Send Orders for Print-Reprints and e-prints to reprints@benthamscience.ae

Current Cancer Drug Targets, 2018, *18*, 1-9

RESEARCH ARTICLE

Metastatic Site Location Influences the Diagnostic Accuracy of ctDNA EGFR- Mutation Testing in NSCLC Patients: a Pooled Analysis

Francesco Passiglia[1,#], Sergio Rizzo[1,#], Christian Rolfo[2], Antonio Galvano[1], Enrico Bronte[1], Lorena Incorvaia[1], Angela Listi[1], Nadia Barraco[1], Marta Castiglia[1], Valentina Calò[1], Viviana Bazan[1,^] and Antonio Russo[1,^,*]

[1]*Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, Palermo University Hospital, Palermo, Italy;* [2]*Phase I- Early Clinical Trials Unit, Oncology Department and Multidisciplinary Oncology Center Antwerp (MOCA), Antwerp University Hospital, Edegem, Belgium*

Abstract: ***Background*:** Recent studies evaluated the diagnostic accuracy of circulating tumor DNA (ctDNA) in the detection of epidermal growth factor receptor (EGFR) mutations from the plasma of NSCLC patients, overall showing a high concordance as compared to standard tissue genotyping. However, it is less clear if the location of the metastatic site may influence the ability to identify EGFR mutations in plasma.

***Objective*:** This pooled analysis aims to evaluate the association between the metastatic site location and the sensitivity of ctDNA analysis in detecting EGFR mutations in NSCLC patients.

***Methods*:** Data from all published studies, evaluating the sensitivity of plasma-based EGFR-mutation testing, stratified by metastatic site location (extrathoracic (M1b) vs intrathoracic (M1a)) were collected by searching in PubMed, Cochrane Library, American Society of Clinical Oncology, and World Conference of Lung Cancer, meeting proceedings. Pooled Odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated for the ctDNA analysis sensitivity, according to metastatic site location.

***Results*:** A total of ten studies, with 1425 patients, were eligible. Pooled analysis showed that the sensitivity of ctDNA-based EGFR-mutation testing is significantly higher in patients with M1b vs M1a disease (OR: 5.09; 95% CIs: 2.93 – 8.84). A significant association was observed for both EGFR-activating (OR: 4.30, 95% CI: 2.35-7.88) and resistant T790M mutations (OR: 11.89, 95% CI: 1.45-97.22), regardless of the use of digital-PCR (OR: 5.85, 95% CI: 3.56-9.60) or non-digital PCR technologies (OR: 2.96, 95% CI: 2.24-3.91).

***Conclusions*:** These data suggest that the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis in detecting EGFR mutations in NSCLC patients.

ARTICLE HISTORY

Received: May 12, 2017
Revised: December 11, 2017
Accepted: January 17, 2018

DOI:
10.2174/1568009618666180308125110

Keywords: EGFR, ctDNA, liquid biopsy, NSCLC, metastasis, intrathoracic, extrathoracic.

1. INTRODUCTION

Targeting the epidermal growth factor receptor (EGFR) by tyrosine-kinase inhibitors (TKIs) has represented a milestone in the treatment of lung cancer. Eight phase III randomized studies have clearly demonstrated that EGFR-TKIs significantly improved response rate (RR), progression-free survival (PFS) and quality of life (QoL) compared to first-line platinum-based chemotherapy in patients with advanced NSCLC harboring EGFR activating mutations [1-8]. Recently a pooled analysis of both LuxLung3 (LL3) and LuxLung6 (LL6) trials showed also an overall survival (OS) benefit in favour of the second generation EGFR-TKI Afatinib in the subgroup of patients with EGFR exon19 deletion [9]. Overall, the results of all such studies convincingly and consistently demonstrated that, for about 40% of Asian and 12% of Caucasian "EGFR-positive" NSCLC patients, the optimal upfront treatment is an EGFR-TKI, as gefitinib, erlotinib, or afatinib. Testing for EGFR mutations in tumor samples DNA is recommended at the time of diagnosis by all the international guidelines for all patients with advanced NSCLC and non-squamous histology in order to decide the proper therapeutic strategy [10]. Even if tissue biopsy remains the current gold-standard, however, it is limited by several features, such as the difficult access to different tumor sites, the invasiveness of procedures, the tumor heterogeneity, and the low patients' compliance. Thus, in the last decade, an alternative approach, known as a liquid biopsy, has been proposed to overcome the aforementioned issues. An increasing number of studies and meta-analysis evaluated the diagnostic accuracy of circulating tumor (ct)DNA in the

*Address correspondence to this author at the Section of Medical Oncology, Department of Surgical, Oncological and Oral Sciences, Palermo University Hospital, Via del Vespro 129, 90127 Palermo, Italy; Tel: + 39-091-6552759; Fax: +39-091-6554529; E-mail: antonio.russo@usa.net
#These authors equally contributed to this work.
^These authors are both last authors of this work.

