**REVIEW ARTICLE**

# Liquid Biopsy as Surrogate to Tissue in Lung Cancer for Molecular Profiling: A Meta-Analysis

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**Abstract:** ***Background***: The accurate microscopic diagnosis of lung cancer has become insufficient due to the concept of personalized medicine. Tissue samples are used not only for microscopic diagnosis but also for the assessment of the different targets. Biopsies are performed in 80% of the patients and they are not sufficient for molecular diagnosis in 30 % of the cases. Liquid biopsy (LB) has been reported as a possible surrogate to tissue samples and has been introduced in the management scheme of the patients since 2014. We aimed to highlight the diagnostic value of liquid biopsy in assessing the molecular profile of non small cell carcinomas in comparison with tissue biopsy.

***Methods***: We retracted eligible articles from PubMed, Embase and Cochrane databases. We calculated the pooled sensitivity (SEN), specificity (SPE), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR). A summary receiver operating characteristic curve (SROC) and area under curve (AUC) were used to evaluate the overall diagnostic performance using the Meta-Disc software 5.1.32. The heterogeneity was assessed using I square statistics. A meta-regression was performed in case of heterogeneity. In case of absence of covariates, a sensitivity analysis was done in order to assess publications that induced a statistical bias.

***Results***: 39 eligible studies involving 4782 patients were included. The overall statistical studies showed heterogeneity in the SEN, SPE, PLR, NLR and DOR. No threshold effect was revealed. The meta-regression incorporating the ethnicity, the test, the technique used in tissue and plasma and the use of plasma or serum as covariates showed no impact of these factors. A sensitivity analysis allowed achieving the homogeneity in the SPE and DOR. The overall pooled SEN and SPE were 0.61 and 0.95 respectively. The PLR was 9.51, the NLR was 0.45 and DOR was 24.58. The SROC curve with AUC of 0,93 indicated that the liquid biopsy is capable of identifying wild type samples from mutated ones with a relatively high accuracy.

***Conclusion***: This meta-analysis suggested that detection of molecular mutations by cfDNA is of adequate diagnostic accuracy in association to tissues. The high specificity and the moderate sensitivity highlight the value of LB as a screening test.

## 1. BACKGROUND

Lung cancer is the leading cause of cancer-related death worldwide [1]. Its positive diagnosis is based on microscopic features and faced a recent change due to the 2015 World Health Organization Classification's [1, 2]. For the first time, this classification introduced molecular pathways and targets especially for adenocarcinomas. In fact, this histologic subtype has become the most frequent non-small-cell lung carcinoma. This classification pointed out the necessity of not only assessing the accurate microscopic diagnosis but also the importance of molecular diagnosis of the most relevant targets. Lung cancer is mainly characterized by its spatial and temporal heterogeneity [3, 4]. Spatial heterogeneity consists in the presence in the same tumor of different molecular drivers. This fact compels to multiply samples in order to assess all the potential relevant pathways involved. On the other hand, temporal heterogeneity consists in the difference of activated pathways between the initial tumour and the metastases or the recurrences. This fact enhances the necessity of sampling the metastases or recurrences even if the initial tumoral profile was assessed. This heterogeneity provides also an explanation to the phenomenon of resistance, which is observed within 3 to 6

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months after the onset of anti-EGFR treatments. This resistance is explained by the activation of secondary pathways that were activated at the onset but concerned a low number of tumour cells. The morphological and molecular tests are performed in 80% of the cases on small samples and molecular testing is impossible in 30% of the patients. This may be due to the unavailability of the specimen, the inaccessibility of the tumoral site or the presence of contraindications to biopsy [3]. This fact made scientists and researchers look for other surrogates to tissue that can be safer and sufficient to establish the molecular profile. In this context, the liquid biopsy was discovered. It consists in the assessment of molecular profile on circulating tumor cells, circulating tumoral DNA, circulating tumoral RNA, exosomes or secretomes [5]. Many studies were published concerning the assessment of these elements with varying techniques of identification. In 2014, the liquid biopsy was introduced in the management scheme of patient candidates for the third generation anti-EGFR in order to assess the presence of the T790M mutation [6, 7]. Besides, in 2016, the first technique of sequencing, the cobas EFGR mutation test, obtained the Food and Drug Administration approval [8, 9]. Even if this technique was approved, there are still many publications dealing with different techniques that may seem less expensive or easier to perform in a Pathology lab. Recently, many authors reported the efficiency of tests performed on free circulating DNA (cfDNA) in comparison with those performed on circulating tumour cells (CTC) [10]. We aimed to highlight the diagnostic value of liquid biopsy in assessing the molecular profile of non small cell carcinomas in comparison with tissue biopsy and we focused on the mutations of the Epidermal Growth Factor Receptor gene (EGFR). Other genes were assessed in only 4 included studies.

## 2. METHODS

### 2.1. Data Source and Search

We conducted this meta-analysis under the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [11]. To retrieve all eligible articles, PubMed and Embase databases and Cochrane Library were comprehensively searched up to 01 June 2017 with limitation to French and English language. The search medical subject heading (MeSH) terms employed for literature retrieval included: lung cancer or lung neoplasm, cell free DNA or cfDNA or circulating DNA and diagnosis or sensitivity or specificity or accuracy. The reference list of eligible articles was also independently searched to obtain other valuable sources.

### 2.2. Study Selection Criteria

To be qualified for inclusion in this meta-analysis, articles must comply with all the following criteria: articles evaluated the diagnosis value of cfDNA in plasma/serum or blood for lung cancer, the diagnosis of lung cancer was confirmed by the gold standard test which is the biopsy and articles provided sufficient data (true negative (TN), true positive (TP), false negative (FN) and false positive (FP)). The major exclusion criteria were as follows: studies with duplicate data reported in other studies and reviews,

technical reports, case reports, comments or letters with invalid data.

