PLEURA
January-December 2015: 1-6

DOI: 10.1177/2373997515616584
plr.sagepub.com
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Adequacy of Malignant Pleural Effusion for Epidermal Growth Factor Receptor Mutation Analysis Using the Pyrosequencing Method

Hassan Hatoum, MD[1], Ylagan Lourdes, MD[2], Samjot S. Dhillon, MD[3,4], Grace K. Dy, MD[1], Kristopher Attwood, PhD[5], Venkata Pokuri, MD[1], and Kassem Harris, MD[3,4]

Abstract

Background: Epidermal growth factor receptor (EGFR) mutation analysis is a standard approach for initial therapeutic decision in patients with metastatic adenocarcinoma of the lung (MAL). The feasibility of performing EGFR mutation testing using pleural fluid specimen is not well characterized. **Objectives:** The aim of this study is to report the percentage of patients eligible for EGFR mutation testing based on the percentage of malignant cells (PMCs) in the pleural fluid using the pyrosequencing method. **Methods:** From our database, we reviewed the clinical data of 61 patients with malignant pleural effusion (MPE) secondary to MAL. The PMCs were divided into 2 categories with a cutoff point of 10% (PMC1 is defined as ≤ 10% and PMC2 is defined as >10%). For the pyrosequencing method, only patients in the PMC2 group were eligible for EGFR mutation testing. **Results:** Of 61 patients with MPE secondary to MAL, 38 (62.3%) were in the PMC2 group, which represents the percentage of patients eligible for EGFR mutation testing. Of these 38 patients, 15 patients had the testing done on the MPE. Quantity was not sufficient for testing only in 1 patient. Therefore, in PMC2 patients group, the rate of successful EGFR mutation testing was 93% (14 of 15). The thoracentesis volume was not significantly different between PMC1 and PMC2. **Conclusion:** Performing EGFR mutation analysis on the MPE in patients with MAL is feasible in 62% of patients. The rate of successful testing on the eligible samples is 93%.

Keywords

epidermal growth factor receptor, yield, pleural effusion, thoracentesis

Introduction

Lung cancer remains the primary cause of death in the United States, with an approximate rate of overall survival (OS) of 16% at 5 years.[1,2] Nonsmall cell lung cancer (NSCLC) is the most common type of lung cancer with a frequency of 85%.[3] Adenocarcinoma constitutes 40% of NSCLC.[4] Epidermal growth factor receptor (EGFR) tyrosine kinase domain mutations are found mainly in this histologic subtype, especially in Asian descents and females.[5,6] In the past, lung cancer treatment was based mainly on the histology. Nowadays, in the era of targeted therapy, testing for EGFR mutation and ALK rearrangement has become the standard approach. The EGFR mutation is found in approximately 10% of caucasian patients with lung adenocarcinoma, and it has been detected in as high as 50% of the Asian patients.[7] This relatively significant percentage of EGFR mutations compared to other sensitizing targets such as ALK gene rearrangement, which is estimated to be in 2% to 7% of patients with NSCLC,[8] has led investigators to vigorously pursue testing for this particular mutation. In addition to being a prognostic biomarker, EGFR mutation has been proven to be a predictive biomarker of response to EGFR tyrosine kinase inhibitors (TKIs). The EGFR

[1] Department of Medicine, Division of Medical Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA
[2] Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY, USA
[3] Department of Medicine, Interventional Pulmonology Service, Roswell Park Cancer Institute, Buffalo, NY, USA
[4] Department of Medicine, Pulmonary, Critical Care and Sleep Medicine, University at Buffalo, State University of New York, Buffalo, NY, USA
[5] Department of Biostatistics and Bioinformatics, Roswell Park Cancer Institute, Buffalo, NY, USA

Corresponding Author:
Kassem Harris, Roswell Park Cancer Institute, Elm and Carlton streets, Buffalo, NY 14623, USA.
Email: kassemharris@gmail.com

