POSTER 2925

An Innovative Evidence-Based Laboratory Medicine (EBLM) Test to Help Doctors in the Assessment of Lung Cancer

Authors: Calleja S. J.[1], Roca A.[2] Affiliations: [1]Kience, Wilmington, Delaware, U.S.; [2]Blueberry Diagnostics, Barcelona, Spain

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BACKGROUND

Introduction

- According to the World Health Organization (WHO), lung cancer (LC) is the leading cause of cancer-related deaths, claiming nearly 1.8 million lives each year[1], and of which the primary risk factor remains tobacco smoking.
- It encompasses two main types: **small cell lung cancer (SCLC)** and **non-small cell lung cancer (NSCLC)**, representing the latter the majority of cases and comprising **adenocarcinoma**, **squamous cell carcinoma (SCC)**, and **large cell carcinoma (LCLC)**.
- The **high incidence and mortality** of this malignancy constitutes a health concern worldwide. Besides, it causes a **profound socioeconomic impact** due to invasive and costly diagnostic procedures.
- The **main risk factors** are: tobacco smoke; exposure to second-hand smoke; exposure to radon gas, exposure to asbestos and other carcinogens; and family history of LC, among others.

Key Facts about Lung Cancer

- Lung cancer (LC)[2]:
  - Incidence: 2.5 M people, 12.4% of total new cases.
  - Mortality: 1.8 M people, 18.7% of total cancer deaths.
- Small cell lung cancer (SCLC):
  - 35 K people, 15% of U.S. total LC patients.
- Non-small cell lung cancer (NSCLC):
  - 199 K people, 85% of U.S. total LC patients.

Risk Factors

- Smoking[3]
- Exposure to second-hand smoke
- Exposure to radon gas
- Exposure to asbestos and other carcinogens
- Inhaled chemicals or minerals
- Radiation therapy to the lungs
- Family history of LC

Prognosis and 5-Year Survival Rate (5-YSR)

In LC, like many other cancers, **mortality is strongly associated with tumor stage at detection**. In this way, the 5-YSR for localized cancers is 55.60%. Besides, the 5-YSR for those cases that have spread to near organs drops to 28.90%. However, **if LC has metastasized to other parts of the body, the 5-YSR is only the 4.50%**. Remaining 7.50% corresponds to unknown 5-YSR.

Current Tests to Diagnose Lung Cancer

They are often invasive, expensive and may give many false positives (FP):

- X-Ray: very low accuracy (high FP rate because scars, cysts or benign lung nodules) and radiation exposure.
- Lung-RADS for CT-scan: expensive and has a high FP rate, which may lead to unnecessary invasive biopsies.
- Bronchoscopy: high invasive and expensive. May have several mechanical complications.
- Lung biopsy: highly invasive and expensive. Risks are not uncommon (pneumothorax may be a usual complication and frequently requires a chest tube). Besides, complications were more common in patients aged 60 to 69, smokers, and patients with chronic obstructive pulmonary disease (COPD).

Upcoming Non-Invasive Lung Cancer Diagnosis

In recent years, tumor markers (TM) have been suggested to help in cancer diagnosis. However, TM can be also increased due to non-malignant diseases, mainly in the liver and kidneys, since TM are catabolized in the liver and excreted through the kidneys, so any disease that affects these two organs will affect TM serum levels[4]. In this way, the **Spanish Society of Laboratory Medicine (SEQC^{ML})** developed in 1994 a set of rules known as the **Barcelona Criteria** to minimize FP as much as possible:

1. Evaluate **serum TM concentration**.
2. Exclude the main source of FP results by **discarding benign pathologies (Table 1)**.
3. When moderate results of TM, **perform follow-up**.
4. Eliminate high results by **avoiding technical interferences**.

