Identification of Circulating Tumor Cells Using 4-Color Fluorescence In Situ Hybridization: Validation of a Noninvasive Aid for Ruling Out Lung Cancer in Patients With Low-Dose Computed Tomography–Detected Lung Nodules

Ruth L. Katz, MD[1]; Tanweer M. Zaidi, MD[1]; Deep Pujara, MBBS[2]; Namita D. Shanbhag, MS[1]; Duy Truong, MS[1]; Shekhar Patil, MD[3]; Reza J. Mehran, MD[1,4]; Randa A. El-Zein, PhD[1,5]; Sanjay S. Shete, PhD[1,6]; and Joshua D. Kuban, MD[1,7]

BACKGROUND: Approximately one third of needle biopsies that are performed to rule out malignancy of indeterminate pulmonary nodules detected radiologically during lung cancer screening are negative, thus exposing cancer-free patients to risks of pneumothorax, bleeding, and infection. A noninvasive confirmatory tool (eg, liquid biopsy) is urgently needed in the lung cancer diagnosis setting to stratify patients who should receive biopsy versus those who should be monitored. **METHODS:** A novel antigen-independent, 4-color fluorescence in situ hybridization (FISH)-based method was developed to detect circulating tumor cells (CTCs) with abnormalities in gene copy numbers in mononuclear cell–enriched peripheral blood samples from patients with (n = 107) and without (n = 100) lung cancer. **RESULTS:** Identification of CTCs using FISH probes at 10q22.3/CEP10 and 3p22.1/3q29 detected lung cancer cases with 94.2% accuracy, 89% sensitivity, and 100% specificity compared with biopsy. **CONCLUSION:** The high accuracy of this liquid biopsy method suggests that it may be used as a noninvasive decision tool to reduce the frequency of unnecessary needle biopsy in patients with benign pulmonary lesions. ***Cancer Cytopathol* 2020;0:1-10**. *© 2020 American Cancer Society*.

KEY WORDS: CTC; FISH; indeterminate lung nodules; LDCT screening; lung cancer.

INTRODUCTION

In the United States, lung cancer accounts for 13% of all new cancer diagnoses, and >234,000 new lung cancer diagnoses are estimated in 2018.[1] Due in part to a lack of effective early detection methods, only 10% to 15% of lung cancers are discovered at an early stage, when surgery or nonsurgical alternatives (eg, stereotactic body radiation therapy) may result in a cure. Consequently, lung cancer has the highest mortality and morbidity rate of all malignancies.

Corresponding Author: Joshua D. Kuban, MD, 1515 Holcombe Boulevard, Unit 1471, Houston, TX 77030 (jdkuban@mdanderson.org).

[1]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas; [2]Department of Neurology, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston, Texas; [3]Department of Internal Medicine, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston, Texas; [4]Department of Thoracic and Cardiovascular Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas; [5]Department of Radiology, Houston Methodist Research Institute, Houston, Texas; [6]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas; [7]Department of Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, Texas

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Low-dose computed tomography (LDCT) scanning is currently one of the most sensitive methods for detecting pulmonary nodules. However, the very high sensitivity of LDCT is shadowed by its high false discovery rate. In the National Lung Screening Trial, "96.4% of the positive screening results and 94.5% in the radiography group were false positive results."[2] Widespread incorporation of LDCT into lung cancer screening protocols has therefore resulted in dramatic increases in nodule detection in adult patients in recent years, without a corresponding increase in lung cancer incidence.[2,3]

Diagnostic decision tools and algorithms are used in conjunction with LDCT results to stratify patient risk. Approximately 80% of patients with positive LDCT results are determined to be at intermediate risk of lung cancer, thus requiring follow-up to rule out malignancy.[4] Of patients in this category, more than one third are not diagnosed with lung cancer, resulting in unnecessary exposure to biopsy-related risks of pneumothorax and bleeding.[5] For low-risk patients, repeated LDCT scans are indicated for long-term follow-up. Collectively, these data highlight the need for a noninvasive tool for ruling out malignancy of pulmonary nodules in patients with positive LDCT results and for conducting follow-up in low-risk patients who have positive LDCT results.

Quantitation of circulating tumor cells (CTCs) is useful in prognosis and monitoring of advanced breast, colon, and prostate cancer[6] as well as prediction of survival in advanced non–small cell lung cancer (NSCLC).[7] CTCs in patients with NSCLC represent malignant cells that continuously extravasate into the bloodstream from the primary tumor or its metastases; they are also the sources of metastases to different organs and are usually rare in the peripheral blood.

