Validation of a Multiprotein Plasma Classifier to Identify Benign Lung Nodules

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Introduction: Indeterminate pulmonary nodules (IPNs) lack clinical or radiographic features of benign etiologies and often undergo invasive procedures unnecessarily, suggesting potential roles for diagnostic adjuncts using molecular biomarkers. The primary objective was to validate a multivariate classifier that identifies likely benign lung nodules by assaying plasma protein expression levels, yielding a range of probability estimates based on high negative predictive values (NPVs) for patients with 8 to 30 mm IPNs.

Methods: A retrospective, multicenter, case-control study was performed using multiple reaction monitoring mass spectrometry, a classifier comprising five diagnostic and six normalization proteins, and blinded analysis of an independent validation set of plasma samples.

Results: The classifier achieved validation on 141 lung nodule-associated plasma samples based on predefined statistical goals to optimize sensitivity. Using a population based nonsmall-cell lung cancer prevalence estimate of 23% for 8 to 30 mm IPNs, the classifier identified likely benign lung nodules with 90% negative predictive value and 26% positive predictive value, as shown in our prior work, at 92% sensitivity and 20% specificity, with the lower bound of the classifier's performance at 70% sensitivity and 48% specificity. Classifier scores for the overall cohort were statistically independent of patient age, tobacco use, nodule size, and chronic obstructive pulmonary disease diagnosis. The classifier also demonstrated incremental diagnostic performance in combination with a four-parameter clinical model.

Conclusions: This proteomic classifier provides a range of probability estimates for the likelihood of a benign etiology that may serve as a noninvasive, diagnostic adjunct for clinical assessments of patients with IPNs.

Key Words: Lung nodule, Proteomics, Molecular diagnostic, Biomarker.

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Lung nodules deemed indeterminate lack the features suggestive of benign etiologies[1] and present clinicians with a diagnostic conundrum. Patient and practitioner balance a desire for the certainty of a diagnosis against the tolerance for the unknown, while assessing the risk and yield of an invasive procedure and the likelihood of malignancy. Achieving an early diagnosis of cancer remains a clinical imperative[2] to improve the dismal 16% 5-year survival of nonsmall-cell lung cancer (NSCLC),[3] and also to assuage the immediate concern and anxiety engendered among both patients and physicians upon the identification of such spots.[4–6] The use of computed tomography (CT) technology has grown annually with the number of nodules identified by chest CT scans approaching millions per year, most of which are benign.[5] Patients with a nodule less than 8 mm in size or having benign radiographic features may be

managed expectantly by serial CT scan surveillance.[1] However, those with larger nodules may embark on a diagnostic odyssey,[7] including positron emission tomography (PET), transthoracic needle aspiration, bronchoscopic biopsy, and/or surgery.[1] Therefore, innovative strategies to identify benign lung nodules may mitigate the diagnostic burden of those considered indeterminate, by providing complementary data for decision-making, minimizing surgical resection of benign processes,[8] and managing more lung nodules by radiographic surveillance.

Extensive efforts to classify pulmonary nodules using molecular biomarkers, such as DNA, RNA, and proteins, have yielded novel insights into lung cancer pathogenesis, with most having been focused largely on identifying malignant rather than benign lung nodules.[2,9–17] Proteins are attractive as biomarkers because they are the dynamic, functional molecules acting in cell communications,[18] with those of greatest interest often being in low abundance in plasma or serum. Therefore, advances in bioinformatics are at the core of recent progress in the development of diagnostic biomarker classifiers.[19] The current enthusiasm for introducing biomarkers into practice has also heightened expectations for rigor in their validation as diagnostic tools for a targeted or intended use population.[20]

Our prior work[21] applied multiple reaction monitoring mass spectrometry[22,23] for the discovery and initial validation of a classifier incorporating plasma protein expression levels to differentiate benign and malignant pulmonary nodules with 90% negative predictive value (NPV). In this study, we performed a validation of a multiprotein plasma classifier that prioritizes the diagnostic parameters of sensitivity and NPV to identify likely benign lesions in patients presenting with 8 to 30 mm lung nodules.

