Comparison of the Yield of Different Diagnostic Procedures for Cellular Differentiation and Genetic Profiling of Non–Small-Cell Lung Cancer

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Introduction: As treatments for non–small-cell lung cancer (NSCLC) become personalized, cellular and molecular differentiation of the tumor is becoming the standard of care. Our objective is to compare the yield of different diagnostic procedures for cellular differentiation of NSCLC and analysis of epidermal growth factor receptor (EGFR) mutation.

Methods: We evaluated all patients diagnosed with NSCLC from January 2004 to September 2010 at the Jewish General Hospital, Montreal. Diagnostic procedures included surgical biopsies, nonsurgical histologic biopsies (endobronchial and core needle), transbronchial needle aspirate (TBNA) and transthoracic needle aspirate (TTNA), bronchoalveolar lavage (BAL), and pleural fluid samples.

Results: We included 702 subjects investigated for histopathologic differentiation of NSCLC. Of these, 269 were also investigated for EGFR mutation. Failure to ascertain the cellular subtype and EGFR mutation status was least likely with surgical specimens (0% and 1.8%, respectively); followed by TTNA (14% and 10%, respectively) and histologic biopsy (18% for both); and was frequent with TBNA (39% and 30%, respectively). Although BAL and pleural fluid specimens provided reasonable yield for cellular differentiation (20 % and 11%, respectively), their results were not accurate in 6% of their samples when compared with concurrent or subsequent surgical specimens (reference standard) performed in a subgroup of patients.

Conclusion: Radiologically guided TTNA and histologic biopsies provided high yield for both molecular and histologic analyses. The yield of unguided TBNA was relatively low. Further studies are needed to assess the adequacy of BAL and pleural fluid samples for EGFR mutation analysis and accurate characterization of cellular subtypes of NSCLC.

Key Words: Lung cancer, cellular differentiation, EGFR, procedures, diagnosis

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Minimally invasive nonsurgical procedures have been successfully used to diagnose lung cancer;[1–4] however, their utility for cellular differentiation and genetic profiling of tumor cells has not been established. As treatments for non–small-cell lung cancer (NSCLC) become increasingly personalized, especially for advanced diseases, the cellular and genetic differentiation of the tumor is becoming essential for the selection of appropriate treatment regimens.[5,6] The American Society of Clinical Oncology stated in 2011 "to obtain tissue for more accurate histologic classification or investigational purposes, update committee supports reasonable efforts to obtain more tissue than that contained in routine cytology specimen."[7] This is sometimes challenging, particularly when dealing with advanced disease in which invasive intervention is less feasible, despite the need for adequate tissue sampling for histologic and molecular analyses.

Recent studies suggest that cytologic specimens, which are obtained using nonsurgical procedures, are sufficient for cellular differentiation and molecular analysis of NSCLC.[8,9] Different cytologic specimens, however, cannot be combined as one entity because the methods of sampling may have great impact on their diagnostic yield. Adequate number of cancer cells relative to normal cells in diagnostic samples is crucial to determine tumor-specific mutations.[10] Therefore, advanced procedures that target lesions under radiology guidance and provide samples with high proportion of abnormal cells are expected to have better diagnostic yield than unguided procedures. To our knowledge, no study has directly compared the utility of different nonsurgical procedures in obtaining accurate cellular differentiation and genetic profile of NSCLC. Our objective is to determine and compare the yield of different diagnostic procedures for accurate cellular differentiation of NSCLC and analysis of epidermal growth factor receptor (EGFR) mutation.

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PATIENTS AND METHODS

Study Population

The study cohort consists of all patients diagnosed with NSCLC between January 2004 and September 2010 at the Jewish General Hospital (JGH), Montreal. Patients were eligible if the diagnostic procedure and the histopathologic and molecular analyses were performed at the JGH.

Demographic data, diagnostic procedures, and disease status were obtained from the pulmonary oncology database at the JGH. Details about the histopathologic and molecular analyses were obtained from the patients' medical records. This study was approved by the Research Ethics Committee of the SMBD-JGH and all study participants signed an informed consent form.