TM	Asc.	BPH	Cho.	CLD	Mod. Jau.	Acu. Jau.	Met.	Pan.	Pem./Pso.	Per./Ple. Eff.	Pro.	CKD
AFP	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	Moderate
b-hCG												
CA 15.3	High	High	High	High	High	High		High	High	High	High	High
CA 19.9	Moderate	Moderate	Very High	Moderate	Very High	Very High		Very High	Moderate	Moderate	Moderate	Moderate
CA 72.4	High	High	High	Very High	High	High		High	High	High	High	Very High
CA 125	Very High	High		High	High	Very High	High		High	Very High	High	High
CEA	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	Very High
CYFRA 21-1	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	High
HE4	Moderate	Mild		High	High	High			Mild	Mild	Mild	N/A
NSE	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate		Moderate	Moderate	Moderate	Moderate	High
ProGRP	High	High	High	High	High	High		High	High	High	High	Very High
PSA	Moderate	Very High	Moderate	Moderate	Moderate	Moderate		Moderate	Moderate	Moderate	Very High	Moderate
SCC	Moderate	High	Moderate	Moderate	Moderate	Moderate		Moderate	Very High	N/A	High	N/A
S100	Mild	Mild	Mild	Mild	Mild	Mild		Mild	Mild	Mild	Mild	N/A

Table 1. Main common non-malignant causes of high serum levels of the major TM that are well-known sources of the most frequent false positives (FP). Mild: up to 2 times upper normality level (UNL). Moderate: between 2 and 5 times UNL. High: between 5 and 10 times UNL. Very High: more than 10 times UNL. N/A: a very high increase in TM, similar to levels found in cancer patients, that should not be used in these patients. TM: Tumor Marker; Asc.: Ascites; BPH: Benign Prostate Hyperplasia; Cho.: Cholestasis; CLD: Chronic Liver Disease; Mod. Jau.: Moderate Jaundice; Acu. Jau.: Acute Jaundice; Met.: Metrorrhagia; Pan.: Pancreatitis; Pem./Pso.; Pemphigus/Psoriasis; Per./Ple. Eff.: Pericardial/Pleural Effusions; Pro.: Prostatitis; CKD: Chronic Kidney Disease.

OBJECTIVES

- To develop a new study based on a previous one conducted by Molina et al.[5] titled "Assessment of a combined panel of six serum tumor markers for lung cancer", published at the American Journal of Respiratory and Critical Care Medicine (AJRCCM) in 2015. The results of this study were acquired by **Bioprognos** (a biotechnology company based in Barcelona, Spain) from the **Hospital Clinic de Barcelona** (Barcelona, Spain) through a Technology Transfer Agreement signed in 2016.
- To check whether new data not included in the previous study (1,152 new patients), as well as some learnings achieved related to the **Barcelona Criteria** (proposed by the **Spanish Society of Laboratory Medicine (SEQC^{ML})**, published first in 1994 by Molina et al.[6], and reviewed later in 2024 by Trapé et al.[7]), about the correct interpretation of the TM and the elimination of false positives (FP) could improve previous performance. The preceding study gave as a result an algorithm to **help doctors in the assessment of lung cancer (LC) from previous suspicious image findings**, which was based on patient's clinical data (gender, age, smoking habits, and nodule size), as well as serum tumor markers (CA 15.3, CEA, CYFRA 21-1, NSE, ProGRP, and SCC). The new algorithm is based on the same parameters but adding new knowledge from the most recent years.
- To validate whether the resulting algorithm could be used as a **non-invasive (only blood or urine) test for LC diagnosis confirmation from previous suspicious image findings**, and potentially **avoid unnecessary biopsies** from FP results.

METHODS

This new study builds upon the same combination of clinical data and tumor markers (TM) used in the previous 2015 study by Molina et al., but incorporates an additional 1,152 patients (**Table 2** and **Table 3**). The expanded patient cohort was examined under similar conditions to the previous study, specifically within a real-world clinical practice setting. Patients were consecutively referred, mostly by their primary care physicians, to the Lung Cancer Diagnostic Unit at the **Hospital Clínic de Barcelona**, a tertiary university hospital. The goal was to rule out lung cancer (LC) due to various symptoms or signs that prompted referral, such as radiographic nodules, hemoptysis, dyspnea, thoracic pain, persistent cough, constitutional symptoms, pleural effusions, persistent fever, pulmonary emboli, dysphonia, or palpable nodes. The diagnosis of LC was confirmed or excluded using standard clinical procedures such as fiber-optic bronchoscopy, CT and PET scans, fine-needle transthoracic aspiration, endobronchial or esophageal ultrasound, and/or surgical resection, all performed by experienced clinicians following international guidelines.

