Multigene Mutation Analysis on Cytologic Samples

To the Editor:

We read with great interest the article by Nakajima et al that was recently published in CHEST (November 2011). The authors demonstrated in a great number of samples that a cytologic specimen obtained by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) could be of particular interest for a successful gene analysis of epidermal growth factor receptor (EGFR), V-Ki-ras Kirsten rat sarcoma viral oncogene homolog (K-ras), or tumor protein 53 (p53).

Other articles have demonstrated already the feasibility of such an approach for EGFR gene analyses on cytologic specimens, particularly using paraffin-embedded cell blocks. Moreover, in the recent publication on the classification of lung cancer through small biopsy specimen and cytology examination, Travis et al recommended to systematically use paraffin-embedded cell blocks not only for immunocytochemistry but also for molecular analysis. In contrast, Nakajima et al emphasized a very important technical point: Artifacts leading to false gene mutation identification can be obtained from small DNA samples extracted from paraffin-embedded cell blocks. Specimens obtained by EBUS-TBNA often are of small size, and optimal processing is essential for their management; therefore, we agree with Nakajima et al that clinicians must pay attention to their treatment. Freezing and storing aliquots of the samples at −80°C in dimethyl sulfoxide according to the same procedure used for cell line preservation is easy to do. Furthermore, doing so enables optimal cell preservation for morphology and a wide range of complementary techniques, such as molecular analyses. In our institution, as already described, we systematically freeze a part of the cytologic specimens obtained by EBUS-TBNA at −80°C. From this frozen material, DNA extraction and sequencing usually are performed with success, whatever the specimen cellularity. These techniques are routinely performed not only on samples obtained by EBUS-TBNA, but also on other cytologic samples such as those obtained by trachebronchic needle aspiration or bronchial brushings and even cerebrospinal fluid. In conclusion, we agree with Nakajima et al that multigene mutation analysis can be performed in EBUS-TBNA samples, promoting freezing cells rather than the cell block and, thus, ensuring optimum technical conditions.

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REFERENCES

Response

To the Editor:

We thank Dr Fleury-Feith and colleagues for their interest in our recent article in CHEST on the use of endobronchial ultrasound-guided transbronchial needle aspiration samples for multigene mutation analysis. Screening for oncogenic gene alteration in non-small cell lung cancer is becoming an important factor in targeted therapy for lung cancer. The ability to acquire surgically resected lung cancer tissue is limited because the majority of patients with lung cancer are inoperable at the time of presentation. Hence, molecular testing using diagnostic biopsy samples is
important for the accurate selection of patients who will benefit from targeted therapy. In our study, the cell pellet derived from a needle wash solution and stored frozen samples were used to avoid the risk of false-positive results due to the fragmented and low amount of DNA. As Dr Fleury-Feith and colleagues suggest, using frozen cells will ensure current optimum technical conditions.

On the other hand, there have been very few articles on the optimal storing methods for samples obtained by needle biopsy techniques for molecular testing. The development of an optimal specimen-handling technique and tumor banking protocol for molecular testing is mandatory for personalized treatment based on biomarker-driven information. Although we agree with the importance of the collection and analysis of fresh-frozen samples from endobronchial ultrasound-guided transbronchial needle aspiration, this may not always be possible in a community hospital. There is a need to develop a reliable, high-throughput analysis using formalin-fixed paraffin-embedded samples. The MassARRAY system (Sequenom Inc; San Diego, California) and molecular inversion probe microarrays may be powerful tools for the detection of comprehensive aberrant gene mutation within lung cancer.14

In conclusion, we are witnessing a paradigm shift in targeted cancer therapy using selective kinase inhibitor in patients with lung cancer. In addition to the exploration of a novel target molecule, we need to continually seek a better way to sample and preserve microsamples for molecular analysis.

Rapid Pleurodesis
An Outpatient Alternative

To the Editor:

We read with great interest in a recent issue of CHEST (June 2011) the report from Reddy et al.1 who describe a pilot study of rapid pleurodesis by combining medical thoracoscopy and talc poudrage with simultaneous tunneled pleural catheter (TPC) placement. In their report, they describe a success rate of 92%, with removal of the TPC at a median of 7.54 days. Furthermore, they have a median hospitalization time of 1.79 days, (mean 3.19). This is clearly a significant advance over the usual 5 to 7 days of hospitalization required during standard chemical pleurodesis.2,3

We used a similar protocol of simultaneous chemical pleurodesis with talc and TPC placement. This procedure evolved as patients with malignant pleural effusions have an average survival of a few weeks to months, so the need for expedient procedures that improve quality of life, minimize discomfort, and decrease the length of stay are of paramount importance.15 To date, we have treated eight cases with a few differences from those described by Reddy et al. (Table 1).

Our standard procedure is a single-port thoracoscopy with parietal pleural biopsies, removal of pleural fluid, and instillation of 4 to 6 g of talc (Sclerosol). We did not leave a thoracostomy tube after the procedure. We placed the TPC on continuous suction for approximately 4 to 12 h, and patients were discharged home the same day of the procedure. We instructed the patients to drain the TPC every day for 5 days and then every other day until the next outpatient visit. Reaching similar results to those described by Reddy et al,1 we had an excellent pleurodesis rate. The catheters were removed at the time of the follow-up visit in seven patients. Pain control was achieved in all cases with the use of oral opioid analgesics.

We agree with Reddy and colleagues1 that rapid pleurodesis with simultaneous chemical and TPC use should be subject to randomized controlled trials in an effort to change the “state of the art” in management of malignant pleural effusions. We hope our experience with a similar protocol and shorter length of stay will contribute in the accumulation of necessary data to support the feasibility, safety, and effectiveness of rapid pleurodesis.

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