Original Article of Interventional Pulmonology Corner

Endobronchial ultrasound-guided fine-needle aspiration for pulmonary carcinomas genotyping: experience with 398 cases including rapid *EGFR/KRAS* analysis in 43 cases

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Background: Endobronchial ultrasound-guided fine-needle aspiration (EBUS-FNA) of mediastinal lymph nodes is a minimally invasive and efficient tool for both diagnosis and staging of lung cancer. EBUS-FNA also permits tumor genotyping. However this critical datum for the therapeutic management is often long to obtain for metastatic patients with short life expectancy.

Methods: From May 2011 to December 2017, 398 lung cancer patients underwent a genetic analysis based on EBUS-FNA samples. EBUS-FNAs were performed with rapid on-site evaluation. Mutations were studied with Sanger or new generation sequencing. Forty-three cases were also tested with a fully automated real-time PCR rapid technique. *ALK* abnormalities were assessed by immunohistochemistry and/or in situ hybridization.

Results: A genotypic result could be obtained in 316 cases (79.4%) and in 180 of the 198 more recent cases (90.9%). Genetic abnormalities were observed in 191 cases (48.0%). Using the rapid technique, *EGFR/KRAS* mutational status was obtained within a few hours following the histological diagnosis and on the same day of the EBUS-FNA by analyzing fresh specimens after intra-operative cytological diagnosis.

Conclusions: In term of molecular diagnosis, EBUS-FNA provides high-quality biological material similar to that of other clinical sampling methods. Furthermore, our study suggests that a rapid molecular diagnostic method could lead to a prompt and appropriate therapeutic management for many advanced stage patients.

Keywords: Endobronchial ultrasound-guided fine-needle aspiration (EBUS-FNA); lung cancer; molecular pathology; fully automated real-time PCR

Submitted Mar 11, 2018. Accepted for publication May 28, 2018.
doi: 10.21037/jtd.2018.06.157
View this article at: http://dx.doi.org/10.21037/jtd.2018.06.157

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Introduction

Endobronchial ultrasound-guided fine-needle aspiration (EBUS-FNA) is an accurate and safe method for establishing a histological diagnosis, for the staging (1), but also for the genotyping (2) of lung carcinomas.

Mediastinal lymph node metastases from pulmonary carcinomas, proven by EBUS-FNA, commonly imply a non-surgical systemic treatment. This treatment must be promptly implemented since lung carcinomas are aggressive tumors (3). The type of treatment depends on possible targetable genetic alterations (4). However tumor genotyping is generally a long and complex process (5) and patients may have to start treatment before biomarkers results are available (6). We have shown that a simple-to-use method could rapidly provide epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene (*KRAS*) status in lung carcinomas (7). We herein report our experience with EBUS-FNA and lung carcinoma genotyping, focusing on how EBUS-FNA is significantly improved by such a rapid molecular diagnostic tool.

Methods

All patients, having an EBUS-FNA that permitted a diagnosis of pulmonary non-small cell carcinoma and that required genotyping, from May 2011 to December 2017 (6 years and 7 months), were included in the study. There were 133 female and 265 male patients aged 34 to 92 years (mean 63.8±10.7).

EBUS-FNAs were performed under moderate sedation or less often under general anesthesia with bronchoscopes fitted with color Doppler ultrasound capability and a 21-gauge needle. Lymph node stations were selected for their positivity on positron emission tomography. Subcarinal and right lower paratracheal lymph nodes were more frequently sampled. However, all other accessible stations (upper paratracheal, left lower paratracheal, hilar, interlobar or lobar) were punctured if needed. Two to eight passes (3 passes on average) per lymph node were performed. A part of the aspirates was smeared on cytology slide and stained with toluidine blue for rapid on site evaluation. Aspirates were afterwards formalin-fixed and paraffin-embedded (FFPE).

Carcinomas were classified according to the 2015 WHO classification (3). Immunohistochemistry and mucin stain (alcian blue) were performed if necessary, according to the current diagnostic recommendations (8). Antibodies against TTF1, p63 and Keratin 7 were used with a Ventana BenchMark GX automated stainer. There were 234 adenocarcinomas, 52 squamous cell carcinomas and 112 non-small cell carcinomas not otherwise specified.

For molecular genotyping, paraffin blocks or blank slides and paraffin shavings were sent to Gustave Roussy University Hospital. Before 2016, mutation assessment was performed by gene amplification with PCR and direct sequencing of exons 18 to 21 of EGFR gene (GeneBank NM005228.3). Exons 2 and 3 of K-RAS gene (NM033360-2), exon 15 of BRAF gene (NM004333.4), exons 10 and 21 of PIK3CA gene (NM006218.2) and exon 20 of HER2 gene (NM 004448.2). Since January 2016, molecular analyses were performed with Vela Diagnostics CE-IVD Sentosa® SQ NSCLC Panel for Next-Generation Sequencing. ALK immunohistochemistry was performed in Gustave Roussy University Hospital, or in Marie Lannelongue Hospital since January 2017, using an anti-ALK antibody (clone 5A4 Zytomed Systems). *In situ* hybridization was performed with Vysis ALK break apart probe kit (Abbott).