### 2.3. Data Extraction and Quality Assessment

One investigator independently reviewed all the articles and extracted data from the selected articles: first authors' name, publication year, characteristics of participants (ethnicity, mean/median, age, source of control, number of cases and controls, sample types), assay methods, assay indicators, sensitivity, specificity and quality assessment information. In addition, based on the revised quality assessment of diagnosis, accuracy studies-2 (QUADAS-2) criteria, the included articles were evaluated as at high risk (H) or low risk (L) independently by four key domains: patient selection, index test, reference standard and flow and timing [12].

### 2.4. Statistical Analysis

We used the Meta-Disc software 5.1.32 to conduct this meta-analysis. The pooled sensitivity (SEN) (TP/TP+FN), specificity (SPE) (TN/TN+FP), negative likelihood ratio (NLR), positive likelihood ratios (PLR) and diagnostic odds ratio (DOR) with the 95% confidence intervals were calculated. At the same time, we constructed the summary receiver operator characteristic (SROC) curve and calculated the area under the SROC curve based on the SEN and SPE of each study.

#### 2.4.1. Threshold Effect

A threshold effect was assessed using the Moses model with calculation of the Spearman correlation coefficient.

#### 2.4.2. Heterogeneity

Q test and $I^2$ statistics were carried out to explore the heterogeneity among studies. P value <0.1 for q test or $I^2$ value >50% represented substantial between study heterogeneity. Besides, based on the characteristics of the included articles, meta-regressions were performed to explore the sources of heterogeneity if necessary.

#### 2.4.3. Sensitivity Analysis

In case of absence of covariates, according to the meta-regression analysis, we performed a sensitivity analysis using the same software. This analysis is performed through a visual inspection of forest plots. Studies causing bias are those that show large deviations from the line corresponding to the pooled accuracy estimation mentioned in the forest plot of specificity. These studies were excluded and considered as possible sources of heterogeneity. The purpose of sensitivity analysis is to stipulate hypothesis about the sources of heterogeneity when metaregression shows no covariates.

## 3. RESULTS

### 3.1. Search Results

Our database research retrieved 839 records. After reviewing the title and abstracts, 729 records were excluded
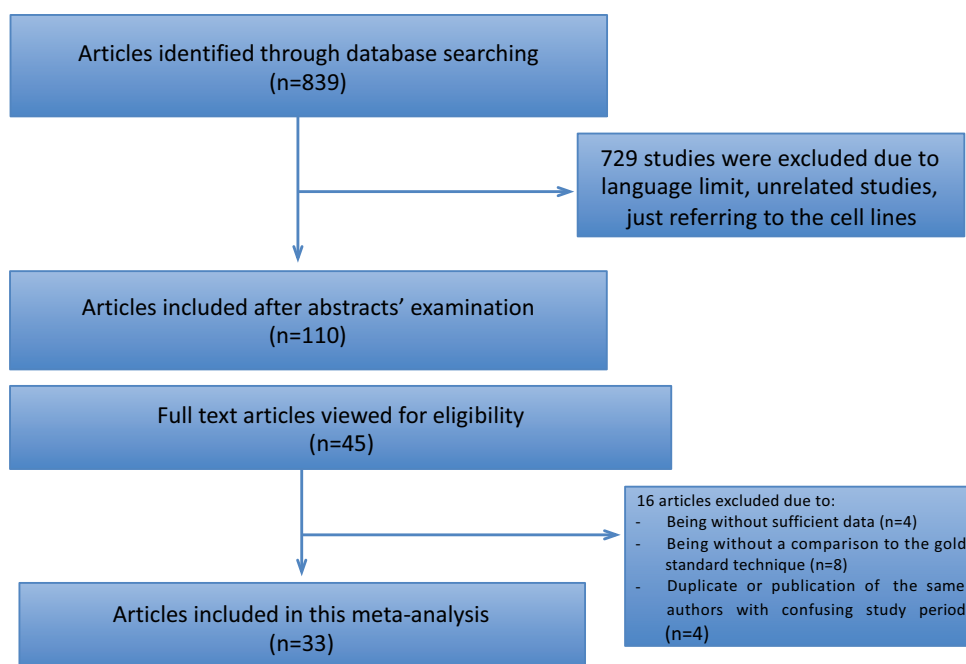
**Fig. (1).** Flow-chart illustrating the literature retrieval.

due to language limit, unrelated studies. By reviewing full-text articles, we excluded further 65 records, leaving 43 eligible articles and 2 international congress abstracts. From these articles, 16 records were excluded due to insufficient data (3 articles and 1 congress abstract), no gold standard (8 articles) and duplicate publications (4 articles). In the study reported by Li and colleagues, EGFR mutation was detected in both plasma and serum and the data of plasma and serum were analyzed as two independent studies [13]. Xu and coworkers described 3 different techniques for the specific analysis of the Exon19 deletion and the L858R mutation of the EGFR gene. So that, the different data were considered as 6 independent studies [14]. After independent review, 39 eligible studies were included in this meta-analysis. The Fig. (**1**) illustrates the flow-chart of the literature retrieval.

All the studies fulfilled the major QUADAS-2 categories with a global low risk of bias and low concerns concerning applicability. The quality assessment of the different studies included is represented in Table **1**.

A total of 4,782 participants were included in the analysis. The majority of the patients presented a late stage lung cancer. All the studies dealt with the sequencing of the EGFR gene in association to the sequencing of TP53, NF1, KRAS, MET in 1 study [15], to BRAF in one study [16], to KRAS in 1 study [17] and 1 study dealt with the screening of the ALK gene [18]. The techniques of sequencing in the liquid biopsy and in the tissue were similar in 20 studies. In the other studies they were different. The molecular diagnosis was performed on liquid biopsy and tissue at the same time in 17 studies and was not specified in 10 studies. Many techniques of sequencing were used in liquid biopsy consisting in PCR-based-sequencing techniques and non PCR-based-sequencing techniques. PCR-based-sequencing techniques consisted in digital PCR (dPCR) [19], amplification refractory mutation system (ARMS) [17], CastPCR [16], peptide-nuclei-acid mediated PCR (PNA-PCR) [20], mutant-enriched PCR (ME-PCR) [21], High

Resolution Melting (HRM) [22], mutant enriched-liquidchip PCR technique [14], PNA-LNA-PCR technique [23]. Non PCR-based tecniques consisted in next-generation sequencing (NGS) [18], Cobas EGFR mutation test [24], Therascreen [25] and denaturing high perforance liquid chromatography (DHPLC) technique [26]. The technique that was the most frequently used in this analysis was the scorpion ARMS technique. The NGS techniques were reported in only 5 studies. The Table **2** summarizes the main characteristics of the included articles.

