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Liquid biopsy in Oncology: Results of a Delphi consensus study endorsed by the AIOM-SIAPEC/IAP-SIBioC-SIF Italian scientific societies

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ABSTRACT

Liquid biopsy (LB) offers a minimally invasive alternative to tissue biopsy by detecting tumor-derived analytes in biological fluids. Nonetheless, its adoption is limited by variability in methodologies. Therefore, this study used a

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modified RAND/UCLA approach involving 23 experts of the field, providing two questionnaires that assessed agreement on 22 items. Consensus was reached for all the pre- and post-analytical phase items, agreeing on the pivotal role of plasma cfDNA. Conversely, opinions varied regarding other biomarkers and biological samples. Furthermore, turnaround time and disease setting resulted as two of the most important analytical parameters for choosing testing methodology. Particularly, a complementary tissue-liquid approach with sampling interval $\leq$2 weeks was preferred. To conclude, a strong consensus on sample handling, biomarker prioritization, and clinical applications is achieved. However, significant heterogeneity remains regarding novel biomarkers, sampling strategies, and costs. Standardization and validation are needed to enhance the clinical adoption of LB.

Abbreviations

AIOM	Italian Association of Medical Oncology
cfDNA	circulating free DNA
CGP	Comprehensive genomic profiling
CHIP	Clonal hematopoiesis of indetermined potential
CSF	Cerebrospinal fluid
CTC	Circulating tumor cells
ctDNA	circulating tumor DNA
ctRNA	circulating tumor RNA
EV	Extracellular vesicles
FDA	Food and Drug Administration
ICER	Incremental Cost-Effectiveness Analysis
IQR	Interquartile range
LB	Liquid biopsy
MRD	Minimal residual disease
MTBs	Molecular tumor boards
NGS	Next generation sequencing
NSCLC	Non-small cell lung cancer
PCR	Polymerase chain reaction
QALY	Quality-Adjusted Life Years
RAND	Research and development (corp.)
SIAPEC/IAP	Italian Society of Pathological Anatomy
SIBioC	Italian Society of Clinical Biochemistry and Clinical Molecular Biology
SIF	Italian Society of Pharmacology
TAT	Turnaround times
TF	Tumor fraction
UCLA	University of California, Los Angeles
WTP	Willingness-To-Pay

1. Introduction

Liquid biopsy (LB) represents a minimally invasive technique designed to detect tumor analytes in biological fluids as a surrogate for neoplastic tissue for diagnostic, prognostic, and predictive purposes [1]. The analysis of circulating tumor DNA (ctDNA), a fraction of cell-free DNA (cfDNA), is currently the most widely adopted approach in clinical practice, although other circulating biomarkers such as extracellular vesicles (EVs), circulating tumor cells (CTCs), and circulating tumor RNA (ctRNA) are gaining attention for their potential applications [2–4]. Typically performed through peripheral blood sampling, LB can also exploit other biological fluids such as pleural effusions, urine, and cerebrospinal fluid (CSF).

To date, five LB-based diagnostic tests have been approved by the U. S. Food and Drug Administration (FDA), guiding treatment decisions for patients with breast, non-small cell lung (NSCLC), prostate, colorectal, and ovarian cancers [5]. These tests are particularly beneficial for patients with inadequate tissue for traditional molecular analysis. While LB is frequently quick and less invasive, minimal residual disease (MRD) analysis based on patient-specific markers can take weeks, which is important to consider during early-stage management [6]. Ongoing research is investigating its broader applicability across various cancers and clinical scenarios, with the technique showing great promise for early cancer detection [7,8]. Beyond its established regulatory framework and its role as an alternative in cases of insufficient tissue, the clinical advantages of LB extend across multiple dimensions of real-world oncology practice. First, its minimal invasiveness enables safe, repeatable sampling over time, allowing true real-time monitoring of tumor dynamics, treatment response, and clonal evolution [9]. Second, LB offers a unique opportunity to overcome spatial and temporal tumor heterogeneity by simultaneously capturing molecular information released from multiple primary and metastatic sites. This feature is particularly relevant in highly heterogeneous malignancies such as NSCLC, colorectal, breast, and prostate cancer, where single-site tissue sampling may fail to reflect the full genomic complexity of disease [10]. Third, LB significantly accelerates molecular reporting, facilitating earlier access to targeted therapies and reducing delays in treatment initiation, which is especially critical in rapidly progressing tumors. Additional advantages include broader patient accessibility, improved feasibility in frail patients with comorbidities, and increasing cost-effectiveness when correctly integrated into diagnostic algorithms. Finally, the expanding applicability of LB across the entire cancer continuum positions this technology not merely as a surrogate to tissue biopsy, but as a central and dynamic pillar of modern precision oncology [2,11].