To date, detection of CTCs has relied on methodologies broadly classified as antigen-dependent and -independent. The only US Food & Drug Administration–approved test for quantitating CTCs, which is based on epithelial cell adhesion molecule (EpCAM) expression, is not approved for detection of early-stage lung cancer.[6,8] Indeed, most CTCs that extravasate into the bloodstream undergo epithelial–mesenchymal transition and are undetectable by this test, as these cells lose EpCAM expression.[6,7] Despite the loss of epithelial marker expression in this CTC population, "sentinel" CTCs were detected by isolation by size of epithelial tumor cells in the blood of patients with nodule-negative LDCT scans 1 to 4 years before the appearance of malignant lung nodules,[9] suggesting that CTCs may be an early marker of lung cancer. To validate use of these CTCs as indicators of lung cancer, however, markers independent of epithelial–mesenchymal transition are needed to distinguish these CTCs from the plethora of leukocytes in the blood.

Previously, we developed an antigen-independent assay to detect a subset of CTCs in blood samples from patients with NSCLC, based solely on copy number variation or aneuploidy of chromosomal regions in individual nuclei.[10] Using comparative genomic hybridization with resected NSCLC samples, we discovered multiple genes that were either deleted or amplified in both squamous cell carcinomas and adenocarcinomas.[11] For the purposes of developing a DNA probe set to detect these chromosomal aberrations, we selected 2 genes that were consistently deleted in both adenocarcinoma and squamous carcinoma, as shown by complementary DNA comparative genomic hybridization and fluorescence in situ hybridization (FISH) performed on NSCLC specimens.[11] We selected genes for surfactant proteins A1 and A2 (SP-A1 and SP-A2) based on the role of these proteins in lung physiology and the innate host defense, as well as GC20/Sui1 at 3p22.1, a general monitor of translational accuracy of proteins, the expression of which is induced by cellular stress. Both genes were previously implicated in the early pathogenesis of lung cancer.[12-14]

Subsequently, using FISH of touch imprints of NSCLC and adjacent normal bronchial cells, we confirmed that SP-A1 and SP-A2, as well as the genes at 3p22.1, were deleted in most primary NSCLCs.[15] Furthermore, we demonstrated a field effect of these deletions ipsilateral to the primary NSCLCs and deletions of 3p22.1 and 10q22.3 in multiple tissues: within normal bronchial cells adjacent to tumors, within the tumors, and within morphologically normal bronchial cells in bronchial brushes[16,17] and in sputum samples from patients with NSCLC.[18] Using 2 separate 2-color FISH probe sets (3p22.1/centromere enumeration probe [CEP3] and 10q22.3/centromere enumeration probe 10 [CEP10]) to analyze a case-control series of blood samples from patients with early-stage or advanced NSCLC, we then showed that the genes located within 3p22.1 (containing eukaryotic translation initiation factor 1B/*GC20*], *RPL14*, *CD39A*, and PMGB) and 10q22.3 (containing SP-A1 and SP-A2) were more frequently

deleted in peripheral blood mononuclear cells (PBMCs) in NSCLC patients than in controls.[10]

Collectively, these data suggest that polysomy or gains of 2 or more FISH probes for 3p22.1/3q29(196F4) and 10q22.3/CEP10 in a single interphase nucleus marks a type of CTC, the presence of which may serve as an indicator of lung cancer. Therefore, using criteria similar to those for a FISH-based assay used to detect malignant cells in urine (UroVysion, Abbott Laboratories),[19] we developed a more convenient and robust assay for detecting these cytogenetic changes, comprising a single cocktail of these 4 FISH probes. We hypothesized that the single 4-color probe cocktail would detect more copy number abnormalities per nucleus than the 2 separate dual-color DNA probe sets for 3p22.1/CEP3 and 10q22.3/CEP10 in PBMCs in NSCLC.[10] The purpose of the present study was to test the accuracy of this "liquid biopsy" in detection of lung cancer compared with the reference standard of needle biopsy.

MATERIALS AND METHODS

Study Population

All participants provided written informed consent, and study procedures were performed in accordance with the Declaration of Helsinki and approved by the MD Anderson institutional review board.

