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Real-world biomarker testing rate and positivity rate in NSCLC in Spain: Prospective Central Lung Cancer Biomarker Testing Registry (LungPath) from the Spanish Society of Pathology (SEAP)

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ABSTRACT

Aim The aim of this study was to describe the testing rate and frequency of molecular alterations observed in the Lung Cancer Biomarker Testing Registry (LungPath).

Methods A descriptive study of NSCLC biomarker determinations collected from March 2018 to January 2019, from 38 Spanish hospitals, was carried out. Only adenocarcinoma and not otherwise specified histologies were included for epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*) and programmed death ligand-1 (PD-L1) expression. The testing rate and the positivity rate were calculated. Multivariate logistic regression was used to explore the joint relationship between independent explanatory factors and both testing and positivity rates. Two models were adjusted: one with sample type and histology as independent factors, and the other adding the testing rate or the positivity rate of the other biomarkers.

Results 3226 patient samples were analysed, where *EGFR*, *ALK*, *ROS1* and PD-L1 information was collected (a total of 12 904 determinations). Overall, 9118 (71.4%) determinations were finally assessed. *EGFR* (91.4%) and *ALK* (80.1%) were the mainly tested biomarkers. Positivity rates for *EGFR*, *ALK*, *ROS1* and PD-L1 were 13.6%, 3.4%, 2.0% and 49.2%, respectively. Multivariate models showed a lower testing rate for *ALK* in surgical pieces, fine-needle aspiration or other types of samples versus biopsies.

Conclusions Despite the high testing rate in *EGFR* and *ALK* in NSCLC, the real-world evidence obtained from the LungPath demonstrates that *ROS1* and PD-L1 were not determined in a significant portion of patients. LungPath provides crucial information to improve the coverage in molecular testing in lung cancer, to monitor the positivity rate and the introduction of new biomarker testing in clinical practice.

INTRODUCTION

Cost-effectiveness will rule oncology practice in the next decade.¹ A key role in establishing cost-effectiveness in clinical cancer management will be played by biomarkers that help screen, detect and diagnose cancers or predict cancer outcomes and influence treatment choice and monitoring.¹

Among patients with cancer, there exists great variability in tumour biology that determines the response to treatments and clinical outcomes.^{2,3} Differences in the biomarker profiles between tumours can explain much of this variability.³ A putative focused use of expensive cancer treatments on diagnosing patients, together with the fact that biomarkers can provide information about the current status or future risk of a disease, has led to an increased interest in biomarker-driven personalised cancer therapy.^{3–5}

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide.^{6–8} There are two major types of LC: small cell lung cancer and non-small cell lung cancer (NSCLC).⁶ NSCLC represents approximately 85% of all LCs and is classified into several histological subtypes including adenocarcinomas (ACs), squamous cell carcinomas (SCCs), large cell carcinomas and other less frequent subtypes.⁸

The complexity and variability of NSCLC—at least 30%–40% of these cancers have a treatable genomic alteration—and the large number of drugs directed against molecular targets, approved or in clinical development, making it one of the best paradigms of targeted therapies.^{8,9}

When choosing a therapeutic option for a patient with LC, the determination of molecular biomarkers plays a key role. According to standardised recommendations¹⁰ and the last consensus of the Spanish Society of Pathology (or Sociedad Española de Anatomía Patológica (SEAP)), molecular determinations for epidermal growth factor receptor (*EGFR*) and *BRAF* mutations, anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) rearrangements, and programmed death ligand-1 (PD-L1) expression are mandatory to be performed in all patients with advanced NSCLC.¹¹

EGFR mutations are present in 8%–11% of advanced NSCLCs. Since *EGFR*-tyrosine kinase inhibitors (TKI) improve progression-free survival (PFS) and quality of life in comparison with platinum doublet chemotherapy, TKIs as first-line therapy are the standard in the main clinical guidelines.¹² In patients with an *EGFR* T790M mutation, osimertinib has shown a higher PFS than platinum/pemetrexed regimen (median, 10.1 months vs 4.4 months, respectively) after progression on

first-line treatment with a first-generation or second-generation EGFR-TKI.¹³

Rearrangements of the *ALK* gene are found in approximately 2%–5% of advanced NSCLC.^{14–15} Patients with *ALK*-positive NSCLC may develop disease resistance and progression, particularly in the central nervous system, resulting in poor prognosis and a negative impact on patients' quality of life.¹⁶ The clinical importance of *ALK* rearrangement and its molecular diagnostic determination has led to the development of new, highly effective *ALK* inhibitor therapies (crizotinib, ceritinib, alectinib and brigatinib).^{17–18} Specific treatment with these therapies has been an important advance in the management of these patients, resulting in response rates of 40%–93.5% and a PFS of up to 8 months.⁸

In approximately 1% of NSCLCs, *ROS1* gene is translocated.¹¹ For patients with stage IV LC with *ROS1* rearrangement, crizotinib is approved as a first-line or second-line monotherapy.^{19–21}

Overexpression of PD-L1 in advanced NSCLC is predictive of clinical benefit with PD-1/PD-L1 inhibitor drugs. In general, there is a correlation between positive testing for the biomarker and efficacy, although this is a marker with a suboptimal negative predictive value.¹¹

Due to the importance and clinical consequences of determining molecular biomarkers in LC, SEAP has developed the Lung Cancer Biomarker Testing Registry (LungPath), an online non-profit tool that permits the Pathology Departments to register, monitor and trace the most important NSCLC biomarkers results in clinical practice, enabling as well, data correlation at a national and global level.

In brief, the ultimate goal of this registry is to ensure that patients with NSCLC are properly diagnosed, thus facilitating the choice of treatment and ensuring that each patient receives the best possible care. In the short term, the aim of this study was to describe the testing rate and positivity rate observed in the first analysis of LungPath. The possible factors associated with the testing rate and positivity rate will also be explored.