1568-0096/18 $58.00+.00 © 2018 Bentham Science Publishers

detection of EGFR activating mutations in plasma of patients with advanced NSCLC, overall showing a sensitivity of 0.62 and a specificity of 0.96 as compared with the standard tissue genotyping, thus suggesting an high concordance rate between these two testing approaches [11-14]. These evidence have led to the analytical validation and the clinical approval of EGFR mutation testing by using ctDNA isolated from plasma or serum for about 25-30% of NSCLC patients whose tissue is not available at the time of diagnosis or tissue analysis results are not evaluable. Following the clinical approval of osimertinib, re-biopsy at progression became mandatory, in order to re-analyze the tumor molecular profile and identify T790M mutation. Oxnard *et al.* first demonstrated an adequate accuracy of plasma genotyping by digital PCR (dPCR) in 180 patients with advanced NSCLC, including 60 patients with acquired resistance to EGFR-TKI [15]. The predictive value of plasma ctDNA genotyping for T790M was prospectively confirmed in Phase III AURA3 trial, showing a longer PFS and higher ORR for osimertinib as compared with platinum-pemetrexed chemotherapy in patients who progressed to first-generation TKI and were T790M positive on plasma [16]. However, because of 30% potential false negative rate associated with this method, the ctDNA analysis is currently recommended as the first step of tumor genotyping, but it must be always followed by tissue biopsy for those patients who are T790M-negative on plasma [10]. Increasing evidence have recently suggested that the ability to identify EGFR activating and resistant mutations by ctDNA in NSCLC is significantly higher in patients who have extra-thoracic metastasis as compared with patients with intra-thoracic disease [15, 17-24], but the low number of patients has limited the scientific reliability of these data. Thus it has not been clearly demonstrated if the metastatic site location may significantly influence the sensitivity of plasma ctDNA analysis in detecting EGFR mutations. Integrating data from multiple studies, in order provide evidence with greater statistical value may lead to practical implications for the management of NSCLC patients. This pooled analysis combined and analyzed simultaneously all the studies which evaluated the sensitivity of ctDNA in the detection of EGFR mutations comparing patients with extrathoracic (M1b) versus intrathoracic (M1a) disease, with the final aim to demonstrate a significant association between the metastatic site location and the diagnostic accuracy of ctDNA analysis in NSCLC patients.

2. MATERIALS AND METHODS

2.1. Search for Clinical Trials

We searched for all published studies reporting the sensitivity of plasma-based EGFR-mutation testing by ctDNA, stratified according to the metastatic site location (extra-thoracic (M1b) *vs* intra-thoracic (M1a) disease). We searched for clinical trials using Medline (PubMed), Embase-databases and Cochrane-Library up to May 2017, with no language restrictions. We used the following search terms: "EGFR" or "Epidermal growth factor receptor", and "T790M", and "circulating tumor DNA" or "ctDNA", and "non-small cell lung cancer", or "NSCLC" or "lung cancer". The search was limited to human studies in the English language. Relevant abstracts from the American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), and World Conference on Lung Cancer (WCLC) were included. We also explored the ClinicalTrials.gov website (www.clinicaltrials.gov) to search for unpublished data and ongoing studies.

2.2. Selection Criteria

According to the aforementioned search, clinical trials were taken into account if they met the following inclusion criteria: 1) patients with histologically-proven diagnosis of advanced NSCLC; 2) studies performing EGFR mutation testing in matched tumor tissue and plasma samples; 3) studies evaluating the sensitivity of EGFR mutation testing by ctDNA analysis; 4) studies reporting the sensitivity of ctDNA EGFR mutation testing according to the metastatic site location (extra-thoracic (M1b) *vs* intra-thoracic (M1a) disease).

2.3. Data Extraction

Data extraction and assessment were performed by two different authors (F.P. and S.R) and disagreements were solved by a discussion with another author (A.R.). The following data were collected from eligible studies: first author name, journal and year of publication, study design, study treatment, baseline characteristics of patients (*i.e.* age, sex, stage, metastatic site location), true positive (TP) and false negative (FN) rates stratified according to the metastatic site location (M1b *vs* M1a disease). The meta-analysis was designed according to the PRISMA - guidelines for reporting of systematic reviews [25].