Participants with lung cancer were recruited from the University of Texas MD Anderson Cancer Center and were eligible for the study if they 1) did not have a recent history of cancer at other sites and 2) underwent a core needle biopsy, fine needle aspiration (FNA), or surgical excision of the suspicious lesion confirming the presence of lung cancer following blood collection. Patients without lung cancer (controls) were eligible if they were at high risk for lung cancer based on their age and smoking history and were self-declared to be lung cancer free. The majority of the controls were matched for age, sex, ethnicity, and smoking status and were recruited from Kelsey-Seybold clinics (a multispecialty physician group) as part of an ongoing molecular epidemiologic study for lung cancer[20] (n = 26) or self-referred through social media because of concern for lung cancer based on age, tobacco use, and/or family history (n = 54). An additional subset of controls comprised patients with indeterminate nodules >6 mm detected by LDCT who were referred to the Department of Interventional Radiology at MD Anderson Cancer Center before their tissue diagnosis but whose lesions demonstrated benign pathology on FNA and/or core biopsy (n = 20) after blood collection.

The first 118 subjects selected between November 2007 and March 2016 served as the discovery (or derivation) cohort for enumeration of CTC and genetic abnormalities. Results for the discovery cohort were then validated in 89 additional patients (ie, the validation cohort).

Data Collection

All patients completed a demographic questionnaire, which included questions about history of smoking and cancer.

As part of routine care, surgical biopsy and FNA samples were subjected to pathological examination. Lung masses were classified according to the World Health Organization classification for lung tumors and staged according to the TNM classification of the Union for International Cancer Control with the American Joint Committee on Cancer 8th Edition Cancer Staging System and the International Staging System for Lung Cancer. Based on histological findings, lung cancer cases were classified according to histologic subtype.

Sample Preparation and Probe Hybridization

FISH was performed using PBMC isolation from fresh blood collected in EDTA tubes (Fig. 1). Blood samples were enriched using Ficoll-Hypaque density medium as described previously.[10] Following centrifugation, interface cells (buffy coat) were collected and counted, and the concentration was adjusted so that 40,000 PBMCs in 100 μL were deposited as cytospins, spray-fixed with alcohol, and stored at −20°C.

A 4-color, custom-made FISH probe set from Cytocell was produced under a material transfer agreement for MD Anderson Cancer center and used to hybridize blood samples overnight. The set consisted of 3q29 (196F4) (Spectrum Green), the locus-specific identifier 3p22.1 (Spectrum Red), CEP10 (Spectrum Aqua), and the locus-specific identifier 10q22.3 (Spectrum Gold). After hybridization, slides were dried, and 10 μL of 4′,6-diamidino-2-phenylindole (DAPI; Boehringer Mannheim) was added to the target

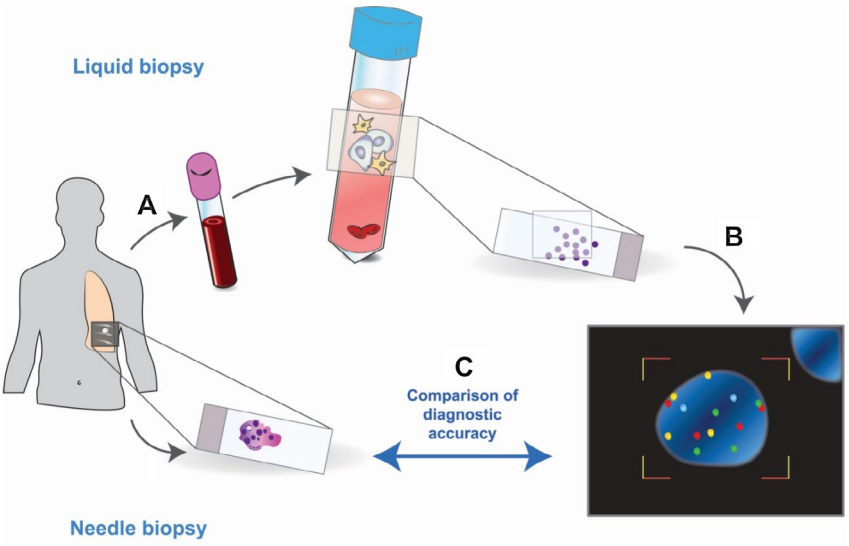

FIGURE 1. Schematic overview of the process by which the study was executed. (A) Blood from patients with indeterminate nodules was processed using Ficoll density separation. (B) Peripheral blood mononuclear cells were deposited on glass slides and probed using a custom 4-color fluorescence in situ hybridization assay. (C) Results from the assay were compared with those from fine needle aspiration biopsy, core biopsy and surgical resection.

area and scanned digitally using a Duet-3 instrument (BioView).

