor marker.

abnormal serum TM values (i.e., false positives). The proper interpretation of this observation requires consideration of the following aspects: (*1*) it is well known that CEA serum levels can increase in smokers, a high LC risk group, and that other TM can be elevated in kidney, skin, or liver diseases (12, 32, 33). Hence, it is important to consider these potential clinical confounders when assessing the risk of LC in a given individual. (*2*) As shown in Table 2, the proportion of false-positive results by individual TMs (and alternative diagnosis) was in most cases well below 5%. (*3*) We observed that abnormal TMs often revert to normal on repeat assessment, suggesting that sequential determination of TM over a short period of time (3–4 wk) might reduce false-positive results. This strategy deserves formal research but is in keeping with previous publications in other cancer types (7, 34).

Patients with LC (true positives and false negatives). The serum concentrations of the TM investigated here were most often abnormal in patients with LC (Table 1, Figure 2) and, when assessed in combination (i.e., ⩾1 abnormal TM value), they had a sensitivity, specificity, NPV, and PPV of 88.5, 82, 83.7, and 87.3%, respectively. It is important to put these figures in perspective by contrasting them with those offered by other TMs currently recommended by clinical guidelines for their routine use in other cancer types. α-Fetoprotein and human chorionic gonadotropin are now mandatory in the clinical management of patients with testicular cancer; yet, their sensitivity ranges between 20 and 60% and 10 and 40%, respectively (5, 35) (ours was 88.5%), and they are not specific for testicular cancer because α-fetoprotein can be false-positively increased in patients with liver disease (36) (our specificity was 82%). Similarly, CEA and CA125 are recommended for the clinical management of patients with colorectal or ovarian cancer; however, their sensitivities range from 15 to 80% and 40 to 80%, respectively, and CA125 values can be falsely elevated in some benign conditions, such as liver disease or endometriosis (5–7, 32, 36). These comparisons suggest that the use of the combined TM panel investigated here may have potential clinical utility, as discussed later.

Table 6. Tumor Marker Serum Levels in Patients with NSCLC by Cell Tumor Type

	Adenocarcinoma (*n* = 758)	*P* Value	Squamous Carcinoma (*n* = 513)	*P* Value	Large Cell Lung Cancer (*n* = 54)	*P* Value	Unspecific NSCLC (*n* = 238)
CEA, ng/ml	11 (4–45.6)*	<0.0001	4.2 (2.4–8.2)	NS	6 (2.5–21.3)*	NS	7.1 (3–27.3)
CYFRA 21-1, ng/ml	3.7 (1.9–8.9)	<0.0001	5.4 (2.6–12.6)	<0.0001	3.1 (2–8.8)	0.01	4.3 (2–9.6)
SCC, ng/ml	0.7 (0.4–1.1)	<0.0001	1.8 (0.8–4.8)	<0.0001	1 (0.5–1.4)	NS	0.8 (0.5–1.7)
CA15.3, U/ml	25 (14–58)	<0.0001	17 (9–26.6)	<0.05	24 (13–35)	NS	25 (15–50)
NSE, ng/ml	14 (11–19)	NS	13 (10.3–17.2)	NS	13 (10–17)	NS	13 (11–19.5)
ProGRP, pg/ml	23 (15–32)	NS	22 (10–32)	NS	20.8 (12–28)	NS	19 (12–31)

Definition of abbreviations: CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NS = not significant; NSCLC = non–small cell lung cancer NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen.
Data are median (interquartile range). *P* values correspond to the comparison of the columns to their right and left.
*$P < 0.05$ between the two marked values.