2.4. Statistical Analysis

Patients were stratified according to the metastatic site location into 2 groups: extra-thoracic (M1b) versus intrathoracic (M1a) metastatic disease. The outcome measure was the sensitivity of ctDNA analysis, defined as the proportion of EGFR-positive patients by tumor tissue testing correctly identified by the ctDNA analysis. We extracted the number of events over total patients included in each arm using odds ratio (OR) as a measure of association between ctDNA sensitivity and the metastatic site location (extra-thoracic (M1b) versus intrathoracic (M1a) disease). Thus, an OR greater than 1 indicates that extra-thoracic (M1b) disease is associated with a higher sensitivity of EGFR-mutation testing by ctDNA analysis in patients with advanced NSCLC. We calculated a pooled OR performing a meta-analysis of ORs emerging from the included studies. Heterogeneity between studies was explored using Chi-square test with a predefined significance threshold of 0.1. We used the random-effect or fixed- effect, based on statistical significance of Q-test, according to Mantel-Haenszel method. We performed a publication bias analysis using both Begg's funnel plots and Egger's test, with $P<0.05$ suggesting a statistically significant publication bias. When publication bias was found, the Duval and Tweedie nonparametric 'trim and fill' method has been adopted to adjust it. The methodological quality of included trials was assessed by QUADAS-2. We used Cochrane RevMan ver. 5.3 statistical software to perform the meta-analysis and Comprehensive Meta-Analysis ver. 2.0 to assess the risk of publication bias.

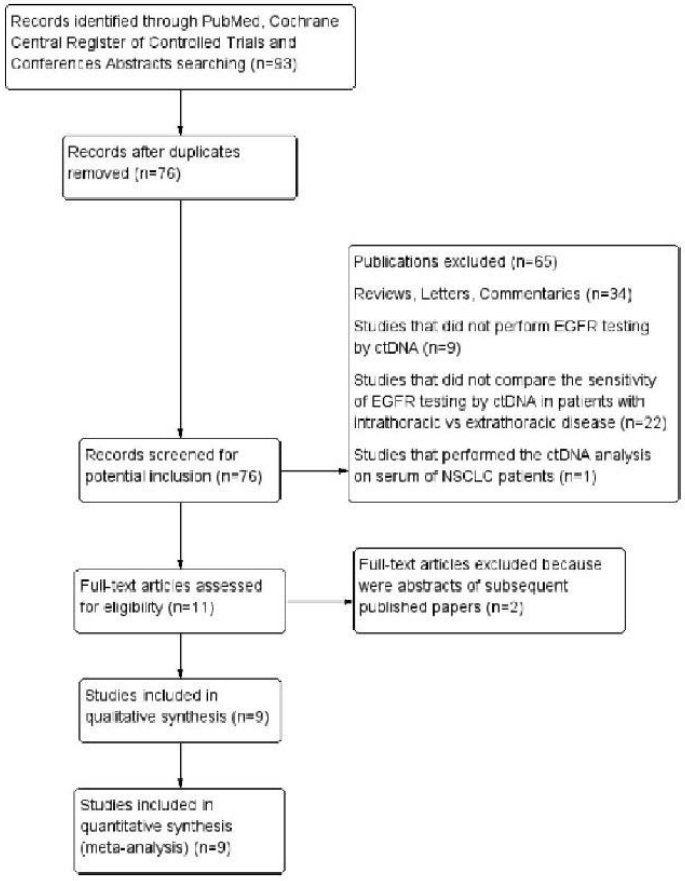

Fig. (1). Flow-chart of trials selection.

3. RESULTS

3.1. Literature Search

The search of literature updated in May 2017, identified a total of 93 records. Among these, nine studies met our inclusion criteria and were included in our pooled-analysis. In the study by Karlovich *et al.* [16] the EGFR mutation analysis by ctDNA was performed both for EGFR-activating and T790M resistant mutations, thus the data were reported as two different studies. Overall ten eligible studies (1425 patients) were included in our pooled analysis (Fig. **1**).

3.2. Studies' Characteristics

All these studies prospectively collected matched blood and tumor tissue from patients with a histologically-confirmed diagnosis of advanced NSCLC. Real-time polymerase chain reaction (RT-PCR) was the most commonly used method to detect EGFR mutations in the plasma of NSCLC patients, while the studies of Oxnard [15], Thress [21], Karlovich [17] and Kasahara [23] used digital PCR technologies. RT-PCR was the most commonly used to detect EGFR mutations in FFPE (formalin-fixed paraffin embedded) tumor tissue. The sample sizes of the analyzed population ranged from 38 to 397, while the percentage of patients with intrathoracic (M1a) disease ranged from 23% to 61% across the different studies. Baseline characteristics of selected studies are described in Table **1**.

3.3. Diagnostic Accuracy of ctDNA

All included studies compared the sensitivity of EGFR mutation analysis by ctDNA in patients with extra-thoracic (M1b) versus intrathoracic (M1a) disease. Pooled analysis showed that the sensitivity of ctDNA was significantly higher in patients with extra-thoracic (M1b) as compared to intrathoracic (M1a) disease (OR: 5.09; 95% CIs: 2.93 – 8.84) (Fig. **2**). The pooled OR for sensitivity was calculated using random-effect model, because of a significant heterogeneity between treatment effects (Q-test: $P < 0.0001$). A significant association between the "M-status" and the diagnostic accuracy of ctDNA analysis was observed for both EGFR-activating (OR: 4.30, 95% CI: 2.35-7.88) (Fig. **3**) and

Table 1. Characteristics of the trials included in the pooled-analysis.