In recent cases, *EGFR* and *KRAS* mutational status was also assessed in the department of pathology at Marie Lannelongue Hospital, using the fully automated real-time polymerase chain reaction based Idylla™ (Biocartis, Mechelen, Belgium) system as previously reported (7). Briefly, this system is intended for the detection of 52 mutations in the *EGFR* oncogene: exon 18 (G719A/S/C), exon 21 (L858R, L861Q), exon 20 (T790M, S768I) mutations, exon 19 deletions and exon 20 insertions, and for the detection of 21 mutations in codons 12, 13, 59, 61, 117 and 146 of *KRAS* oncogene. Tests were performed on sections of FFPE material. These tests could also be performed on fresh material (20 µL of fluid) after making a diagnosis on intra-operative cytology. *KRAS* and *EGFR* were tested first separately in smoker and non-smoker patients, respectively. The second gene was not tested in case of positivity since *EGFR* and *KRAS* mutations are generally mutually exclusive (9). Among the 43 patients who benefited from this rapid technique, 17 were stage III, 22 were stage IV, 10 were nonsmokers and 29 were smokers (more than 10 pack-years).

Genetic diagnoses were compared with those obtained similarly with tissue from the pulmonary tumor when available.

This study had the approval of the Ethics Committee

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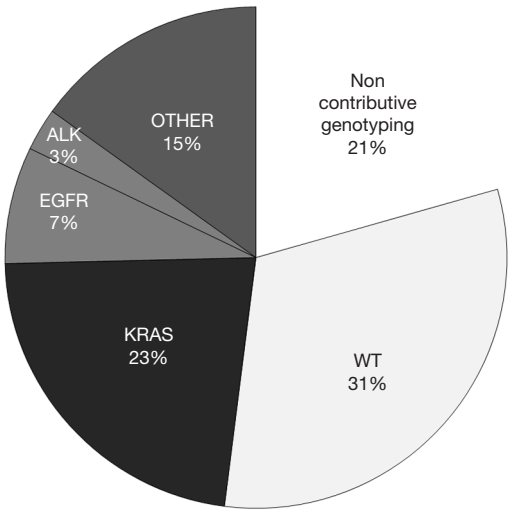

Figure 1 Percentages of genetic abnormalities observed in the series of 398 pulmonary lung carcinomas based on material obtained from endobronchial ultrasound-guided fine-needle aspiration. WT, wild type for all tested genetic abnormalities.

of Marie Lannelongue Hospital (CCML 25-2016, 5th September 2016).

Results

A genetic diagnosis was obtained in 316 of the 398 cases (79.4%) and in 180 of the 198 (90.9%) more recent cases (2015 to 2017). Noncontributory cases were related to insufficient tumor cellularity or to unsuccessful DNA amplification. Abnormalities were detected in 191 cases (48.0%), including 30 cases with *EGFR* mutations, 90 cases with *KRAS* mutations and 11 cases with *ALK* rearrangement (*Figure 1*). In 35 cases, a genetic analysis was also performed on tissue sample from the pulmonary lesion. Results were identical in 24 cases. In 8 cases with noncontributory EBUS-FNA samples, a diagnosis was obtained on surgical samples (n=6), bronchial biopsy (n=1) or transthoracic needle biopsy (n=1). On the contrary, in three cases, the genetic diagnosis was obtained with EBUS-FNA sample while transthoracic needle biopsy was noncontributory.

With the usual procedure (external platform), the first molecular diagnosis (including *EGFR*) was obtained within 3 weeks or less in 275 cases (69%) but never in less than 8 days after sampling of the patient (*Figure 2*).

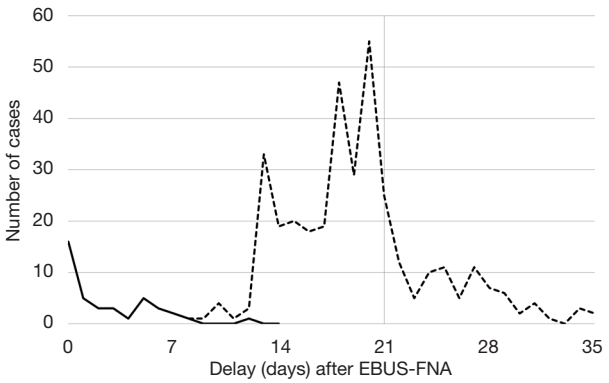

Figure 2 Comparison of the delay for *EGFR/KRAS* mutational status by the on-site rapid Idylla™ system (continuous line) with the delay for the first molecular result including *EGFR* by the external biopathological platform (line with dashes).

With the rapid technique performed locally in 43 cases, the *EGFR/KRAS* mutational status could be obtained within a few hours after histological diagnosis; in fact it was obtained in 1 week or less in 38 cases (88.4%) and even on the very day of the EBUS-FNA procedure in 16 cases (*Figure 2*). In the 43 cases, our sequential testing method permitted to avoid 13 *EGFR* tests and 6 *KRAS* tests, which the external platform later proved to be negative and were thus useless. Specificity and sensitivity of this rapid technique were 100% and 80% for EGFR and 100% and 87% for KRAS, respectively. In 3 cases, a mutation was not detected by the Idylla™ system: *EGFR* T790M, *KRAS* G13C (mutation that is not tested by this technique) and *KRAS* G12C. Conversely, in 1 case the Idylla™ system identified a *KRAS* G12C mutation that had not been detected initially by the routine reference method.