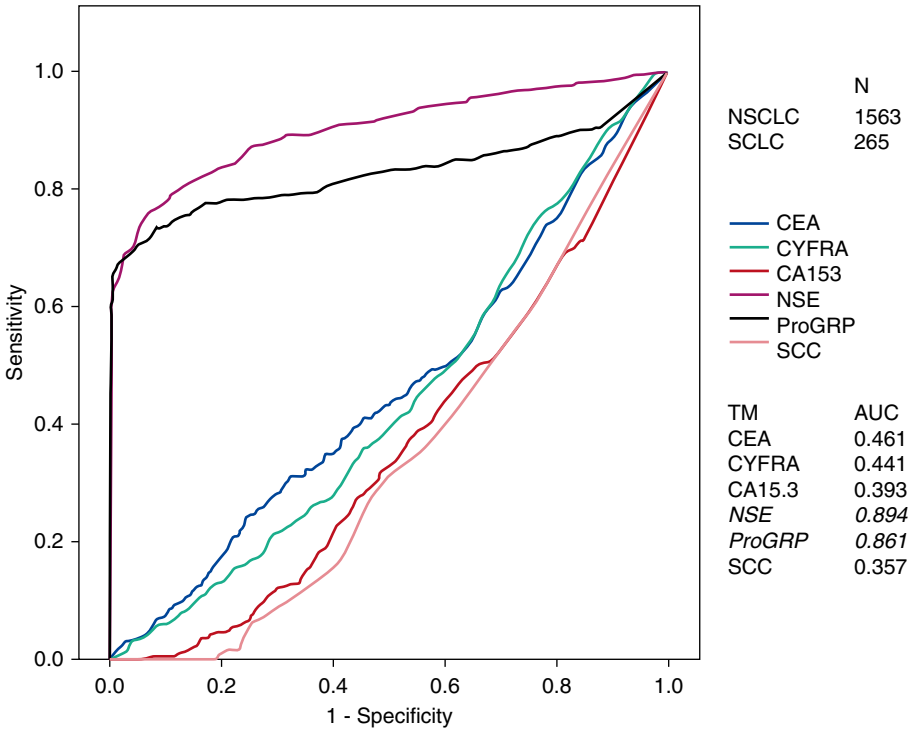


Figure 6. Individual receiver operating characteristic curves of the six tumor markers investigated here to discriminate non–small cell from small cell lung cancer. For further explanations, *see* text. AUC = area under the curve; CA15.3 = carbohydrate antigen 15.3; CEA = carcinoembryonic antigen; CYFRA 21-1 = cytokeratin-19 fragment; NSCLC = non–small cell lung cancer; NSE = neuron-specific enolase; ProGRP = pro–gastrin-releasing peptide; SCC = squamous cell carcinoma–associated antigen; SCLC = small cell lung cancer; TM = tumor marker.

Nodule size. Nodule size is a strong predictor of LC because lesions less than 1 cm have a very low pretest probability of being malignant, whereas those greater than 3 cm have a greater than 90% probability of being LC (18). Our results confirmed these previous observations because the prevalence and size of lung nodules were significantly higher in patients with LC (Table 1). Besides, they also showed that within each nodule size category individual TM serum levels (Table 4; *see* Table E1) and the percentage of participants with abnormal TM levels (Figure 3; *see* Figure E1) were higher in patients with LC; and the combined assessment of these TM (i.e., ⩾1 abnormal value) improved markedly the sensitivity, specificity, PPV, and NPV of each individual TM (Table 5; *see* Table E2). Besides, as shown in Figure 4 and Figure E2, the prevalence of LC was more than twofold higher ($P < 0.001$) in patients with greater than or equal to one abnormal TM (*red columns*) than in those with normal TM values (*green columns*). Finally, the high NPV (91.4%) of the combined TM assessment in patients with less than 1 cm nodules (Table 5; *see* Table E2) provides some support for a more conservative diagnostic approach (37). Yet, because it might stem from a relatively low prevalence of LC in this group of patients (12.4%, Table 4), this possibility requires prospective research, as discussed later.

TM pattern in different LC types. Our results show, for the first time to our knowledge, that the pattern of TM abnormalities is different in NSCLC (where CEA, CYFRA 21-1, SCC, and CA15.3 levels are particularly elevated) and SCLC (characterized by high NSE and ProGRP levels) (Table 1, Figure 4). Furthermore, ROC analysis identified NSE and ProGRP as the best TM for the discrimination of NSCLC and SCLC (Figure 4).

