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The accuracy of laboratory studies in differentiating pulmonary nodules due to Coccidioidomycosis from lung cancer

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Abstract

Background: Lung nodules are frequently identified during chest imaging. Coccidioidomycosis may represent up to 30% of nodules in endemic areas. Fungal nodules can be difficult to distinguish from lung nodules due to lung cancer. There is limited data evaluating Coccidioides serology, CT biopsies, bronchoscopies or galactomannan antigen assay in diagnosing nodules due to Coccidioidomycosis.

Methods: We established a Lung Nodule Clinic in Fresno, CA, an endemic Coccidioidomycosis region. We reviewed nodule cases seen between October 2009 and December 2012. In addition, we prospectively evaluated a separate cohort of twenty-three patients referred with a nodule using urine, serum and BAL fluid for Coccidioides galactomannan antigen.

Results: We retrospectively evaluated 1079 patients. 192 patients had a diagnosis of bronchogenic carcinoma, 110 had a diagnosis of Coccidioidomycosis, and 109 had another specific diagnosis. The sensitivity of Coccidioides serology was 77% by immunodiffusion (ID) and 54% by complement fixation (CF). The specificity for ID was 93% and for CF was 98%. Sensitivity for granulomas on biopsy by CT was 78% but much lower by bronchoscopy at 12%. Sensitivity of cultures from CT biopsy and by BAL ranged from 30-33%. Of the 23 patients prospectively evaluated for Coccidioides antigen by EIA of BAL, sensitivity was only 12.5%.

Conclusions: In lung nodule patients, Coccidioides serology has insufficient sensitivity to exclude Coccidioidomycosis but has high specificity. Coccidioides galactomannan antigen testing and culture have poor sensitivity in this population. We need better noninvasive diagnostic tools to differentiate lung nodules due to Coccidioidomycosis from those due to lung cancer.

Abbreviations: BAL: bronchoalveolar lavage; CF: complement fixation; CTNA: CT-guided needle aspiration; EIA: enzyme-linked immunoassay; ID: immunodiffusion; LN: lung nodule; VATS: video-assisted thoracic surgery

Introduction

Solitary pulmonary nodules (SPNs) are common radiologic findings, typically discovered incidentally during routine chest radiographs or computed tomography of the chest. SPNs have been noted in approximately 0.09 - 0.2% of all chest radiographs [1]. With the improved resolution of computed tomography (CT), solitary pulmonary nodules are identified even more frequently [2]. In the recent screening study for lung cancer using low dose CT scans in smokers, nearly 40% of the patients who had a CT scan had at least one positive screening result [3]. However, only a small fraction of these positive results were due to early lung cancer. The screening trial did, however, demonstrate for the first time that screening resulted in fewer lung cancer deaths. While there are no clear guidelines for managing lung nodules found on screening CT scans, the Fleischner Society has developed a set of guidelines for managing noncalcified nodules detected on nonscreening CT scans [4-6]. However, these guidelines may not apply to all the patient populations, especially in regions with endemic fungal infections that can mimic lung cancer [7]. One of these endemic fungal infections is Coccidioidomycosis.

Coccidioidomycosis is a dimorphic fungus that is endemic to the American Southwest including the Central Valley in California [7]. The primary route of infection is the lung, and Coccidioidomycosis can present as an asymptomatic lung nodule. Using chest X-ray imaging, Coccidioidomycosis may be responsible for up to 30% of suspicious lung nodules occurring in an endemic area [8].

Both invasive cultures and serology have been useful in diagnosing patients with acute pneumonia due to Coccidioidomycosis [8-11]. Serology has very high sensitivity in diagnosing acute pneumonias due to Coccidioidomycosis [10]. The sensitivity for finding granulomatous inflammation on transthoracic needle biopsy was 73% but the sensitivity of cultures was only 4% [8]. By contrast, bronchoscopy has been less useful. A retrospective study found 24% sensitivity of cytology from broncho-alveolar lavage (BAL) in patients with active pulmonary Coccidioidomycosis and only 30% in patients with focal disease [9]. However, there are few studies directly evaluating the diagnostic performance of serology, histology and cultures in patients with lung nodules due to Coccidioidomycosis.

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Recently, tests for fungal antigen detection have proved successful in detecting both serum and urine galactomannan isolated from C. immitis in immunocompromised patients with pneumonia due Coccidioides [12-14]. Again, there are no studies to evaluate the sensitivity or specificity of Coccidioides antigen detection in patients presenting with lung nodules due to Coccidioidomycosis.