Study (Reference)	EGFR Mutation (ctDNA)	Sensitivity (M1b) n.(%)	Sensitivity (M1a) n.(%)	Odds Ratio (95% CI)
Oxnard *et al.* 2016 *(15)*	Del19/L858R	139/161 (86%)	36/48 (75%)	2.11 (0.95-4.66)
Normanno *et al.* 2016 *(18)*	Del19/L858R	52/82 (63%)	13/57 (23%)	5.87 (2.73-12.6)
Yi-Long Wu *et al.* 2016 *(19)*	Del19/L858R	180/234 (77%)	63/105 (60%)	2.22 (1.35-3.65)
Tseng *et al.* 2015 *(20)*	Del19/L858R	32/41 (78%)	5/21 (24%)	11.28 (3.27-39.6)
Kumar *et al.* 2017 *(22)*	Del19/L858R	21/28 (75%)	15/27 (55%)	2.40 (0.76-7.53)
Kasahara *et al.* 2017 *(23)*	Del19/L858R	26/33 (79%)	8/16 (50%)	3.71 (1.03-13.46)
Karlovich *et al.* 2016 *(17)*	Del19/L858R	52/55 (95%)	7/18 (39%)	27.24 (6.07-122.17)
Karlovich *et al.** 2016 *(17)*	T790M	47/49 (96%)	4/15 (27%)	64.63 (10.47-398.8)
Thress *et al.* 2015 *(21)*	T790M	21/27 (78%)	2/11 (18%)	15.75 (2.65-93.46)
Jenkins *et al.* 2017 *(24)*	T790M	111/154 (72%)	123/243 (51%)	2.52 (1.63-3.88)

The number of patients reported corresponds to the number of patients evaluable

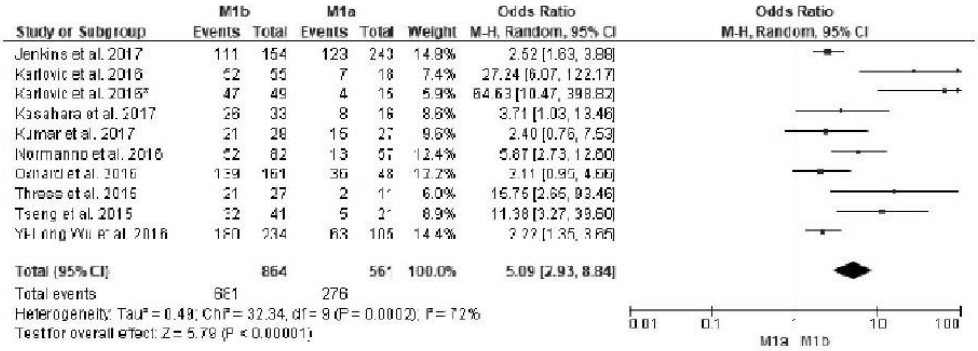

Fig. (2). Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR-mutation testing by metastatic sites location (M1b *vs* M1a).

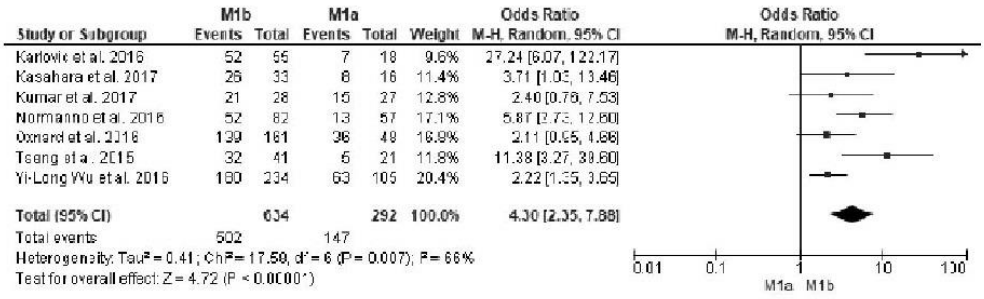

Fig. (3). Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR-activating (Del19, L858R) mutations testing by metastatic sites location (M1b *vs* M1a).

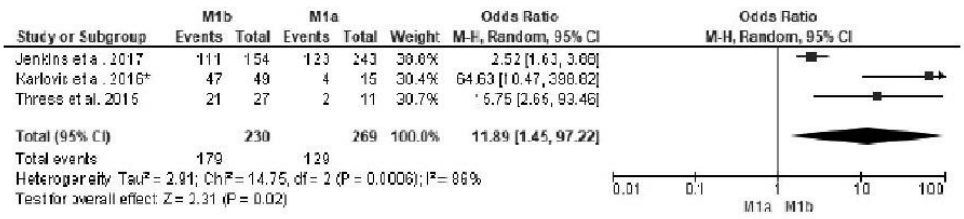

Fig. (4). Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR-T790M mutation testing by metastatic sites location (M1b *vs* M1a).

