ORIGINAL ARTICLE

Real-World Data of the Correlation between EGFR Determination by Liquid Biopsy in Non-squamous Non-small Cell Lung Cancer (NSCLC) and the EGFR Profile in Tumor Biopsy

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Abstract

EGFR-mutated non-small cell lung cancer (NSCLC) has significant improved outcomes when treated with EGFR-tyrosine kinase inhibitors (TKI). Thus, EGFR-mutational status should be assessed at diagnosis and in the course of treatment with TKI. However, tissue samples are not always evaluable, and molecular profiling has been increasingly performed in cell-free tumor DNA (ctDNA) from blood samples. Our objective is to evaluate the reliability of ctDNA profiling in plasma samples in a real-world setting. We retrospectively analyzed the patients diagnosed with non-squamous NSCLC from May 2016 to December 2017 at Hospital Universitario Doctor Peset who had been tested for EGFR mutations in tissue and plasma samples. Both samples were sent to an external laboratory to perform the analysis by the cobas® EGFR assay. Percentage of agreement and concordance were calculated by kappa statistic. Of 102 patients reviewed, 89 were eligible. The overall EGFR mutation frequency was 18.6% for the evaluable tissue samples and 19.6% for evaluable plasma samples. Mutation status concordance between matched samples was 87.4%. Cohen's kappa index (κ) = 0.6 (sensitivity 70.6%, specificity 91.7%, positive predictive value 66.7%, negative predictive value 93%). When concordance was stablished only in stage IV tumors κ = 0.7, suggesting a higher agreement in advanced disease. This real-world data suggest that plasma is a feasible sample for ctDNA EGFR mutation assessment. Results of ctDNA molecular profiling are reliable when using a validated technique such as the cobas® EGFR assay, especially in patients that cannot undergo a tissue biopsy.

Keywords EGFR mutation status · Liquid biopsy · ctDNA · Cobas EGFR assay · Non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with approximately 1.6 million deaths in 2012 [1]. Non-small cell lung cancer (NSCLC) accounts for around 85% of all cases, and despite the recent development of new treatments for this disease in advanced setting, the prognosis remains poor and overall survival is only 15% after 5 years of diagnosis [2]. However, that is somehow different for tumors with driver mutations.

One of the recent greatest achievements in expanding knowledge on the natural history of NSCLC was the discovery of oncogenes that work as driver mutations for the origin and development of cancer, because they have shown to be prognostic and predictive factors. Among them, EGFR mutations are found in 10–15% of cases of NSCLC in our environment, especially in

Table 3 Distribution of EGFR mutation types

	TISSUE	PLASMA
Del19	11	16
Mut21	8	4

Del19: deletions in exon 19; Mut21: mutation L858R in exon 21

Table 4 Concordance of EGFR mutation status between matched tissue/cytologic and plasma samples in the evaluable population

Characteristic	%	95% CI
Sensitivity	70.6	48.9–92.2
Specificity	91.7	85.8–97.5
Positive predictive value	66.7	43.7–83.7
Negative predictive value	93.0	84.6–97.0

Unfortunately, patients that are initially sensitive to first and second-generation EGFR-TKIs acquire resistance in the course of treatment, with a median PFS reported to be around 9–11 months [9, 10] and about half of these resistances are due to the presence of the T790 M mutation in EGFR [11]. In recent years, third-generation molecules have been developed to inhibit both EGFR sensitizing mutations and T790 M resistance mutation [12]. Consequently, it is important to re-assess EGFR mutation status when a progressive disease is observed in order to be able to choose the most appropriate therapy. However, assessing the EGFR mutation status in tissue sample is not always possible because re-biopsing the tumor is an invasive procedure with risk of complications, and even when it can be performed, obtaining sufficient sample to perform molecular testing is a challenge. That is why analysis of ctDNA is gaining relevance as a minimally invasive alternative method for the detection of EGFR mutations [13].

Several technologies have been evaluated for the detection of EGFR mutations using plasma ctDNA. In the present study we analyzed the correlation between EGFR mutation status in plasma and tissue samples using the Roche cobas® EGFR assay in a real-world setting.

The cobas® EGFR assay is a validated allele-specific quantitative PCR-based method which uses selective oligonucleotide probes for targeted mutations located in exons 18, 19, 20 and 21 labeled with a fluorescent reporter [14]. Its workflow is based on two major procedures: firstly, manually extracting genome DNA from tissue or plasma samples and secondly, PCR amplification to detect mutations in the EGFR gene by measuring fluorescence. This has proved to be a simple and fast method for detecting the most frequent clinically significative EGFR mutations and that is why it was implemented in our hospital, being performed by an external laboratory.

In randomized clinical trials, the cobas® EGFR mutation test in plasma samples has shown concordance rates higher than 90% compared with tissue samples and a sensitivity ranging from 85 to 100% [14, 15]. However, it is uncertain if these promising results are alike in a real-world setting. We are aware that this series is limited by its retrospective nature and being based on a single center experience. Nevertheless, there is a need to confirm the reported findings reported in controlled clinical trials [16, 17] and in larger centers experience to improve local practice and to make us able to provide our patients with the most accurate diagnosis and personalized treatment options. Moreover, real-world data studies have shown differences between results in ctDNA testing depending on the geographic region [18], which demonstrates that ctDNA assessment methodology needs reassurance.