### 3.2. Diagnostic Accuracy of the Liquid Biopsy

The overall pooled SEN and SPE were 0.63 (95% CI, 0.61-0.65) and 0.92 (95% CI, 0.91-0.93) respectively (Figs. **2** and **3**). Our results showed that PLR was 8.123 (95% CI, 5.13-12.84), NLR was 0.456 (95% CI, 0.383-0.543) and DOR was 20.50 (95% CI, 12.61-33.30) (Fig. **4**). Between-study heterogeneity was significant in the SEN, SPE and the DOR (I-square estimated to respectively 84.9%, 89.1% and 74.9%). We did not find any evidence of threshold effect (Spearman correlation coefficient: 0.029 and p=0.861). Fig. (**5**) shows the corresponding SROC curve with AUC of 0,82 indicating that the liquid biopsy is capable of identifying wild type samples from mutated ones with a relatively high accuracy.

### 3.3. Subgroup Analysis

Sub-group analyses based on the use of the NGS technique, the use of scorpion ARMS technique, the use of DHPLC technique, the use of the same technique in the liquid biopsy and tissue and the analysis of specific mutations of the EGFR gene were also conducted. The NGS tehniques seem to have the highest sensitivity of 0.75 and the highest specificity was recorded in the group of the ARMS Scorpion technique. Even when we analyzed the group of studies using the same techniques in the tissue and the liquid

**Table 1. The quality assessment of the different studies included.**

| Studiess | | | Risk of Bias | | | | Applicability Concerns | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Patient Selection | Index Test | Reference Standard | Flow and Timing | Patient Selection | Index Test | Reference Standard |
| Kimura *et al.* 2007 [29] | 2007 | 42 | L | L | L | L | L | L | L |
| Bai *et al.* [26] | 2009 | 230 | L | L | L | L | L | L | L |
| Que *et al.* [30] | 2016 | 121 | L | L | L | L | L | L | L |
| Cui *et al.* [18] | 2017 | 39 | L | L | L | L | L | L | L |
| Rachiglio *et al.* [31] | 2016 | 44 | L | L | L | L | L | L | L |
| Santos *et al.* [32] | 2016 | 63 | L | L | L | L | L | L | L |
| Goto *et al.* [33] | 2012 | 86 | L | L | H | L | L | L | L |
| Douillard [34] | 2013 | 652 | L | L | H | L | L | L | L |
| He *et al.* [35] | 2016 | 120 | L | L | H | L | L | L | L |
| Yang *et al.* [16] | 2017 | 107 | L | L | H | L | L | L | L |
| Huang *et al.* [36] | 2012 | 822 | L | L | L | L | L | L | L |
| Liu *et al.* [10] | 2013 | 86 | L | L | L | L | L | L | L |
| Kim *et al.* [37] | 2013 | 40 | L | L | L | L | L | L | L |
| Zhao *et al.* [21] | 2012 | 111 | L | L | L | L | L | L | L |
| Wang *et al.* [38] | 2014 | 134 | L | L | L | L | L | L | L |
| Jing *et al.* [22] | 2014 | 120 | L | L | L | L | L | L | L |
| Weber *et al.* [39] | 2014 | 196 | L | L | L | L | L | L | L |
| Zhang *et al.* [19] | 2016 | 215 | L | L | L | L | L | L | L |
| Zhu *et al.* [40] | 2015 | 172 | L | L | L | L | L | L | L |
| Mack *et al.* [17] | 2009 | 14 | L | L | L | L | L | L | L |
| Kuang *et al.* [41] | 2009 | 43 | L | L | L | L | L | L | H |
| He *et al.* [42] | 2009 | 18 | L | L | L | L | L | L | L |
| Brevet *et al.* [29] | 2011 | 31 | L | L | L | L | L | L | L |
| Jiang *et al.* [43] | 2011 | 58 | L | L | H | L | L | L | L |
| Sriram *et al.* [44] | 2011 | 64 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |
| Xu *et al.* [14] | 2012 | 34 | L | L | L | L | L | L | L |

| Studiess | | | Risk of Bias | | | | Applicability Concerns | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Patient Selection | Index Test | Reference Standard | Flow and Timing | Patient Selection | Index Test | Reference Standard |
| Kim *et al.* [20] | 2013 | 57 | L | L | L | L | L | L | L |
| Sequist *et al.* [25] | 2015 | 227 | L | L | L | L | L | L | L |
| Wu [45] | 2015 | 24 | L | L | L | L | L | L | L |
| Mok [24] | 2015 | 238 | L | L | L | L | L | L | L |
| Li [13] | 2014 | 141 | L | L | H | L | L | L | L |
| Li [13] | 2014 | 108 | L | L | L | L | L | L | L |
| HE [35] | 2017 | 120 | L | L | L | L | L | L | L |
| Yung *et al.* [29] | 2009 | 35 | L | L | L | L | L | L | L |