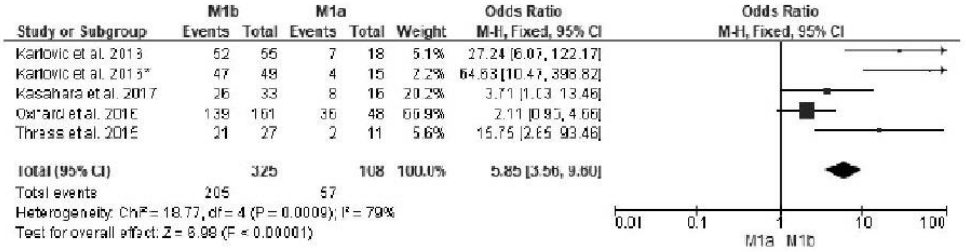

Fig. (5). Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR mutations testing by digital-PCR technologies according to the metastatic sites location (M1b *vs* M1a).

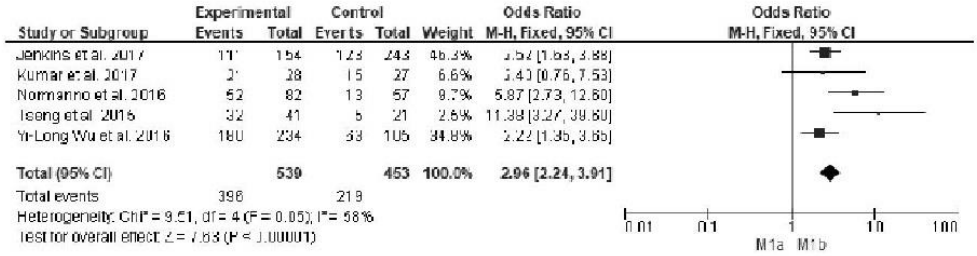

Fig. (6). Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR mutations testing by RT-PCR technologies according to the metastatic sites location (M1b *vs* M1a).

resistant T790M mutation (OR: 11.89, 95% CI: 1.45-97.22) (Fig. **4**), and it was confirmed regardless of the use of digital-PCR (OR: 5.85, 95% CI: 3.56-9.60) (Fig. **5**) or non-digital PCR technologies (OR: 2.96, 95% CI: 2.24-3.91) (Fig. **6**).

3.4. Risk of Bias Assessment

Publication bias has been found either by Begg's and Egger's tests (P<0.01) (Fig. **7**). However, the 'trim and fill' analysis did not show significantly different results, confirming that the sensitivity ctDNA analysis is associated to the metastatic site location (adjusted OR: 3.19, 95% CI: 1.73–5.88). The methodological quality of each trial was assessed by QUADAS-2, showing a good average quality of all included trials (Fig. **8**).

4. DISCUSSION

This meta-analysis included ten studies which evaluated the diagnostic accuracy of EGFR mutation testing by ctDNA in patients with advanced NSCLC comparing patients with extra-thoracic *vs* intra-thoracic disease. The results of this work have shown a significant association between the metastatic site location and the sensitivity of the ctDNA analysis. Indeed the ability to identify EGFR mutations in the plasma of NSCLC patients is significantly higher in patients with extra-thoracic disease (M1b) as compared to patients with intrathoracic (M1a) disease. Since the clinical application of ctDNA in patients with lung cancer is rapidly evolving, it is important to establish both the accuracy and feasibility of this tool in the practical management of advanced disease. Published studies and meta-analysis revealed an adequate diagnostic accuracy of ctDNA in the detection of EGFR activating mutations, showing a sensitivity of 0.62 and specificity of 0.96, with an overall mutation status concordance of about 90% with the standard tissue genotyping [11, 13]. The promising diagnostic performance of ctDNA observed in these controlled studies has been confirmed also in a real-world diagnostic setting. The multicenter ASSESS study has recently showed a similar concordance rate of 89%