To address the performance of available diagnostic tests for Coccidioidomycosis presenting with lung nodules, we evaluated our patients referred to a Multidisciplinary Lung Nodule Clinic. We postulated that a significant number of the patients would be found with nodules due to Coccidioidomycosis, but that Coccidioidomycosis serology would be highly sensitive but nonspecific in our patients due to the high prevalence of infection in our region [15].

Materials and methods

We retrospectively reviewed the clinical records of all the patients referred to the Lung Nodule Program at Community Regional Medical Center, located in Fresno, California, an area of endemic Coccidioidomycosis, between 2009 and 2012. All subjects had CT chest images which were reviewed with a radiologist.

Because we anticipated that Coccidioidomycosis would be common in our population, Coccidioides serology by both immunodiffusion and complement fixation was recommended and performed at an outside laboratory as previously described [16]. The lab performing the tests was blinded to the patients' clinical presentation and to the ultimate diagnosis.

After all subjects were evaluated by a pulmonologist, a multidisciplinary physician team consisting of radiology, pulmonary, oncology, thoracic surgery, and pathology reviewed every new patient and agreed on a clinical plan. The decision to proceed with invasive studies was based on an assessment of clinical risk and radiographic appearance. The decision reached was based on consensus among the specialists. The cases that required an invasive diagnostic procedure had either a bronchoscopy including BAL or CT-guided needle aspiration (CTNA). If these were non-diagnostic, the subjects were referred for video-assisted thoracic surgery (VATS). In this study, proven Coccidioidomycosis was based on the finding of positive cultures for C. immitis or by morphologic identification of C. immitis from pulmonary samples. Probable Coccidioidomycosis was diagnosed based on finding granulomatous inflammation on the biopsy and a clinical course compatible with Coccidioidomycosis including radiographic stability, improvement or resolution of the lung nodule over a two-year period.

In a smaller subset of the 1079 subjects, we prospectively evaluated the subjects for Coccidioides antigen. Twenty three subjects with a non-calcified nodule larger than 1 cm were referred for bronchoscopy with BAL. Subjects' serum and urine, along with BAL samples, were collected at the time of bronchoscopy and sent for C. immitis galactomannan antigen testing. Coccidioides galactomannan antigen testing was performed by EIA at MiraVista Diagnostics in Indianapolis, using the techniques previously reported [13,17]. The lower limit of detection for Coccidioides galactomannan was 0.07 ng/ml [17]. The investigators at MiraVista Diagnostics were blinded to the results of Coccidioides serology or clinical features.

Bronchoscopy with BAL was performed using a flexible fiberoptic bronchoscope. BAL was performed by "wedging" the bronchoscope into a clinically relevant lung subsegment and lavaging with 100 ml of sterile saline. The samples were sent for culture and cytologic examination. For cytologic examination, BAL samples and bronchial washings were centrifuged and the pellet stained using the Papanicolaou and Gomori silver-methenamine methods, then examined microscopically by an experienced pathologist.

CTNA was performed by the radiologist using a coaxial technique. Briefly, a Greene (Cook, Bloomington, IN) 20-gauge needle in a 19-gauge introducer needle was inserted under CT-guidance. Local anesthesia was obtained by subcutaneous injection of 1% lidocaine.

VATS was performed if bronchoscopy or CT-guided biopsies were non-diagnostic but the suspicion of malignancy remained high.

Data were analyzed and sensitivity, specificity, positive and negative predictive values were calculated using SPSS software. All studies were reviewed and approved by the local Community Medical Centers Institutional Review Board (IRB #2011075).

Results

We reviewed 1079 records of patients referred to the Lung Nodule Program between 2009 and 2012. After evaluation by the multidisciplinary team, 474 subjects underwent invasive diagnostic studies. Of these 474 subjects, 192 were diagnosed with bronchogenic carcinoma, 110 were diagnosed with Coccidioidomycosis, and 109 with another specific pathological diagnosis. A total of 63 subjects had nonspecific findings on biopsy. The subject characteristics for the Coccidioidomycosis and lung cancer subjects are described in Table 1.

The average nodule size for all patients was 3.5 cm ± 2.4 cm (mean ± SD). While serologic testing was requested on all subjects, some did not follow through or the results could not be located. Of the 411 eligible subjects with a specific histologic diagnosis, 319 had serology by immunodiffusion and 317 by complement fixation. The sensitivity, specificity, positive and negative predictive values for serology from these subjects are displayed in Table 2. The sensitivity for immunodiffusion (ID) was only 77% and for complement fixation (CF) was 51%. For invasive studies, 193 subjects underwent transthoracic needle biopsy under CT guidance, 147 subjects underwent bronchoscopy, 52 subjects underwent endobronchial ultrasound-guided biopsy and 40 subjects required VATS or thoracotomy. These numbers add up to greater than the 411 subjects because some subjects underwent multiple procedures to make their diagnosis. Histology, fungal smear and fungal culture were obtained from the biopsy specimens. The sensitivity, specificity, positive and negative predictive values for these studies from CT biopsy and bronchoscopy are displayed in Table 3.