This research also classifies LC histological types according to the 1999 WHO recommendations[8]. The differential diagnosis between SCLC and NSCLC was based on the morphological characteristics plus a positive CD56 and/or synaptophysin immunohistochemistry of the tumor. LC staging (TNM) was established according to international recommendations[9].

In this way, following the same process as in the study by Molina et al., blood samples were obtained by peripheral venipuncture in all participants before the final diagnosis had been established and any anticancer therapy had been initiated.

Variable	Value	Percentage
Sample size (n)	3,144	100.00
Controls	1,316	41.85
Cases	1,828	58.14
Small cell lung cancer (SCLC)	265	8.42
Non-small cell lung cancer (NSCLC)	1,563	49.71
Adenocarcinoma	758	24.10
Squamous cell carcinoma (SCC)	513	16.31
Unspecified NSCLC	238	7.56
Large cell carcinoma (LCLC)	54	1.71

Table 2. 2015 study sample by controls and cases. Cases are further classified by histological type and subtype. Non-small cell lung cancer (NSCLC) includes adenocarcinoma, squamous cell carcinoma (SCC), unspecified NSCLC, and large cell carcinoma (LCLC). Unspecified NSCLC corresponds to those cases for which it could not be established whether the histologic subtype was adenocarcinoma or SCC. LCLC was included in total NSCLC for statistical purposes.

Variable	Value	Percentage
Sample size (n)	4,296	100.00
Controls	1,614	37.56
Cases	2,682	62.43
Small cell lung cancer (SCLC)	465	10.82
Non-small cell lung cancer (NSCLC)	2,217	51.61
Adenocarcinoma	1,104	25.70
Squamous cell carcinoma (SCC)	740	17.23
Unspecified NSCLC	301	7.01
Large cell carcinoma (LCLC)	72	1.67

Table 3. 2024 study sample by controls and cases. Cases are further classified by histological type and subtype. Non-small cell lung cancer (NSCLC) includes adenocarcinoma, squamous cell carcinoma (SCC), unspecified NSCLC, and large cell carcinoma (LCLC). Unspecified NSCLC corresponds to those cases for which it could not be established whether the histologic subtype was adenocarcinoma or SCC. LCLC was included in total NSCLC for statistical purposes.

Yet, because this was a real-life clinical investigation, some patients were receiving treatment for other common chronic conditions, including chronic obstructive pulmonary disease (COPD), cardiovascular diseases and/or diabetes, among others.

After centrifugation, serum TM were quantified in less than 5 hours from sampling, except for ProGRP and SCC which were quantified in less than 2 days. The serum levels of CA 15.3, CEA, CYFRA 21-1, and NSE were measured with a commercially-available electro-chemiluminiscent assay (Elecsys, ROCHE Diagnostics, Rotkreuz, Switzerland), and those of ProGRP and SCC were measured with an Architect automated assay (ABBOTT Laboratories, Chicago, Illinois, U.S.). According to previously published results[10,11], **the following thresholds were considered as the upper normality level (UNL) of: CA 15.3, 35 U/mL; CEA, 5 ng/mL; CYFRA 21-1, 3.3 ng/mL; NSE, 25 ng/mL; ProGRP, 50 pg/mL; and, SCC, 2 ng/mL.**

Accordingly, any individual TM value above these values was considered abnormal. When these six TM were assessed in combination, we considered abnormal the presence of ≥1 abnormal TM values, as in the 2015 study (**Figure 1** and **Figure 2**).

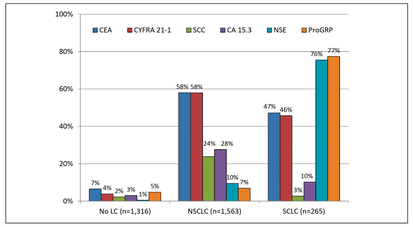

Figure 1. Proportion of participants with abnormal individual TM values was remarkably low among those without LC but significantly higher in those with LC.