Definition of a CTC

A CTC was defined as an intact round or oval cell, having polysomy of at least 2 out of 4 DNA probes per nucleus (Supporting Fig. 1). This definition was used to quantify the sensitivity and specificity of FISH-based CTC detection in lung cancer detection. To qualify for quantitation, slides had to have at least 85% of interphase nuclei with optimum hybridization in the target area. Two experienced FISH technologists who were blinded to participants' cohort assignments analyzed each slide using BioView software optimized to display only mononuclear cells with a diameter greater than that of the average lymphocyte within the cytospin. Five hundred oval to round, intact, nonoverlapping cells with excellent hybridization signals were analyzed. Loss and/or gain for CEP10 and subtelomeric 3q29 (1964F) probes were used as internal control probes.

Use of Spike in Controls

To validate the system, we used PBMCs from healthy controls and different concentrations of A549 lung cancer cells spiked into PBMCs at 1% and 10%, and then undiluted, and quantitated these via FISH using the same 4-color probe set and using the same criteria for signal scoring and cell selection, as described in the Supporting Information.

Statistical Analyses

All statistical analyses were performed using STATA 14 (StataCorp). All P values were 2-sided, and $P < 0.05$ was considered statistically significant.

Categorical variables were assessed using the Pearson chi-square test or Fisher exact test where appropriate. Results for categorical data are presented as medians and interquartile ranges or as counts and percentages. Continuous variables were analyzed using the Student t test or Wilcoxon rank-sum test where appropriate and are presented as means and standard deviations.

Survival estimates for participants with lung cancer were produced using the product-limit (Kaplan-Meier) method using CTC result (positive [≥3 CTC] vs negative [<3 CTC]) as the subgroup.

The CTC threshold for differentiating a positive or negative result was selected iteratively using the area under the receiver operating characteristic (ROC) curve in multiple subgroups. Sensitivity, specificity, and accuracy of

the CTC threshold for detection of lung cancer were analyzed first for the discovery cohort and were then verified in the validation cohort.

RESULTS

Results of Spike in Experiments

Following 4-color FISH quantitation, actual tumor cell recovery versus expected tumor cell recovery were similar for spiked A549 lung cancer cells at both the 1% and 10% levels. Unspiked PBMCs and undiluted A549 cells also yielded expected results with close to 100% demonstration of aneuploidy for 4-color FISH in the latter, and no demonstration of CTCs in the former (Supporting Table 1).

Participant Demographics

A total of 207 patients (107 lung cancer cases and 100 controls) were recruited from the University of Texas MD Anderson Cancer Center. The discovery cohort enrolled 118 participants (61 cases and 57 controls), and the validation cohort enrolled 89 patients (46 cases and 43 controls).

Most lung cancer cases were early stage (67% were stage I or II). The predominant histological subtype of NSCLC was adenocarcinoma (Table 1). Compared with controls, lung cancer cases had significantly more smoking pack-years ($P < .001$).

CTC Characteristics in the Study Population

Compared with patients in the validation cohort, patients in the discovery cohort had significantly fewer 3q29 deletions ($P < .001$), 3p22.1 gains ($P < .01$), and CEP10 gains ($P < .001$) (Supporting Table 2). The median (interquartile range) CTC count per 500 PBMCs counted was 4 (3-5) for all NSCLC patients compared with 0 (0-1) for the controls, 4 (3-5) for the patients with stage I disease, 7 (4-8) for those with stage II disease, 4 (4-6) for those with stage III disease, and 5 (4-5) for those with stage IV disease (Supporting Table 2). The median total abnormalities per 500 PBMCs counted were significantly different between all NSCLC patients compared with controls (Supporting Table 2). There were no significant differences in the distribution of CTC or other cellular abnormalities according to histological subtype or NSCLC stage (Supporting Fig. 2A, Supporting Table 2).

Developing, Refining, and Validating the Assay

For the confirmation of detection of CTC using the 4-probe cocktail, PBMC samples from an individual found to have indeterminate nodules by LDCT (Fig. 2) were analyzed and representative image results show the positive identification of CTC based on polysomy of DNA probes by FISH (Fig. 2A-G). For the quantification of single nuclei positive FISH results, single channel images were scored showing the independent probe results for quantitation of 3p22.1/3q29 and 10q22.3/CEP10 within a single nucleus (Fig. 2B-G, arrows). Subsequent FNA biopsy results combined with immunohistochemistry (Fig. 2H-M) from this case confirmed cells of neuroendocrine tumor within the mass identified in the LDCT (Fig. 2).

