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Application of Different Body Fluid Specimen Biopsies in Minimal Residual Disease Monitoring in Lung Cancer: A Postprint

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Abstract

Lung cancer is one of the malignant tumors with the highest mortality rate worldwide, and its primary challenge is post-treatment recurrence. Minimal Residual Disease (MRD), as the "bridgehead" for solid tumor recurrence, is described as the persistent presence of free circulating tumor cells or other tumor cell derivatives in patients' biological fluids after treatment of the primary tumor, in the absence of any clinical symptoms of cancer. Recently, China has also established the first "Consensus on Detection and Clinical Application of Lung Cancer MRD," aiming to evaluate MRD status through liquid biopsy monitoring, thereby improving postoperative individualized treatment for lung cancer patients. This article reviews the progress of several promising liquid specimens (peripheral blood, urine, saliva, sputum, pleural effusion) in lung cancer MRD detection and explores their application value in guiding precision therapy for lung cancer MRD.

Full Text

Preamble

Application of Different Body Fluid Biopsy in Monitoring Minimal Residual Disease of Lung Cancer

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Abstract Lung cancer is one of the world' s deadliest malignancies, with treatment recurrence representing its primary challenge. Minimal Residual Disease (MRD), described as a "bridgehead" for solid tumor recurrence, refers to the presence of free circulating tumor cells or other tumor cell derivatives in a patient' s biological fluids following primary tumor treatment, in the absence of any clinical cancer symptoms. Recently, China reached its first "Consensus on Detection and Clinical Application of Lung Cancer MRD," aiming to evaluate MRD status through liquid biopsy monitoring to improve postoperative individualized treatment for lung cancer patients. This review summarizes progress in detecting lung cancer MRD using several promising fluid specimens (peripheral blood, urine, saliva, sputum, and pleural effusion) and discusses their application value in guiding precision therapy for lung cancer MRD.

[**Keywords**] liquid biopsy; circulating tumor DNA; minimal residual disease; lung cancer

1 Introduction

After decades of exploration, the management of lung cancer patients has undergone profound transformation. It has become increasingly clear that disease monitoring is fundamental to treatment success. Nevertheless, the percentage of patients who die from postoperative recurrence remains high in current clinical practice (45% of Stage IB patients recur within two years, rising to 76% for Stage IIIA patients) [1]. Consequently, Minimal Residual Disease (MRD)—the "culprit" behind tumor recurrence—has garnered increasing clinical attention. MRD refers to cancer-derived molecular abnormalities detectable through liquid biopsy after treatment, which cannot be identified by conventional imaging or laboratory methods, representing persistent disease and potential clinical progression [2]. In the TRACERx study, liquid biopsy analysis confirmed that over 99% of non-small cell lung cancer patients without recurrence were MRD-negative, while MRD was detectable in patients who later recurred before standard imaging could identify disease. Liquid biopsy has emerged as a novel method for MRD detection, analyzing circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, and other solid tumor-derived biomarkers from blood and other bodily fluids. Unlike tissue biopsy, this non-invasive approach can be performed repeatedly and continuously to identify tumor driver mutations, track tumor evolution, and monitor disease recurrence, with greater convenience and dynamic feedback on disease progression. Reports indicate that liquid biopsy can help patients avoid approximately 5% of major complications associated with CT-guided lung tissue biopsy [3]. Moreover, liquid biopsy can fully reflect tumor heterogeneity, providing more opportunities for postoperative individualized targeted therapy and improved prognosis.

2 Advantages and Challenges of Liquid Biopsy for Lung Cancer MRD

Traditional examinations (including tissue biopsy, imaging, and biochemical markers) have long played crucial roles in monitoring lung cancer development and treatment, serving as the gold standard for pathological diagnosis, staging, and treatment decisions for malignant tumors including primary lung cancer before liquid biopsy entered clinical practice. However, traditional detection methods are limited by procedural and temporal constraints that hinder effective MRD monitoring. Liquid biopsy offers several advantages. First, as a non-invasive alternative to tissue biopsy, patient body fluids can be easily and repeatedly sampled, enabling continuous monitoring of tumor characteristics during treatment (“real-time biopsy”) while overcoming sampling limitations related to tumor heterogeneity. A study by Rothwell et al. [4] demonstrated that next-generation sequencing (NGS) of cfDNA from 39 advanced solid tumor patients revealed 30% more mutations in plasma samples than in corresponding tumor tissue DNA. Second, liquid biopsy offers temporal superiority over conventional detection and higher specificity. The TracerX study found that postoperative ctDNA predicted 36-month recurrence with 48% sensitivity and 100% specificity [5], with a median lead time of 167 days between ctDNA detection and radiographic recurrence, compared to only 61 days between CEA elevation and radiographic recurrence [6]. Interestingly, one report described a Stage IIb non-small cell lung cancer patient who received radiotherapy with a residual mass visible on imaging. Despite undetectable ctDNA post-treatment, continuous ctDNA monitoring deemed the patient disease-free at 22 months; in retrospect, the mass represented radiation-induced inflammatory changes, raising concerns about high false-positive rates and radiation risks associated with imaging-based screening [6].