Otherwise, two cases of pneumothorax and one case of transitory respiratory failure occurred after bronchoscopy. These minor complications only required medical management of the patients. There was no major complication, no significant bleeding and no death.

Discussion

EBUS-FNA is a minimally invasive method for tissue sampling of mediastinal lymph nodes (10). Our study confirms its importance in the genotyping of pulmonary carcinomas. Indeed, this technique has been shown to be

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as effective as surgical procedures for complete mutational testing of non-small cell carcinomas (11-13). With the development of immunotherapy other biomarkers are routinely required and samples obtained by EBUS-FNA also appear suitable for the evaluation of PD-L1 expression in lung cancer (14). The diagnostic accuracy rate of EBUS-FNA has been reported to increase with time according to a learning curve (1). In our experience the implementation of more performing techniques also certainly contributes to the improvement of the diagnostic quality.

In a recent study, we have shown that the Idylla™ system could be used in everyday practice for assessing *EGFR* and *KRAS* status in lung carcinomas (7) even in routine diagnostic units of any hospital, without molecular expertise and infrastructure, without complex training and with very good specificity and sensitivity. This was confirmed in another series (15) and for *EGFR* on cytological specimens (16). Unfortunately, the *KRAS* Idylla™ cartridge has been initially designed for colorectal cancer (17) and this made us miss a non-targeted G13C mutation, which represent up to 3% of *KRAS* mutations in lung carcinomas (9). In our experience the main advantages of the Idylla™ system are rapidity and ease of use, making molecular analysis even simpler than immunohistochemistry with explicit results requiring no specialized interpretation. IdyllaTM system for *EGFR* and *KRAS* status must be used in association with immunohistochemistry for ALK, ROS1 and PDL1. These rapid techniques can provide the most needed biomarkers at the time of histological diagnosis of lung carcinoma and their results can be included in the initial pathological report. Complex and time-consuming techniques (in situ hybridization, DNA sequencing) can be performed secondarily, usually by external biopathological platforms. Sequential testing is supposed to cause delay but this is not the case for rapid techniques that can be used in combination with multiplex platforms. Indeed, *EGFR* and *KRAS* mutations sequential testing in lung carcinoma, by selecting the gene that is most likely to be mutated, can be a pragmatic more cost-effective approach (8,18) since *EGFR* and *KRAS* mutations occur in different clinical and histological settings (7) and mutations of these genes are virtually always mutually exclusive (19).

Such a rapid molecular diagnostic procedure is totally fitted to EBUS-FNA: EBUS-FNA is itself a rapid technique and when confirming mediastinal involvement is generally an indication to prompt medical treatment. During our study, the rapidity of the diagnosis was very appreciated by clinicians. However, we could not assess the real impact on the therapeutic management since both clinicians and patients were informed of pending results by the usual reference method.

Rapid on-site evaluation is an intra-operative cytological assessment of EBUS-FNA samples. It has been reported to be associated with lesser number of needle passes (20), to be helpful in providing a preliminary diagnosis (21) and to improve the adequacy for molecular testing (22). This rapid on-site evaluation is a current practice in our hospital and we have found it very useful for selecting fresh material for immediate molecular analysis, thus also reducing the time for a definite diagnosis. The smear can be used for the pre-analysis. Molecular testing of fresh specimens rather than formalin fixated and paraffin embedded material (19) permits analysis of non-deteriorated DNA (23). It was very convenient and useful to have an available system ready to use in our department.

In conclusion, our study shows that, with the help of rapid, cost-effective and easy-to-use molecular techniques, EBUS-FNA has the potential to become even more efficient to meet the need of oncologists for enabling a more rapid initiation of appropriate therapy in lung cancer patients.

Acknowledgements

We thank Mrs. Nathalie Baussart, Mrs. Caroline Communaux, Mr. Philippe Dumet, Mrs. Dominique Gimond, Mrs. Sylvie Planté, Mrs. Idalina Ramos and Mrs. Jocelyne Taiclet for their skilled technical and secretarial assistances.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Ethics Committee of Marie Lannelongue Hospital (No. CCML 25-2016).

Disclaimer: This study has been selected as an oral presentation at the Carrefour Pathologie congress 2017, Paris, France, November 21, 2017.

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Cite this article as: Ghigna MR, Crutu A, Florea V, Feuillet-Soummer S, Baldeyrou P, Adam J, Lacroix L, Besse B, Mercier O, Fadel E, Dorfmuller P, El Ayoubi R, Thomas de Montpréville V. Endobronchial ultrasound-guided fine-needle aspiration for pulmonary carcinomas genotyping: experience with 398 cases including rapid *EGFR/KRAS* analysis in 43 cases. J Thorac Dis 2018;10(7):4653-4658. doi: 10.21037/jtd.2018.06.157

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