Despite its many advantages, LB clinical application is hampered by a range of pre-analytical, analytical, and post-analytical issues [12]. Firstly, the collection, processing, and storage of biological samples represents critical steps. Tumor biology itself poses challenges, particularly in differentiating proper ctDNA among the vast majority of cfDNA [13]. The short half-life of cfDNA and its extremely low concentration further complicate detection, necessitating highly sensitive techniques for accurate analysis. Another major issue is the contamination by clonal hematopoiesis of indetermined potential (CHIP), a condition where somatic mutations accumulate in hematopoietic stem cells introducing genomic alterations that are unrelated to the tumor while leading to false positive interpretations if not correctly identified [14]. In the diagnostic setting, targeted single-plex approaches such as polymerase chain reaction (PCR)-based technologies have emerged as powerful tools for identifying mutations with high sensitivity and specificity. New multi-plex technologies, including next-generation sequencing (NGS) and comprehensive genomic profiling (CGP), have improved detection accuracy, although often costly and not yet widely accessible for routine clinical use [15,16]. In the post-analytical phase, data interpretation and reporting pose further challenges. The rapid evolution of genomic information and the need for standardized reporting formats add complexity to the integration of LB into clinical practice. Furthermore, the issue of false-negative results due to low cfDNA shedding levels necessitates repeated testing or confirmatory tissue biopsy in some cases, complicating the clinical decision-making process [17].

In the rapidly evolving LB field, the Delphi method is an ideal approach to address such challenges by systematically aggregating the

opinions of diverse experts to achieve consensus on complex or controversial issues for the consolidation of LB expertise across disciplines [18,19]. This approach is critical for developing practical recommendations that are not only scientifically sound but also implementable across diverse patient settings. This study aimed to facilitate consensus among experts from academic and institutional backgrounds related to the discipline of LB where there is variable or insufficiently robust evidence in the clinical practice.

2. Methods

2.1. Study design

In this study, we used a modified Delphi technique (RAND/UCLA Delphi method [20]) which included three different phases: i) a steering committee meeting to select the topics to be addressed in the study and define the items to be included in the questionnaire; ii) two sequential rounds of questionnaires to obtain the opinion of a scientific committee of experts on the items defined in the first phase; iii) analysis and discussion of the results with subsequent definition of conclusions.

Expert participants were asked to respond to questionnaires administered in sequential rounds, developed based on the feedback provided in the previous rounds. The feedback from earlier rounds encouraged participants to re-evaluate and modify their opinions to achieve consensus, where possible [21].

2.2. Committee definition

Three different committees were involved in this study: a steering committee, a scientific committee, and a technical committee. The steering committee was responsible for conducting an extensive review of the scientific literature to define the topics to be addressed in the study and, subsequently, the items for the questionnaire to be submitted to the scientific committee. The scientific committee, chosen by the steering committee, was responsible for answering the items created by the steering committee based on their clinical and research experience. Finally, the technical committee, which directed and supervised the entire process, was responsible for the implementation of the Delphi method (literature search, questionnaire distribution to the scientific committee, response analysis, and statistical interpretation of the consensus).

The scientific committee was composed of 23 members (Supplementary Information A): 14 medical oncologists, 4 pathologists, 2 pharmacologists, 2 molecular biologists, and 1 clinical biochemist with recognized experience in the field of LB in oncology. The members of the committee were chosen based on their clinical experience and scientific interest in the use of LB in patients with cancer, according to the recognized expertise in oncology, molecular pathology, laboratory medicine, and translational research with documented scientific contributions and active involvement in national scientific societies with a specific focus on LB. All panelists had substantial clinical and/or methodological experience relevant to the scope of the recommendations. Potential conflicts of interest were systematically collected and disclosed prior to participation; panel members with relevant financial or professional conflicts did not influence the formulation or voting on recommendations in areas where a conflict could be perceived.

2.3. Questionnaire

A questionnaire consisting of 22 items was created by the steering committee (Supplementary Information B). Items were grouped into 3 different domains regarding the applications of LB in oncology: pre-analytical phase (5 items), analytical phase (12 items), and post-analytical phase (5 items). Two types of items were used: i) questions requiring a score based on a 5-point Likert scale (“strongly agree”, “agree”, “uncertain”, “disagree”, and “strongly disagree”); ii) questions requiring ranking of the available answers. For each item, there was a blank space where each member of the scientific committee could express comments or suggestions to improve the statement/question.

2.4. Data analysis

Responses to the questionnaires were evaluated based on the type of item. For questions with a Likert scale response, the absolute and percentage frequencies of favorable answers (sum of "strongly agree" and "agree" responses), uncertain answers ("uncertain" response), and unfavorable answers (sum of "strongly disagree" and "disagree" responses) were calculated. If a question had a percentage frequency of agreement $\geq$75 %, it was accepted; if the frequency of agreement was <25 %, it was rejected. Additionally, if a question had a percentage frequency of agreement $\geq$25 % and <75 %, it was resubmitted in a second round as an open-ended question. For questions requiring ranking of the available responses, the number of times each item was ranked in a certain position (e.g., number of times ranked in the first position, second position, etc.) was calculated. The results of the analyses were presented based on their related domains (pre-analytical, analytical, and post-analytical phases). All statistical analyses and plots were performed using R version 4.4.0 (*The R Foundation for Statistical Computing*, 2022).