Diagnostic Procedures

The initial procedure for obtaining adequate tissue for diagnosis of NSCLC was considered the diagnostic procedure. Based on the method of obtaining the tissue samples, these diagnostic procedures were classified as surgical and nonsurgical procedures. The surgical procedures included primary and secondary tumor resections. The nonsurgical procedures included:

- Histologic biopsy (i.e., procedures that provide tissue samples, including endobronchial biopsy and transthoracic core needle [16–18 gauge needle size]);
- Bronchoalveolar lavage (BAL);
- Pleural fluid;
- Conventional transbronchial needle aspirate (TBNA; 22 gauge needle size);
- CT guided transthoracic needle aspirate (TTNA; 22 gauge needle size).

Samples obtained by surgeries and nonsurgical biopsies (histologic biopsies) provided histologic (or tissue-based) specimens. BAL, pleural fluid, TBNA, and TTNA provided cytologic (or cellular-based) specimens.

Histopathologic and Molecular Analyses

Using the electronic database from the pathology department, all information regarding histological and cytological analysis was retrospectively gathered for each case, including immunocytochemical/immunohistochemical data whenever available. All cases were re-classified into four groups: adenocarcinoma, squamous cell carcinoma, NSCLC not otherwise specified (NOS), and others (large cell, neuroendocrine, sarcomatoid, and mixed types). NSCLC-NOS was defined when the analysis of histologic and/or cytological specimens show neither clear evidence of glandular or that of squamous differentiation, due to either the nature of the tumor (poor differentiation) or scarcity of tumor sampling. Poorly differentiated NSCLC was defined when the cellular subtype was not determined by nonsurgical specimens despite adequate immunohistochemistry (IHC) staining. The diagnosis of large cell carcinoma was only accepted in resected tumor, that had undergone thorough histologic examination, with the aid of ancillary techniques (special stains and IHC) to exclude either squamous or adenocarcinoma differentiation.[11]

Fragment analysis on a DNA sequencer for exon 19 and real-time polymerase chain reaction for exon 21 were performed in a proportion of samples with nonsquamous histology. All slides pertaining to cases where EGFR was ordered were reviewed by a pathologist. A new section from the selected paraffin block to be tested was taken, stained with H&E, and re-evaluated by the pathologist for documentation of tumor viability, and evaluation of tumor percentage in relation to the nontumoral component. As per our own validation process, exon 19 and exon 21 analysis can be performed successfully in samples with a minimum of 5% tumor and/or at least 100 tumor cells. We do not microdissect for enrichment, since most of our samples are either too small and because a significant percentage are aspiration biopsies, resulting in tumor cells being admixed with non-neoplastic ones. Moreover, during the validation process, we were unable to detect significant sensitivity differences between enriched and nonenriched samples. For cytology (aspiration biopsy samples), we have only validated our EGFR detection method for specimens that have been collected in cell blocks. In fact, in our hospital, aspiration biopsies are seldom prepared with smears; we have opted to give preference to preparation of cell blocks for most aspiration biopsies, using formalin solution at 10% as fixative, with aggregation of the cell pellet with Histogel after centrifugation. Unsatisfactory EGFR mutation analysis was defined in specimen with either insufficient number of cells, as determined by the pathologist, or failed assay, in which no results (either positive or negative) was obtained.

Statistical Analysis

Descriptive and regression analyses were performed to investigate potential confounding factors related to subjects' demographic and clinical characteristics. The yield for histologic and molecular differentiation of the different procedure groups was assessed by comparing the proportions of NSCLC-NOS and unsatisfactory EGFR mutation analysis using χ^2 test. In addition, univariate and multivariate logistic regression analyses were performed, and the results were expressed as odds ratios (OR) and 95% confidence intervals (CI). From a multicategory variable that contains the different nonsurgical specimens (histologic biopsy, BAL, pleural fluid, TBNA, and TTNA), the histologic biopsy category was used as a reference category to construct four dummy variables, each representing one of the other nonsurgical (cytologic) specimens. To deal with missing values, multiple imputation was performed using Bayesian statistic approach with noninformative prior values.

Sensitivity Analysis

To estimate the proportion of specimens with undetermined cellular subtype due to inadequate sampling, we repeated the comparative analysis after excluding specimens with poorly differentiated NSCLC (defined as undifferentiated cellular subtype in nonsurgical specimens after adequate IHC staining).