MATERIALS AND METHODS

Validation

The study conforms to Institute of Medicine guidelines[20] (Supplemental Table 4, Supplemental Digital Content, http://links.lww.com/JTO/A773) and the Standards for Reporting of Diagnostic Accuracy (STARD) criteria for reporting studies of diagnostic accuracy (Supplemental Table 5, Supplemental Digital Content, http://links.lww.com/JTO/A773).[24] Protein expression analyses and computational procedures were performed in a clinical laboratory adhering to the Clinical Laboratory Improvement Amendments of 1988.[20]

Study Design and Oversight

The overall objective was to validate the performance of an 11-protein classifier in identifying lung nodules with likely benign (i.e., nonmalignant) etiologies (Supplemental Materials, Supplemental Digital Content, http://links.lww.com/JTO/A773), yielding a range of probability estimates for use as a diagnostic adjunct in clinical assessments. A retrospective, case-control study utilized multiple reaction monitoring mass spectrometry to analyze archival plasma samples from subjects enrolled in clinical studies approved by the Ethics Review Board or Institutional Review Boards at multiple institutions, using a blinded data analysis strategy. Management of clinical data complied with the Health Insurance Portability and Accountability Act of 1996.

Study Inclusion and Exclusion Criteria

The subject inclusion criteria were a minimum age of 40 years and any smoking history. The radiologic and pathologic criteria for lung nodule inclusion were a diameter between 8 to 30 mm, a histopathologic diagnosis of NSCLC or a benign process, or a clinical diagnosis of a benign etiology based on stability in size and appearance for 2 years after the baseline CT scan. The subject exclusion criteria included the lack of nodule size or histopathologic diagnosis, follow-up for less than 2 years, or a diagnosis of small-cell lung cancer. The subjects' spirometry data and the global initiative for chronic obstructive lung disease criteria[25] were used to define the presence and severity of chronic obstructive pulmonary disease (COPD). The cancer and benign subgroups were matched for age, gender, smoking history, and nodule size.

Lung Nodule Protein Expression Classifier and Proteomic Analysis

The classifier consists of five diagnostic and six normalization proteins (Table 1), which were fully defined, or "locked-down," before sample analysis. The five diagnostic proteins were refined from the 13 proteins previously shown to discriminate benign and malignant lung nodules[21] using stable isotope standards (Supplemental Materials, Supplemental Digital Content, http://links.lww.com/JTO/A773). The six normalization proteins were identified to reduce preanalytical and analytical variations in mass spectroscopic protein quantification.[25a] Plasma protein expression assays were performed as previously described[21] using methods incorporating stable isotope standards (Supplemental Materials, Supplemental Digital Content, http://links.lww.com/JTO/A773).

Data Analysis

The first objective was to validate the overall performance of the classifier (Table 1) in identifying benign nodules, using the method of the partial area under the curve (pAUC).[26] This objective required that the lower 95% confidence bound of the pAUC bounded by a sensitivity of 0.8 be higher than the corresponding pAUC (0.02) of a nonperforming classifier. The second objective was to validate the performance of the classifier in identifying benign nodules at predefined reference values using binomial testing. This objective required that the lower 95% confidence bound of the fraction of benign samples among samples whose scores were less than or equal to the corresponding reference values be higher than the fraction of benign samples in the study. The fixed-sequence procedure[27,28] was used to control the overall multitesting error rate ($\alpha = 0.05$) in the study. Statistical analyses were performed using the MannWhitney and Fisher's exact tests.

RESULTS

Study Cohort

Plasma specimens from 195 subjects with lung nodules at four institutions in different geographic regions of North America initially satisfied the study inclusion criteria, which included a minimum subject age of 40 years, but no stipulated smoking status or pack-year history. Thirty-two candidate