2.5. Consensus process

In each round, the questionnaire was submitted to the scientific committee members in electronic format using “Microsoft Forms”. The experts were invited to respond to the questions anonymously. To submit the questionnaire, it was mandatory to answer all items. In the first round, the members of the scientific committee were asked to respond to the questionnaire sent via email on May 15, 2024. At the end of the first round, the responses provided by the scientific committee were analysed by the technical committee. In the second round, a new questionnaire was sent to the expert members on July 05, 2024, consisting of the items that did not reach consensus in the first round. Items were converted into open-ended questions to allow for an understanding of the reasons for the lack of agreement.

3. Results

In the first round, all 23 members of the scientific committee (100 %) responded to the questionnaire. The median time taken to complete the questionnaire was 22 days, with an interquartile range (IQR) of 4–36 days. Of the 20 items requiring a Likert scale response, 17 (85 %) reached an agreement frequency of 75 % or higher and were accepted in the initial round. No items were rejected; however, 3 items (15 %) had an agreement frequency between 25 % and 75 %, and were therefore referred to the technical committee. In the second round, 19 members (83 %) responded to the three items under review. The median completion time for this follow-up questionnaire was zero days, with an IQR of 0–2 days.

3.1. Pre-analytical phase

In the pre-analytical phase, all items (n. 1, 2, 3, 4, and 5) submitted to the scientific committee reached a consensus in the first round (Table 1). Particularly, all the experts unanimously confirmed that the isolation of cfDNA is currently one of the most commonly used analyte among LB applications in cancer patients (item 1), specifying that plasma should be favored over serum (item 2). Additionally, the scientific committee stated that there is currently no scientific evidence to determine the amount of blood needed to isolate ctDNA (item 3) and that plasma processing to obtain cfDNA should follow specific centrifugation protocols (item 4). Finally, the experts confirmed that other extraction methods validated on tissue samples or other biological matrices should not be used for LB (item 5).

Table 1
List of items related to the pre-analytical phase.

Item		Agree n (%)	Uncertain n (%)	Not Agree n (%)
1	In the context of circulating tumor DNA (ctDNA) analysis using liquid biopsy, do you agree that the isolation of circulating free DNA (cfDNA) from peripheral blood represents one of the most commonly used procedures?	**23 (100)**	0	0
2	In the context of circulating tumor DNA (ctDNA) analysis using liquid biopsy, do you agree that the use of plasma is preferable to serum?	**20 (86.96)**	3 (13.04)	0
3	Do you agree that there are currently no clear and conclusive guidelines on the amount of blood to be used for isolating cfDNA?	**21 (91.30)**	1 (4.35)	1 (4.35)
4	Do you agree that specific centrifugation protocols should be used to isolate plasma for obtaining a suitable cfDNA sample for subsequent analyses?	**23 (100)**	0	0
5	Among the various methods for extracting cfDNA, do you agree that extraction techniques validated on tissue samples or other biological matrices should not be used?	**22 (95.65)**	1 (4.35)	0

3.2. Analytical phase

In the analytical phase, 9 (75 %) items reached consensus (n. 6, 7, 8, 9, 10, 11, 12, 13 and 14) after the first round (Table 2). The scientific committee confirmed that the quality of samples for LB analysis is influenced by factors such as temperature, method, and time of cfDNA preservation (item 6) and that turnaround times (TAT) and disease setting are two of the most important parameters for choosing the method of analysis and quantification of cfDNA (items 7 and 8). In terms of LB accuracy, the experts emphasized that both the disease burden and its distribution across different anatomical sites are significant factors influencing the outcome (items 9 and 10). Specifically, item 22 showed the order of importance assigned by the scientific committee to a list of eight factors that can influence the LB analysis outcome (Fig. 1A, Supplementary Information C). Regarding the different analytes that can be assessed with LB, the experts confirmed that the use of DNA or RNA is a significant factor in determining the outcome (item 11) and that the only analyte that can currently provide useful information in clinical practice is ctDNA (item 12). Finally, the expert committee confirmed that peripheral blood and pleural fluid are the only biological fluids that can provide helpful information in clinical practice (item 13).

Three items did not reach a consensus in the first round (Table 3). Particularly, two items (n. 15 and 16) were re-proposed as open-ended questions in the second round. We did not include item 14 since the data obtained from item 21 (Fig. 1B) allowed an understanding of the opinion of the scientific committee (Supplementary Information C). Although all the experts reached a complete consensus for item 12 on the importance of ctDNA in clinical practice, the question was re-proposed as open-ended to gather insights from the scientific committee regarding the potential role of the other biomarkers for clinical practice. As a result of the second round, most of the experts stated the importance of having both the data from liquid and tissue biopsy ("complementary approach") to integrate information from the two distinct methods, potentially yielding the maximum diagnostic accuracy and offering insights into the spatial-temporal heterogeneity of the disease. In contrast, a smaller number of experts agreed on the "liquid first" approach due to the possibility of preserving diagnostic tissue and

Table 2
List of items related to the analytical phase that were accepted after the first round.