Diagnostic Accuracy

In a subgroup of patients who underwent both surgical and nonsurgical procedures, the surgical procedure was used as a reference standard (standard procedure) to evaluate the accuracy of each corresponding nonsurgical procedure for

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determining the cellular subtypes (i.e., comparing the result from the nonsurgical procedure to the result from the standard surgical procedure done for the same patient). This was assessed by determining the sensitivity (number of cases correctly diagnosed using nonsurgical specimens/number of cases diagnosed using surgical specimens) and the proportion of incorrect diagnosis (number of cases with discordant diagnosis). When a diagnosis of large cell carcinoma, which cannot be made by small biopsies as per our definition, was made by the surgical specimen, the expected (accurate) diagnosis by the corresponding nonsurgical specimen was NSCLC-NOS. All analyses were conducted using STATA (version 12) and WinBUGS14 softwares.

RESULTS

Between January 2004 and September 2010, a total of 912 subjects were identified; of whom, 702 were included in the analysis (Fig. 1). All 702 subjects had a confirmed tissue diagnosis of NSCLC. Diagnostic tissue specimens from all 702 subjects were analyzed for cellular subtype of NSCLC. A subgroup of 269 subjects was also assessed for EGFR mutation status. Table 1 shows patient demographics and disease characteristics.

The proportions of adenocarcinoma and squamous cell carcinoma were comparable between the surgical and nonsurgical specimens. However, the nonsurgical specimens yielded a higher proportion of NSCLC-NOS (25% vs. 0%, $p < 0.001$; Fig. 2).

Among the nonsurgical specimens, the proportion of NSCLC-NOS was 18% (95% CI, 12–25%) in histologic biopsies, 20% (95% CI, 9–35%) in BAL samples, 11% (95% CI, 4–23%) in pleural fluid samples, 39% (95% CI, 32–46%) in TBNA, and 14% (95% CI, 7–24%) in TTNA ($p < 0.001$). Comparing the different cytologic specimens to the histologic biopsy, the yield of conventional TBNA for determining the cellular subtype of NSCLC was significantly worse (adjusted OR, 3.4; 95% CI, 1.9–5.7). Otherwise, the yield of BAL (adjusted OR, 1.1; 95% CI, 0.4–2.5), pleural fluid (adjusted OR, 0.6; 95% CI, 0.2–1.6), and TTNA (adjusted OR, 0.7; 95% CI, 0.3–1.4) were not significantly different (Table 2).

After excluding samples with poorly differentiated NSCLC (defined as undifferentiated cellular subtype in nonsurgical specimens after adequate IHC staining), the remaining nonsurgical specimens with undetermined cellular subtypes were 2% (95% CI, 0.4–5.7%) in histologic biopsies, 17% (95% CI, 7–32%) in BAL, 6% (95% CI, 1.3–17.5%) in pleural fluid, 15% (95% CI, 10–21%) in TBNA, and 8% (3–17%) in TTNA ($p = 0.001$).

There was a subgroup of 93 subjects who underwent both surgical and nonsurgical procedures. The sensitivity of the nonsurgical procedures in this subgroup of patients was 88% (95% CI, 70–98%) for histologic biopsies, 81% (95% CI, 54–96%) for BAL, 76% (95% CI, 50–93%) for pleural fluid, and 71% (95% CI, 53–85%) for fine needle aspirates (TBNA and TTNA combined). All diagnoses from nonsurgical specimens were correct (concordant to surgical diagnosis) except for one (6% [95% CI, 0.2–30%]) from BAL and one (6% [95% CI, 0.1–29%) from pleural fluid samples.

The diagnostic yield for determination of the EGFR status varied substantially between the different procedures. The proportion of unsatisfactory results (defined in the methods section) was 1.8% in surgical and 23.6% in nonsurgical specimens ($p < 0.001$). Among the nonsurgical specimens, this proportion was 18% (95% CI, 8.6–31.4) in histologic biopsies, 30% (95% CI, 18.5–42.6%) in TBNA, and 10% (95% CI, 2.2–27.4%) in TTNA. Because of the small number of subjects, we could not adjust for multiple covariates; however, age-adjusted estimates did not change the trend of the diagnostic yields of the different diagnostic procedures (Table 3). The proportion of positive EGFR mutation result was 15% (95% CI, 8.6–22%) in surgical specimen, 20% (95% CI, 6.9–32%) in histologic biopsy, 23% (95% CI, 10–36%) in TBNA, and 31% (95% CI, 12–50%) in TTNA. The number of BAL and pleural fluid samples analyzed for EGFR mutation was too small (nine and eight samples, respectively) to provide accurate estimates.