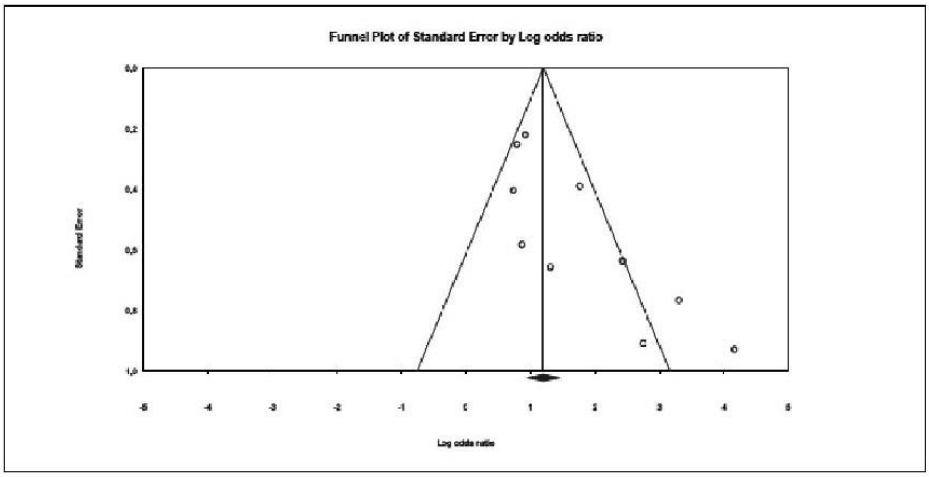

Fig. (7). Funnel plot of odds ratio (OR) for overall sensitivity of plasma ctDNA EGFR-mutation testing by metastatic sites location (M1b *vs* M1a). Each study is represented by one circle- the vertical line represents the pooled effect estimate.

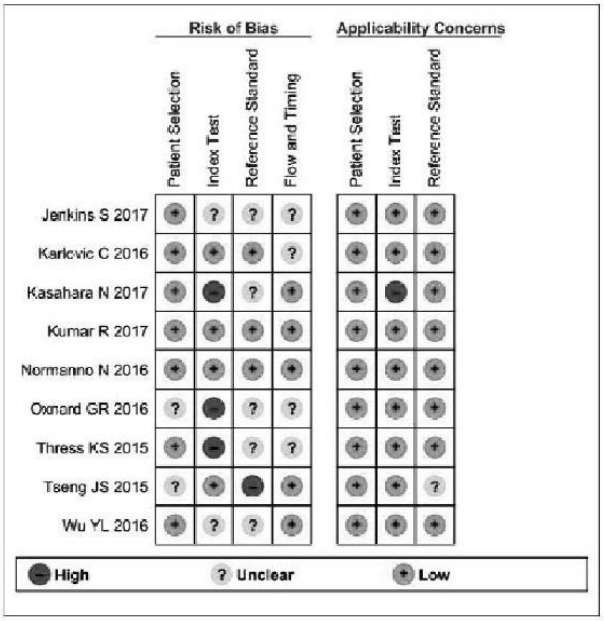

Fig. (8). Quality assessment of studies by QUADAS-2.

(sensitivity 46%, specificity 97%) between plasma and tissue-based EGFR mutation analysis in 1162 patients with advanced NSCLC [18], suggesting the clinical utility and reliability of ctDNA for the detection of EGFR activating mutations in clinical practice. These data have led to the recommendation of EGFR mutation analysis by ctDNA in patients with newly diagnosed, advanced NSCLC when tumor tissue is not available [10] and this was also stated in the approval of Gefitinib. However, some efforts should be needed to raise the sensitivity of this tool, in order to reduce the number of false negative patients who would be wrongly excluded from receiving EGFR-TKI treatment. In this scenario, our work provides additional evidence with potential practical implications for the management of NSCLC patients. Indeed the results of this pooled analysis clearly demonstrated that the ability to detect EGFR activating mutations in the plasma of patients with advanced NSCLC is significantly influenced by the location of tumor metastasis.