Item		Agree n (%)	Uncertain n (%)	Not Agree n (%)
6	Do you agree that the concentration, temperature, method, and duration of cfDNA storage are important factors in determining sample quality?	**23 (100)**	0	0
7	Do you agree that the turnaround time (TAT) is among the most important parameters in determining the choice of method for analyzing and quantifying cfDNA?	**18 (78.26)**	3 (13.04)	2 (8.70)
8	Do you agree that disease staging is among the most important parameters in determining the choice of method for analyzing and quantifying cfDNA?	**22 (95.65)**	1 (4.35)	0
9	Do you agree that disease burden is among the factors that most significantly influence the accuracy of liquid biopsy results?	**20 (86.96)**	3 (13.04)	0
10	Do you agree that distribution across different anatomical sites is among the factors that most significantly influence the accuracy of liquid biopsy results?	**21 (91.30)**	2 (8.70)	0
11	Do you agree that the choice between using DNA or RNA in liquid biopsy is an important parameter that can influence the outcome of the liquid biopsy?	**21 (91.30)**	1 (4.35)	1 (4.35)
12	Do you agree that the following biomarkers, assessable through liquid biopsy, provide useful information in clinical practice?			
	Circulating tumor DNA	**23 (100)**	0	0)
	Circulating tumor RNA	11 (47.83)	12 (52.17)	0
	Circulating tumor cells	6 (26.09)	11 (47.83)	6 (26.09)
	Extracellular vesicles derived from the tumor	7 (30.43)	12 (52.17)	4 (17.39)
13	Do you agree that performing a liquid biopsy on the following biological fluids provides useful information for clinical practice?			
	Peripheral blood	**23 (100.0)**	0	0
	Cerebrospinal fluid	17 (73.91)	6 (26.09)	0
	Urine	14 (60.87)	9 (39.13)	0
	Ascitic fluid	15 (65.22)	7 (30.43)	1 (4.35)
	Pleural fluid	**19 (82.61)**	4 (17.39)	0

shortening the time to receive information on the molecular characteristics of the disease. Although some experts stated that a single best approach could not be defined, most of them specified that the best approach should be evaluated on a case-by-case basis in light of the patient's clinical history.

Finally, experts emphasized that the assessment of ctRNA, CTCs, and tumor-derived EVs does not provide helpful information for clinical practice due to critical issues in the pre-analytical phase. Nevertheless, they represent potential resources for the future that should continue to be explored.

3.3. Post-analytical phase

In the post-analytical phase, all items (n.17, 18, 19, and 20) submitted to the experts reached a consensus in the first round (Table 4). Specifically, the scientific committee confirmed that LB should be performed at the time of disease recurrence/progression (item 17), highlighting its usefulness for therapeutic monitoring in case of response and