METHODS

A descriptive study of NSCLC biomarker determinations collected from March 2018 to January 2019, from 38 Spanish hospitals through the LungPath, was carried out. The LungPath includes samples of patients with advanced or metastatic NSCLC undergoing biomarker determination. Figure 1 represents the flowchart, where first external samples (from another centre) were excluded to avoid double counting. After, other histologies than AC and non-small cell lung cancer– not otherwise specified (NSCLC-NOS) were excluded. Finally, the frequency of EGFR, *ALK*, *ROS1* and PD-L1 were analysed based on testing results according to recommendations of scientific societies.¹¹

The LungPath compiles information such as date of registration, source of the sample analysed (it is analysed in the centre itself or in an external reference centre), type of sample analysed and histology of the LC, and variables related with the biomarker (type of biomarker, diagnostic techniques, determination and reason for no determination if appropriate, and test result that can be positive, negative or invalid).

Statistical analysis and presentation of results

Frequencies and percentages of all study variables (sample type, histology, assessment and result of biomarkers testing) were obtained.

Main objective analysis

The testing rate of the selected biomarkers (*EGFR/ALK/ROS1/PD-L1*) was calculated and, when not determined, the frequencies of the reasons for non-assessment were described. Then, for each biomarker assessed, the positive rate (positivity based on standardised methods) was obtained excluding invalid results. Invalid cases are considered those samples which, due to different circumstances during the testing process, were not finally available to perform the biomarker test or the test results were not conclusive enough for determining if they were positive or negative.

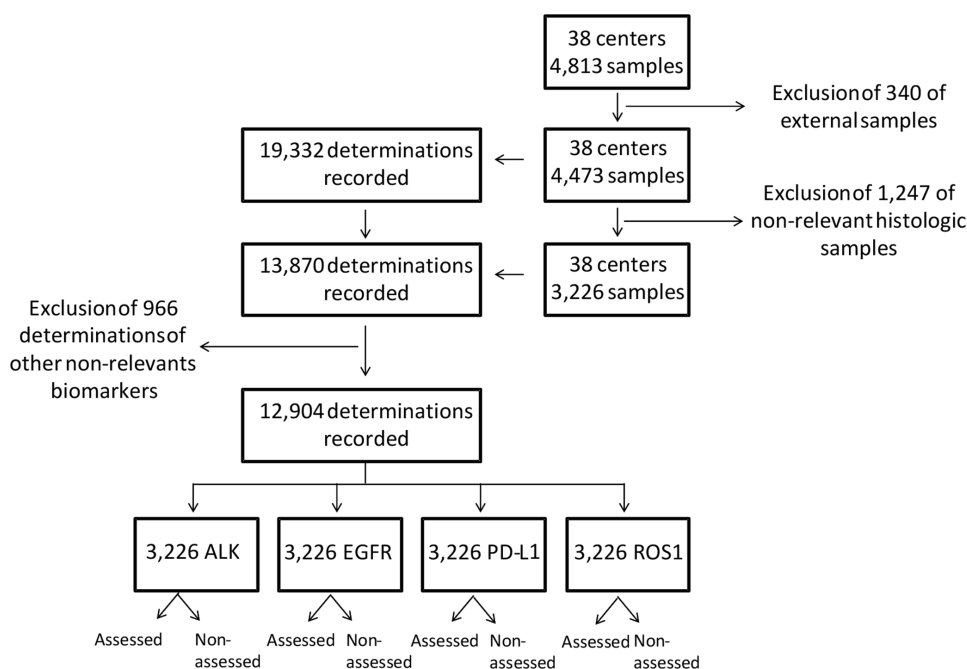


Figure 1 Flowchart followed during the analysis of the LungPath. *ALK*, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; LungPath, Lung Cancer Biomarker Testing Registry; *PD-L1*, programmed death ligand-1; *ROS1* c-ros oncogene 1.

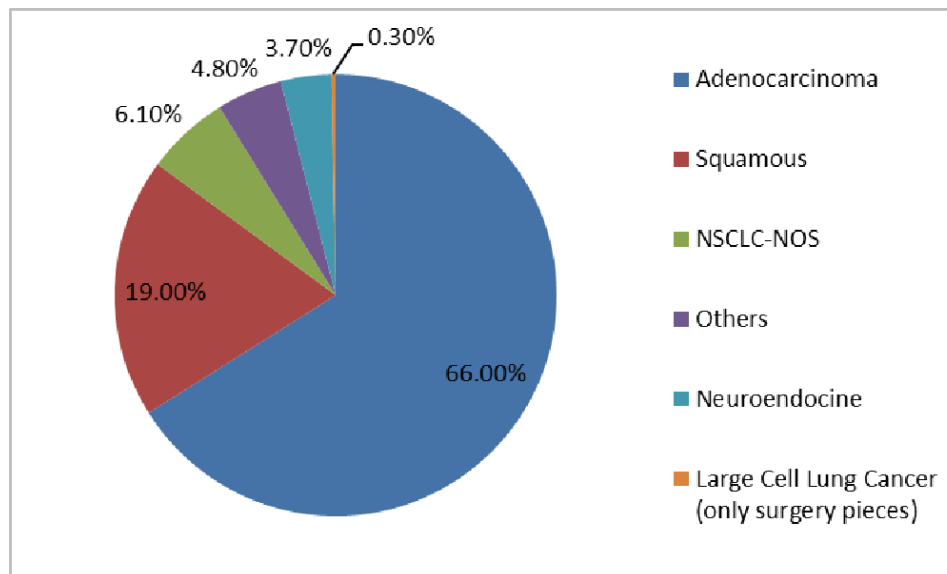


Figure 2 Type of histology collected in the registry excluding only external samples (all histologies). NSCLC-NOS, non-small cell lung cancer—not otherwise specified.