ROC curves were used to refine the definition of a positive test result (ie, the threshold CTC count). In the discovery cohort, using ≥3 CTC as the definition for a positive result produced 95.1% sensitivity, 100% specificity, and 97.5% accuracy compared with biopsy results (Fig. 3A). In the validation cohort, the same threshold produced lower sensitivity (80%), the same specificity (100%), and 89.9% accuracy (Fig. 3B). In the overall cohort, the threshold detected lung cancer with 88.8% sensitivity, 100% specificity, and 94.2% accuracy (Fig. 3C). Based on the results of the ROC analysis, a CTC-positive blood sample was defined as having at least 3 CTCs; fewer than 3 CTCs was considered a negative result.

In addition to CTC quantity, total number of abnormalities (gains plus deletions) shows promise as a biomarker. CTC count showed positive correlation with total number of abnormalities (Supporting Fig. 2B). In the overall cohort, when counts of copy number abnormalities were ≥19, a sensitivity of 78.5% and a specificity of 88% were achieved with an area under the ROC curve of 0.9179 (Supporting Fig. 2C).

Prognostic Utility of CTC Detection for Lung Cancer

To determine whether CTC number can predict prognosis for lung cancer, survival in lung cancer cases was compared by CTC result (Supporting Fig. 2D). Follow-up data were available for 95 lung cancer patients and 1 control enrolled in the study. After dichotomizing based on the CTC threshold of 3, survival did not

TABLE 1. Participant Demographics

Characteristic	Controls (n = 100)	Patients With Lung Cancer (n = 107)	Cancer Stage				Discovery Cohort (n = 118)	Validation Cohort (n = 89)
			I[a] (n = 55)	II (n = 10)	III (n = 18)	IV (n = 17)		
Age, y, mean (SD)	65.11 (9.51)	66.45 (10.41)	65.93 (11.23)	66.90 (5.24)	68.00 (8.47)	64.53 (10.11)	64.34 (9.88)	67.74 (9.84)*
Sex, n (%)								
Men	47 (47)	47 (44)	22 (40)	5 (50)	9 (50)	6 (35)	52 (46)	42 (47)
Women	53 (53)	60 (56)	33 (60)	5 (50)	9 (50)	11 (65)	66 (54)	47 (53)
Race, n (%)								
White	53 (72)	84 (79)**	45 (82)	7 (70)	15 (83)	12 (71)	65 (71)	72 (81)
Black	6 (8)	11 (10)**	5 (9)	1 (10)	1 (6)	2 (12)	9 (10)	8 (9)
Hispanic	1 (1)	8 (7)**	5 (9)	2 (20)	1 (6)	0 (0)	7 (8)	2 (2)
Asian	14 (19)	4 (4)**	0 (0)	0 (0)	1 (6)	3 (18)	11 (12)	7 (8)
Histology, n (%)								
Adenocarcinoma	NA	65 (61)	33 (60)	6 (60)	9 (50)	13 (76)	38 (62)	27 (59)
Squamous cell carcinoma	NA	20 (19)	8 (15)	4 (40)	5 (28)	1 (6)	11 (18)	9 (20)
Other[b]	NA	22 (21)	14 (25)	0 (0)	4 (22)	3 (18)	12 (20)	10 (22)
Ever-smokers, n (%)	61 (67)	84 (79)	42 (76)	9 (90)	14 (78)	12 (71)	76 (70)	69 (78)
No. of pack-years, mean (SD)	20.00 (23.88)	37.22 (31.05)***	30.66 (28.72)	50.25 (14.79)	39.03 (31.67)	36.81 (29.09)	26.76 (27.42)	34.42 (31.46)
Deaths, n (%)	NA	22 (21)	4 (7)	3 (30)	11 (61)	4 (24)***	18 (15)	4 (4)*

Abbreviations: NA, not applicable; SD, standard deviation.

[a]Lung cancer stage could not be ascertained in 7 cases.

[b]Includes neuroendocrine carcinoma of the lung (n = 9), small cell lung cancer (n = 4), non–small cell lung cancer not otherwise specified (n = 4), well-differentiated neuroendocrine tumor of the lung (carcinoid tumor; n = 2), sarcomatoid lung cancer (n = 2), and adenosquamous carcinoma of the lung (n = 1).