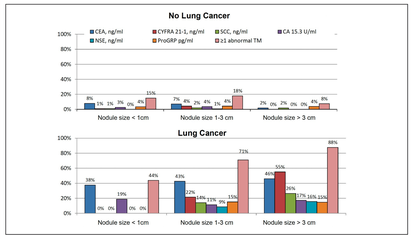

Figure 2. Proportion of participants (with and without LC) with abnormal TM values. Participants were stratified by nodule size category (<1 cm, 1–3 cm and >3 cm). In general, the proportion of participants with abnormal TM levels was higher in those with LC.

Results were measured as n, proportion, median and interquartile range [IQR] as appropriate. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using standard formulas. TM levels were compared using non-parametric (Wilcoxon, Mann–Whitney U, Kruskal–Wallis) or parametric tests (Student's t-test), as appropriate. Proportions between groups were compared using the chi-square (X^2) test. Receiver operator characteristic (ROC) curves were constructed using the DeLong model[12]. A p value <0.05 was considered statistically significant.

We computed the whole data with an improved version of our **Multi-Biomarker Disease Activity Algorithm (MBDAA)** used in the previous study.

Besides, we also included a couple of new self-developed machine learning algorithms, such as **Evidence-Based Laboratory Medicine Algorithm (EBLMA)** and **Artificial Intelligence Recursive Algorithm (AIRA)**, to improve both sensitivity and specificity. EBLMA is the algorithm that includes the **Barcelona Criteria** basis already updated with more knowledge regarding the correct interpretation of the TM and the elimination of false positives (FP). This resulted in an **increase of the specificity from 0.82 to 0.96**. AIRA is an algorithm that computes in a recursive way all the values from 0 to 100% for both sensitivity and specificity to find the optimal cutoff values for low, moderate (moderate-low and moderate-high), and high-risk results.

Finally, we repeated all the steps mentioned above to **determine the histological type (SCLC or NSCLC)** and, in the case of NSCLC, its **histological subtype (adenocarcinoma or squamous cell carcinoma)**. In this way, all the previous objectives stated above were achieved (mainly to **help doctors in the correct assessment of lung cancer from previous suspicious image findings**).

RESULTS

As can be seen in **Table 4**, this new approach improved the results obtained in the previous study conducted by Molina et al. in 2015. Also, as shown in **Figure 3**, the area under the receiver operating characteristic (AUROC) curve achieved in 2024 is higher than that of 2015. Thus, it suggests an improvement in diagnostic accuracy due to all the knowledge added in the 2024 study.

In addition to a notable increase in the sample size, the main reason for this improvement is due to the implementation of the learnings obtained during the years 2016–2024 in the operation of the **Barcelona Criteria** of 1994[6] (and their revision of 2024[7]) regarding the elimination of false positives (FP).

As mentioned above, TM serum levels can increase in several non-malignant conditions that mainly affect the liver and the kidneys.

In this sense, the interpolation of the learnings about the impact of **non-alcoholic fatty liver disease (NAFLD)**, **non-alcoholic steatohepatitis (NASH)**, **liver fibrosis** or **liver cirrhosis** on a few tumor markers (mainly CEA and CYFRA 21-1, but also CA 19.9 when jaundice), is a very useful method to discard FP due to liver disease.

In the same way, taking advantage of previous knowledge with the estimated glomerular filtration rate (eGFR) from the **Kidney Disease Improving Global Outcomes (KDIGO) guidelines**[13], as well as the **urine albumin-to-creatinine ratio (ACR)**, elevated serum levels of CEA, CYFRA 21-1, NSE, and ProGRP can be studied in depth since kidney disease affect these TM.

The application of all these learnings translates in an overall increase of the algorithm's performance (**Table 4**, **Figure 3**). Specially, in the values of specificity and the negative predictive value (NPV), which are the most important ones in diagnosis confirmation approaches, because they depend on the FP. So, the reduction in the FP results translates into an increase of the specificity and the NPV (**Table 4**).