mutation analysis is a major determinant in the selection of first-line therapy in advanced adenocarcinoma of the lung.[9-13] It has been shown to confer susceptibility to treatment by small-molecule EGFR TKIs such as Gefitinib, Erlotinib, and Afatinib.[9-15] Several trials have shown that in patients with sensitizing EGFR mutation, the use of EGFR TKI as a first-line therapy compared to chemotherapy has led to improvement in progression-free survival (PFS), with OS benefit seen in patients with exon 19 deletion who receive Afatinib.[16,17] Therefore, identification of EGFR mutation status before starting first-line treatment in metastatic NSCLC has become the standard of care. It is well known that the tumor tissue is the optimal source of DNA for performing this test. However, tumor tissues are not always available and other sources for EGFR mutation testing should be explored. For instance, only 36% in the Iressa Pan-Asia Study (IPASS) study and 20% in the IRESSA NSCLC Trial Evaluating Response and Survival against Taxotere (INTEREST) study had adequate tumor tissue for testing.[12,18] In another study by Vanderlaan et al, the failure rate for EGFR mutation analysis using image-guided percutaneous transthoracic core-needle biopsies to collect tissues was 31.8%, and it was as high as 23.1% when using samples obtained from metastatic bone tissue.[19] Although testing for EGFR mutation in malignant pleural fluid in lung cancer has been reported in previous studies,[20-23] none of these studies reported the rate of successful testing based on the percentage of malignant cells (PMCs) in pleural fluid. One report included comparison of different diagnostic procedures such as transthoracic needle biopsy, bronchoalveolar lavage, surgical biopsies, and thoracentesis for pleural fluid samples to look at the yield of genetic profiling in NSCLC.[24] In another study by Yi Liu et al, comparison between 2 different mutation testing approaches, amplification refractory mutation system (ARMS) and direct sequencing using samples from different body fluids, was reported.[25] In this study, we report the chances of patients with malignant pleural effusion (MPE) secondary to metastatic adenocarcinoma of the lung (MAL) to be eligible for EGFR molecular testing based on the requirement of the presence of PMCs of more than 10% in the MPE for pyrosequencing method as well as the rate of successful EGFR mutation testing in the eligible group.

Methods

This is a retrospective study of 61 patients with diagnosis of MPE and MAL who underwent diagnostic thoracentesis at Roswell Park Cancer Institute (RPCI) between January 2009 and July 2014. The study was approved by the Institutional Review Board at RPCI. The primary objectives of this study are to report the percentage of patients eligible for EGFR mutation testing based on the PMCs in the pleural fluid of more than 10% to satisfy the requirement of pyrosequencing method used for testing and to determine the rate of successful EGFR mutation testing in this group of patients. If the patient had more than 1 thoracentesis, he was included in the study only if any of the pleural fluid samples obtained was positive for malignant cells (Figure 1). Data collection included age, gender, past lung cancer history, site of thoracentesis, PMCs, immunohistochemistry (IHC), EGFR mutation testing result, and the volume of pleural effusion collected from thoracentesis. All the cytology slides for the pleural fluid samples were reviewed by the cytologist (LY), and the PMC was reported (Figure 2). The PMCs were divided into 2 categories with a cutoff point of 10% (PMC1 defined as $\leq$10% and PMC2 as >10%). This was because the minimal requirement for molecular testing using pyrosequencing method is more than 10%. Pyrosequencing is a sensitive and accurate method for detection of EGFR mutations, and it uses a nonelectrophoretic real-time sequencing technology with luminometric detection.[26,27] In addition to mutation detection, pyrosequencing has the ability to accurately quantify the percentage of mutated alleles and characterize these mutations in the sample.[28]

In the MAL cohort, the thoracentesis status and corresponding results are reported as frequencies and relative frequencies. Within the patients with MPE, the patient

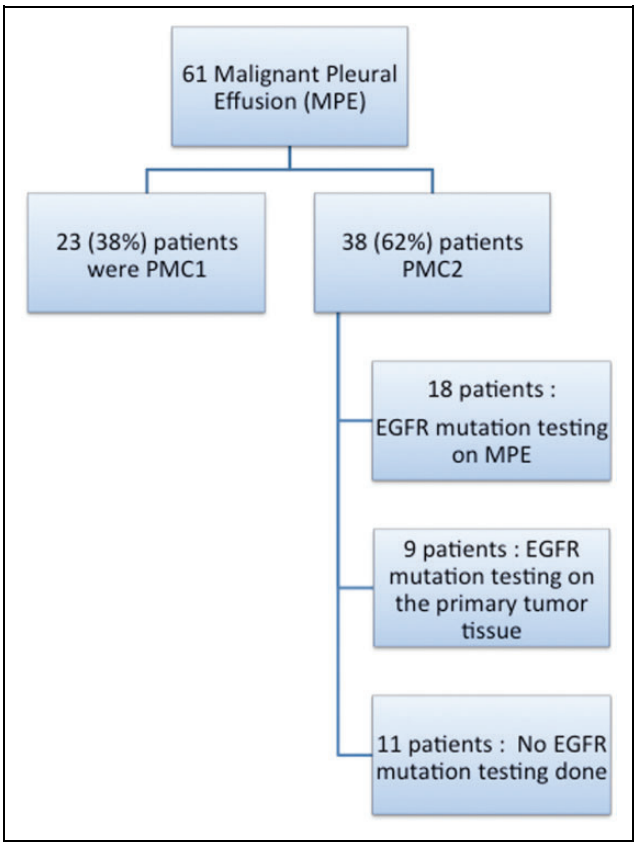

Figure 1. Algorithm showing the patients' distribution and the diagnostic thoracentesis results.