Particularly, the sensitivity of ctDNA is significantly lower in patients with intra-thoracic (50%) as compared to extra-thoracic disease (79%), suggesting the "M" status as a clinical predictor of ctDNA accuracy, used to identify the best candidates for plasma EGFR-testing. As consequence, oncologists should carefully evaluate any negative result obtained in patients with M1a disease. Indeed, the very low sensitivity of ctDNA analysis in M1a stage could lead to 50% of potentially false-negative patients who would be wrongly excluded from receiving the best first-line treatment with an EGFR-TKI. Even if they represent only 30% of the overall metastatic population, every effort should be made by clinicians to detect EGFR-mutations in tumor tissue in this subgroup of patients. Conversely, the ctDNA analysis may be considered as a reliable test for NSCLC patients with the extra-thoracic disease, since it showed a sensitivity of about 80% and it could be peacefully recommended when tumor tissue is not available. It would be interesting to compare also the sensitivity of ctDNA in M1b (≤1 extrathoracic metastasis) versus M1c (>1 extrathoracic metastasis) disease, as defined by the last version of the IASLC-TNM staging project [26]. Unfortunately, the majority of the studies included in this pooled analysis did not perform this kind of evaluation, since they were conducted before the publication of the last version of IASLC-TNM staging. Furthermore, none of the included trials evaluated how the number and the specific site of extra-thoracic tumor metastasis could influence the sensitivity of the ctDNA analysis. The detection of EGFR-T790M mutation by ctDNA is currently recommended as a routinary test for all patients with advanced NSCLC who failed first-generation EGFR-TKI and are a candidate to receive osimertinib as second-line treatment [10]. To date, three published studies [17, 21, 24] revealed a significantly lower sensitivity, ranging from 20%-50%, of plasma ctDNA analysis in the subgroup of patients with M1a intrathoracic disease as compared to 70%-90% in patients with M1b extra-thoracic metastasis. The results of our work confirmed a significantly higher sensitivity nearly to 80% in patients with M1b as compared to 50% in patients with M1a disease, suggesting that T790M mutation testing by ctDNA could result negative in half of the patients without extra-thoracic metastasis, thus requiring further tissue biopsy and second-line treatment delay. For this reason, tissue biopsy should be recommended upfront in all EGFR-positive NSCLC patients with M1a disease who progressed after first-generation TKIs in order to increase the chance to detect T790M mutation and avoid any subsequent treatment delay. Interestingly the subgroup analysis performed to evaluate the influence of detection technology on ctDNA analysis sensitivity, revealed that the use of digital-PCR increased ctDNA sensitivity (87%) as compared to non-digital PCR (73%) only in patients with the M1b disease while no differences have been observed in M1a patients. As previously reported in different studies, these data confirm that the chances to detect EGFR mutations in the blood of NSCLC patients with intra-thoracic disease may not be increased by the use of more accurate technologies and that the significant association between M-status and ctDNA accuracy is the result of a greater release of DNA into the blood of patients with extra-thoracic metastasis. Interestingly the combination of exosomal RNA and ctDNA significantly increased the sensitivity for EGFR mutation detection in plasma of NSCLC patients with intrathoracic disease [27], suggesting exoNA-based liquid biopsy platform as an innovative and promising approach for this subgroup of patients. This literature-based analysis highlights the potential role of the metastatic site location as a clinical predictor of ctDNA ability to identify EGFR mutations in the plasma of patients with advanced NSCLC. Nevertheless there are also some limitations: the cohorts of patients enrolled in the analyzed studies are heterogeneous; indeed different selection criteria as well as the different practices for EGFR mutation testing in plasma samples have been used among included trials. Genotyping of ctDNA was performed by RT-PCR, including QIAGEN therascreen, Roche Cobas and others in the real word ASSESS study, while the adoption of the BEAMING digital PCR allowed to increase the sensitivity of ctDNA analysis in the Oxnard trial. Furthermore the local practices for plasma sample testing used in real word setting could have influenced the inferior ctDNA performance observed in the ASSESS study as compared to the expert laboratories included in the academic controlled clinical trials. Even if the previously described heterogeneity doesn't reduce both the clinical and statistical value of results, further prospective clinical studies are needed to investigate if and how the "M status" should be considered by oncologists to identify patients more suitable for plasma EGFR testing in clinical practice.

CONCLUSION

In conclusion, the results of this pooled-analysis have clearly demonstrated that the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis, suggesting that the ability to identify both EGFR activating and resistant T790M mutations in plasma of NSCLC patients is significantly higher in presence of extra-thoracic disease, regardless of the use of more accurate diagnostic technologies, like digital-PCR. These observations should be carefully considered to optimize the clinical management of EGFR-mutated NSCLC patients.

LIST OF ABBREVIATIONS

ASCO	=	American Society of Clinical Oncology
95% CIs	=	95% confidence intervals
CMA	=	Comprehensive Meta-Analysis software
ctDNA	=	Circulating tumor
EGFR	=	Epidermal growth factor receptor
ELCC	=	European Lung Cancer Conference
ESMO	=	European Society of Medical Oncology
FFPE	=	Formalin-fixed paraffin embedded
FN	=	False negative
LL3	=	LuxLung3
LL6	=	LuxLung6
M1b	=	Extra-thoracic disease
M1a	=	Intrathoracic disease
NSCLC	=	Non-small cell lung cancer
OR	=	Odds ratio

ORR	=	Objective response rate
PFS	=	Progression free survival
PRISMA	=	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
QoL	=	Quality of life
RevMan5.3.5	=	Review Manager 5.3.5
RR	=	Response rate
RT-PCR	=	Real-time polymerase chain reaction
TKIs	=	Tyrosine-kinase inhibitors
TP	=	True positive
WCLC	=	World Conference on Lung Cancer

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Dr. Chiara Drago contributed to the revision of the English language of this manuscript

REFERENCES

[1] Mok TS, Wu YL, Thongprasert S, *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* 2009;361(10):947-957.

[2] Rosell R, Carcereny E, Gervais R, *et al.* Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2012;13(3):239-246.

[3] Maemondo M, Inoue A, Kobayashi K, *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med.* 2010;362(25):2380-2388.