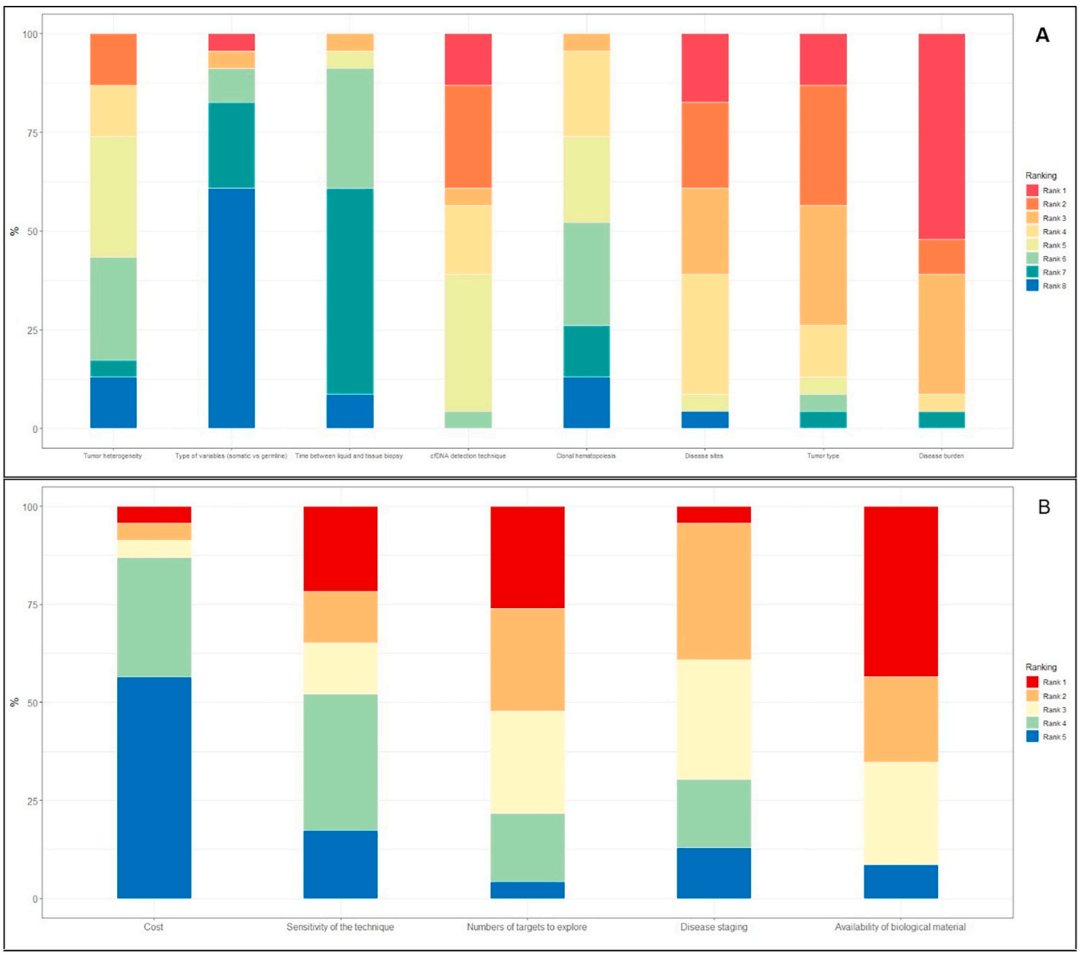

Fig. 1. **A** – Item 22: "In your opinion, rank the main factors that can influence the outcome of a liquid biopsy in order of importance*". **B** – Item 21: "In your opinion, rank the factors in order of importance that can influence the choice of NGS method to be employed (e.g., DNA, DNA and RNA, real-time PCR, etc.) among those listed below*".
*The percentages are reported in Supplementary Information C.

Table 3
List of items related to the analytical phase that were re-proposed in the second round.

Item		Agree n (%)	Uncertain n (%)	Not Agree n (%)
14	Do you agree that the cost of the method is among the most important parameters in determining the choice of method for analyzing and quantifying cfDNA?	14 (60.87)	2 (8.70)	7 (30.43)
15	Please state your grade of confidence in complementary or sequential approach as the optimal strategy:			
	Complementary approach (liquid biopsy + tissue biopsy)	15 (65.22)	5 (21.74)	3 (13.04)
	Sequential approach (tissue/ plasma)	15 (65.22)	5 (21.74)	3 (13.04)
	Sequential approach (plasma first)	9 (39.13)	9 (39.13)	5 (21.74)
16	In the context of a "complementary" approach, do you agree that the time elapsed between tissue sampling and liquid sampling can influence the outcome of liquid biopsy?	17 (73.91)	4 (17.39)	2 (8.70)

progression to treatment (item 18). Additionally, the experts confirmed that TAT is a relevant parameter in evaluating MRD and the diagnostic accuracy of the biomarker, but not in the assessment of therapeutic monitoring (item 19). Finally, the scientific committee unanimously emphasized that the LB report should indicate: the biological sample used for the analysis, the method employed for conducting the analysis, pre-analytical and analytical variables, and any treatments (item 20).

4. Discussion

This Delphi consensus study provided critical insights into the current understanding, practical challenges, and expert opinions on the standardization of LB use in oncology. By conducting two rounds of questionnaires, opinion leaders were encouraged to refine their opinions and reconsider their positions considering collective feedback, resulting in more robust and widely accepted recommendations. The results of the study demonstrated a strong consensus on several key areas while also revealing significant divergencies that warrant further investigation in the LB field.

4.1. Pre-analytical phase

This study achieved a unanimous agreement on the importance of using plasma as biological source (item 1), preferring it over serum for ctDNA extraction (86.9 %, item 2). This concurred with the literature showing that serum usually presents larger DNA fragments deriving

Table 4
List of items related to the post-analytical phase.

Item		Agree n (%)	Uncertain n (%)	Not Agree n (%)
17	Do you agree that liquid biopsy should be performed at the time of disease recurrence/ progression?	**21 (91.30)**	2 (8.70)	0
18	Do you agree that liquid biopsy is useful for therapeutic monitoring both in cases of treatment response and treatment progression?	**20 (86.96)**	2 (8.70)	1 (4.35)
19	The "Tumor Fraction" is a relevant parameter for the:			
	Assessment of minimal residual disease	**19 (82.61)**	3 (13.04)	1 (4.35)
	Assessment of therapeutic monitoring	17 (73.91)	4 (17.39)	2 (8.70)
	Assessment of diagnostic accuracy of the biomarker	**18 (78.26)**	3 (13.04)	2 (8.70)
20	It is important that the liquid biopsy report indicates:			
	Biological sample used for the analysis	**23 (100.0)**	0	0
	Technique used for the analysis	**23 (100.0)**	0	0
	Pre-analytical and analytical variables	**23 (100.0)**	0	0
	Any treatments	**20 (86.96)**	2 (8.70)	1 (4.35)

from leukocyte lysis with consequent release of germline DNA that would dilute tumor DNA [22, 23]. There was also a consensus that no clear guidelines exist for determining the optimal blood volume required for cfDNA isolation (91.3 %, item 3). This reflects the variability in existing protocols, where commercially available tests have different plasma volume requirements [24,25]. Importantly, the required plasma volume may also vary depending on the clinical context (such as pre-surgical staging, post-radical surgery, or longitudinal monitoring during therapy) and tumor characteristics. Therefore, tailoring the volume to both the tumor type and clinical scenario could be crucial to maximize analytical sensitivity and clinical utility of LB assays. Additionally, a unanimous agreement was endorsed on the necessity of centrifugation protocols (item 4), indicating recognition of its impact on cfDNA yield and integrity [26]. Furthermore, extraction techniques validated for tissue samples should not be used for circulating biological matrices (95.6 %, item 5). This distinction is critical since cfDNA, compared to DNA extracted from tissue biopsies, is highly fragmented, thus requiring specialized extraction techniques [27].

