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Next-Generation Sequencing for Identifying Actionable Mutations in Non-Small Cell Lung Cancer: A Protocol for a Systematic Review and Meta-Analysis

Secuenciación de nueva generación para identificar mutaciones accionables en el cáncer de pulmón de células no pequeñas: un protocolo para revisión sistemática y metanálisis

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ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) is the most prevalent phenotype of pulmonary neoplasm. This study aims to review the efficacy of Next-generation sequencing (NGS) compared to other techniques for identifying actionable mutations in tissue tumor

samples or circulating tumor DNA (ctDNA) blood samples from NSCLC patients eligible for systemic therapy. **Methods and Analysis**: We will identify eligible diagnostic test studies using a predefined search strategy in Medline and PubMed. We will extract individual study results to calculate the diagnostic performance values of the test, such as sensitivity, specificity, and predictive value. NGS's diagnostic performance will be compared with techniques recommended by NCCN and ESMO guidelines. The review and meta-analysis will adhere to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA-P) guidelines. **Discussion:** Identifying actionable mutations in tumor samples is crucial for managing advanced NSCLC. NGS offers benefits by detecting multiple genetic alterations simultaneously, though its high cost and time requirements hinder widespread use. Our systematic review will evaluate NGS's diagnostic accuracy and clinical benefits compared to other techniques, considering factors like test turnaround time. To address challenges such as the difficulty in obtaining detailed patient data and interpreting mutations of uncertain clinical significance, we will seek evidence from clinical studies and guidelines to provide comprehensive insights.

Keywords: carcinoma non-small-cell lung; next-generation sequencing (NGS); diagnostic accuracy; actionable mutations; non-small cell lung cancer (NSCLC); metanalysis and systematic review

Resumen

Introducción: El cáncer de pulmón de células no pequeñas (NSCLC) es el fenotipo más prevalente de neoplasia pulmonar. Este estudio tiene como objetivo revisar la eficacia de la secuenciación de nueva generación (NGS), en comparación con otras técnicas para identificar mutaciones accionables en muestras de tejido tumoral o muestras de sangre con

ADN tumoral circulante (ctDNA) de pacientes con NSCLC elegibles para terapia sistémica. **Métodos y análisis:** Se identificaron estudios de prueba diagnóstica elegibles utilizando una estrategia de búsqueda predefinida en Medline y PubMed. Los resultados de los estudios individuales se extrajeron para calcular los valores de rendimiento diagnóstico de la prueba, como sensibilidad, especificidad y valor predictivo. El rendimiento diagnóstico de la NGS se comparó con las técnicas recomendadas por las guías de NCCN y ESMO. La revisión y el metanálisis se adherirán a las guías de los Ítems de Reporte Preferidos para Revisiones Sistemáticas y Meta-análisis (PRISMA-P). **Discusión:** Identificar mutaciones accionables en muestras tumorales es crucial para el manejo del NSCLC avanzado. La NGS ofrece beneficios al detectar múltiples alteraciones genéticas simultáneamente, aunque su alto costo y los requisitos de tiempo dificultan su uso generalizado. La revisión sistemática evaluará la precisión diagnóstica y los beneficios clínicos de la NGS en comparación con otras técnicas, considerando factores como el tiempo de respuesta de la prueba. Para abordar desafíos como la dificultad de obtener datos detallados de los pacientes e interpretar mutaciones de significado clínico incierto, se buscará evidencia de estudios clínicos y guías para proporcionar información integral.

Palabras clave: carcinoma pulmonar no microcítico; secuenciación de nueva generación (NGS); precisión diagnóstica; mutaciones procesables; cáncer pulmonar no microcítico (CPNM); metanálisis y revisión sistemática.

Introduction

Lung cancer is one of the leading contributors to cancer-related deaths worldwide and a high disease burden, resulting in approximately thirty-one million years lost due to premature death (1–3). However, there are also other relevant aspects, such as its economic impact,

affecting governments, insurers, caregivers, and society. The direct costs of lung cancer care have been estimated to reach $45,364.48 USD PPP, and these costs have been observed to increase as the disease progresses, particularly high in advanced stages (1,4,5).