**Table 2.** **The major characteristics of the different studies included.**

| Study | Year | Number | TP | FP | FN | TN | Test | Genes | Ethnicity | Test of Biopsy | Time Point of Biopsy and Liquid Biopsy | Stage | Plasma/ Serum | CR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yung *et al.* [29] | 2009 | 35 | 11 | 0 | 1 | 23 | Microfluidics digital PCR | Ex 19, L858R | Asian | Direct sequencing | No mention | No mention | plasma | 97% |
| Kimura *et al.* 2007 [29] | 2007 | 42 | 6 | 1 | 2 | 33 | ARMS | Ex 18, 19, 21 | Asian | Direct sequencing | BT liquid biopsy, not at the same time | III or IV | serum | 92% |
| Bai *et al.* [26] | 2009 | 230 | 63 | 16 | 14 | 137 | DHPLC | Ex19, 21 | Asian | DHPLC | No mention | IIIb ou IV | plasma | 87% |
| Que *et al.* [30] | 2016 | 121 | 34 | 10 | 10 | 67 | DHPLC | Ex19, 21 | Asian | ARMS | BT the same time | I-IIIa :17 IIIb-IV :104. | plasma | 83% |
| Cui *et al.* [18] | 2017 | 39 | 13 | 0 | 11 | 15 | NGS | ALK | Asian | NGS | Not at the same time | I-IIIa :7 IIIb-IV :32 | plasma | 72% |
| Rachiglio *et al.* [31] | 2016 | 44 | 17 | 2 | 5 | 20 | NGS | EGFR | European | NGS | Not at the same time | IV | Plasma | 84% |
| Santos *et al.* [32] | 2016 | 63 | 33 | 10 | 15 | 5 | NGS | EGFR, TP53, NF1, KRAS, MET | European | Not mentionned | Not specified | Not specified | plasma | 60% |
| Goto *et al.* [33] | 2012 | 86 | 22 | 0 | 29 | 35 | Scorpion ARMS | EGFR | Asian | ARMS | Pre-TT both | Not specified | serum | 66% |
| Douillard [34] | 2013 | 652 | 69 | 1 | 36 | 546 | Scorpion ARMS (ex19 del, L858R, T790M) | EGFR | European | Scorpion ARMS | Both BT. | Stage IIIa, b, IV | plasma | 94% |
| He *et al.* [35] | 2016 | 120 | 80 | 0 | 26 | 14 | Targeted (ddPCR) (Ex19 del, L858R, T790M) | EGFR | Asian | | | | | 78% |

**(Table 2) contd….**

| Study | Year | Number | TP | FP | FN | TN | Test | Genes | Ethnicity | Test of Biopsy | Time Point of Biopsy and Liquid Biopsy | Stage | Plasma/ Serum | CR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yang *et al.* [16] | 2017 | 107 | 31 | 3 | 24 | 49 | Cast PCR | EGFR, BRAF | Asian | Not mentionned | Not the same time | I-III:42 IV:65 | plasma | 74% |
| Huang *et al.* [36] | 2012 | 822 | 188 | 81 | 108 | 445 | DHPLC | EGFR | Asian | DHPLC | THE SAME TIME | IIIb, IV: 744 I-IIIa: 78 | plasma | 77% |
| Liu *et al.* [10] | 2013 | 86 | 27 | 0 | 13 | 46 | Scorpion ARMS | EGFR | Asian | ARMS | No mention | III ET IV | plasma | 85% |
| Kim *et al.* [37] | 2013 | 40 | 6 | 0 | 29 | 5 exclus | PNA-mediated real-time PCR | EX19 del, L858R | Asian | Direct sequencing | Not the same time | advanced | plasma | 87% |
| Zhao *et al.* [21] | 2012 | 111 | 16 | 3 | 29 | 63 | ME-PCR(19 del, L858 R) | EGFR | Asian | ME-PCR | Same time BT | Not mentionned | plasma | 71% |
| Wang *et al.* [38] | 2014 | 134 | 15 | 0 | 53 | 64 | (ARMS SCORPION) | EGFR VP : 15, FP : 2, VN : 4, FN : 53 | Asian | ARMS | After TT | 115 IV, 19 IIIb | plasma | 59% |
| Jing *et al.* [22] | 2014 | 120 | 29 | 2 | 16 | 73 | HRM + direct sequencing | EGFR | Asian | HRM + direct sequencing | During surgery for liquid biopsy. Not at the same moment, | I-II : 38 III-IV :82 | plasma | 85% |
| Weber *et al.* [39] | 2014 | 196 | 17 | 6 | 11 | 162 | NGS (cobas) | EGFR | European | cobas | BT liquid biopsy, not at the same time | I, II :2 III, IV : 197 | plasma | 91% |
| Zhang *et al.* [19] | 2016 | 215 | 57 | 4 | 36 | 118 | ddPCR | Ex19 del, L858R | Asian | ARMS | The same, AT | IIIb :36 IV :179 | plasma | 81% |
| Zhu *et al.* [40] | 2015 | 172 | 30 | 4 | 7 | 131 | Targeted (ddPCR) | Ex19 del, L858R | Asian | ARMS | No mention | Not mention | plasma | 93% |
| Mack *et al.* [17] | 2009 | 14 | 4 | 4 | 2 | 4 | (scorpion ARMS) | EGFR, KRAS | American | Nested PCR assay | BT liquid biopsy not mentionned for tissue | IIIb et IV | plasma | 57% |
| Kuang *et al.* [41] | 2009 | 43 | 21 | 9 | 2 | 11 | Scorpion ARMS | Ex18, 19, 20 | American | Direct DNA sequencing or DNA endonuclease-based method (local) | AT liquid biopsy not the same time as biopsy | III or IV | plasma | 74% |
| He *et al.* [42] | 2009 | 18 | 8 | 0 | 1 | 9 | ME-PCR | Ex19del, Ex 21 L858R | Asian | Direct sequencing | BT liquid biopsy, not at the same time. | Not specified | plasma | 94% |