4.2. Analytical phase

A notable point of discussion identified TAT as a crucial determinant of the method choice for cfDNA analysis, with more than two-thirds of the experts emphasizing its importance (78.2 %, item 7). Especially for guideline-recommended biomarkers, LB NGS offers a faster TAT compared to tissue-based testing [28]. Faster results help prevent delays in clinical trial enrollment and treatment decisions, addressing the growing demand for rapid molecular profiling in oncology. However, the only moderate level of agreement among different health professionals involved in this study suggests that, while TAT is essential, other factors such as sensitivity and specificity must also be prioritized to ensure effective clinical application, mostly considering that molecular biologists and clinicians may have differing perspectives on TAT significance. In this way, molecular tumor boards (MTBs) have been crucial in bridging the gap between molecular biology, pathology, and clinical oncology. This highly collaborative environment also facilitates a mutual understanding of the difficulties and limitations of tests, as well as aligning expectations regarding TAT. Ultimately, this supports and validates therapeutic decisions in a more effective and timely manner.

A consensus was reached on the role of disease burden (86.9 %, item 9) and its distribution across different anatomical sites (91.3 %, item 10) in significantly affecting the accuracy of LB results. As expected, this is consistent with literature evidence indicating that tumor DNA shedding rates vary according to disease staging, cancer histotype, and treatment response. Larger, metastatic, and treatment-resistant tumors tend to shed more ctDNA, whereas a localized or indolent disease may release minimal plasma cfDNA increasing the risk of false negatives [29–31]. Moreover, brain tumors may have limited cfDNA plasma shedding due to the blood-brain barrier, limiting its detection [28]. This variability highlights the importance of adjusting read-depth and assay sensitivity based on both tumor and patient characteristics to optimize detection.

Interestingly, while ctDNA was unanimously recognized as the most informative analyte, expert opinions were considerably divided regarding the clinical utility of other biomarkers of the LB family (items 11–12). Only nearly half of the panel agreed on ctRNA clinical significance, while two-thirds of experts were uncertain or disagreed on the clinical utility of CTCs and EVs. This divergence highlighted the evolving landscape of LB research, where ctRNA along with CTCs and EVs shows promise but still faces technical and validation challenges. In this vein, the quality and quantity of literature evidence supporting their routine use remain insufficient, with studies often constrained by small cohorts, heterogeneous methodologies, and variable analytical sensitivity [32,33]. Indeed, although CTCs offer unique advantages by providing intact, viable tumor cells that enable phenotypic, genomic, transcriptomic, and functional analyses, their extreme rarity in peripheral blood (often 1–10 cells per mL), high inter- and intra-patient heterogeneity, and lack of standardized enrichment platforms remain major technical barriers. Even if CTC enumeration is an FDA-approved prognostic biomarker in metastatic breast, prostate, and colorectal cancers, their widespread clinical adoption is still limited by pre-analytical variability, false positive signals in inflammatory conditions, and false negatives related to epithelial–mesenchymal transition and antigen loss during capture [34]. Similarly, EVs represent a biologically attractive class of biomarkers due to their remarkable stability in biological fluids and their ability to transport tumor-derived DNA, RNA, and proteins involved in immune modulation, angiogenesis, and metastatic colonization. However, strong heterogeneity among vesicle subtypes, contamination with non-EV particles, and the absence of universally accepted isolation and characterization pipelines severely limit inter-study reproducibility and hinder regulatory-grade validation [35]. With regard to ctRNA, available evidence indicates that circulating RNA species can provide complementary information to ctDNA by capturing gene expression programs, splicing events, and immune-related signaling. ctRNA shows particular promise in scenarios characterized by low ctDNA shedding and for tissue-of-origin attribution. Nevertheless, its intrinsic instability, very low abundance, susceptibility to RNase degradation, and lack of standardized extraction and normalization protocols currently preclude wide clinical deployment [33,36].

Taken together, the uncertainty expressed by experts regarding ctRNA, CTCs, and EVs support a hierarchical view of LB biomarkers, in which ctDNA currently represents the only analyte fully ready for “prime-time” clinical implementation, while ctRNA, CTCs, and EVs should be considered promising complementary tools whose routine adoption will depend on the results of large, prospective, standardized validation studies.

