Selection or Work-Up Bias: A Recurrent Caveat in Evaluation of New Diagnostic Modalities

To the Editor:

I read with great interest the recent manuscript by Ernst et al. on the comparison of endobronchial ultrasound (EBUS) versus mediastinoscopy (MS) and the accompanying editorial.[1,2] The authors report a sensitivity of 87% for EBUS versus 68% for cervical MS and conclude that "endobronchial ultrasound may be preferred in the histologic sampling of paratracheal and subcarinal mediastinal adenopathy because the diagnostic yield can surpass mediastinoscopy."

The recently introduced minimally invasive staging procedures—endoscopic esophageal ultrasound and EBUS—represent promising staging and restaging techniques for lung cancer although their specific role in relation to invasive techniques remains controversial and has not been clearly established yet. Unfortunately, when these newer diagnostic procedures are compared with the more classic techniques as cervical MS, certain biases repeatedly show-up in most studies which lead to inaccurate conclusions.[3] Also the present study clearly suffers from a so-called work-up or selection bias where a new technique is only applied in a specific, predefined subset of patients in whom the diagnostic yield is known to be very high. Patients evaluated for lung cancer were first selected by computed tomography scan, which is rather inaccurate for primary staging, and only patients with enlarged lymph nodes in some specific stations were included. The newer technique—EBUS—was only applied in these pathologically enlarged lymph nodes and only these stations were compared with MS. No information is provided on the other lymph node stations sampled at MS which were not evaluated by EBUS; so, the reported sensitivity of EBUS is artificially high. Moreover, as in a previous comparative study, only suboptimal results are present for cervical MS.[4,5] The latter study concluded that esophageal ultrasound was superior to MS, but sensitivity of MS was only 24%. Although this study was published in 2005, no further prospective studies were published confirming these results. In a prospective study from our institution comparing MS with the findings on chest computed tomography published in 1997, sensitivity of cervical MS was 89% and for the subcarinal lymph node station seven, where the largest disagreement was noted by Ernst et al., sensitivity was 87%. Even for repeat MS, sensitivity has been reported to be higher than for the primary MS in the present study. Four centers participated in this trial including a total of 66 patients. There were only three endoscopists performing EBUS versus eight surgeons performing MS. In the methods section only a very short paragraph is dedicated to MS. What was the experience of the surgeons performing MS? Were they all thoracic surgeons? Were specific guidelines followed? What are the overall results for routine MS in each participating center? In fact, EBUS results in a selected group of patients are compared with a suboptimal performed cervical MS, questioning the final conclusion.

From its introduction MS has been constantly criticized by radiologists, nuclear and invasive pulmonary physicians. However, by providing large tissue samples and a complete mapping of the superior mediastinum, it remains an invaluable tool for staging and restaging of lung cancer. The value of minimally invasive techniques still has to be demonstrated in prospective studies with a clear design in which selection biases that artificially raise sensitivity, are eliminated. The beginning of the end of MS seems far away . . .

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REFERENCES

1. Ernst A, Anantham D, Eberhardt R, Krasnik M, Herth FJ. Diagnosis of mediastinal adenopathy–real-time endobronchial ultrasound guided needle aspiration versus mediastinoscopy. *J Thorac Oncol* 2008;3:577–582.
2. Wigle DA. The beginning of the end of mediastinoscopy? *J Thorac Oncol* 2008;3:561–562.
3. Blackstone EH, Lauer MS. Caveat emptor: the treachery of work-up bias. *J Thorac Cardiovasc Surg* 2004;128:341–344.
4. Larsen SS, Vilmann P, Krasnik M, et al. Endoscopic ultrasound guided biopsy versus mediastinoscopy for analysis of paratracheal and subcarinal lymph nodes in lung cancer staging. *Lung Cancer* 2005;48:85–92.
5. Van Schil P. Accuracy of mediastinoscopy compared to endoscopic ultrasound with fine needle aspiration. *Lung Cancer* 2005;50:273–274.

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Rapid Detection of Hotspot Mutations in Epidermal Growth Factor Receptor by Polymerase Chain Reaction Facilitates the Management of Non-small Cell Lung Cancer

To the Editor:

Histopathologic diagnosis is critical for lung cancer treatment, but sometimes it could be challenging when the

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primary lesion is not easily accessible or the biopsy fails to differentiate the histology. Because recent studies have shown that lung cancer, especially adenocarcinoma, exclusively harbors hotspot mutations in exon 18 to 21 of epidermal growth factor receptors and cancer cells with epidermal growth factor receptor (EGFR) mutations are more likely to be sensitive to tyrosine kinase inhibitors than those without.[1,2] Therefore, using molecular technique to identify these mutations in cancer cells has both diagnostic and predictive value for lung cancer.[3] We report a case of lung adenocarcinoma with vertebral metastases whose diagnosis and treatment were facilitated by the detection of EGFR mutations.