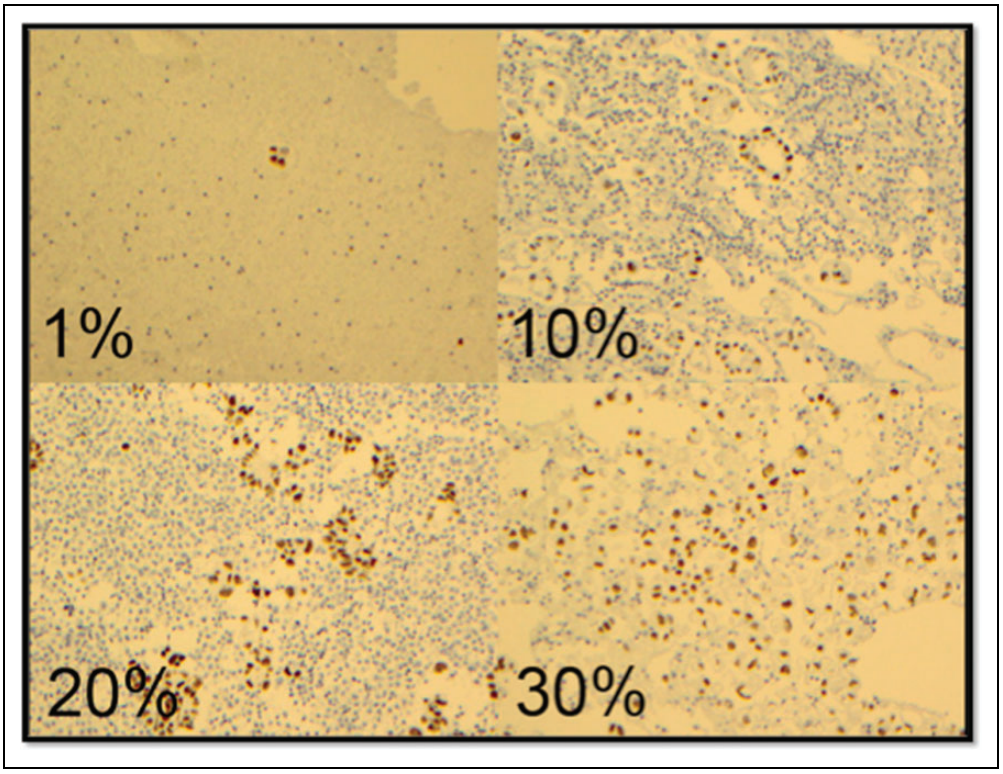

Figure 2. This figure shows nuclear staining for thyroid transcription factor 1 (TTF-1) where approximately 1%, 10%, 20%, and 30% of lung adenocarcinoma cells show positive staining in the pleural effusion.

characteristics (age, gender, history of cancer, and fluid volume) and testing outcomes (molecular testing order status and EGFR mutation testing results) are reported by percentage of positive malignant cells (PMC $\leq$ 10% vs >10%) using the median and range for continuous variables and using frequencies and relative frequencies for categorical variables. Comparisons are made using the Wilcoxon rank sum and Fisher exact tests for continuous and categorical variables, respectively. The EGFR eligibility is defined as the number of patients with PMC2 divided by the total number of patients with MPE in the study. The success rate of EGFR mutation testing on the MPE is defined as EGFR mutation testing that came back with a result whether positive or negative in the PMC2 group. Confidence intervals about the yield are obtained using Jeffrey prior method. A significance level of .05 is considered, therefore a P value less than .05 is considered statistically significant. All analyses are conducted in SAS v 9.4 (Cary, North Carolina).