[4] Mitsudomi T, Morita S, Yatabe Y, *et al.* Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol.* 2010;11(2):121-128.

[5] Zhou C, Wu YL, Chen G, *et al.* Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* 2011;12(8):735-742.

[6] Sequist LV, Yang JC, Yamamoto N, *et al.* Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol.* 2013;31(27):3327-3334.

[7] Wu YL, Zhou C, Hu CP, *et al.* Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol.* 2014;15(2):213-222.

[8] Han JY, Park K, Kim SW, *et al.* First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol.* 2012;30(10):1122-1128.

[9] Yang JC, Wu YL, Schuler M, *et al.* Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol.* 2015;16(2):141-151.

[10] Novello S, Barlesi F, Califano R, *et al.* Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2016;27(suppl 5):v1-v27.

[11] Qiu M, Wang J, Xu Y, *et al.* Circulating tumor DNA is effective for the detection of EGFR mutation in non-small cell lung cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2015;24(1):206-212.

[12] Wu Y, Liu H, Shi X, Song Y. Can EGFR mutations in plasma or serum be predictive markers of non-small-cell lung cancer? A meta-analysis. *Lung Cancer.* 2015;88(3):246-253.

[13] Qian X, Liu J, Sun Y, *et al.* Circulating cell-free DNA has a high degree of specificity to detect exon 19 deletions and the single-point substitution mutation L858R in non-small cell lung cancer. *Oncotarget.* 2016;7(20):29154-29165.

[14] Luo J, Shen L, Zheng D. Diagnostic value of circulating free DNA for the detection of EGFR mutation status in NSCLC: a systematic review and meta-analysis. *Sci Rep.* 2014;4:6269.

[15] Oxnard GR, Thress KS, Alden RS, *et al.* Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol.* 2016;34(28):3375-3382.

[16] Mok TS, Wu YL, Ahn MJ, *et al.* Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N Engl J Med.* 2017;376(7):629-640.

[17] Karlovich C, Goldman JW, Sun JM, *et al.* Assessment of EGFR Mutation Status in Matched Plasma and Tumor Tissue of NSCLC Patients from a Phase I Study of Rociletinib (CO-1686). *Clin Cancer Res.* 2016;22(10):2386-2395.

[18] Normanno N, Brown H, Haddad V, *et al.* Clinical and demographic features that influence EGFR mutation detection in plasma from patients (pts) with aNSCLC: The ASSESS experience. Journal of Thoracic Oncology (2016) 11 (supplement 4): S57-S166. S1556-0864(16)X0004-4

[19] Wu YL, Sequist LV, Hu CP, *et al.* EGFR mutation detection in circulating cell-free DNA of lung adenocarcinoma patients: analysis of LUX-Lung 3 and 6. *Br J Cancer.* 2017;116(2):175-185.

[20] Tseng JS, Yang TY, Tsai CR, *et al.* Dynamic plasma EGFR mutation status as a predictor of EGFR-TKI efficacy in patients with EGFR-mutant lung adenocarcinoma. *J Thorac Oncol.* 2015;10(4):603-610.

[21] Thress KS, Brant R, Carr TH, *et al.* EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer.* 2015;90(3):509-515.

[22] Kumar R, *et al.* Diagnostic EGFR Testing with ctDNA versus Tumour in patients with Advanced Non-Small Cell Lung Cancer (NSCLC): The Royal Marsden Experience. Annals of Oncology (2017) 28 (suppl_2): ii28-ii51. 10.1093/annonc/mdx091.

[23] Kasahara N, Kenmotsu H, Serizawa M, *et al.* Plasma epidermal growth factor receptor mutation testing with a chip-based digital PCR system in patients with advanced non-small cell lung cancer. *Lung Cancer.* 2017;106:138-144.

[24] Jenkins S, Chih-Hsin Yang J, Jänne PA, *et al.* EGFR Mutation Analysis for Prospective Patient Selection in Two Phase II Registration Studies of Osimertinib. *J Thorac Oncol.* 2017;12(8):1247-1256.

[25] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg.* 2010;8(5):336-341.

[26] Chansky K, Detterbeck FC, Nicholson AG, *et al.* The IASLC Lung Cancer Staging Project: External Validation of the Revision of the TNM Stage Groupings in the Eighth Edition of the TNM Classification of Lung Cancer. *J Thorac Oncol.* 2017.

[27] Krug A.K, Enderle D, Karlovich C, *et al.* Improved EGFR mutation detection using combined exosomal RNA and circulating tumor DNA in NSCLC patient plasma. *Annals of Oncology 2017.* https://academic.oup.com/annonc/advance-article/doi/10.1093/annonc/mdx765/4693822?rss=1

DISCLAIMER: The above article has been published in Epub (ahead of print) on the basis of the materials provided by the author. The Editorial Department reserves the right to make minor modifications for further improvement of the manuscript.

PMID: 29521235