In **Figure 4** the performances of each TM to distinguish between SCLC and NSCLC by themselves are shown. NSE and ProGRP stand out above the rest, exhibiting an AUROC of 0.89 and 0.86, respectively. The other TM (CA 15.3, CEA, CYFRA 21-1, and SCC) achieved only an AUROC between 0.35 and 0.46. However, the combination of this six TM panel improves the performances of both NSE and ProGRP by themselves.

Finally, the combination of this six TM panel (always under the umbrella of the Barcelona Criteria postulates to minimize false positives as much as possible), not only allows the classification of the main types of LC (SCLC and NSLCLC), but also allows the classification of the main histological subtypes of NSCLC (adenocarcinoma and squamous cell carcinoma).

Variable	2015	2024	Improvement
Sample Size (n)	3,144	4,296	1,152
Sensitivity	0.88	0.93	+0.05
Specificity	0.82	0.96	+0.14
AUROC	0.89	0.92	+0.03
PPV	0.87	0.95	+0.08
NPV	0.83	0.93	+0.10

Table 4. Comparison of the results obtained in the 2024 and the 2015 studies, where the increase in the sample size as well as new algorithms included show an important improvement in all variables (mainly specificity and NPV).

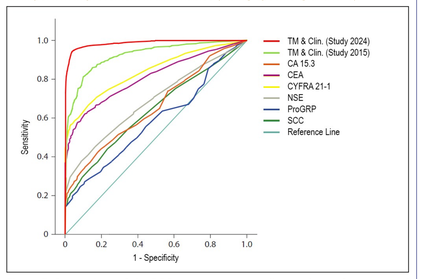

Figure 3. Comparison of the ROC curves corresponding to the performances of the algorithms obtained in 2015 and 2024, as well as all TM alone.

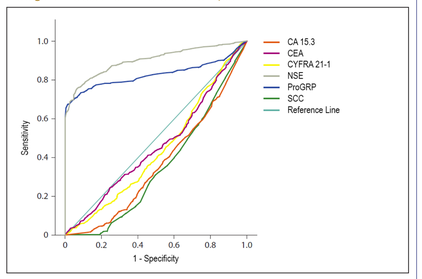

Figure 4. Comparison of the ROC curves corresponding to the performances of all TM alone for discriminating between SCLC and NSCL.

CONCLUSIONS

- As it is already known, **TM are very good indicators of cancer**. However, many causes can also increase their levels.
- The **Barcelona Criteria are the best tool to discard FP results**, but further working is still needed in the understanding of other causes that may increase TM levels. Mainly, these other causes are non-malignant conditions, but also medication and technical interferences (such as hemolyzed, lipemic, or icteric samples, among others). This is also the case of many of the other laboratory clinical determinations. For example, an elevated consumption of biotin[14,15] (vitamin B7) without medical prescription to strengthen hair and nails, produces the well-known "hook effect", which affects the results by saturating the testing antibodies, among others.
- This data suggests that the innovative **non-invasive blood and urine-based biomarker algorithm holds promise in providing timely and accurate diagnosis confirmation of LC**. It also has the **ability to categorize its types (SCLC and NSCLC) and its subtypes (adenocarcinoma or SCC)**, particularly among individuals aged 40 and above with previous image suspicious findings, as the best tool to avoid unnecessary biopsies that patients should undergo (mainly given the current epidemiological state of this malignancy).
- These results advocate for further exploration, prompting our intention to conduct a new clinical study involving 10,000 participants with additional analytes, like serum hepatic enzymes (ALP, ALT, AST, GGT, and LDH), renal function tests (serum creatinine, urine albumin and urine creatinine), total and direct bilirubin, and CA-62 (a promising new tumor marker for epithelial carcinomas), for a better confirmation of LC and determination of its type and subtype (SCLC, NSCLC, adenocarcinoma, SCC, or neuroendocrine tumor), and offer the physicians a **non-invasive test with minimum false positives (FP) and false negatives (FN) results**.
- Although we have been able to achieve great results, **there is still a lot of information to be obtained from blood and urine samples**, and a lot of work to be done to understand it.

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Disclosures: S. J. Calleja is a shareholder of Kience and Blueberry Diagnostics. S. J. Calleja has financial interests in Bioprognos.

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Correspondence: sergio.calleja@kience.com; adria.roca@blueberrydx.com.