There was some variability in expert opinions regarding clinically useful information provided by the diverse biofluids (item 13). Peripheral blood was unanimously accepted as the gold standard, with the pleural fluid being widely endorsed (82.6 %). In contrast, CSF together with ascitic fluid and urine were viewed with more skepticism, mostly considering that the literature evidence was based on case reports or rare prospective cohorts [37,38]. Notably, pleural fluid is a promising source for LB for its richness in tumor-derived products, particularly facilitating both the morphological diagnosis and molecular

characterization of metastatic pleural effusions. However, pleural fluid sampling is much more invasive than a simple blood draw, limiting its routine use according to specific clinical settings and patient performance status [39]. Accordingly, although emerging as a promising method for detecting and monitoring neoplastic processes of the central nervous system (such as gliomas or leptomeningeal carcinomatosis), the implementation of CSF-based liquid biopsies in clinical practice remains in its early stages with CSF collection, primarily performed via lumbar puncture, being inherently invasive while posing procedural risks and potentially limiting the feasibility of serial sampling [38]. Dealing with urine samples, challenges include limited utility in non-urological cancers due to filtration barriers in the kidney, as well as variability in cfDNA concentrations caused by factors like hydration, renal function, and medications [40]. This is consistent with the TIGER-X phase I/II study, where matched urine and plasma samples yielded a weak positive percentage agreement cut-off lower than 80 %. Urine-based LB further suffers from low specificity, with cfDNA levels increasing in non-malignant conditions such as inflammation, pregnancy, and autoimmune diseases, which raises the risk of false positives and overdiagnosis, especially in pre-symptomatic stages [41]. Further, the heterogeneous and often low concentration of tumor-derived DNA in CSF, urine, and ascitic fluid might necessitate the use of highly sensitive detection methodologies that, by detecting low-frequency and sub-clonal genomic alterations, would introduce challenges in data interpretation, overcomplicating the detection of clinically relevant alterations from background noise or sequencing artifacts. Another area of divided opinion regarded the cost as a determinant aspect for selecting the analytical methodology, where only 60 % of the panelists agreed that this could prove crucial (item 14). In the CGP era, this response likely reflected the tension between the push for cost-effective diagnostics and the clinical imperative for high-sensitive assays. While plasma NGS offers a significant advantage over single-plex methods by detecting not only known mutations but also rare or previously unknown variants, initial limitations included higher costs, longer processing times, and the need for advanced bioinformatics. Recent technological advancements have largely addressed these issues by reducing TAT and making costs comparable to or even lower than sequential or hotspot panel testing [42]. In addition to commercially available assays, academic institutions have also developed in-house tests for local assessments [43,44]. As the list of guideline-recommended genes for advanced NSCLC expands, the use of broader NGS panels is expected to increase both in tissue and plasma, proving to be more cost-effective than sequential testing. However, health-economic evidence regarding LB in other cancer types and clinical contexts remains limited [45]. As previously discussed [46], available data largely support the cost-effectiveness of cfDNA-based testing, particularly in NSCLC. Systematic evaluations have shown that LB generally falls below commonly accepted willingness-to-pay (WTP) thresholds and is often associated with a modest budget impact or even cost savings when appropriately integrated into diagnostic pathways [45]. Importantly, these analyses consistently indicate that LB is most cost-effective when used in combination with standard diagnostic strategies rather than as an exclusive plasma-only approach. A paradigmatic example is provided by the German analysis by Englmeier et al. [47], which demonstrated that adding ctDNA-based LB to standard diagnostic workflows in metastatic NSCLC resulted in only a minimal increase in average costs (+€394 per patient), while yielding a favorable incremental cost-effectiveness ratio (ICER) of approximately €53,900 per quality-adjusted life year (QALY) gained. Notably, in *EGFR*-mutated patients—where plasma testing shows particularly high diagnostic accuracy—the ICER became negative, indicating that LB was not only effective but also cost-saving. Conversely, short-term economic models focused exclusively on direct testing costs have sometimes favored tissue-first strategies. For instance, Yang et al. [48] reported that tissue-first NGS minimized immediate financial losses compared with plasma-first approaches; however, such analyses did not account for downstream consequences, including delays in treatment initiation, prolonged exposure to ineffective therapies, or long-term survival benefits. In contrast, longer-horizon models, such as that by Liu et al. [49], demonstrated that a sequential tissue–plasma NGS strategy generated additional QALYs with a favorable ICER and a positive net monetary benefit over a 20-year time horizon, supporting its economic sustainability in newly diagnosed advanced NSCLC. Taken together, these data help explain why experts may legitimately diverge in their weighting of cost as a primary determinant for LB implementation. While NSCLC represents a setting in which the clinical and economic value of cfDNA testing is now relatively robust, evidence remains far more limited in other tumor types and clinical scenarios. Consequently, the cost-effectiveness of LB remains context-dependent, being influenced by local reimbursement policies, access to targeted therapies, laboratory organization, and patient case-mix.

The debate over the best approach for integrating LB into tissue biopsy was also notable (item 15). Both the complementary and the sequential tissue-plasma approach received equal support (65 %). In contrast, the plasma-first approach garnered less consensus with less than 40 % agreement among panelists, although a recently published retrospective study suggested that LB through CGP could significantly reduce the time to initiate therapy (21 vs. 35 days; $P < .001$) [50]. It is conceivable that this reflected a lack of robust evidence from prospective randomized clinical trials directly comparing complementary and sequential approaches, leaving this debate open to further investigation.

The time interval between liquid and tissue biopsy sampling was identified as a significant confounding factor, with more than 70 % of experts agreeing that prolonged gaps between sample collections can lead to discrepancies in LB results (item 16). This issue is particularly relevant in the context of tumor spatial and temporal heterogeneity [51]. This is consistent with an individual patient data meta-analysis on ctDNA in breast cancer, confirming that longer intervals between tissue and plasma sampling correlate with lacking concordance between the two methods [52].