*$P < .05$;

**$P < .01$;

***$P < .001$.

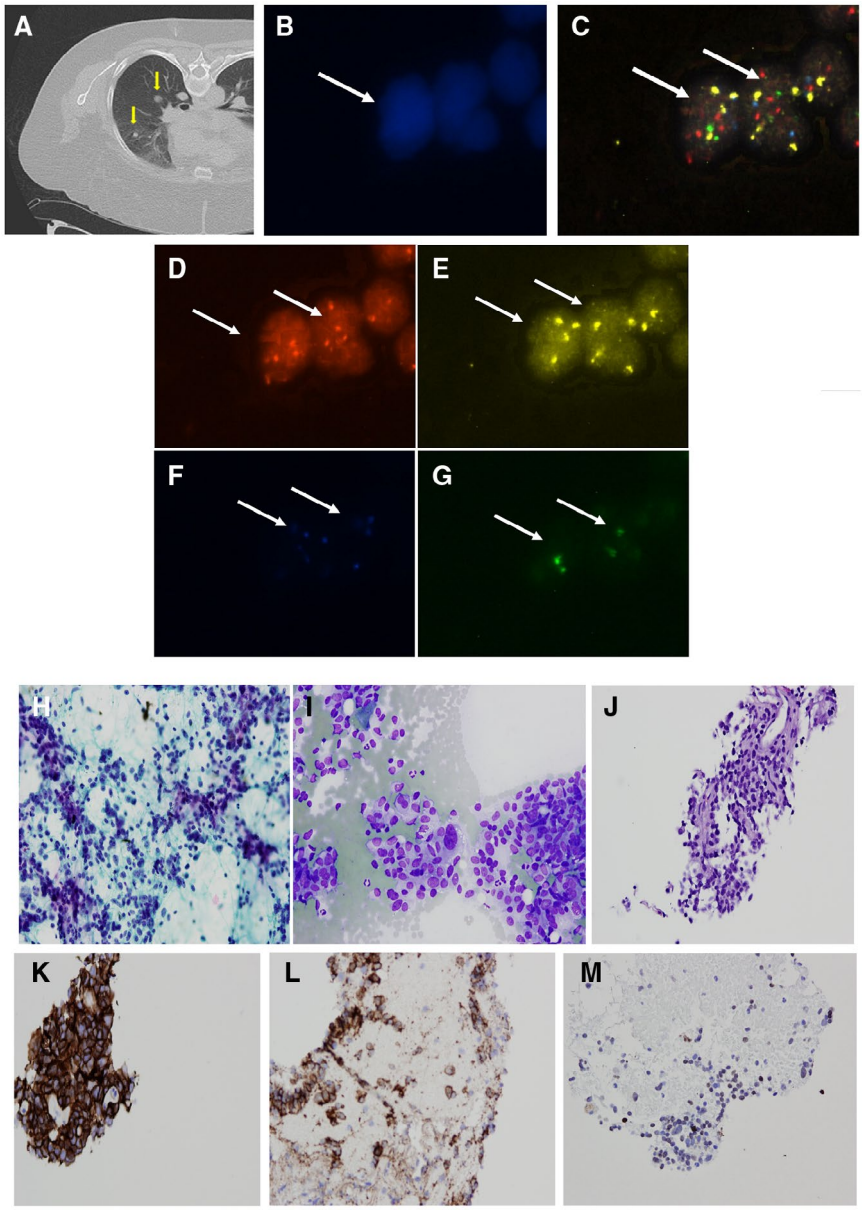

FIGURE 2. Representative positive case showing clinical workflow from computed tomography scan to nodule biopsy. (A) Low-dose computed tomography image of multiple noncalcified lung nodules in a 55-year-old nonsmoking woman that were suspicious for fungal disease versus malignancy. (B-G) Two of 8 circulating tumor cells (CTCs) that were detected in the patient's peripheral blood prior to biopsy are displayed as merged and individual fluorescent channels. (B) Cells with 4′,6-diamidino-2-phenylindole (DAPI) stain (original magnification ×100). (C) Merged images of CTCs showing polysomy/gain of 3p22.1 (red) and polysomy/gain of 10q22.3 (gold), whereas CEP10 (aqua, 2 copies) and 3q29 (green, 2 copies) are diploid; genetic abnormalities were identified using a 4-color cocktail of FISH probes on a BioView Duet-3 instrument (original magnification ×400). (D) Three red signals consistent with 3 copies of 3p22.1 (arrows). (E) Three gold signals consistent with 3 copies of 10q22.3 (arrows). (F) Two aqua signals representing 2 copies of CEP10 (arrows). (G) Two green signals representing 2 copies of 3q29 (arrows). (H-M) Pathology from a fine needle aspiration (FNA) biopsy and cell block of 1 of the indeterminate nodules showed a low-grade neuroendocrine tumor of the lung. (H) Papanicolaou stain (original magnification ×200). (I) Diff-Quik stain (original magnification ×200). (J) Hematoxylin and eosin stain (original magnification ×100). (K, L) IHC showed strong membranous staining for CD56 on cell block and FNA (original magnification ×200). (M) Focal nuclear staining for TTF1 (original magnification ×200).

differ significantly between the 2 groups ($P = .2526$), although we observed a trend of decreased survival over time in patients with at least 3 CTCs. Of 75 patients for whom follow-up data regarding recurrence were available, 13 had lung cancer recurrence, all of whom had at least 3 CTCs.