For each biomarker, possible associations between the testing rate or positivity rate according to histology, sample type and result in other biomarkers were explored using contingency tables and the χ^2 test. Then, multivariate logistic regression was used to explore the joint relationship between independent explanatory factors and both response variables (testing rate and positivity rate). For each outcome, two models were adjusted: one with sample type and histology as independent factors (model 1) and the other one adding the testing rate or the positivity rate of the other biomarkers (model 2). Results are presented as odds-ratio (OR) and 95% CI. Model calibration was assessed using the Hosmer-Lemeshow test. Discrimination ability was measured by means of the area under the receiver operating characteristic curve.

All tests were performed at a significant level of 5%. All analysis was carried out with the R statistical programme.

RESULTS

Previously to the selection of the histologies of interest, as shown in figure 1, overall frequencies of histological types were analysed. The main histological type collected was AC (2951 out of 4473, 66%), followed far behind by SCC (851 out of 4473, 19%), NSCLC-NOS (275 out of 4473, 6.1%), large cell neuroendocrine carcinoma (166 out of 4473, 3.7%) and large cell carcinoma accounting for 0.3% (15 out of 4473) (figure 2). Over the 3226 patient samples finally analysed (AC and NSCLC-NOS, as defined in the study objective), AC is by far the main histological type (a total of 2951 samples, 91.5%).

Regarding the type of samples obtained, the biopsy was the most commonly used (1859 samples, 57.6%), followed by the surgical resection specimen with 552 samples (17.1%), cell block cytology with 348 samples (10.8%), fine-needle aspiration (FNA) with 103 samples (6.0%), others with 190 samples (5.9%) and peripheral blood with 84 samples (2.6%) (figure 3).

Of these 3226 samples, the four biomarkers selected (*EGFR/ALK/ROS1/PD-L1*) have been registered in all cases even if the determination was not finally performed, which means a total of 12904 determination entries (3226 for each biomarker). Of the overall of 12904 determinations recorded, 9118 (71.4%) were finally assessed, and the remaining 3686 (28.6%) were not

finally performed. When determination was not performed, the reason was not recorded in almost half (49%). The absence of requirement/requests (42%) and not enough amount of sample (9%) were the reasons for not determining the biomarkers in the recorded cases.

Figure 4 shows the testing rate for each biomarker. As noted, *EGFR* (91.4%) and *ALK* (80.1%) were the mainly tested biomarkers, following by far by *ROS1* (58.1%) and *PD-L1* (56.2%).

Overall, information about techniques performed in 8.934 sample determination were collected. More than half of the determinations were performed by immunohistochemistry (56.3%), followed by fluorescence in situ hybridisation (13.1%) and others techniques (30.6%), such as next-generation sequencing (NGS) or real-time polymerase chain reaction (RT-PCR).

In table 1 are shown the positivity rates of each biomarker after excluding invalid results and the comparison between the two histological types selected (AC and NSCLC-NOS). Statistically significant differences should be noted between the positivity rate of *EGFR* biomarker in AC or NSCLC-NOS histology (14.6% vs 2.8%, respectively; $p < 0.001$).

Bivariate analysis for the testing and positivity rates as outcomes are shown in online supplemental material 1. Statistically significant differences were observed by sample type for the testing rate, but no for tumour histology (AC or NSCLC-NOS).

Multivariate

Tables 2 and 3 show the logistic regression models for the testing rate for each of the four biomarkers of interest. Regarding *ALK*, both models capture a lower testing rate in FNA (model 1: OR 0.16, 95% CI 0.12 to 0.22; model 2: OR 0.29, 95% CI 0.19 to 0.44), surgical piece (model 1: OR 0.66, 95% CI 0.52 to 0.85; model 2: OR 0.58, 95% CI 0.41 to 0.82) and other sample types (model 1: OR 0.20, 95% CI 0.15 to 0.26; model 2: OR: 0.23, 95% CI 0.16 to 0.33) compared with biopsies as reference sample type. On the contrary, for *EGFR* testing rate, both models showed a higher testing rate in FNA (model 1: OR 2.89, 95% CI 1.44 to 6.88; model 2: OR 3.83, 95% CI 1.74 to 9.69) and other sample types (model 1: OR 2.2, 95% CI 1.28 to 4.10; model 2: OR 2.78, 95% CI 1.50 to 5.55) compared with