Table 1. Subject characteristics

	Coccidioidomycosis (N = 110)	Lung Cancer (N = 192)
Age	51.6 ± 13.6 years	67.9 ± 11.6 years
Sex		
Male	65%	48%
Female	35%	52%
Race or ethnic group		
White	53 (48%)	137 (71%)
African American	6 (5%)	10 (5%)
Hispanic	47 (43%)	37 (19%)
Asian	4 (4%)	8 (4%)
Smoking status		
Never smoker	46%	17%
Current	17%	42%
Former	29%	42%

Table 2. Sensitivity, specificity, PPV, NPV of serology

	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Coccidioides serology by immunodiffusion	77% (68-84)	93% (89-96)	86% (77-91)	89% (84-92)
Coccidioides serology by complement fixation	51% (42-61)	98% (96-99)	92% (82-96)	79% (74-84)

Table 3. Sensitivity, Specificity, Positive and Negative Predictive Value for CT Biopsy and Bronchoscopy

	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
CT Transthoracic Biopsy				
• **Granulomas**	78% (63-88)	98% (94-100)	93% (80-98)	93% (88-96)
• **Spherules**	53% (38-67)	100% (97-100)	100% (85-100)	87% (80-91)
• **Smear Positive**	0% (0-15)	100% (87-100)	-	55% (41-69)
• **Culture positive**	33% (17-55)	100% (91-100)	100% (65-100)	73% (59-83)
Bronchoscopy				
• **Granulomas on Biopsy**	12% (4-29)	97% (92-99)	50% (19-81)	81% (73-87)
• **Spherules on Biopsy**	12% (4-29)	100% (97-100)	100% (44-100)	81% (72-87)
• **Smear Positive on BAL**	3% (1-17)	100% (96-100)	100% (21-100)	77% (68-83)
• **Culture Positive on BAL**	30% (17-48)	100% (96-100)	100% (70-100)	82% (74-88)

Given the limited performance of serology, pathology and microbiology in making the diagnosis, we evaluated antigen detection in our subjects. Twenty-three subjects underwent bronchoscopy with BAL and had urine and serum collected for antigen assay. Of the 23 subjects, two had proven Coccidioidomycosis (cultures or pathology positive) and 5 subjects had probable disease (serology positive or granulomatous inflammation seen on cytopathologic exam but without identification of C. immitis). Six of these 7 subjects had positive Coccidioides serology, by both immunodiffusion and complement fixation. Among those subjects without a diagnosis of Coccidioidomycosis, 3 had malignancy, 1 had sarcoidosis, 4 had possible Coccidioidomycosis and 2 had TB. There were 6 patients for which the bronchoscopy was non-diagnostic. Coccidioides antigen measured by EIA was detected in the BAL specimens of two subjects, one with proven Coccidioidomycosis and one without a clear diagnosis. The EIA antigen test did not correlate well with Coccidioides serology (five subjects with positive serologies had a negative EIA antigen test). No antigen was detected in the serum or urine specimens from any subjects.

Discussion

Evaluating solitary pulmonary nodules remains a challenge for clinicians. This challenge is especially acute in areas with endemic infections such as Coccidioidomycosis where it can be difficult to distinguish Coccidioides nodules from early-stage lung cancers. Two recent findings suggest this challenge may increase. First, emerging epidemiological data suggest that there has been a statistically significant increase in the incidence of Coccidioidomycosis in endemic states during 1998–2011 [18]. Second, the recent National Lung Screening Trial results have led to multiple organizations recommending low dose chest CT screening for smokers between the ages of 55 and 74 years [19]. The NLST reported that 39% of the CT patients had at least one abnormality, and this rate may be even higher in areas endemic for inhaled fungi such as Histoplasmosis [20]. Thus, the challenge for clinicians working in endemic fungal regions of the country will be to develop evidence-based approaches to evaluating an increased number of patients presenting with lung nodules.

Our data clearly demonstrate that despite improvements in radiographic imaging with thin-slice chest CT scans, about 1/3 of the lung nodules presenting to us were ultimately demonstrated to be due to Coccidioidomycosis. This is the same fraction as reported almost 30 years ago when plain chest X-rays were the most commonly used chest imaging modality [8]. This figure may underestimate the total burden of lung nodules due to Coccidioidomycosis because we only investigated nodules that were suspicious for lung cancer with invasive studies. More than 60% of our subjects only had radiographic follow up, and in many of those subjects Coccidioidomycosis could have been responsible for their nodules.