However, several challenges must be addressed for liquid biopsy to achieve broader clinical utility. Clonal hematopoiesis (CH) represents a significant issue—hematopoietic stem cells may acquire mutations during the random process of hematopoiesis with age, detectable through cfDNA in non-small cell lung cancer patients without known driver mutations. Most JAK2 mutations and some TP53 mutations may result from clonal hematopoiesis, potentially leading to misinterpretation of CH-related mutations as indicators of postoperative MRD. Various approaches are being adopted to distinguish CH-related mutations from true tumor DNA-derived mutations. The most direct method involves deep sequencing of nucleated white blood cells (CAPP-Seq) to identify clonal hematopoiesis mutations for exclusion from samples [7]. While technically feasible and operationally simple, this approach doubles costs. Other challenges include small fragment size, short half-life, and substantial decreases in tumor DNA proportion as treatment efficacy becomes apparent. Given high detection costs and lack of analytical consensus, liquid biopsy cannot yet completely replace traditional detection but serves as a valuable adjunct. Future research must integrate both approaches to monitor and evaluate MRD status, thereby

improving postoperative individualized treatment for lung cancer patients. provides a comparison of different fluid biopsies.

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3 Body Fluid Specimen Biopsy

3.1 Peripheral Blood

As solid tumors progress, metastasize, and recur, large numbers of tumor cells and their derivatives enter peripheral circulation, making blood a reliable specimen for accurately identifying MRD and guiding subsequent treatment decisions before radiological and serological findings emerge. Blood collection is less invasive than tissue biopsy, facilitating easy acquisition and near-real-time cancer monitoring. Primary biopsy targets in peripheral blood include circulating tumor cells, circulating tumor DNA, and exosomes, with comparative characteristics shown in . Extensive clinical validation studies and established analytical protocols have made blood-based biopsy the most commonly used method. In contrast, tumor cells and their derivatives in certain specific bodily fluids cannot reflect cancer metastasis as comprehensively as systemic blood circulation, remaining in the research and exploration phase for clinical application. Additionally, other bodily fluids have more complex microbial environments than blood, where microorganisms and their metabolites can unpredictably affect detection results. Current blood biopsy technologies can be broadly classified by genomic coverage, ranging from targeted allele-specific polymerase chain reaction (PCR) to next-generation sequencing (NGS) technologies such as hybrid capture NGS, whole exome sequencing, and whole genome sequencing. Common challenges across all detection methods include low ctDNA content in plasma, abundant background cfDNA from normal (non-tumor) cells, and uncertain origins of discovered genetic variants. However, technological advances enabling personalized gene sequencing to track more mutations are improving sensitivity for detecting low-volume MRD.

3.1.1 Circulating Tumor Cells (CTCs) CTCs are malignant cells that detach from primary tumors or metastatic lesions and undergo epithelial-mesenchymal transition to enter peripheral blood circulation [8]. Tumor recurrence requires numerous pathophysiological cascades, with CTCs potentially playing a crucial role in the metastatic phase of cancer expansion, growth, and spread. CTCs are extremely rare in blood vessels, with only approximately 1–10 CTCs present in 1 mL of whole blood [9]. Literature review indicates that CTCs can be found in nearly all lung cancer patients [10]. In early-stage lung cancer, thyroid transcription factor-1 (TTF-1) positive CTCs can be detected from peripheral blood and correlate with poor prognosis and shorter progression-free intervals [11]. For lung cancer patients with confirmed distant metastasis or recurrence, higher CTC counts predict worse prognosis, and Lindsay et al. [12] reported that CTCs serve as independent prognostic indicators for progression-free survival (PFS) and overall survival

(OS) in advanced non-small cell lung cancer. Therefore, CTCs can serve as a dynamic monitoring tool to observe changes in tumor kinetics before and after treatment. In one study of 26 early-stage lung cancer patients, CTC detection was performed preoperatively and on postoperative days 0, 1, and 3. Recurrent patients showed lower CTC count decline slopes, while non-recurrent patients exhibited steeper declines. For extrapulmonary malignancies with lung metastasis, CTCs decreased after tumor resection but rebounded to higher levels by postoperative day 3, even when imaging showed complete lesion removal. These findings demonstrate that CTCs can serve as an MRD detection tool and predict recurrence months after curative surgery.