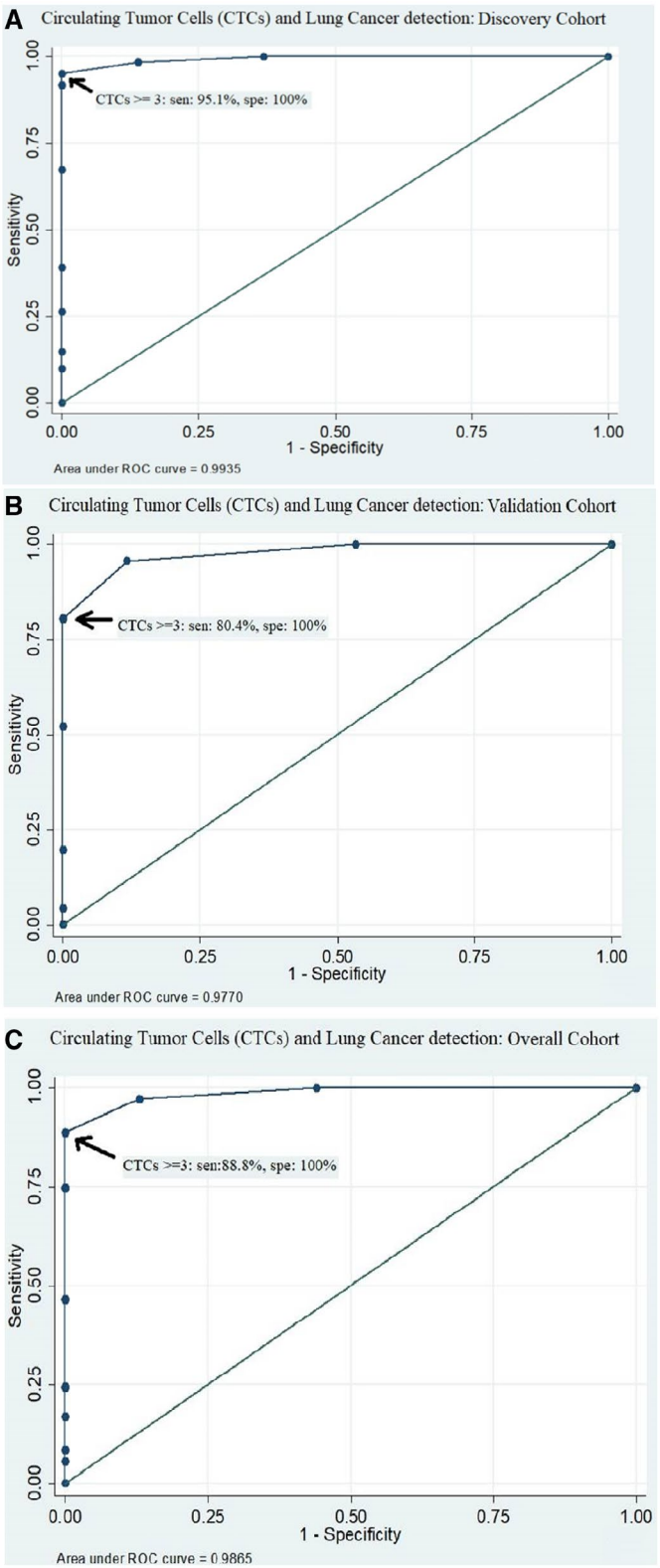

FIGURE 3. Analytical results from discovery and validation datasets. The sensitivity and specificity of a circulating tumor cell (CTC) count of ≥3 as a threshold for lung cancer detection are shown for the (A) discovery cohort, (B) validation cohort, and (C) overall cohort using at least 3 CTCs as the threshold. ROC, receiver operating characteristic; sen, sensitivity; spe, specificity.