The mutation status concordance observed in our population (87.4%) and the assessed inter-test agreement ($\kappa = 0.6$) suggest that plasma is a feasible sample for real-world EGFR mutation analysis even though tissue sample analysis is still considered the gold standard [13]. Furthermore, the results are even more encouraging if we only consider stage IV NSCLC, where $\kappa = 0,7$, indicating a substantial agreement between tumor and plasma EGFR mutation status [19].

Notwithstanding these promising results, we analyzed possible reasons for the low positive predictive value obtained (PPV = 66.7%). Firstly, in almost 20% of patients, EGFR

Fig. 1 Percentage of concordant (wild-type and mutations) and discordant mutations in matched tissue and plasma mutations
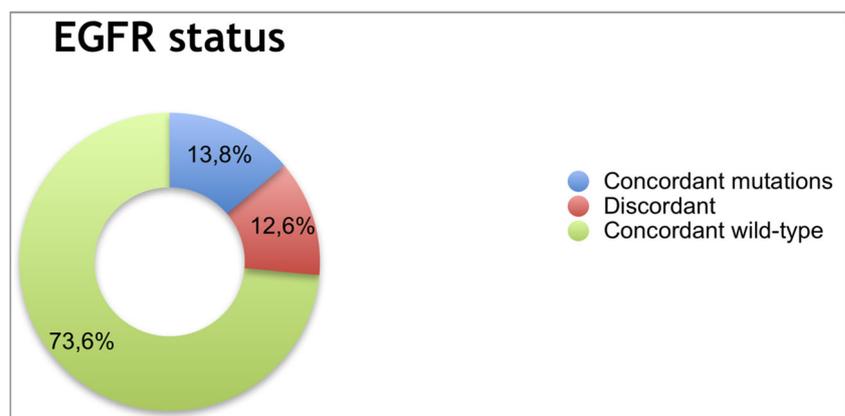
mutational tissue status was determined in cytologic samples, which may not be representative of the disease; and the percentage was higher in discordant results (42.9%). Also, some of the differences observed among tissue and plasma samples could be due to the different timing of the diagnostic procedures. Therefore, the low PPV could be due to false-negative results in tissue samples, that can occur because of tumor heterogeneity or initial inexperience in the workflow procedure, rather than because of false-positive results in plasma samples.

Our results were more consistent with other studies of real-world basis [18, 20]. They reported values of sensitivity around 50% and specificity around 95%. PPV was 82% in the Asian and Russian study and 78% in the Japanese and European study (specifically, 70% in the European cohort), and NPV was shown to be around 80–90%.

This study however, has limitations. Besides its retrospective nature and the small sample size, not all of the patients diagnosed with non-squamous NSCLC in our hospital during 2016 and 2017 were tested for EGFR mutations in plasma, and patients that did not have both determinations (tissue and plasma) were not included in our analysis, and these may explain why the prevalence of EGFR mutation is higher in our series than in previous publications [21]. Molecular profiling in ctDNA was not initially included in our diagnostic protocol, and it was performed according to the physician's criteria, but it was added subsequently.

Finally, as our samples are externally studied, our major concern is not he DNA extraction, but the pre-analytical workflow. In our case, all these procedures are performed by the same trained personnel following manufacturer instructions, which reduces the possibility of compromising the correct workflow. Importantly, in 2017 we started using BD Vacutainer® Plasma Preparation Tubes (PPT™) which allow a longer interval between the extraction of the blood sample and its processing. Shipping is also carefully scheduled, and plasma samples are transported on dry ice. Although shipping is also a source of possible mismanagement of the samples, we did not record any problems in this area during the time of collecting the data.

More sensitive techniques such as next generation sequencing (NGS) are being used in clinical trials and in large hospitals to evaluate the mutational status in patients diagnosed with NSCLC [22–24]. Nevertheless, these systems are not widely available, and therefore, we have to make an effort to offer our patients the optimal mutation analysis of both tissue and plasma samples that is accessible in our centers. With validated allele-specific quantitative PCR-based methods like the Roche cobas® mutation test that can be performed also in ctDNA [14] we are given the potential of real-time monitoring of tumor mutation status using a minimally invasive procedure.

Conclusions

Taken together, our results suggest that assessing the mutational status of the EGFR gene in plasma ctDNA is an equally robust method in detecting the most common EGFR mutations as performing the same technique in tumor DNA, which is considered the gold standard. Furthermore, it is a feasible technique highly useful in patients in which an invasive procedure such as a tissue biopsy cannot be performed because of anatomic difficulties, impaired performance status or comorbidities.

Compliance with Ethical Standards

Informed Consent The study was conducted in accordance with the Declaration of Helsinki, written informed consent was exempted because of the retrospective nature of the study.
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Conflict of Interest The authors declare that they have no conflicts of interest.

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References

1. Cancer Incidence IARC (2012) Mortality and prevalence worldwide. In: GLOBOCAN
2. Malvezzi M, Carioli G, Bertuccio P, Boffetta P, Levi F, La Vecchia C et al (2017) European cancer mortality predictions for the year 2017, with focus on lung cancer. Ann Oncol 28:1117–1123. https://doi.org/10.1093/annonc/mdx033
3. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sánchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M (2009) Screening for epidermal growth factor receptor mutations in lung Cancer. N Engl J Med 361:958–967. https://doi.org/10.1056/NEJMoa0904554
4. Janku F, Angenendt P, Tsimberidou AM, Fu S, Naing A, Falchook GS, Hong DS, Holley VR, Cabrilo G, Wheler JJ, Piha-Paul SA, Zinner RG, Bedikian AY, Overman MJ, Kee