3.1.2 Circulating Tumor DNA (ctDNA) ctDNA is defined as short DNA sequences shed by tumor cells into systemic circulation, derived from degraded CTCs or active secretion by tumor cells, representing a small fraction of circulating cell-free DNA (cfDNA). Despite comprising less than 1% of cfDNA, sequencing technology advances have enabled detection of mutations and chromosomal alterations relevant to tumor biology [13], with ctDNA' s half-life of approximately 2 hours being shorter than other tumor markers [14]. During lung cancer progression, lung tumor tissues may acquire a series of somatic mutations. Certain mutations, such as EGFR T790M, confer drug resistance and affect overall survival. In 2016, the U.S. Food and Drug Administration (FDA) approved the cobas EGFR Mutation Test v2 [15] as the first liquid biopsy test for MRD detection in lung cancer. Due to ctDNA' s potential to detect occult cancer and dynamically track tumor-specific mutations, sequential plasma sample analysis for tumor gene mutations has been used for clinical decision-making in advanced non-small cell lung cancer patients [16]. Furthermore, postoperative ctDNA positively correlates with later recurrence rates. In a study of 230 postoperative lung cancer cases [17], ctDNA quantified by the highest mutant allele fraction (MAF) in plasma samples relative to healthy controls identified significant differences in postoperative recurrence-free survival (RFS) between ctDNA-positive and ctDNA-negative patients. Postoperative ctDNA status was found to influence RFS more than any single clinicopathological risk factor or combination of factors, with higher sensitivity and specificity than other detection methods. Current limitations of ctDNA detection include clonal hematopoiesis and genomic differences from tissue biopsy, though advances in next-generation sequencing (NGS) and digital PCR (dPCR) are progressively improving ctDNA detection sensitivity.

3.1.3 Exosomes Johnstone et al. [18] first discovered and named exosomes while studying reticulocyte maturation. Exosomes are spherical vesicles measuring 40–100 nm in diameter with a density of 1.13–1.19 g/mL. Their primary nucleic acid contents include microRNA (miRNA), tRNA, long non-coding RNA (lncRNA), and numerous fragmented mRNAs. Exosome isolation methods primarily involve techniques based on physical properties (size, density, molecular weight), precipitation, microfluidics, and immunoaffinity capture, with ultracen-

trifugation and commercial kit extraction being the most widely used conventional methods [15]. Although all cell types release exosomes, they are particularly abundant in tumor cells. Research demonstrates that tumor cell-derived exosomes play important roles in tumor biology, promoting angiogenesis, invasion, and proliferation in recipient cells by transferring their contents to target cells in the lung cancer microenvironment, thereby participating in lung cancer formation and progression [20]. Cancer cell exosomes contain various cancer-related proteins; for example, epidermal growth factor receptor (EGFR) is the primary membrane-bound protein in lung cancer exosomes, with approximately 80% of exosomes extracted from lung cancer being EGFR-positive [21]. Tumor-derived exosomal miRNAs may also serve as independent predictors of survival in non-small cell lung cancer patients [22]. Rabinowits et al. [23] evaluated the diagnostic and prognostic potential of circulating tumor exosome levels in plasma samples from 27 lung adenocarcinoma patients and 9 healthy controls, finding that both exosome levels (mean 2.85 mg/mL) and exosomal miRNA concentrations (158.6 ng/mL) were higher in the adenocarcinoma group than in healthy controls (mean 0.77 mg/mL and 68.1 ng/mL, respectively).