| Study Year Number | | TP | FP | FN | TN | Test | Genes | Ethnicity | Test of Biopsy | Time Point of Biopsy and Liquid Biopsy | Stage | Plasma/ Serum | CR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Brevet *et al.* [29] | 2011 | 31 | 7 | 2 | 11 | 11 | Mass spectrometry genotyping assaya | Ex19 del et Ex21 L858R | American | PCR-RFLP | BT liquid biopsy not always at the same time. | III or IV | plasma | 58% |
| Jiang *et al.* [43] | 2011 | 58 | 14 | 0 | 4 | 40 | ME-PCR | Ex19, 21 | Asian | Not specified | BT the same time | IIIb, IV | serum | 93% |
| Sriram *et al.* [44] | 2011 | 64 | 3 | 0 | 3 | 58 | ME-PCR and HRM | EGFR: Ex19 et 21 | European | ME-PCR et HRM | THE SAME TIME | Not specified | serum | 95% |
| Xu *et al.* [14] | 2012 | 34 | 4 | 4 | 4 | 23 | ARMS | EGFR 19 del | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 79% |
| Xu *et al.* [14] | 2012 | 34 | 4 | 0 | 4 | 26 | ARMS | EGFR L8585R | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 88% |
| Xu *et al.* [14] | 2012 | 34 | 0 | 1 | 7 | 26 | DHPLC | EGFR 19 del | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 76% |
| Xu *et al.* [14] | 2012 | 34 | 2 | 2 | 6 | 24 | DHPLC | EGFR L8585R | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 76% |
| Xu *et al.* [14] | 2012 | 34 | 2 | 5 | 5 | 22 | ME-liquidchip | EGFR 19 del | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 70% |