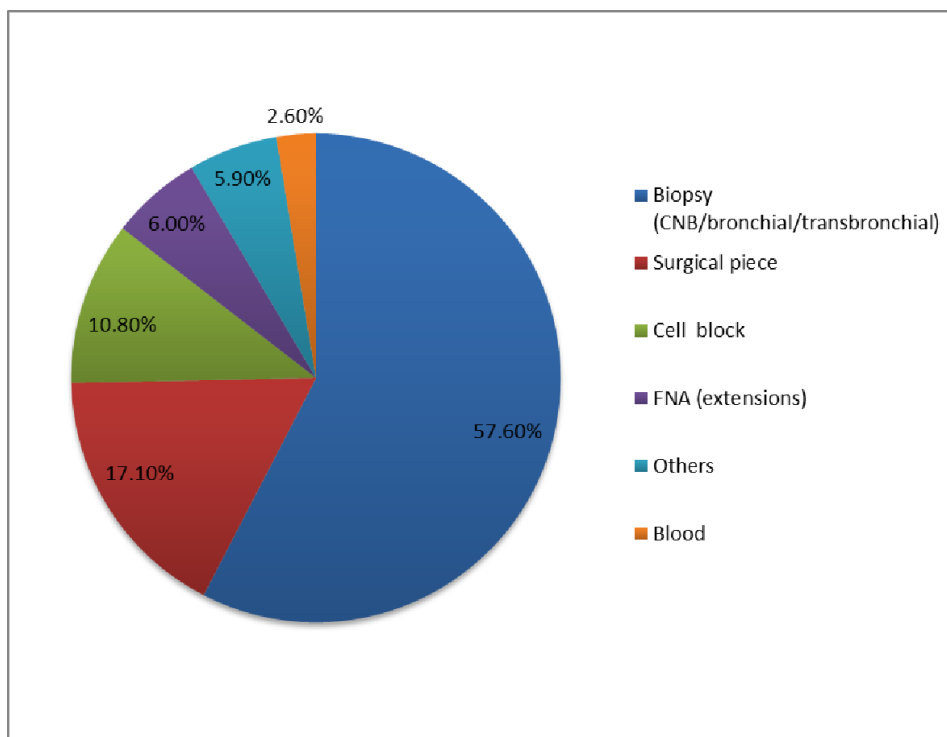


Figure 3 Origin of the samples analysed. CNB, core needle biopsy; FNA, fine-needle aspiration.

biopsies as reference. Regarding the testing rate for PD-L1, all sample types showed a considerably lower testing rate compared with biopsies with both models. Finally, for *ROS1*, similarly to *ALK*, model 1 shown a lower testing rate in cell block (OR 0.77, 95% CI 0.61 to 0.97), FNA (OR 0.24, 95% CI 0.17 to 0.33) and other sample types (OR 0.33, 95% CI 0.26 to 0.44) compared with biopsies, while model 2 does not capture statistically significant differences.

Model 2 captures a strong association between the testing rate of every biomarker and the testing rate of other biomarkers; this is logical since in most cases the determinations are performed in parallel (*ALK*, *EGFR*, *ROS1* and PD-L1 are determined simultaneously).

Tables 4 and 5 show the logistic regression models for the positivity rate for the selected biomarkers, except for *ROS1*,

since both models presented stability problems due to the lack of data. Concerning *ALK*, both models show no association with histology and sample type. The second model shows a significant reduction in *ALK* positivity for samples with positive *EGFR* result (OR 0.10, 95% CI 0.01 to 0.45), and this relationship is captured in turn by model 2 for *EGFR* positivity (OR 0.10, 95% CI 0.01 to 0.47). Model 2 for *EGFR* positivity also showed a higher positivity in FNA (OR 0.58, 95% CI 0.35 to 0.93) and blood samples (OR 0.42, 95% CI 0.22 to 0.76) compared with biopsies as reference. As described previously, both models showed a statistically significant higher positivity rate of *EGFR* in AC compared with NSCLC-NOS as reference histology (model 1: OR 0.16, 95% CI 0.07 to 0.33; model 2: OR 0.15, 95% CI 0.06 to 0.31). Lastly, model 2 for PD-L1 revealed a slightly

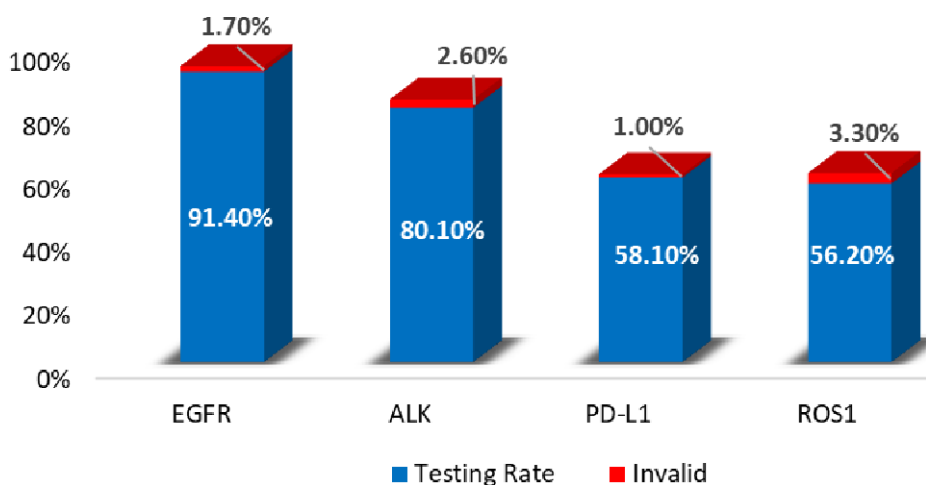


Figure 4 Testing rate and invalid samples in adenocarcinoma and NSCLC-NOS histology cases. ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC-NOS, non-small cell lung cancer–not otherwise specified; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

Table 1 Positivity rate of both AC and NSCLC-NOS histology cases

Biomarker	Result	AC		NSCLC-NOS		P value*	AC+NSCLC-NOS	
		n	%	n	%		n	%
ALK	Positive	79	3.4	6	2.9	0.854	85	3.4
	Negative	2232	96.6	200	97.1		2432	96.6
EGFR	Positive	387	14.6	7	2.8	<0.001	394	13.6
	Negative	2261	85.4	244	97.2		2505	86.4
PD-L1	Positive	822	48.7	91	54.2	0.204	913	49.2
	Negative	866	51.3	77	45.8		943	50.8
ROS1	Positive	33	2.0	2	1.5	0.909	35	2.0
	Negative	1585	98.0	132	98.5		1717	98.0

*P value determined by the χ^2 test.

AC, adenocarcinoma; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC-NOS, non-small cell lung cancer—not otherwise specified; PD-L1, programmed death ligand-1; ROS1, c-ros oncogene 1.

lower positivity rate if the result of *EGFR* biomarker is positive (OR 0.70, 95% CI 0.51 to 0.97).