Clinical Implications

We envisage that the assessment of this TM panel can be clinically useful in two different scenarios: the diagnostic setting of patients with suspected LC and in LC screening programs. First, several TM are currently recommended for the management of cancer types other than LC, such as α-fetoprotein and human chorionic gonadotropin for testicular cancer (5, 35) or CEA and CA125 for colorectal or ovarian cancer (5–7, 32, 36). None of them aims at substituting the histologic diagnosis of cancer, but to provide additional information to the attending physician to better estimate the presence of cancer (5–7). Given that the sensitivity, specificity, PPV, and NPV of the TM panel studied here were less than 90%, we also propose that its main clinical utility is not to substitute the histologic diagnostic of cancer but to provide complementary information to the attending physician to better estimate the risk of LC presence and, thus, to select a more aggressive or conservative diagnostic strategy in the individual patient (37).

This added performance value is illustrated in Figure 5, which compares the AUC of a traditional clinical model that considers nodule size (centimeter), age (years), and smoking status (current, former, ever) (18) with that resulting from the combination of this clinical model and the assessment of the TM panel investigated here. The former is able to classify correctly 85% of patients in terms of LC presence (AUC, 0.85 [95% confidence interval, 0.83–0.88]), whereas the latter increased this proportion up to 93% (AUC, 0.93 [0.91–0.94]; $P < 0.001$). In practice, there are individuals in whom the risk of LC is clinically too high for a normal TM panel to dissuade the physician from pursuing an aggressive diagnostic workup. Likewise there are patients in whom the risk of LC is too low for a positive TM panel to precipitate further workup. However, in clinically doubtful cases (e.g., small radiographic nodules) or in those individuals at high-risk for invasive diagnostic procedures, the assessment of this TM panel can potentially support a more conservative (follow-up) or invasive diagnostic strategy (7, 34, 37).

Second, because our study was not a LC screening program among smokers (1, 8–10), results cannot be extrapolated directly to this scenario. However, we propose that the TM panel studied here deserves further research in this setting

because it compares favorably with the diagnostic performance offered by other TMs currently in use in other cancer screening programs, including prostate-specific antigen for prostate cancer (specificity, 25–45%; sensitivity, 50–90%) (7, 38, 39) or occult fecal blood for colon cancer (specificity, 20–75%; sensitivity, 30–60%) (6, 40). Furthermore, in individuals with small radiographic nodules (<1 cm), which are most often encountered in LC screening programs (1, 8–10), the NPV of our TM panel was quite high (91.4%). Finally, in this context it is also relevant to highlight that the sequential measurement of TM can be an effective strategy to reduce false-positive rates (7, 34).

Strengths and Limitations

The large sample size and the independent and real-life nature of our cohort are clear strengths of our study. It has, however, several limitations that deserve comment. First, it can be argued that most participants in whom LC was excluded had diseases (Table 2) that would not have been confused with LC. In real life, however, the diagnosis and/or exclusion of LC are not always straightforward. For instance, patients with LC can present initially without any radiographic abnormality (e.g., smoker with hemoptysis) or that, conversely, that smokers with radiographic abnormalities (e.g., former tuberculosis) end up not having LC. Second, the diagnostic strategy used in each participant was at the discretion of the treating physician and was not based on TM results. Hence, our results require formal validation in future interventional studies aimed at determining the risks and benefits of the steps the physician will take based on the result of the laboratory test by comparing a standard of care group with a TM panel guided group.

Conclusions

This study shows that the assessment of a panel of six serum TMs (CEA, CA15.3, SCC, CYFRA 21-1, NSE, and ProGRP) is a more accurate marker for LC presence as compared with individual TM. Further studies are needed to evaluate the potential of these TMs in the diagnostic and screening settings. ■

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