| Xu *et al.* [14] | 2012 | 34 | 2 | 1 | 6 | 25 | ME-liquidchip | EGFR L8585R | Asian | ARMS | Liquid AT and tissue BT | IIIb a IV | Plasma | 79% |
| Kim *et al.* [20] | 2013 | 57 | 8 | 3 | 4 | 42 | PNA-LNA PCR (EGFR), sequencing (KRAS) | EGFR, KRAS | Asian | Direct sequencing | The same time BT | IIIb, IV | serum | 87% |
| Sequist *et al.* [25] | 2015 | 227 | 155 | 23 | 37 | 12 | NGS ( cobas or therascreen) | EGFR | American | Cobas or therascreen | The same time | IV after progreesion | plasma | 73% |
| Wu Ya-Lan [45] | 2015 | 24 | 7 | 2 | 10 | 5 | ARMS | EGFR T 790M | Asian | ARMS | Yes after treatment | IV | plasma | 50% |
| Mok [24] | 2015 | 238 | 72 | 5 | 24 | 137 | cobas | EGFR | Asian | Cobas | Yes before TT | IIIb, IV | plasma | 87% |
| Li [13] | 2014 | 141 | 27 | 3 | 29 | 62 | ARMS | EGFR 19 del, L858R, T790M | Asian | ARMS | Not specified | IIIb, IV | plasma | 63% |
| Li [13] | 2014 | 108 | 19 | 2 | 29 | 42 | ARMS | EGFR 19 del, L858R, T790M | Asian | ARMS | Not specified | IIIb, IV | serum | 56% |
| HE [35] | 2017 | 120 | 80 | 0 | 26 | 14 | ddPCR | EGFR, Ex19del, L858R, T790M | Asian | ddPCR | At the same time, BT | Advanced stage | plasma | 78% |

CR: concordance rate.
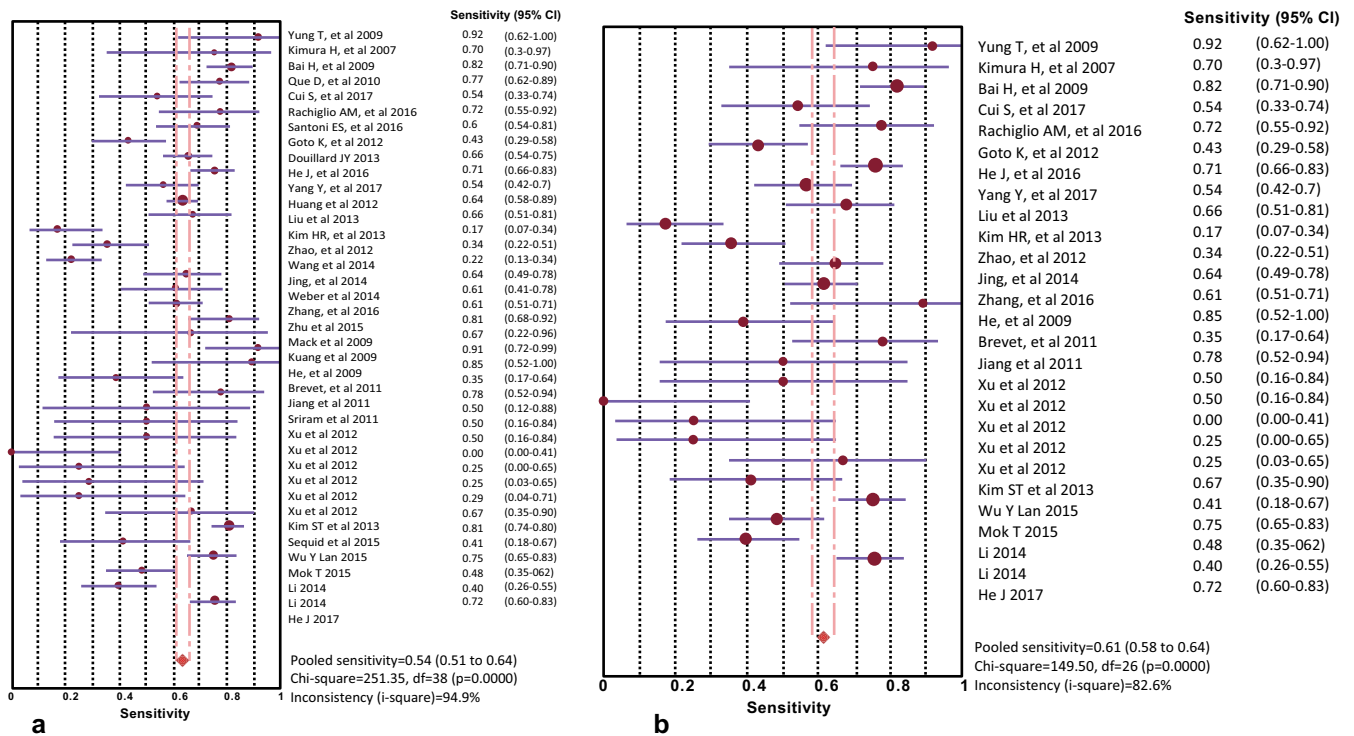
**Fig. (2). a**) Forrest plot of sensitivity of all studies, **b**) Forrest plot of sensitivity after sensitivity analysis.
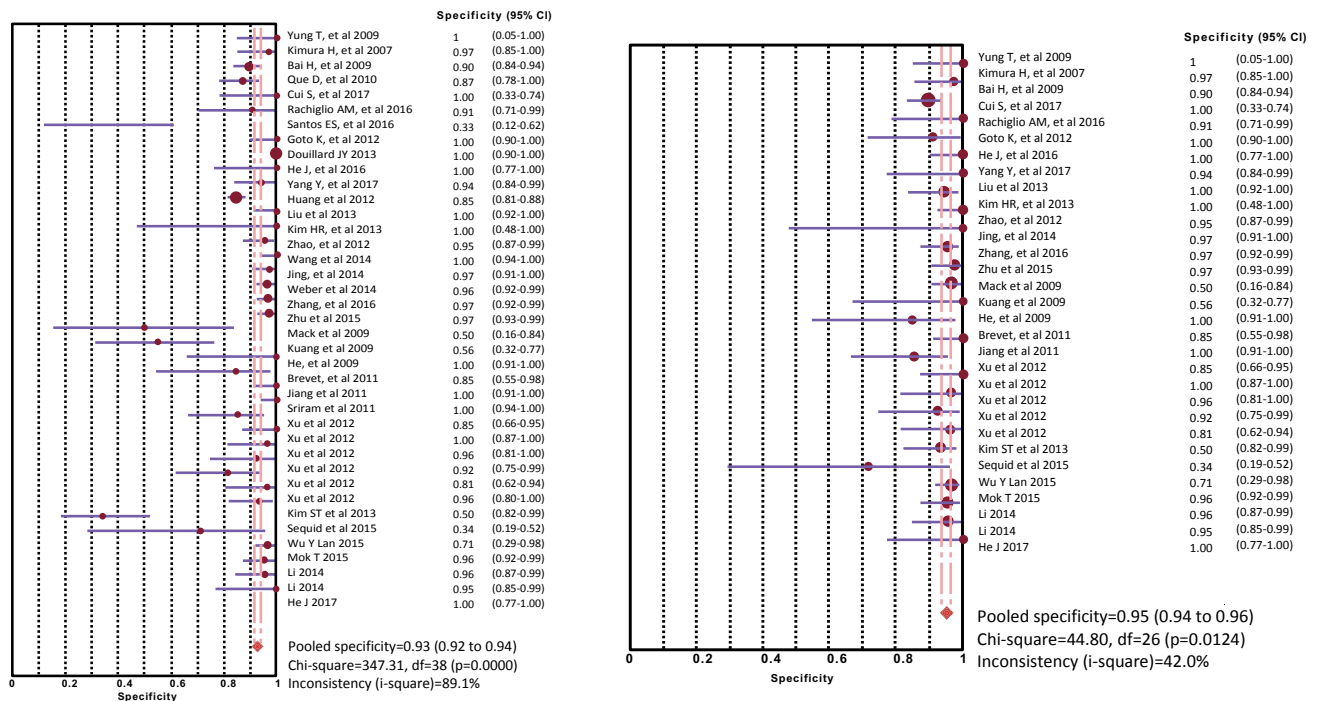