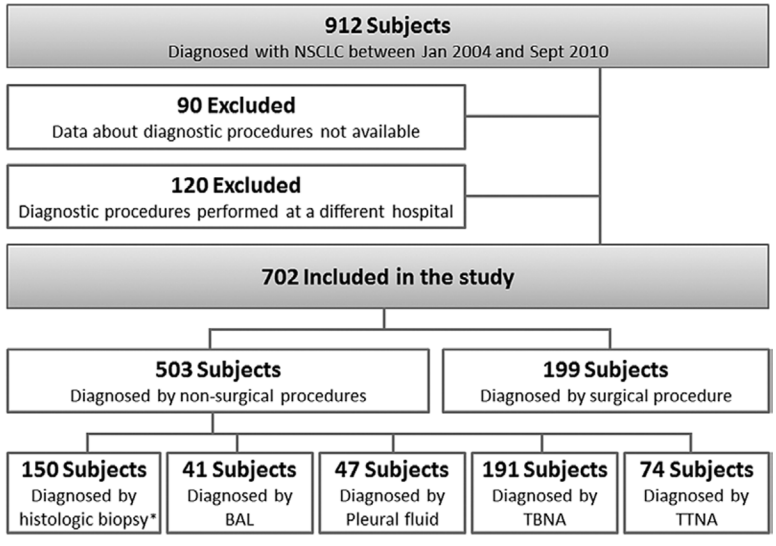

FIGURE 1. Population selection. BAL, bronchoalveolar lavage; TBNA, conventional transbronchial needle aspirate; TTNA, CT guided transthoracic needle aspirate. *Includes endobronchial and core needle biopsies.

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TABLE 1. Demographic and Disease Characteristics among Subjects Who Underwent Different Diagnostic Procedures

		Surgery	Histologic biopsy[a]	BAL	Pleural fluid	TBNA	TTNA
		No. = 199	No. = 150	No. = 41	No. = 47	No. = 191	No. = 74
Subject characteristics							
Age, mean (SD)		66 (10.3)	68 (11.8)	70 (11.3)	72 (11.5)	69 (10.6)	72 (9.3)
Male gender, No. (%)		109 (54.8)	84 (56.0)	22 (53.7)	26 (56.5)	103 (54.5)	40 (54.1)
Smoking (ever), No. (%)		168 (85.7)	124 (87.3)	30 (79.0)	33 (75.0)	167 (89.3)	63 (85.1)
Performance status,[b] No. (%)	0–1	165 (82.9)	100 (67.6)	27 (65.9)	32 (68.1)	144 (75.8)	55 (75.3)
	2–4	34 (17.1)	48 (32.4)	14 (34.2)	15 (31.9)	46 (24.2)	18 (24.7)
Tumor characteristics							
Cancer stage No. (%)	I-II	115 (57.8)	13 (8.8)	11 (26.8)	4 (8.5)	19 (10.0)	18 (24.3)
	III-IV	84 (42.2)	135 (91.2)	30 (73.2)	43 (91.5)	171 (90.0)	56 (75.7)
Tumor size (cm),[c] mean (SD)		3.4 (2.0)	5 (2.5)	4.8 (1.9)	3.8 (2.0)	4.7 (2.2)	4.4 (2.3)

[a]Includes endobronchial and transthoracic core biopsies.
[b]ECOG performance status scale.
[c]Size of the primary tumor.
BAL, bronchoalveolar lavage; TBNA, transbronchial needle aspirate; TTNA, transthoracic needle aspirate; SD, standard deviation.

DISCUSSION

In our population, we compared the yield of different diagnostic nonsurgical procedures for accurate cellular differentiation and EGFR mutation analysis. Radiologically guided TTNA and histologic biopsies (i.e., endobronchial and transthoracic core biopsies) provided relatively high yield for both cellular and molecular characterization of NSCLC. The yield from nonguided (conventional) TBNA was relatively low.

When compared with concurrent or subsequent surgical procedures, all nonsurgical procedures provided reasonable sensitivity and accurate histopathologic analysis results except BAL and pleural fluid samples which provided incorrect results in 6% of samples.