Results

The median age of the cohort studied (61 patients) was 67 years (range 31-87 years). Forty-three (71.7%) patients had prior lung cancer history, while the rest had their initial clinical presentation with MPE. The majority of the patients, 55 (90.2%), underwent only 1 diagnostic thoracentesis, while 5 (8.2%) patients had 2 and 1 (1.6%) patient had 3 procedures done. The IHC was done on the pleural effusion sample in 76.7% of the patients, while all the patients had IHC done on the tissues from the primary lung cancer as part of their diagnosis. The sites of thoracentesis were divided almost equally between the right and the left, 32 (52.2.%) and 29 (47.5%), respectively. There was a significant association between PMC and age (P = .031) and molecular testing ordered (P = .038; Table 1). Patients in the PMC2 were younger compared to those in the PMC1 group (median age 65 vs 74 years), and there was a higher rate of molecular testing for EGFR mutation ordered for patients in the PMC2 group. The initial reporting of the cytology percentage at diagnosis was done by different cytologists of our cytology laboratory. Of the 61 patients in this cohort, 38 (62.3%) were in the PMC2 group, which represents the percentage of patients eligible for EGFR testing (EGFR eligibility). The EGFR mutation testing was requested in 15 patients on the MPE in the PMC2 group; 14 samples had successful testing and 1 sample failed testing (quantity not sufficient). The rate of successful EGFR mutation testing in the PMC2 group was 93% (14 of 15).

Discussion

The treatment of metastatic NSCLC has evolved over time with targeted therapy playing a significant role in first-line and subsequent lines of therapy. The identification of sensitizing mutations such as certain EGFR mutations has become

Table 1. Characteristics of Patients Based on Percentage of Malignant Cells in the Pleural Effusion.[a]

		PMC ≤ 10%	PMC > 10%	Overall	*P* Value
Overall	N	23 (37.7)	38 (62.3)	61 (100%)	
Age		74 (51-87)	65 (31-84)	67 (31-87)	.031
Gender	Male	12 (52.2)	19 (50)	31 (50.8)	1.000
	Female	11 (47.8)	19 (50)	30 (49.2)	
Past lung cancer history	No	7 (30.4)	10 (27)	17 (28.3)	.777
	Yes	16 (69.6)	27 (73)	43 (71.7)	
Volume of pleural effusion, mL		850 (275-1800)	1100 (13-3100)	1000 (13-3100)	.331
EGFR results on pleural fluid[a]	Neg	3 (60.0)[b]	8 (36.4)	11 (40.7)	.651
	Pos	0	6 (27.3)	6 (22.3)	
	QNS	0	1 (4.5)	1 (3.7)	
EGFR results on tumor tissue		2 (40%)	7 (31.8)	9 (33.3)	
IHC		No		14 (23.3)	
		Yes		46 (67.7)	

Abbreviations: QNS, quantity not sufficient; IHC, immunohistochemistry; EFGR, epidermal growth factor receptor; PMCs, percentage of malignant cells.
[a]Total number of patients sent for EGFR testing on pleural fluid is 18 (3 patients in PMC ≤ 10% and 15 patients in PMC >10%).
[b]These 3 patients were reported as >10% initially but on review of the slides by the head of cytology laboratory during the study, it was reported to be <10%. The EGFR results for these patients could be false negative, as they do not satisfy the requirement for pyrosequencing method which is sensitive for PMC >10%.

an essential part of the decision making on the therapeutic approach for newly diagnosed NSCLC. The EGFR mutations include the exon19 deletion, which is found in 45% of patients, and exon21 mutation, which is found in about 40% of patients. While these 2 common mutations in the EGFR region is associated with good response to TKIs,[29] exon 20 insertion mutations confer resistance to TKIs. Response to TKI as first-line treatment in patients with NSCLC has been avidly documented in several randomized studies[11-13,16,30,31]; Erlotinib has been shown to have superior PFS when compared to chemotherapy in the first-line setting in patients with The EGFR mutation. This has been shown in the EURTAC trial where median PFS was 9.7 months and 5.2 months in the Erlotinib group and the standard chemotherapy group, respectively.[16] These findings strongly support the recommendation for initial EGFR mutation testing for patients with metastatic NSCLC.

Our study is a retrospective study to assess the adequacy of MPE for EGFR mutation analysis. Thoracentesis has been successfully used to obtain malignant pleural fluid for EGFR mutation analysis. Nevertheless, there is no data about the rate of successful EGFR testing using pleural fluid as a source. In this study, we described the yield or the success of the EGFR testing based on the PMCs in the pleural fluid. Advantages of using MPE as specimen include ease of performance of the thoracentesis procedure, minimal invasiveness, and the ability to perform it multiple times. Another appealing reason for considering EGFR testing on MPE is that EGFR mutations are found in higher numbers in patients diagnosed with MAL having MPE compared to those who did not have MPE.[23] In their retrospective study, Jain et al reported the finding of EGFR mutation in 28.6% (81 of 283) of patients with lung adenocarcinoma; however, the incidence of EGFR mutation was reported to be 38% in patients with MPE versus 20% in patients without MPE ($P = .028$).[23] In our study and after reviewing all the slides on malignant pleural fluid on the 61 patients with MAL, we revealed that 62.3% (38 of 61) of the patients with MPE secondary to MAL are eligible for EGFR mutation testing on MPE samples using pyrosequencing method. The rate of successful testing on the eligible samples is 93% (14 of 15). Accordingly, patients with MAL can be well informed about their chances of being eligible for EGFR mutation testing on MPE using pyrosequencing method, and this will help them making a better decision.