**Fig. (3). a**) forrest plot of specificity of all studies, **b**) forrest plot of specificity after sensitivity analysis.

biopsy, we noticed a heterogeneity between the different studies. The studies screening the deletion in the exon19 and those reporting mutations of the different exons of the EGFR gene presented quite similar sensitivities and specificities with heterogeneity in all cases. Table **3** illustrates the different results.

### 3.4. Heterogeneity and Meta-Regression Analysis

The meta-regressions were also performed to further explore potential sources of heterogeneity (Table **4**). Our meta-regression analysis characteristics included 'ethnicity (Asian or not)', 'the technique (Next generation sequencing
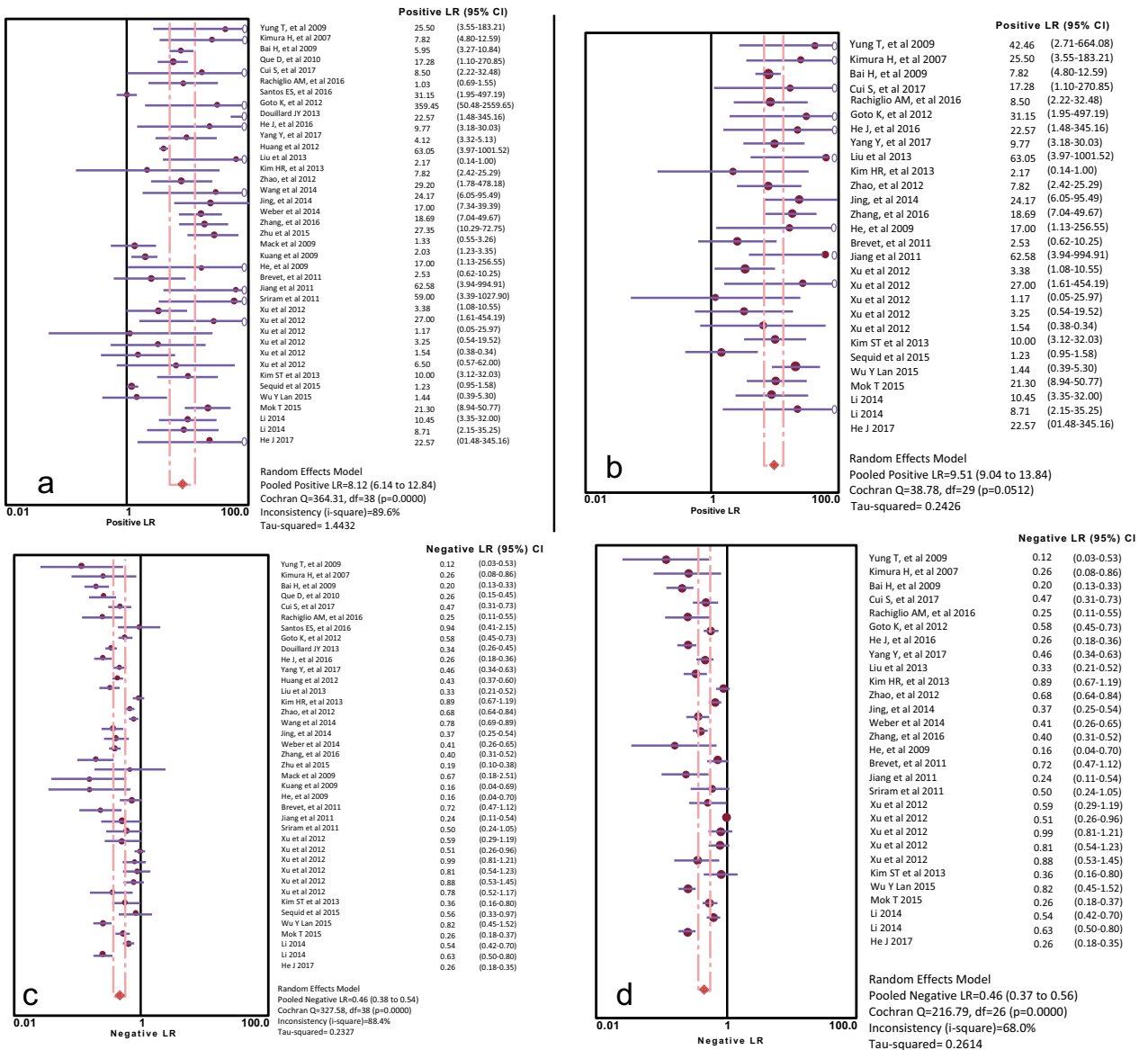
**Fig. (4). a)** Forrest plot of likelihood ratios for positive test results of all studies, **b)** Forrest plot of likelihood ratios for positive test results after sensitivity analysis, **c)** forrest plot of likelihood ratios for negative test results of all studies, **d)** forrest plot of likelihood ratios for negative test results after sensitivity analysis.
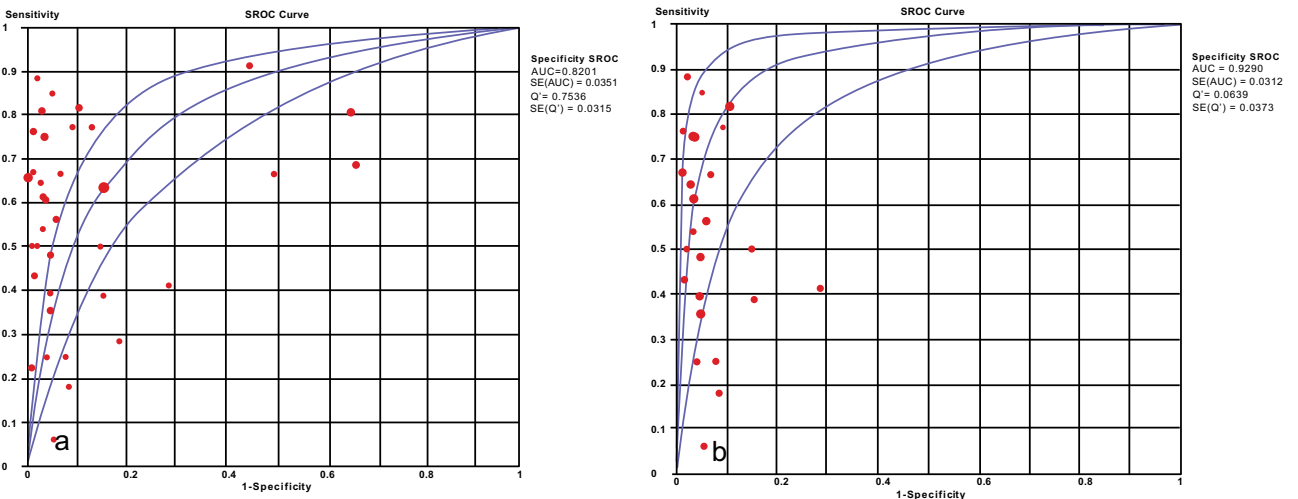


**Fig. (5). A)** The summary operative receiver characteristic curve indicating the area under curve of all studies, **B)** Forrest plot of dOR after sensitivity analysis.

**Table 3.** The pooled sensitivities, specificities and I-square of the sub-groups : NGS technique, ARMS technique, DHPLC technique, same technique in tissue and liquid plasma, screening of Ex19 deletion and L858R mutation, screening of Exons 18, 19, 20.

| Sub-groups | Pooled-SEN | Pooled-SPE |
|---|---|---|
| NGS | | |
| Cui S *et al.* <br> Rachiglio *et al.* <br> Santos *et al.* <br> Sequist *et al.* <br> Mok *et al* | 0.75 [0.71-0.801] <br><br> I2: 56.9% | 0.82 [o.77-0.87] <br><br> I2:95.6% |
| ARMS | | |
| Goto *et al.* <br> Douillard *et al.* <br> Liu *et al.* <br> Wang *et al.* <br> Mack *et al.* <br> Kuang *et al.* <br> Xu *et al.* <br> Xu *et al.* <br> Wu *et al.* <br> Li *et al.* <br> Li *et al* | 0.509 [0.461-0.558] <br><br> I2: 83.5% | 0.972 [0.95-0.98] <br><br> I2:90.3% |
| DHPLC | | |
| Bai *et al.* <br> Que *et al.* <br> Huang *et al.* <br> Xu *et al.* <br> Xu *et al* | 0.66 [0.618-0.709] <br><br> I2: 88.1% | 0.86 [0.83-0.88] <br><br> I2:40.6% |
| Same technique Tissue/Biopsy | | |
| Bai *et al.* <br> Cui *et al.* <br> Rachiglio *et al.* <br> Goto *et al.* <br> Douillard *et al.* <br> Huang *et al.* <br> Liu *et al.* <br> Zhao *et al.* <br> Wang *et al.* <br> Jing *et al.* <br> Weber *et al.* <br> Sriram *et al.* <br> Xu *et al.* <br> Xu *et al.* <br> Sequist *et al.* <br> Wu *et al.* <br> Mok *et al.* <br> Li *et al.* <br> Li *et al.* <br> He *et al* | 0.63 [0.6-0.65] <br><br> I2:87.3% | 0.93 [0.91-0.94] <br><br> I2:92.4% |