Many studies have assessed the performance characteristics of different diagnostic modalities for lung cancer diagnosis in general. Comparison between these modalities, however, was mainly done across studies, using meta-analysis methods, despite significant heterogeneity of results.[12] Some studies have assessed the adequacy of nonsurgical samples to determine both cellular subtype and genetic mutation status of NSCLC. Similar to our finding, the yield of TTNA in one study was high (over 90%) for both cellular differentiation and EGFR mutation analysis.[9] In another study, the cellular subtype of NSCLC was determined in 85% of cytology specimens that included both TBNA and TTNA.[8] The yield of endobronchial ultrasound guided TBNA (EBUS-TBNA) for EGFR mutation analysis varied between studies—ranging from 72%[13] to 90%.[14] Most comparative studies[15–17] assessed the difference between cytologic specimens in general and histologic (tissue) specimens, although, to our opinion, the sampling technique of cytologic specimens may have great impact on the yield for cellular and molecular analyses. Many studies conclude that cytologic specimens provide high accuracy for cellular and molecular analyses. The majority of these studies, however, included either small number of subjects[16] or a selected sample of specimens, most of which are radiologically guided,[9] which may not be generalizable to all cytologic specimens performed in clinical practice. Based on a consensus agreement at the International Association for the Study of Lung Cancer meeting, "cytology samples may be suitable for analysis but further research is needed to fully understand the clinical reliability of mutational data obtained from these samples."[10] To our knowledge, our study is the first study that compared the yield of different diagnostic procedures performed in the usual clinical practice for cellular and molecular differentiation of NSCLC.

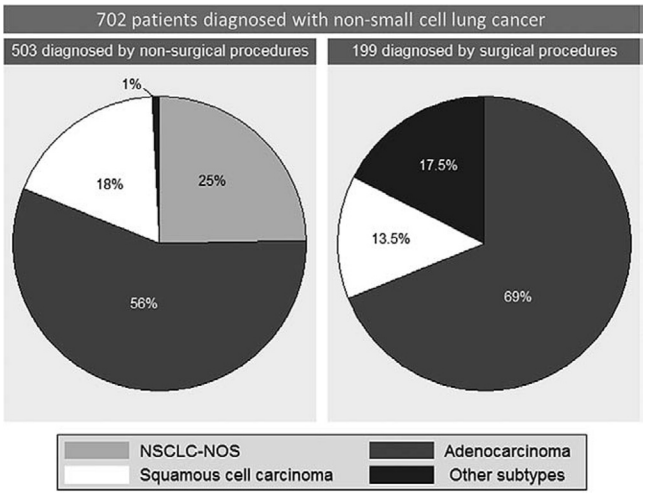

FIGURE 2. Proportions of cellular subtypes determined in surgical and nonsurgical specimens. NSCLC-NOS, non–small-cell lung cancer not otherwise specified.

One limitation of this study is that a reference sample (surgical biopsy) was not available for all the patients. However, due to the fact that the majority of lung cancer patients are diagnosed in advanced stage, it is difficult to achieve comparable sample size of subjects who underwent both surgical and each type of nonsurgical procedures. In addition, limiting the study to subjects who underwent

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TABLE 2. Odds Ratios (OR) of Having NSCLC-NOS, Comparing Different Nonsurgical Procedures

	NSCLC-NOS		
Diagnostic Procedure	**Proportion (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**
Histologic biopsy[b] (No. 150)	18% (12.2–25.1%)	1 (Ref.)	1 (Ref.)
BAL (No. 41)	19.5% (8.8–34.9%)	1.1 (0.46–2.66)	1.1 (0.37–2.53)
Pleural fluid (No. 47)	10.6% (3.5–23.1%)	0.54 (0.2–1.5)	0.64 (0.17–1.57)
TBNA (No. 191)	38.7% (31.8–46%)	2.9 (1.7–4.8)	3.41 (1.9–5.7)
TTNA (No. 74)	13.5% (6.7–23.5%)	0.71 (0.32–1.56)	0.68 (0.26–1.44)

[a]Adjusted for age, sex, smoking history, primary tumor size, performance status, and cancer stage
[b]Includes endobronchial and transthoracic core biopsies. Missing values were treated with multiple imputation, using Bayesian approach with noninformative prior values.
NSCLC-NOS, non–small-cell lung cancer not otherwise specified; BAL, bronchoalveolar lavage; TBNA, conventional transbronchial needle aspirate; CI, confidence interval; TTNA, CT guided transthoracic needle aspirate.