3.2 Saliva

Saliva is produced by acinar cells in salivary glands, which are highly permeable and surrounded by abundant capillaries, allowing free exchange of molecules between blood and adjacent salivary cells [24]. Currently, approximately 40% of blood tumor markers can also be found in saliva [25], and saliva collection is rapid, simple, inexpensive, and non-invasive, making it an ideal liquid biopsy specimen. Gu et al. [26] first combined plasma CTCs and salivary mRNA biomarkers for non-invasive detection of non-small cell lung cancer, achieving 92.1% sensitivity and 92.9% specificity in distinguishing early-stage lung cancer patients from healthy controls. No significant quantitative or concentration differences in salivary cfDNA were observed between non-small cell lung cancer patients and healthy populations [27]; however, the concordance rate for EGFR mutations between plasma cfDNA and salivary cfDNA was 83.78%, suggesting that salivary cfDNA can serve as a supplementary source for gene mutation analysis [28]. EGFR is a membrane receptor frequently expressed in NSCLC that influences cell proliferation, angiogenesis, MRD recurrence, chemoresistance, and metastasis [29]. Frequent biopsy monitoring of EGFR mutations in postoperative lung cancer patients is impractical, making salivary biopsy a promising diagnostic supplement for lung cancer MRD. The University of California, Los Angeles (UCLA) School of Dentistry developed a technology called electric field-induced release and measurement (EFIRM) to detect EGFR mutations in lung cancer patient body fluids. Li et al. [30] detected circulating tumor DNA (sctDNA) EGFR mutations in saliva samples from 13 non-small cell lung cancer patients using this technology with 100% sensitivity. Other tumor biochemical indicators significantly correlate with lung cancer patient survival [31], such as imidazole compound (IC) concentration and salivary lactate dehydrogenase (LDH) activity. The combination of these two parameters proves more

effective for evaluating lung cancer prognosis and survival rates, with patients having favorable prognoses (IC < 0.311 mmol/L and LDH > 1133 U/L) showing 2-fold higher 1-, 3-, and 5-year survival rates than those with poor prognoses. C-reactive protein (CRP) concentrations may also increase with tumor size and regional metastasis.

3.3 Urine

Plasma ctDNA and CTC content is relatively low in MRD after treatment, requiring relatively large blood volumes for continuous disease monitoring, which remains uncomfortable for patients despite being minimally invasive. Studies show that cell-free DNA from peripheral blood can cross the renal barrier and be excreted in urine [32]. Chen et al. [33] analyzed matched 3 mL peripheral blood and 8 mL urine samples from 150 non-small cell lung cancer patients, finding no statistical difference in the quantity of free DNA (fDNA) obtained. Urine is easier to store and transport, facilitating large-volume sample collection and providing additional options to supplement traditional tumor sampling methods. MRD positivity indicates detectable tumor-derived DNA in blood after cancer treatment; similarly, DNA levels detected in urine can indicate tumor burden correlation. Previous reports demonstrated clinical applicability of urinary DNA for tracking tumor-specific mutations and personalizing treatment for drug resistance in advanced non-small cell lung cancer patients. Li et al. [34] found that detectable urinary DNA after treatment significantly correlated with disease recurrence in lung cancer patients. Results showed that patients with undetectable urinary DNA had better disease-free survival, while those with detectable DNA had recurrence probabilities of 15.6%, 6.6%, and 5.1% at 3, 6, and 9 months, respectively. This indicates that urinary ctDNA has good clinical utility for identifying patients with low disease recurrence risk, particularly those with undetectable mutant DNA. Lee et al. [35] noted that persistent EGFR mutation positivity in NSCLC patients after treatment suggests possible residual disease requiring further therapy or enhanced recurrence monitoring, with the T790M mutation particularly associated with shortened time to disease progression and reduced overall survival. Chen et al. [33] stratified 150 non-small cell lung cancer patients and found that the urinary DNA T790M-positive group had significantly worse overall survival outcomes (median survival 30 months) compared to the T790M-negative group (median survival 34 months), further validating the clinical utility of urinary DNA for post-treatment risk stratification and disease monitoring.

3.4 Sputum

The National Cancer Institute (NCI) [36] conducted dual screening with low-dose spiral computed tomography and sputum cytology for lung cancer, finding that 18 of 90 patients (20%) diagnosed through dual screening had cancer-positive sputum specimens but negative imaging, demonstrating sputum' s temporal superiority over imaging for diagnosing clinically quiescent or occult can-

cers. However, most lung cancer patients produce limited sputum samples containing few tumor cells, and the viscous mucus component makes tumor-derived DNA extraction more difficult—one reason why sputum is relatively underutilized in liquid biopsy. Wang et al. [37] developed a methanol-free mucolytic solution (MS2) to improve isolation of tumor-derived cfDNA from sputum, demonstrating significantly higher sensitivity for EGFR mutation detection in post-treatment patient sputum specimens using MS2 compared to traditional methanol-containing mucolytic solution (MS1) in the same cohort. In a study of 102 lung adenocarcinoma patients using qRT-PCR for sputum cfDNA detection, 30 patients (29.4%) were EGFR mutation-positive, with overall sensitivity and specificity of 46.2% and 100%, respectively. Mao et al. [38] detected K-ras and p53 mutations in sputum samples from 8 of 10 patients before clinical diagnosis of lung cancer, which is significant for improving tumor genotyping, targeted precision therapy, and developing perioperative individualized treatment. Multiple studies have demonstrated that overexpression of miRNAs, mir-21, and mir-155 are negative factors for postoperative recurrence, prognosis, and overall survival. Sputum contains bronchial epithelial cells from the lungs and lower respiratory tract, where miRNAs are resistant to RNase activity, exist in a remarkably stable form, and remain detectable in sputum samples stored for up to 7 days [39]. Roa et al. [40] found that miRNA panels from sputum samples could detect NSCLC with significant sensitivity and specificity. Adenocarcinomas from smaller airways are difficult to detect through bronchoscopy or sputum cytology; miRNA expression in sputum will provide a highly accurate specific marker for lung cancer MRD monitoring, enabling early diagnosis and treatment on a non-invasive basis.