A 54-year-old male nonsmoker was admitted to our hospital because of aggravating low back pain accompanied by progressive weakness in right lower extremity for 3 months and body weight loss of 5 kg in 6 months. Magnetic resonance imaging of the lumbar spine showed lytic lesions in the L3–4 region, together with a mass lesion in the left upper lung on both chest radiograph and computerized tomography. Carcinoembryonic antigen level was elevated to 25.2 ng/ml. The patient then received surgical decompression by laminectomy and the surgical specimen was sent for pathologic examination and for screening of EGFR mutations. The pathologic examination only revealed necrotic tissue, without identifiable cancer cells. Nevertheless, screening of EGFR mutations by polymerase chain reaction (PCR) revealed an exon 20 insertion, suggestive of primary lung cancer (Figure 1*A*). Direct sequencing of the PCR product proved the presence of a 15-nt insertion (Figure 1*B*). Subsequent computerized tomography-guided biopsy of the chest lesion revealed adenocarcinoma with the same insertion. The patient subsequently received local radiotherapy and systemic chemotherapy.

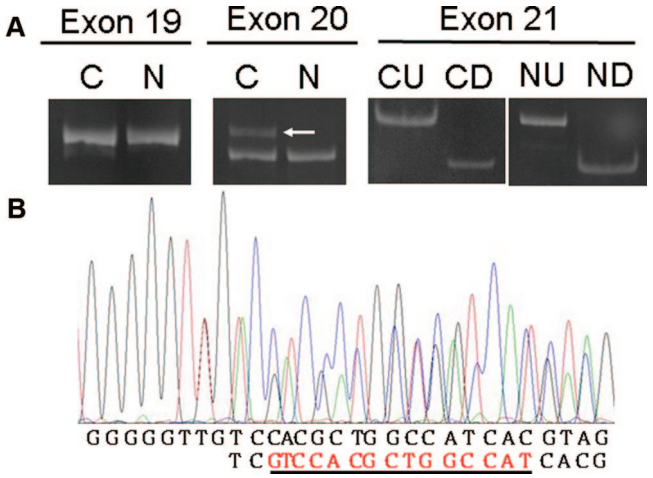

FIGURE 1. Screening of EGFR mutations. Genomic DNA isolated from laminectomized tissue and PCR was used to amplify exon 18 to 21 in EGFR. Mutations in exon 18 were detected by direct sequencing and exon 19 deletions and exon 20 insertions were resolved by length differences on gel electrophoresis. Exon 21 L858R point mutations were identified by first digesting PCR product with MscI, followed by gel electrophoresis. Mutant allele remains undigested after enzyme treatment. *A*, Gel electrophoresis of PCR products for exon 19 to 21. C, cancer tissue; N, normal control; CU, cancer, undigested; CD, cancer, digested; NU, normal, undigested; and ND, normal, digested. Arrow, mutant band. *B*, Sequencing of exon 20 mutant band. Underlined, inserted nucleotides. Primer sequences and detailed PCR conditions are available on request.

This case illustrates the usefulness of including routine EGFR mutation screening to facilitate lung cancer diagnosis. Although the presence of EGFR mutations in exon 19 and 21 indicates the high response rates to tyrosine kinase inhibitors with improved survival, the presence of exon 20 mutations indicates the resistance.[2,4] Therefore, a screening of EGFR mutations by conventional DNA-based PCR methods could greatly improve the diagnosis and treatment design of lung cancer and should be considered as a part of routine pathologic examinations.

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REFERENCES

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
2. Yang CH, Yu C-J, Shih JY, et al. Specific EGFR mutations predict treatment outcome of stage IIIb/IV patients with chemotherapy-naive non-small-cell lung cancer receiving first-line gefitinib monotherapy. *J Clin Oncol* 2008;26:2745–2753.
3. Hsieh RK, Huang MJ, Li CN, Cheng YC, Lin S, Liu HE. Rapid detection of epidermal growth factor receptor mutations in lung cancer by conventional polymerase chain reaction and prediction of treatment response to gefitinib. *Ann Oncol* 2006;26(suppl 9):877P.
4. Sasaki H, Endo K, Takada M, Kawahara M, et al. EGFR exon 20 insertion mutation in Japanese lung cancer. *Lung Cancer* 2007; 58:324–328.