surgery may not be generalizable to nonsurgical candidates who may also benefit from targeted therapies. Alternatively, we compared the yields across subjects and adjusted for potential confounding factors; however, residual confounding by unmeasured factors is a possibility. To validate our results, we also analyzed the yield of nonsurgical procedures through direct comparison to the reference surgical procedure in a subgroup of patients who underwent both procedures. Although IHC staining has been done routinely in our center to characterize the cellular subtype of NSCLC, it may have not been performed during the work-up of some samples analyzed earlier before cellular differentiation of NSCLC became a standard of care. This, however, is expected to similarly reduce the sensitivity of different nonsurgical specimens without significantly affecting the comparative inferences. We also did not have adequate number of samples to accurately estimate the yield of BAL and pleural fluid sampling procedures for EGFR mutation analysis. Another consideration to be discussed is that the denominator in our analysis did not include all subjects who underwent the procedures, but only those diagnosed with NSCLC. However, IHC staining and EGFR mutation analyses are considered only when cancer cells are identified. Therefore, similar to other studies that assessed the utility for histopathologic and molecular differentiation of NSCLC,[8,9,13] we limited the denominator to subjects who were considered for the same diagnostic evaluation.

Despite these limitations, this study has a number of strengths. To our knowledge, this study constitutes the largest cohort of patients with NSCLC who were assessed to determine the sampling utilities for histologic and molecular differentiation of the tumor. Unlike other studies that handled different cytologic specimens as one entity, we assessed them separately, based on the sampling procedure (BAL, pleural fluid, TBNA, and TTNA), and identified important differences between their utilities. Finally, all of the procedures were performed, and their samples were analyzed, in one institution, which reduced potential variability related to differences in procedure equipments and laboratory resources, thereby allowing for equitable comparison.

This study suggests that the yield of cytologic specimens for cellular and molecular differentiation of NSCLC varies according to the performed procedure. Unlike other cytologic specimens, TTNA provided high yield, which was equivalent to the yield of nonsurgical histologic biopsy specimens. This can be explained by the anticipated higher number of cancer cells in proportion to normal cells when the samples are obtained through direct or radiologic visualization of the tumor. Correspondingly, EBUS-TBNA may provide high yield; however, this requires direct comparison to other cytologic specimens in further studies. Our study also suggests that BAL and pleural fluid specimens may occasionally provide incorrect cellular classification of NSCLC. These findings can have important clinical implications for selecting the diagnostic modality that will likely provide enough information to appropriately treat lung cancer patients.

We conclude from our study that the adequacy for cellular differentiation of NSCLC and EGFR mutation analysis depends not only on the type of specimen (i.e., histologic versus cytologic), but also on the procedure used to obtain this specimen. Further studies are required to assess the adequacy of BAL and pleural fluid samples for determining EGFR mutation status and accurately differentiating the cellular subtype of NSCLC.

TABLE 3. Odds Ratios (OR) of Having Unsatisfactory EGFR Mutation Status, Comparing Different Nonsurgical Procedures

	Unsatisfactory EGFR Mutation Analysis		
Diagnostic Procedure	**Proportion (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**
Histologic biopsy[b] (No. 50)	18% (8.6–31.4)	1 (Ref.)	1 (Ref.)
TBNA (No. 61)	29.5% (18.5–42.6)	1.9 (0.77–4.7)	2 (0.8–5.3)
TTNA (No. 29)	10.3% (2.2–27.4)	0.5 (0.13–2.1)	0.5 (0.12–2.1)

Estimates for bronchoalveolar lavage and the pleural fluid samples were excluded because of small number of subjects (nine and eight, respectively).
[a]Adjusted only for age, no enough power to adjust for other covariates.
[b]Includes endobronchial and transthoracic core biopsies.

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Authors' contributions: J.A. designed the study. G.K., C.R., and A.S.A collected and processed the data. A.S.A. analyzed the data, interpreted the results, and wrote the manuscript. G.K., C.R., V.C., G.B., C.P., D.S., and J.A. regularly discussed different aspects of the study and critically revised the manuscript.

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