The identification of various actionable mutations in all patients with advanced NSCLC is recommended (6–8), allowing for the provision of targeted therapies with significant clinical benefits (9–11). The assessment of the tumor profile is conducted on tissue samples collected through biopsy, the same ones used for the diagnosis and determination of the histological subtype of NSCLC. Therefore, the priority is to preserve as much tissue as possible to ensure sufficient material for future studies. When the collected material is exhausted, the number of subsequent studies that can be performed may be limited. This requires collecting new material for additional studies, increasing exposure to procedure-related risks, and delaying treatment times. To maximize the use of available samples, techniques that can identify the greatest number of alterations and ensure the rational use of resources should be preferred (6).

NGS DNA sequencing is the recommended option for the simultaneous evaluation of mutations and to facilitate the early initiation of personalized therapy or clinical trials (6,12); however, the use of such tools has been limited by the high cost of tests and the time required for genomic diagnostic analyses. Recent advances in the implementation of this technology have made genomic studies more accessible for clinical use.

This systematic review will review the diagnostic accuracy of NGS in comparison to other techniques for identifying actionable mutations in tissue tumor samples or ctDNA blood

samples when the tissue is not available of patients diagnosed with NSCLC who are eligible for systemic therapy of treatment. Also, this review will let us evaluate important variables such as the turnaround time of the test. By examining these variables alongside diagnostic accuracy, we aim to provide comprehensive insights into the utility of NGS in guiding treatment decisions for patients with advanced NSCLC.

Methods

Aim and objectives

This systematic review aims to synthesize and report the existing knowledge regarding the diagnostic accuracy of NGS compared to other techniques for identifying actionable mutations in tissue tumor samples or ctDNA blood samples, particularly when the tissue is not feasible for patients diagnosed with NSCLC who are eligible for systemic therapy of treatment.

To achieve this goal we will systematically search, select, and synthesize the available information from relevant studies. By comprehensively examining and analyzing the existing literature, we aim to provide a clear understanding of the diagnostic accuracy of NGS and its comparative effectiveness in identifying actionable mutations in NSCLC patients. We will extract individual study results to calculate the diagnostic performance values of the test, such as sensitivity, specificity, and predictive value.

Our specific objectives are:

1. Synthesize the existing evidence from studies assessing the diagnostic performance of NGS in tissue or liquid biopsy for the detection of actionable mutations in advanced NSCLC patients.
2. Synthesize the existing evidence on the turnaround time of the evaluated diagnostic tests whenever possible.
3. Evaluate whether there is a difference in diagnostic performance between commercially available NGS platforms and in-house NGS testing in tissue or liquid biopsy.

Study design

We designed the protocol in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P). Figure 1

Figure 1. Methods according to the PRISMA protocol (13)

Search strategy

The search for studies will be conducted by developing search strategies for each of the following databases: MEDLINE-PubMed, Embase. The reference lists of the identified studies will be reviewed to obtain additional studies. A proposed list of terms for the different databases is attached (Table 1).

PICO

- **Population**: Patients aged 18 and above who have been diagnosed with advanced stage NSCLC and require actionable mutation studies.

- **Intervention**: Identification of actionable mutations utilizing NGS technique on either tumor tissue samples or ctDNA in blood. Both in-house and commercial assay will be considered and specific details regarding the tests utilized will be provided.

- **Comparator**: All techniques that allow the identification of different actionable mutations recommended by the NCCN and ESMO guidelines to define targeted treatment in patients with NSCLC will be considered. Among the techniques for the identification of targeted therapies are immunohistochemistry, in situ hybridization techniques, and PCR-based methods. Additionally, comparisons between NGS applied to tissue samples versus liquid biopsy, as well as commercial NGS panels versus in-house assays, will also be analyzed to assess differences in diagnostic performance.

- **Outcomes**: The analytic and diagnostic sensitivity, specificity, and likelihood ratio of each technique for detecting actionable mutations. This will involve comparing the number of patients diagnosed with each technique and comparing it with the number of patients diagnosed through NGS in tissue or blood, as appropriate. Additionally, when available, we include the turnaround time for each technique, and make relevant comparisons.

Eligibility criteria

We will include studies that involve patients diagnosed with non-small cell lung cancer and report outcomes related to the specified measures of interest. There will be no restrictions

based on language or publication date. Eligible studies will encompass all evaluations of sensitivity and specificity involving NGS on tissue biopsy or liquid biopsy for targeted therapy in NSCLC.