DISCUSSION

Biomarkers are becoming an essential requirement to properly treat patients with NSCLC.²² This, coupled with recent advances in genomic and proteomic technologies plus bioinformatic tools that have allowed the discovery of several new biomarkers, has led to the fast and substantial accumulation of new biomarker-related data.^{1,23} Nevertheless, while the research of new biomarkers is ongoing, the number of biomarkers for which a recommendation for testing is included in clinical guidelines is limited, as many of these promising biomarkers reported require reliable determination processes, validation and correlation to clinical outcomes.^{24–26} In this context, registries may play a key role ensuring a more balanced evaluation of proposed biomarkers, a harmonisation and comparability of the collected data, and enabling the translation of the scientific literature to biomarker analysis of tumour tissues to assist biomarker–drug association evidence useful in clinical decision making.^{25,27,28}

The main objective of this study was to analyse all the information recorded in the largest multicentre, prospective registry in NSCLC in Spain, the LungPath. In line with the literature, the descriptive analysis underscored that AC was the main histological type collected (66% of all histology types). Indeed, as previously mentioned, more than 85% of LC cases are currently classified as NSCLC, with AC being the predominant NSCLC histological phenotype (~50%).²⁹ Lung biopsies accounted for 57.6% of sample types, a figure that falls far from the other

sample types, such as surgical resection, cell block FNA and blood. The analysis of some molecular biomarkers requires the extraction of nucleic acids from different samples (tumour tissue or cells, and/or blood samples).³⁰ The fact that the quality of nucleic acids in blood samples, in particular RNA, can vary and that FNA could not be effective for small lesions as small nodules as it cannot provide enough tissue for an accurate diagnosis may be at the root of the preference for lung biopsies that are currently the gold standard.³⁰ It is important to highlight that, although its use is currently not widespread, in the future, an increasing number of patients with NSCLC could benefit from liquid biopsy to identify their disease mutation instead of tissue samples, as recently reported in the Blood First Assay Screening Trial (BFAST) that demonstrated the clinical utility of blood-based NGS as a method to inform clinical decision-making in *ALK*+NSCLC.³¹

Regarding biomarker determinations, even though the mandatory test for each patient with advanced NSCLC are *EGFR* and *BRAF* mutations, *ALK* and *ROS1* rearrangements and PD-L1 expression, the analysis of LungPath focused only in four main biomarkers (*EGFR/ALK/ROS1/PD-L1*).^{10,11} One aspect to be highlighted from the analysis of the registry is that the determination of these biomarkers was not always performed, probably because the determination in some laboratories is sequential, and there is not enough sample material or samples were of poor quality containing insufficient tumour cell percentage to determine all biomarkers.

A concern raised by this study is the large proportion of non-assessment of biomarkers in samples from patients with AC and

Table 2 Logistic regression model 1 for the testing rate

	ALK		EGFR		PD-L1		ROS1	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Sample type* (ref. biopsy)								
Cell block	0.84 (0.62 to 1.17)	0.291	1.41 (0.93 to 2.23)	0.121	0.44 (0.35 to 0.56)	<0.001	0.77 (0.61 to 0.97)	0.026
FNA	0.16 (0.12 to 0.22)	<0.001	2.89 (1.44 to 6.88)	0.007	0.03 (0.02 to 0.05)	<0.001	0.24 (0.17 to 0.33)	<0.001
Surgical piece	0.66 (0.52 to 0.85)	0.001	1.10 (0.80 to 1.55)	0.568	0.62 (0.51 to 0.75)	<0.001	0.97 (0.79 to 1.18)	0.726
Others	0.20 (0.15 to 0.26)	<0.001	2.2 (1.28 to 4.10)	0.008	0.17 (0.13 to 0.22)	<0.001	0.33 (0.26 to 0.44)	<0.001
NSCLC-NOS (ref. adenocarcinoma)	0.93 (0.68 to 1.29)	0.666	1.06 (0.68 to 1.72)	0.806	1.31 (0.99 to 1.75)	0.062	0.87 (0.68 to 1.13)	0.296
Hosmer-Lemeshow, p value	>0.999		0.957		0.968		0.998	
AUROC (95% CI)	0.65 (0.62 to 0.67)		0.559 (0.530 to 0.589)		0.673 (0.655 to 0.691)		0.588 (0.569 to 0.606)	

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.

*Category 'others' includes blood samples.

ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer—not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

Table 3 Logistic regression model 2 for the testing rate

	ALK		EGFR		PD-L1		ROS1	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Sample type* (ref. biopsy)								
Cell block	1.20 (0.79 to 1.83)	0.393	0.94 (0.57 to 1.60)	0.807	0.47 (0.37 to 0.60)	<0.001	0.85 (0.65 to 1.12)	0.242
FNA	0.29 (0.19 to 0.44)	<0.001	3.83 (1.74 to 9.69)	0.002	0.05 (0.03 to 0.09)	<0.001	0.71 (0.47 to 1.08)	0.112
Surgical piece	0.58 (0.41 to 0.82)	0.002	1.02 (0.68 to 1.55)	0.909	0.65 (0.52 to 0.80)	<0.001	1.27 (1.00 to 1.63)	0.051
Others	0.23 (0.16 to 0.33)	<0.001	2.78 (1.50 to 5.55)	0.002	0.27 (0.20 to 0.37)	<0.001	0.80 (0.56 to 1.13)	0.198
NSCLC-NOS (ref. adenocarcinoma)	0.86 (0.57 to 1.31)	0.477	1.13 (0.67 to 2.00)	0.660	1.40 (1.04 to 1.90)	0.029	0.79 (0.59 to 1.07)	0.128
Testing rate ALK (yes vs no)	–	–	20.61 (13.96 to 31.01)	<0.001	3.61 (2.71 to 4.87)	<0.001	61.50 (36.97 to 111.36)	<0.001
Testing rate EGFR (yes vs no)	24.9 (16.8 to 37.4)	<0.001	–	–	0.12 (0.08 to 0.17)	<0.001	3.07 (1.99 to 4.76)	<0.001
Testing rate PD-L1 (yes vs no)	4.16 (3.07 to 5.69)	<0.001	0.12 (0.08 to 0.17)	<0.001	–	–	2.34 (1.95 to 2.80)	<0.001
Testing rate ROS1 (yes vs no)	72.3 (42.7 to 133.3)	<0.001	3.56 (2.29 to 5.54)	<0.001	2.41 (2.01 to 2.90)	<0.001	–	–
Hosmer-Lemeshow, p value	<i>0.313</i>		<i><0.001</i>		<i><0.001</i>		<i>0.234</i>	
AUROC (95% CI)	<i>0.93 (0.92 to 0.94)</i>		<i>0.854 (0.825 to 0.884)</i>		<i>0.744 (0.727 to 0.762)</i>		<i>0.793 (0.777 to 0.810)</i>	