DISCUSSION

There is currently no noninvasive test for ruling out malignancy of LDCT-identified indeterminate nodules of the lung. However, such a test would permit a reduction in the frequency of biopsies ordered for nonmalignant nodules. In the present study, a blood-based, 4-probe FISH assay accurately identified lung cancer cases from patients without lung cancer, suggesting that this test can be applied as an adjunct decision tool when evaluating the need for needle biopsy.

The FISH cocktail used in this study probed genes that are likely to be associated with the tumorigenesis of NSCLC. Surfactant proteins are the major transcriptional products of type 2 pneumocytes, which likely are the cells of origin for NSCLC. Recurrent somatic insertions and deletions in the noncoding regions of the *SP-A*, *SP-B*, and *SP-C* genes occur in adenocarcinomas of the lung.[21] Previously, we demonstrated that deletion of the *SP-A1* and *SP-A2* genes in normal bronchial cells adjacent to stage I NSCLCs was associated with poor prognosis.[22] Similarly, a single-nucleotide polymorphism on 3p22.1 in the β-catenin gene *CTNNB1*, a key component of the Wnt signaling pathway, has been associated with poor survival in NSCLC.[23,24] We have also previously reported a significant correlation between deletion of 3p22.1 and reduced β-catenin levels, suggesting that 3p22.1 drives β-catenin expression and perhaps tumorigenesis secondary to deregulated cell adhesion.[23,24]

In this study, we detected higher numbers of CTCs detected by the 4-probe FISH assay compared with other reports. This may be due to detection of an immunophenotypically diverse set of CTCs that undergo lineage plasticity while traversing the peripheral bloodstream. Previously, in patients with NSCLC, we detected aneuploidies of 10q22.3/CEP10 in multiple phenotypes of PBMCs displaying partial epithelial–mesenchymal transition and stemness traits, including CD45−/CK−, ALDH1+/CK+, ALDH1+/CK− (stem cells), ALDH1+SNAIL− (stem cells CD45+/CK+,CD45−/CK+,CK+/SNAIL+,CK-/SNAIL+), or ALDH1+SNAIL+, (epithelial–mesenchymal transition) cells, using immunocytochemistry combined with FISH.[25] Alternatively, differences in assay performance between the current study and other reports may stem from the elusive definition of a CTC. A previous report by Wendel et al[26] found no significant differences in CTC (cytokeratin-positive and CD45-negative cells) between categories of tumor stages in blood samples from 78 chemotherapy-naïve NSCLC patients. An advantage of the current study, and perhaps a reason for the higher CTC count than that observed by Wendel et al, is that the FISH probes used are independent of epithelial–mesenchymal transition and can therefore be used to detect CTCs that lack cytokeratin.

A component of the assay that requires refinement is optimization of CTC retention during the gradient enrichment and counting process. However, in this study, the loss of CTCs during processing was likely offset by the use of software that selectively presented cells with larger nuclei than coexisting lymphoid cells, effectively enriching the samples for cells displaying genetic abnormalities.

In conclusion, we describe a novel FISH-based blood test used to detect a population of CTC that accurately differentiates between patients with and without lung cancer. With further refinement, the assay presents a potential adjunct decision-making tool for assessing risk of malignancy in patients with intermediate-risk pulmonary nodules detected via LDCT, and may present an alternative to serial LDCT scans for monitoring patients with low-risk pulmonary nodules. Currently, a negative CTC result is insufficient to guide decisions regarding resection or needle biopsy; however, future studies will guide the use of this test as a method of risk stratification or an adjunct confirmatory tool.

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CONFLICT OF INTEREST DISCLOSURES

Ruth Katz has received personal fees from San Med Bio and hold patents related to the technology described in the article, and the Katz laboratory has been sponsored by LungLife AI, which has licensed the technology described in the article from MD Anderson Cancer Center. Tanweer Zaidi holds a patent related to the technology described in the article. Joshua D. Kuban has received research support from Cynvenio Biosystems and has served as a consultant for Argon, Boston Scientific, and BTG.

AUTHOR CONTRIBUTIONS

Ruth L. Katz: study design; study experimentation; data analysis; writing–review and editing. **Tanweer M. Zaidi:** study

experimentation; data analysis; writing–review and editing. **Deep Pujara:** study experimentation; data analysis; writing–review and editing. **Namita D. Shanbhag:** study experimentation; data analysis. **Duy Truong:** study experimentation; data analysis. **Shekhar Patil:** data analysis. **Reza J. Mehran:** study experimentation. **Randa A. El-Zein:** study experimentation; writing–review and editing. **Sanjay S. Shete:** study design; data analysis. **Joshua D. Kuban:** study experimentation.

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