Inclusion criteria are as follows:

- All patients with NSCLC must have a cytological or histopathological diagnosis.
- Tissue or blood biopsies should be paired within the same patient.
- The targeted mutation status should be detected by the standard test in tissue tumor for each mutation.
- The type of study conducted must be specified for both the tumor sample and plasma.
- Sufficiently reported data should be available to construct the diagnostic 2 × 2 table.

Exclusion criteria are as follows:

- Studies involving cell lines or artificial samples were subsequently excluded.
- If they presented data in a manner that did not permit proper extraction.
- Patients intending to undergo exclusive, non-oncology-specific palliative care.
- Different histological pattern

We will include diagnostic test studies to determine the sensitivity and specificity of each test.

Quality Assessment

For the evaluation of the quality of the selected studies, the investigator (AG and NT) will use the QUADAS-2 tool (14) developed by the University of Bristol to assess the risk of bias in studies that evaluate diagnostic accuracy through 4 domains: patient selection, index test, reference standard, and flow and timing. The evaluation will be conducted in 4 phases, and each domain will be assigned a risk score of low, high, or unclear bias.

Additionally, the QUADAS-C tool will be used to assess the risk of bias in comparative precision studies. The results of both tools will be synthesized in a bar graph to provide an overview of the quality assessment. In case of discrepancies, the researchers will convene to reach an agreement.

Data Management

The search results will be uploaded to Mendeley, and the Rayyan tool will be used to organize the studies, remove duplicate articles, and facilitate discussions regarding the inclusion/exclusion of each article. Two members of the research team (NT and AG) will independently select publications based on their title and abstract. In cases of discrepancies, a third member will review the articles to arrive at a final selection.

For the selected articles, the full text will be read to verify eligibility criteria, and the reasons for exclusion will be recorded. Data from the included studies will be extracted using a structured form and presented in tables by the review authors (NT and AG). In case of insufficient data in a study, the authors of the respective study will be contacted, and a data collection form will be sent to them for completion of the required information.

Analysis of the results

The studies that evaluate the performance of different techniques for assessing actionable mutations will be included and evaluated together. The selected studies will be presented in evidence tables and will be extracted by two researchers (NT and AG) and analyzed together with the rest of the participants. Preference will be given to the available data under the intention-to-treat principle. When feasible, missing standard deviations will be calculated from other statistics such as standard errors, confidence intervals, or p-values. The data extracted from the selected articles will be recorded in a manner that allows for the generation of a 2x2 table to calculate relevant effectiveness outcomes.

When the studies included in the analysis are comparable to each other, the results will be combined using two types of models: fixed-effects and random effects. The choice between these two reporting models will consider the assessment of heterogeneity present in the studies included in the analysis. If heterogeneity is low, the fixed-effects model may be used. The level of heterogeneity among the studies will be quantified using the I2 index. For the purpose of the study, a value of zero is considered as true homogeneity, values of 25 represent mild heterogeneity, 50 moderate heterogeneity, and 75 high heterogeneities.

To analyze differences in the percentage of valid results (tissue vs. tissue and tissue vs. liquid biopsy) according to the sample type, a hypothesis test for the difference in proportions will be performed, considering a p-value <0.05 as statistically significant. To analyze differences in turnaround time between the standard and the comparator, a t-test for the difference in means will be conducted, with a p-value <0.05 considered statistically significant.

All analyses will be conducted using Stata 17®.

Subgroup Analysis

A subgroup analysis is planned to be conducted but not limited based on the following criteria:

- Tumor stage.
- Type of collected sample (tumor tissue vs. cytology vs. Liquid biopsy).
- Sample collection method.
- Genes analyzed.

The aim is to determine whether the data collected from the different subgroups are significantly different by inspecting the overlap of confidence intervals and using the subgroup differences test.

Meta-analysis

A meta-analysis will be conducted to analyze the sensitivity and specificity in the detection of actionable mutations that enable targeted therapy implementation in patients with NSCLC using tissue biopsy evaluated by NGS compared to standard techniques performed on tumor tissue. The goal is to determine the best technique for detecting actionable mutations and enabling targeted therapy implementation. The statistical analysis will be performed using the "metadta" command in STATA 17® for each mutation, evaluation test, and reference standard. This function will allow us to conduct a meta-analysis of diagnostic tests, combining the sensitivity and specificity reported in the included studies. Additionally, forest

plots will be generated to facilitate the visualization of individual and combined study estimates.