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.

*Category 'others' includes blood samples.

ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer—not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

NSCLC-NOS (28.6%), despite guidelines regarding the mandatory molecular analysis of *EGFR/ALK/ROS1/PD-L1* biomarkers for these patients. It should be also noted that in 42% of cases, the absence of biomarker determination was due to the lack of request, which suggests the need to create more awareness on the benefits that biomarker determinations add to the patients, and also increase multidisciplinary collaboration to improve molecular diagnosis.^{32 33}

When analysing the positivity rate, significantly more EGFR mutations were found in the AC (14.6%) versus NSCLC-NOS subgroup (2.85%). This statistically significant difference seen in *EGFR* biomarker for the positivity comparison between the two selected histological types is similar to previously published data.³⁴

As previously described in the literature, the results of the bivariate and multivariate analyses for the testing rate of each biomarker show the importance of the type of sample in the realisation or non-realisation of the biomarker determinations.^{35 36} This can be observed in the multivariate analysis, where a lower testing rate for the *ALK* biomarker is evident when the samples are surgical pieces, FNA or other types of samples versus biopsies as reference sample type. This lower rate of *ALK* assessment observed in FNA-derived samples could be due to the fact that the use of the method for detecting *ALK* gene rearrangement

in cytology smears is quite controversial¹¹ to recent studies, however, have proven the suitability of the method.³⁷ Nevertheless, these results may be subjected to change in the future due to an upward trend towards minimally invasive sampling procedures, such as liquid biopsies.³¹

In line with several previous literature reports showing that *ALK* rearrangement tend to be mutually exclusive with mutations in *EGFR*, an absence of positive *ALK* results in *EGFR*-positive samples was observed in the logistic regression model 2 for *ALK* with the positivity as an outcome.^{38–40}

In summary, the LungPath has allowed obtainment and analyses, for the first time in Spain, of the largest amount of real-world data of biomarker determinations, thus aiding a better understanding of the national diagnostic practices in LC biomarkers. It should be noted, however, that LungPath is an online-based data entry registry which can lead to missing data, inaccuracies or human errors in data entry.

To date, no other similar registries about real diagnostic practice and performance of biomarkers test in a sample this large of patients with LC have been described in Spain. There is, however, a Thoracic Tumours Registry in Spain, created by the Spanish Lung Cancer Group, in its commitment to improving the prognosis and the treatment of NSCLC and other thoracic tumours, which included data about biomarkers tested (from

Table 4 Logistic regression model 1 for the positivity rate

	ALK		EGFR		PD-L1	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Sample type* (ref. biopsy)						
Cell block	0.77 (0.31 to 1.60)	0.513	0.95 (0.66 to 1.36)	0.802	1.00 (0.72 to 1.37)	0.984
FNA	1.72 (0.59 to 4.04)	0.261	1.20 (0.76 to 1.84)	0.417	0.86 (0.28 to 2.60)	0.785
Surgical piece	1.13 (0.62 to 1.96)	0.682	0.94 (0.69 to 1.25)	0.661	0.80 (0.63 to 1.03)	0.081
Blood	–	–	1.54 (0.85 to 2.65)	0.134	–	–
Others	1.48 (0.60 to 3.12)	0.341	1.47 (0.96 to 2.19)	0.068	1.17 (0.73 to 1.88)	0.515
NSCLC-NOS (ref. adenocarcinoma)	0.85 (0.33 to 1.83)	0.707	0.16 (0.07 to 0.33)	<0.001	1.20 (0.87 to 1.66)	0.256
Hosmer-Lemeshow, p value	<i>0.997</i>		<i>0.995</i>		<i>0.997</i>	
AUROC (95% CI)	<i>0.543 (0.486 to 0.601)</i>		<i>0.565 (0.538 to 0.592)</i>		<i>0.526 (0.503 to 0.549)</i>	

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.

*Category 'others' includes blood samples for *ALK* and *PD-L1* models.

ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNA, fine-needle aspiration; NSCLC-NOS, non-small cell lung cancer—not otherwise specified; PD-L1, programmed death ligand-1; ref., reference.