4.3. Post-analytical phase

The study results demonstrated a high consensus on performing LB at the time of disease recurrence or progression (91.3 %, item 17). This is well-aligned with its increasing use in the current clinical practice, especially and not only in those patients associated with multiple mechanisms of acquired resistance to molecularly targeted agents [42].

Consensus was also achieved regarding the LB role in therapeutic monitoring, supporting its growing role in assessing tumor response to treatment (86.9 %, item 18). However, only nearly 74 % of experts agreed that tumor fraction (TF), defined as the proportion of total cfDNA in a tumor-derived ctDNA sample from a CGP assay, is relevant for therapeutic monitoring, indicating some uncertainty regarding its clinical application (item 19). Recent studies, including a large-scale analysis of 3854 patients across multiple cancer types, have demonstrated an increased positive percent agreement and negative predictive value between liquid and tissue samples for driver alterations in samples with TF $\geq$ 1 %, thus increasing confidence in negative LB results with high ctDNA TF. For samples with TF <1 %, the reduced concordance between liquid and tissue samples should imply confirmatory tissue testing [53]. Accordingly, a consensus was reached on TF as a potential tool for assessing both minimal residual disease and the diagnostic accuracy of LB biomarkers, mostly considering that ctDNA TF calling from specific panels would avoid confounding signals from germline and CHIP variants [54,55].

Finally, the study showed unanimous agreement on the necessity for standardized reporting, including details on biological sample type, use of analytical techniques, and relevant pre-analytical/analytical variables (item 20). This is crucial for ensuring cross-study comparability and clinical reproducibility. In addition to reporting, implementing a standardized LB request report could improve communication between

clinicians and patients, ensuring that the test outcome meets expectations and can efficiently answer the clinical question. This would facilitate a more targeted management of the oncological scenario.

5. Conclusions

This Delphi expert study on the use of LB in patients with solid tumors achieved a strong consensus on sample handling, biomarker prioritization, and clinical applications. While these agreements reflect significant advancements, harmonizing parameters across laboratories and clinical settings remains challenging with significant uncertainty involving areas such as the impact of cost and the clinical reliability of emerging analytes and biofluids. While proving clarity, variability among expert opinions highlighted the need for the standardization of assessment methods to ensure analytical and clinical utility.

Importantly, the consensus achieved in this Italian Delphi study appears largely aligned with current international regulatory frameworks and major European guidelines. The strong prioritization of plasma cfDNA/ctDNA as the only biomarker currently ready for routine clinical implementation is fully consistent with international recommendations, as well as with the present FDA-approved companion diagnostics landscape. Conversely, the cautious positioning of ctRNA, CTCs, and EVs as promising but not yet prime-time biomarkers reflects the same prudence endorsed by international societies, where these analytes remain confined to research or exploratory multi-omics settings [2,5]. This alignment supports the external validity of our conclusions, suggesting that such uncertainties are not country-specific but rather reflect shared global gaps in standardization, analytical validation, and regulatory maturity. In this context, the present consensus does not propose alternative paradigms, but rather reinforces the need for harmonized, evidence-based implementation of LB across different healthcare systems.

In some cases, the framing of the questions might have had an impact on the degree of agreement. For instance, rather than agreement on particular procedures, the broad consensus regarding the lack of guidelines for blood volume in cfDNA isolation probably indicates a common understanding of a technological deficiency. This emphasizes how the Delphi technique maps uncertainty rather than offering therapeutic advice. More robust protocols based on prospective evidence must be established to move the LB world toward broader clinical adoption, ensuring accurate, reproducible, and actionable results.

Ethical approval

[Not applicable]

Author contributions

VG: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration. **UM**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **GD**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **GMI**: Software, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **TDBR**: Software, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **RB**: Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **GDB**: Investigation, Writing – Original Draft, Writing – Review & Editing. **EDC**: Writing – Original Draft, Writing – Review & Editing, Visualization. **MC**: Writing – Original Draft, Writing – Review & Editing, Visualization. **RD**: Writing – Original Draft, Writing – Review & Editing, Visualization. **MDR**: Writing – Original Draft, Writing – Review & Editing, Visualization. **MF**: Writing – Original Draft, Writing – Review & Editing, Visualization. **GG**: Writing – Original Draft, Writing – Review & Editing, Visualization. **SG**: Writing – Original Draft, Writing – Review & Editing, Visualization. **LI**: Writing – Original Draft, Writing – Review & Editing, Visualization. **AM**: Writing – Original Draft, Writing – Review & Editing, Visualization. **NN**: Writing – Original Draft, Writing – Review & Editing, Visualization. **CP**: Writing – Original Draft, Writing – Review & Editing, Visualization. **DS**: Writing – Original Draft, Writing – Review & Editing, Visualization. **ASB**: Writing – Original Draft, Writing – Review & Editing, Visualization. **NS**: Writing – Original Draft, Writing – Review & Editing, Visualization. **PT**: Writing – Original Draft, Writing – Review & Editing, Visualization. **GT**: Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **MDM**: Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **FP**: Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **AG**: Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **CR**: Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **AR**: Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2025.100453.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information file.

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