Discussion

This systematic review aims to address these limitations by evaluating the diagnostic accuracy of NGS compared to other techniques to identify actionable mutations in tissue tumor samples or ctDNA blood samples. By summarizing the available evidence, we aim to provide comprehensive insights into the clinical benefit of NGS for the management of NSCLC. Key considerations in our review include not only diagnostic accuracy but also important variables such as the turnaround time of the test. This holistic approach aims to inform clinicians and policymakers about the clinical applicability and practical considerations associated with implementing NGS in routine practice for patients with advanced NSCLC.

We propose a thorough and meaningful review; however, we can find some limitations for the conduction of the study. To obtain detail data of clinical characteristics of the patients might be challenging hindering potential analysis regarding the relationship between clinical characteristics and mutation outcomes. In addition, the identification of mutations with uncertain clinical significance presents another challenge in interpreting our review results. To address these issues, we will seek available evidence from clinical studies to determine the actual value of these mutations, particularly when not specified in the NCCN or ESMO guidelines.

Conclusion

This systematic review has the potential to significantly contribute to optimizing diagnostic strategies and personalized treatment approaches for patients with advanced NSCLC. By elucidating the diagnostic accuracy and practical considerations of NGS, we aim to facilitate informed decision-making and improve clinical outcomes for this patient population.

Conflict of interests

None.

References

1. Yousefi M, Jalilian H, Heydari S, Seyednejad F, Mir N. Cost of lung cancer: a systematic review. Value Health Reg Issues. 2023 Jan 1;33:17–26. https://doi.org/10.1016/j.vhri.2022.07.007
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May 4;74(3):229–63. https://doi.org/10.3322/caac.21834
3. Bray F, Colombet M, Aitken J, Bardot A, Eser S, Galceran J, et al. Cancer incidence in five continents, vol. XII (IARC CancerBase No. 19) [Internet]. Lyon; 2023 [cited 2024 May 31]. Available from: https://ci5.iarc.who.int
4. Wood R, Taylor-Stokes G. Cost burden associated with advanced non-small cell lung cancer in Europe and influence of disease stage. BMC Cancer. 2019 Dec 8;19(1):214. https://doi.org/10.1186/s12885-019-5428-4
5. Ezeife DA, Morganstein BJ, Lau S, Law JH, Le LW, Bredle J, et al. financial burden among patients with lung cancer in a publically funded health care system. Clin Lung

Cancer [Internet]. 2019 Jul 1;20(4):231–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525730418303346

6. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2023 Apr;34(4):339–57.
7. Kristina Gregory N, Miranda Hughes O, Aisner DL, Akerley W, Bauman JR, Bruno DS, et al. NCCN guidelines version 3.2023 non-small cell lung cancer continue NCCN Guidelines Panel Disclosures [internet]. 2023. Available from: https://www.nccn.org/home/member-
8. Grodzka A, Knopik-Skrocka A, Kowalska K, Kurzawa P, Krzyzaniak M, Stencel K, et al. Molecular alterations of driver genes in non-small cell lung cancer: from diagnostics to targeted therapy. EXCLI J [Internet]. 2023 Jan 2;22:415–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/37346803
9. Nesline MK, Knight T, Colman S, Patel K. Economic burden of checkpoint inhibitor immunotherapy for the treatment of non–small cell lung cancer in US clinical practice. Clin Ther [Internet]. 2020 Sep 1;42(9):1682-1698.e7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149291820303283
10. Haslem DS, Van Norman SB, Fulde G, Knighton AJ, Belnap T, Butler AM, et al. A Retrospective analysis of precision medicine outcomes in patients with advanced cancer reveals improved progression-free survival without increased health care costs. J Oncol Pract. 2017 Feb 1;13(2):e108–19. https://doi.org/10.1200/JOP.2016.011486
11. Villaruz LC, Socinski MA, Weiss J. Guidance for clinicians and patients with non-small cell lung cancer in the time of precision medicine. Front Oncol. 2023 Feb 1;13. https://doi.org/10.3389/fonc.2023.1124167

12. Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Annals of Oncology [Internet]. 2020 Nov 1;31(11):1491–505. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0923753420399713
13. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009 Dec 4;339(jul21 1):b2700–b2700.
14. Whiting PF. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011 Oct 18;155(8):529.