Table 5 Logistic regression model 2 for the positivity rate

	ALK		EGFR		PD-L1	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Sample type* (ref. biopsy)						
Cell block	0.74 (0.30 to 1.56)	0.474	0.86 (0.58 to 1.23)	0.417	1.01 (0.74 to 1.40)	0.931
FNA	1.82 (0.60 to 4.52)	0.237	0.58 (0.35 to 0.93)	0.029	0.78 (0.25 to 2.38)	0.665
Surgical piece	1.15 (0.63 to 2.01)	0.633	0.80 (0.58 to 1.08)	0.156	0.80 (0.62 to 1.02)	0.075
Blood	–	–	0.42 (0.22 to 0.76)	0.005	–	–
Others	1.52 (0.62 to 3.25)	0.313	1.22 (0.78 to 1.87)	0.366	1.20 (0.75 to 1.93)	0.457
NSCLC-NOS (ref. adenocarcinoma)	0.77 (0.29 to 1.66)	0.547	0.15 (0.06 to 0.31)	<0.001	1.19 (0.86 to 1.65)	0.289
Result ALK: positive	–	–	0.10 (0.01 to 0.47)	0.025	1.63 (0.94 to 2.86)	0.084
Result ALK: missing	–	–	4.12 (2.97 to 5.72)	0.000	1.21 (0.83 to 1.77)	0.318
Result EGFR: positive	0.10 (0.01 to 0.45)	0.022	–	–	0.70 (0.51 to 0.97)	0.035
Result EGFR: missing	0.76 (0.18 to 2.10)	0.652	–	–	1.38 (0.95 to 2.01)	0.089
Result PD-L1: positive	1.61 (0.94 to 2.83)	0.088	0.71 (0.51 to 0.98)	0.039	–	–
Result PD-L1: missing	1.22 (0.69 to 2.19)	0.501	1.07 (0.80 to 1.43)	0.651	–	–
Result ROS1: positive	–	–	2.03 (0.80 to 4.54)	0.103	0.97 (0.41 to 2.29)	0.952
Result ROS1: missing	–	–	0.91 (0.68 to 1.20)	0.498	0.88 (0.70 to 1.11)	0.274
Hosmer-Lemeshow, p value	<i>0.68</i>		<i>0.904</i>		<i>0.929</i>	
AUROC (95% CI)	<i>0.613 (0.555 to 0.672)</i>		<i>0.679 (0.650 to 0.708)</i>		<i>0.562 (0.536 to 0.587)</i>	

The results of the Hosmer-Lemeshow test and the AUROC are in italics to differentiate them from the ORs.

*Category 'others' includes blood samples for ALK and PD-L1 models.

ALK, anaplastic lymphoma kinase; AUROC, area under receiver operating characteristic curve; EGFR, epidermal growth factor receptor; FNPA, fine-needle aspiration; NSCLC-NOS, non-smallcell lung cancer—not otherwise specified; PD-L1, programmed death ligand-1; ref., reference; ROS1, c-ros oncogene 1.

2012 to 2018) considering all stages of LC and whose data are aligned with the data obtained in LungPath.⁴¹ In Europe, there are differences between different countries in the availability of diagnoses of molecular alterations in NSCLC,⁴² so in this context, LungPath can be a useful online tool to monitor the availability and the incorporation of new biomarkers testing in LC. Other biomarker-based registries have been developed, such as the Caris Registry promoted by Caris Life Sciences, and intended to become a robust library of tumour biomarker results, covering not only NSCLC but also breast, ovary, colon, endometrium and other cancers.⁴³ Thus, LungPath represents a great step forward to ensure the quality of biomarker determination and results homogenisation, provided by SEAP. Development of more central biomarker databases, such as LungPath, or promoting the use and content expansion of LungPath in the future, could

be useful to monitor, correlate results between different centres at a national and international level, and improve the available knowledge regarding biomarkers in NSCLC.

Given the incremental importance of biomarkers in guiding the treatment of patients with NSCLC and the cost saving that optimal biomarker determination could mean in drug spending-related health expenditure, having this information available could be also essential to elaborate future analysis in this area, being therefore, specifically interesting to the pathology departments and, generally, to the scientific society.^{9 44}

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Take home messages

- For first time, Lung Cancer Biomarker Testing Registry (LungPath) allows obtainment and analysis of the large amount of real-world data of biomarker determinations in Spain.
- The real-world evidence obtained from LungPath demonstrates that epidermal growth factor receptor (*EGFR*) (91.4%) and anaplastic lymphoma kinase (*ALK*) (80.1%) were the mainly tested biomarkers, while c-ros oncogene 1 (*ROS1*) (58.1%) and PD-L1 (56.2%) were not determined in almost half of the patients.
- Multivariate models explored the different associations between the response variables (testing and positivity rates) and the different explanatory factors such as sample type or histology.
- This study aids to a better understanding of the national diagnostic practices in lung cancer biomarkers and to continue with future analysis in this area.