The current approach to diagnosing Coccidioidomycosis relies heavily on serologic testing [10]. However, most of the prior studies evaluating serology did not independently evaluate subjects who were presenting with lung nodules. To our knowledge, our study is the first one to investigate serology when focusing only on subjects referred with lung nodules. Our findings were somewhat unexpected with low sensitivity for ID and CF but high specificity. Data based on skin testing when it was available, demonstrated a high prevalence of infection in subjects living in the endemic area for Coccidioidomycosis [15]. However, we did not find similar results when using serology. In fact, only two subjects with a pathologic diagnosis of lung cancer had positive serology for Coccidioidomycosis. Thus, the presence of positive serology was helpful, but its absence was not. The lower sensitivity of Coccidioides serology in our study, compared with the 90% sensitivity in acute disease, may reflect lower disease burden. We also found an unexpected high specificity for serology in our population. The diagnosis of lung cancer may have affected the immune response in our subjects and contributed to this finding.

Evaluating high risk lung nodules often requires invasive studies including transthoracic needle biopsy, bronchoscopy or surgery. All of our subjects required at least one invasive study. Our sensitivity for granulomas on transthoracic needle biopsy was similar to the only previous study of needle biopsy [8], but our sensitivity for spherules on biopsy was significantly higher. The prior study was published in 1986 when the dominant modality was fine needle aspiration under fluoroscopic guidance while our biopsies yielded core biopsies under CT guidance. Our sensitivity for culture from CT biopsy was also higher than reported in the previous study, but remained fairly poor at 33%. Several of our subjects also underwent bronchoscopy with both BAL and transbronchial biopsies. The sensitivity for granulomas and spherules on transbronchial biopsy was very poor at 12% but the results of culture were equivalent to the yield previously reported in non-HIV

infected subjects with focal Coccidioidomycosis [11]. These values, however, are insufficient to exclude an infectious cause for the nodules.

Given the limited performance of serology and invasive cultures, we evaluated the ability of EIA to detect Coccidioides antigen. Prior studies, mainly done in immunocompromised populations with Coccidioidomycosis, found good sensitivity for Coccidioides antigen EIA with up to 73% in EDTA-heat-treated samples [17]. Our results, however, showed a poor sensitivity for C. immitis galactomannan by EIA in all three samples (urine, blood and BAL). Two factors should be considered when interpreting these results. First, all the subjects had incidentally found lung nodule(s) and they were not selected based on the pretest probability of Coccidioidomycosis. Second, the antigen load may be very low, and even very sensitive detection methods like EIA may not detect it.

We acknowledge several potential limitations in our study. First, this is a retrospective analysis. However, our protocol for all new patients included recommending Cocci serology in all our patients. Second, Cocci serologies were not completed on all subjects. This limitation, however, will not affect the sensitivity and negative predictive value because we have 109 serology results among our 110 confirmed cases of lung nodules due to Coccidioides. Among the patients with diagnoses other than Coccidioidomycosis, we have serology results on 73%. In the worst case scenario, if all of the missing values are false positives (a highly unlikely occurrence), the specificity for ID falls to 64%. Given the consistency of the specificity between patients with lung cancer and other specific diagnoses, this seems highly unlikely.

Conclusions

In summary, none of the currently available laboratory tests for Coccidioidomycosis were sufficiently discriminating when evaluating patients presenting with lung nodules. Improved laboratory methods or better clinical and radiographic discriminators are needed to assist us in differentiating lung nodules due to Coccidioides from those due to lung cancer without requiring expensive and risky invasive procedures in these patients.

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Guarantor statement

Michael W. Peterson, M.D. takes responsibility for the content of the manuscript, including the data and analysis.

Author contributions

- Catalin Nicola, M.D., PhD has made substantial contributions to concept and design, data acquisition, and analysis of data. He has drafted and revised the manuscript for critically important intellectual content and has provided final approval of the version to be published.
- Paul K. Mills, PhD, MPH has made substantial contributions to concept and design and analysis of data. He has drafted and revised the manuscript for critically important intellectual content and has provided final approval of the version to be published.
- Ali Rashidian, M.D. has made substantial contributions to analysis of data. He has drafted and revised the manuscript for critically important intellectual content and has provided final approval of the version to be published.
- Michael W. Peterson, M.D. has made substantial contributions to concept and design, data acquisition, and analysis of data. He has drafted and revised the manuscript for critically important intellectual content and has provided final approval of the version to be published. He has also agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Other contributions

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