3.5 Pleural Effusion

Malignant pleural effusion (MPE) is a common complication in intermediate and advanced-stage lung cancer, resulting from tumor-blocked lymphatic glands causing fluid exudation and accumulation in the pleural cavity, significantly correlating with tumor recurrence and metastasis. MPE is easily collected compared to other invasive techniques like tissue biopsy. Moreover, mutation rates are much higher in lung cancer-related MPE than in surgical resection specimens [41], and pleural effusion biopsy biomarkers may originate from multiple tumor clones, simultaneously reflecting tumor and disseminated lesion heterogeneity. Abundant MPE provides a rich opportunity for assessing tumor genomics [42]. Molecular analysis of pleural effusion represents a minimally invasive method for detecting tumor driver gene mutations, particularly for clinical decision-making when tumor tissue is unavailable. It may serve as an alternative source of useful information for gene mutation status such as EGFR. If EGFR mutation detection can be achieved through more readily available pleural effusion samples, it would become a superior specimen for exploring MRD' s role in both driver gene-positive and driver gene-negative patients, investigating resistance mechanisms, and identifying resistance before radiographic detection, making targeted drug therapy possible for advanced non-small cell lung cancer patients

with important clinical and practical value.

3.5.1 Pleural Effusion Supernatant (MPEs) Cell-free DNA (cfDNA) from malignant pleural effusion supernatant in advanced non-small cell lung cancer patients shows significantly higher tumor gene mutation abundance than effusion tumor cells and plasma cfDNA samples, proving superior for detecting therapeutic targets and tumor mutation burden (TMB) [43]. In advanced lung cancer MRD, driver gene EGFR mutation detection guides targeted therapy, while TMB values can evaluate immunotherapy efficacy. Wang et al. [44] compared EGFR mutation status in tumor tissue, plasma, and MPEs, correlating results with EGFR-TKI therapy. The study demonstrated high concordance in EGFR mutation sensitivity and specificity between tumor tissue and MPEs, while plasma showed the lowest EGFR mutation rate. EGFR mutations in MPEs could predict efficacy of first-generation EGFR-TKI treatment, with EGFR-mutant patients receiving TKI therapy showing longer median overall survival than wild-type EGFR patients. Objective response rate (ORR) and disease control rate (DCR) were 56% and 94%, respectively, in MPEs EGFR-mutant patients receiving first- or second-line EGFR-TKI therapy, consistent with tissue-based detection results. Therefore, when both samples are available, cfDNA extracted from MPEs may be a better biomarker than plasma for predicting tumor response to TKIs. Additionally, Yang et al. [45] observed significantly longer median PFS in MPEs EGFR-mutant patients compared to wild-type EGFR patients (7.33 vs. 2.07 months). Song et al. [46] investigated the feasibility of gene detection using exosomal DNA from lung adenocarcinoma patient MPEs, finding that 78% of mutations identified in MPEs exosomal DNA matched those found in MPEs ctDNA, supporting its reliability for genetic testing. Multi-channel determination of original tumor mutation status and monitoring mutation changes is crucial for lung cancer treatment.

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4 Summary and Outlook

Biomarkers derived from liquid samples are urgently needed clinically for monitoring MRD, predicting tumor response, and exploring therapeutic resistance. Liquid biopsy, as an alternative method for analyzing variations, provides not only a non-invasive approach for early detection of lung cancer changes but also complements tissue biopsy results, enabling more cancer patients to receive precise treatment. However, many questions regarding MRD itself remain to be resolved. Future research will need to determine how best to integrate tumor tissue biopsy, clinical examination, and medical imaging with liquid biopsy genomics and MRD information. Ultimately, in this era of individualized precision medicine, applying diversified liquid biopsy specimen analysis to clinical oncology will become a novel pathway for guiding clinical decisions and improving patient outcomes.

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Note: Figure translations are in progress. See original paper for figures.

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