The utilization of thoracentesis to obtain malignant pleural fluid sample for EGFR mutation analysis has been described in literature recently[20-23]; however, none of these studies has described the percentage of patients who could be eligibility for EGFR mutation testing on MPE using pyrosequencing method. For instance, the study by Liu et al has described the comparison between ARMS and direct sequence for assessment of EGFR mutation status in 50 patients treated with TKI using 32 pleural fluid and 18 plasma samples.[25] They showed that positive mutation results using any of the above-mentioned methods were associated with better outcome compared to patients who had no mutation.[25] Soh et al reported the value of EGFR mutation analysis in MPE as a predictor to the outcome to Gefitinib treatment.[22] In their study, they reported that patients with EGFR mutation on pleural fluid samples had higher overall response rate (ORR) ($P < .0001$), OS ($P < .0092$), and PFS (.018).[22] In another trial, Buttitta et al reported the sensitivity of Next Gen Sequencing (NGS) method in assessment of EGFR mutation in bronchoalveolar lavage (BAL) and pleural fluid compared to Sanger sequencing, where Sanger detected only 16% of the EGFR mutation versus 81% detection by NGS.[20]

The limitation of our study is the small number of patients reviewed for the eligibility for EGFR testing depending on PMCs (61 patients) and the even smaller number of patients where EGFR testing was ordered (15 patients). We are aware

that there is selection bias which could not be controlled, as this is a retrospective study. We are also cognizant of the fact that the eligibility for EGFR mutation testing may change based on the sensitivity of the method used for EGFR mutation testing. For instance, ARMS has a higher sensitivity and can be applied reliably on a PMC as low as 1%,[32] Sanger technique has a lower sensitivity and can be applied on a PMC of at least 20%, while pyrosequencing can be applied on a PMC of at least 10%.[25,28,33,34] Nevertheless, the results of this study are significant for patient care and can be applied to centers that use the pyrosequencing method for EGFR mutation analysis.

Conclusion

This study presents an estimate of the percentage of patients with MPE secondary to MAL who are eligible for EGFR mutation testing on the pleural fluid (62.3%). In addition, the rate of successful testing in this group of patients using pyrosequencing method was 93%. This information has a significant impact on clinical practice where, now, it is possible to inform patients with MPE about the expected yield of their EGFR mutation testing using thoracentesis. Malignant pleural effusion has been reported to be present in up to 40% of patients with lung cancer at certain point during their disease.[35] Because of this considerable incidence and the ease of performing thoracentesis, we suggest using MPE samples for EGFR mutation analysis, with expected eligibility for EGFR testing of 62.3%. Prospective studies are recommended to identify the factors affecting PMCs and hence eligibility for testing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Author Biographies

Hassan Hatoum finished his Hematology-Oncology fellowship from Roswell Park Cancer Institute/SUNY at Buffalo, NY. Currently, he is an assistant professor at Stephenson Cancer Center/University of Oklahoma Health Sciences Center. He is interested in academic medicine and Research. His main interest is in solid tumors, mainly GI malignancies and sarcoma.

Ylagan Lourdes is an associate professor of pathology and cytopathology laboratory director. Her area of interest is in the detection of malignant cells in all body sites using light microscopy, immunohistochemistry and biomarker assays.

Samjot S. Dhillon is an associate professor of oncology and chief of pulmonary medicine. His research interest is in early detection of lung cancer and pre-malignant lung lesions.

Grace K. Dy is a physician scientist with disease focus on thoracic malignancies. Her areas of research interests are in early drug development, cell signaling and immunotherapy. He is also interested in the bronchoscopic techniques for management of lung cancer.

Kristopher Attwood is an Assistant Professor of Biostatistics at Roswell Park Cancer Institute; with research interests in classification problems, decision theory, and statistics education.

Venkata Pokuri is a clinical oncology fellow at Roswell Park Cancer Institute, Buffalo, New York. He completed medical training in 2012 from St. Luke's-Roosevelt Hospital Center affiliated to Columbia University College of Physicians and Surgeons.

Kassem Harris is an interventional pulmonologist with research interests in diagnostic and therapeutic pleural and bronchoscopy development.