| Sub-groups | Pooled-SEN | Pooled-SPE |
|---|---|---|
| Ex19 del and L858R mutation | | |
| Yung *et al*<br>Bai *et al*<br>Que *et al*<br>Kim *et al*<br>Zhang *et al*<br>Zhu *et al*<br>He *et al*<br>Brevet *et al*<br>Jiang *et al*<br>Sriram *et al* | 0.66 [0.61-0.71]<br>I2:86.6% | 0.94 [0.92-0.96]<br>I2:72% |
| Screening exons 18, 19, 20 | | |
| Kuang *et al*<br>Kimura *et al*<br>Li *et al*<br>Li *et al*<br>He *et al* | 0.63 [0.57-0.69]<br>I2:88.1% | 0.91 [0.86-0.95]<br>I2:84.4% |

**Table 4.    Meta-regression analyzing 3 covariates: the test (NGS or not), the ethnicity (Asian or not), the test used in the tissue and the liquid biopsy (the same or not), the use of plasma or serum (serum or not).**

| Covariates | Coefficients | P value |
|---|---|---|
| **Ethnicity** | 0.01 | 0.98 |
| **Test** | -0.49 | 0.4 |
| **Test tissue versus liquid biopsy** | 0.45 | 0.37 |
| **Plasma versus serum** | 0.48 | 0.53 |

or not)', 'tissue/plasma (same technique use in the tissue and plasma or not)', 'plasma/serum (studies performed on serum or not). We didn't include the smoking status as a possible covariate because of its subjective estimation by the patients. The meta-regression results suggested that no covariates might be responsible for this heterogeneity.

Sensitivity analysis: A sensitivity analysis was performed because of the presence of heterogeneity with no covariates highlighted by the meta-regression. We focused on the specificity forest plot because liquid biopsy is considered as a diagnostic test and mustn't induce the treatment of patients with no mutations. The sensitivity analysis excluded the studies of Que D, *et al*, Santos, *et al*, Douillard, *et al*, Huang, *et al*, Wang, *et al*, Weber, *et al*, Zhu, *et al*, Mack *et al*, Kuang *et al*, Sriram, *et al*, Xu, *et al.* and Sequist, *et al.* [30, 32, 34, 36, 38, 39, 40, 17, 36, 44, 14, 25]. The overall pooled SEN and SPE were 0.61 (95%CI, 0.58-0.64) and 0.95 (95%CI, 0.94-0.96) respectively (Figs. **2a** and **2b**). Our results showed that PLR was 9.51 (95%CI, 6.66-13.58), NLR was 0.45 (95%CI, 0.37-0.56) and DOR was 24.58 (95%CI, 15.23-39) (Figs. **3a**, **3b** and **4**). We noticed no heterogeneity between studies in the SPE, PLR and the DOR (I-square estimated to respectively 42%, 33% and 43.9%). The area under curve was estimated to 0.93. We did not find any evidence of threshold effect (p=0.159).

## 4. DISCUSSION

This meta-analysis highlighted the efficacy of liquid biopsy in determining the EGFR gene mutation status in non-small cell carcinoma. According to the suggested guidelines for interpretation of AUC, ctDNA had high accuracy (0.9<AUC<1) for detection of EGFR mutation status in NSCLC. The value of DOR ranges from 0 to infinity with higher values indicating better discriminatory test performance. Our results showed a high diagnostic performance with a DOR of 20.5 even without sensitivity analysis. The likelihood ratios provided information about the likelihood that a patient with a positive or negative result has EGFR mutation or not. In our study, the PLR of 8 and the NLR of 0.45 were quite high. The meta-regression proved that the nature of the liquid used (plasma or serum), the ethnicity, the similarity of the techniques used in the tissue and the liquid biopsy are not the potential sources of the heterogeneity observed. Few meta-analyses have been reported about the diagnostic value of liquid biopsy and they were published in late 2014. They described also an important heterogeneity between the different techniques. Qiu *et al.* investigated the effect of the detection methods, TNM stages, collection time and format of blood sample and treatment of tumor tissues as potential confounding factors without proving significant results [27]. In this meta-

analysis, the majority of the studies were about late-staged carcinomas. Our sub-group analysis revealed a better sensitivity of next generation sequencing techniques with a better specificity of ARMS technique. Besides, even the stratified analysis of individual mutation, when applicable, showed relatively the same SEN and SPE with a significant heterogeneity between studies. Our sub-group analysis showed a heterogeneity even if studies were grouped based on the technique, the use of plasma or serum or the punctual mutation of the EGFR gene. The sensitivity analysis allowed achieving homogeneity by excluding 12 studies. The final group was characterized by the Asian ethnicity and the use of PCR-based techniques as diagnostic tests. It was quite surprising to exclude the study of Sequist *et al.* [25] which was based on NGS techniques. In their meta-analysis, Li and coworkers studied the importance of the country, the random or consecutive patient selection and test method and reported that test method was the unique contributing factor with p=0.00354 [28]. This meta-analysis included only 13 studies and the authors didn't perform a subgroup analysis.

We would like to discuss the potential limitations of this work. The fact that we didn't assess confounding factors highlights the multiplicity of these factors including the technical steps that are not discussed in the different studies, the percentage of tumor cells, the histologic subtype of the tumours, the collection time of blood sample, the detailed chemotherapy regimens that may be different sources of bias. Besides, most studies included tissue samples formalin-fixed paraffin-embedded which lead to significant DNA degradation and increase detection bias. This fact enhances further studies to investigate these issues.

## CONCLUSION

This meta-analysis suggested that detection of molecular mutations by cfDNA is of adequate diagnostic accuracy in association with tissues. The high specificity and the moderate sensitivity highlight the value of liquid biopsy as a screening test.

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## CONSENT FOR PUBLICATION

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## CONFLICT OF INTEREST

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

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