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REFERENCES

- Chatterjee SK, Zetter BR. Cancer biomarkers: knowing the present and predicting the future. *Future Oncol* 2005;1:37–50.
- De Palma M, Hanahan D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol Oncol* 2012;6:111–27.
- Baird R, Caldas C. Biomarker-driven approach in cancer therapy. *Medicographia* 2015;37:287–95.
- Bailey AM, Mao Y, Zeng J, et al. Implementation of biomarker-driven cancer therapy: existing tools and remaining gaps. *Discov Med* 2014;17:101–14.
- Pletcher MJ, Pignone M. Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. *Circulation* 2011;123:1116–24.
- Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. *Ann Glob Health* 2019;85:8.
- Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, et al. The global burden of cancer 2013. *JAMA Oncol* 2015;1:505–27.
- Sampsonas F, Ryan D, McPhillips D, et al. Molecular testing and personalized treatment of lung cancer. *Curr Mol Pharmacol* 2014;7:22–32.
- SEAP IAP. Libro Blanco de la Anatomía Patológica en España, 2019. Available: https://www.seap.es/documents/10157/1760706/Libro_Blanco_Anatomia_Patologica_2019.pdf/87fe0625-9dc9-4170-a0ea-353d1cf06a66
- Melosky B, Blais N, Cheema P, et al. Standardizing biomarker testing for Canadian patients with advanced lung cancer. *Curr Oncol* 2018;25:73–82.
- Garrido P, Conde E, de Castro J, et al. Updated guidelines for predictive biomarker testing in advanced non-small-cell lung cancer: a national consensus of the Spanish Society of pathology and the Spanish Society of medical oncology. *Clin Transl Oncol* 2020;22:989–1003.
- Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology* 2018;29:iv192–237.
- Mok TS, Wu Y-L, Ahn M-J, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive lung cancer. *N Engl J Med* 2017;376:629–40.
- Remon J, Besse B. Brain metastases in Oncogene-Addicted non-small cell lung cancer patients: incidence and treatment. *Front Oncol* 2018;8:88.
- Gómez JJ, De Castro J, Á C. Recomendaciones para La determinación de biomarcadores en El carcinoma de pulmón no microcítico avanzado. Consenso nacional de la Sociedad Española de Anatomía Patológica Y de la Sociedad Española de Oncología Médica. *Rev Esp Patol* 2012;45:14–28.
- European Medical Agency. European public assessment report de Alecensa® (alectinib), 2018. Available: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/004164/WC500241099.pdf
- Issuu. IASLC atlas of ALK testing in Lung cancer - Spanish translation by IASLC - issuu, 2020. Available: https://issuu.com/iaslc/docs/alk_atlas_spanish_lo-res_final/54
- Bedi S, Khan SA, AbuKhader MM, et al. A comprehensive review on Brigatinib - A wonder drug for targeted cancer therapy in non-small cell lung cancer. *Saudi Pharm J* 2018;26:755–63.
- Mazières J, Zalcman G, Crinò L, et al. Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. *J Clin Oncol* 2015;33:992–9.
- Moro-Sibilot D, Faivre L, Zalcman G, et al. Crizotinib in patients with advanced ROS1-rearranged non-small cell lung cancer (NSCLC). Preliminary results of the ACSé phase II trial. *J Clin Oncol* 2015;33:8065.
- Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014;371:1963–71.
- Lopez JM, Alexandre LA, Caldero SG, et al. EP1.09-17 Spanish lung cancer biomarker testing registry (Lungpath): descriptive analysis focus in ALK Translocation results. *J Thorac Oncol* 2019;14:S1004–5.
- Chang N-W, Dai H-J, Shih Y-Y, et al. Biomarker identification of hepatocellular carcinoma using a methodical literature mining strategy. *Databse* 2017;2017:bax082.
- Diamandis EP. Cancer biomarkers: can we turn recent failures into success? *J Natl Cancer Inst* 2010;102:1462–7.
- Voith von Voithenberg L, Crocetti E, Martos C, et al. Cancer registries - guardians of breast cancer biomarker information: A systematic review. *Int J Biol Markers* 2019;34:194–9.
- Aazmi O, Akhtari M, Amnekar R V. Epigenetic biomarkers in Cance. In: Sharma S, ed. *Prognostic epigenetics*. 15. 1st edition. London: Elsevier Inc, 2019.
- Andre F, McShane LM, Michiels S, et al. Biomarker studies: a call for a comprehensive biomarker study registry. *Nat Rev Clin Oncol* 2011;8:171–6.
- Selleck MJ, Senthil M, Wall NR. Making meaningful clinical use of biomarkers. *Biomark Insights* 2017;12:1177271917715236.
- Chen Z, Fillmore CM, Hammerman PS, et al. Non-Small-Cell lung cancers: a heterogeneous set of diseases. *Nat Rev Cancer* 2014;14:535–46.
- Hofman P. The challenges of evaluating predictive biomarkers using small biopsy tissue samples and liquid biopsies from non-small cell lung cancer patients. *J Thorac Dis* 2019;11:S57–64.
- Gadgeel SM, Mok TSK, Peters S, et al. Phase III/II blood first assay screening trial (BFAST) in patients (PTS) with treatment-naïve NSCLC: initial results from the ALK+ cohort. *Ann Oncol* 2019;30:v918.
- Leary AF, Castro DGde, Nicholson AG, et al. Establishing an EGFR mutation screening service for non-small cell lung cancer - sample quality criteria and candidate histological predictors. *Eur J Cancer* 2012;48:61–7.
- von Dinkelge JJ, Ball D, Silvestri GA. A review of clinical practice guidelines for lung cancer. *J Thorac Dis* 2013;5 Suppl 5:S607–22.
- Sandelin M, Berglund A, Sundström M, et al. Patients with non-small cell lung cancer analyzed for EGFR: adherence to guidelines, prevalence and outcome. *Anticancer Res* 2015;35:3979–85.
- Kerr KM, Bubendorf L, Edelman MJ, et al. Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer. *Ann Oncol* 2014;25:1681–90.
- Brega E, Brandao G. Non-Small cell lung carcinoma biomarker testing: the pathologist's perspective. *Front Oncol* 2014;4:182.
- Lozano MD, Echeveste JJ, Abengozar M, et al. Cytology smears in the era of molecular biomarkers in non-small cell lung cancer: doing more with less. *Arch Pathol Lab Med* 2018;142:291–8.
- Gainor JF, Varghese AM, Ou S-HI, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* 2013;19:4273–81.
- Wang Z, Zhang X, Bai H, et al. EML4-ALK rearrangement and its clinical significance in Chinese patients with advanced non-small cell lung cancer. *Oncology* 2012;83:248–56.
- Wallander ML, Geiersbach KB, Tripp SR, et al. Comparison of reverse transcription-polymerase chain reaction, immunohistochemistry, and fluorescence in situ hybridization methodologies for detection of echinoderm microtubule-associated proteinlike 4-anaplastic lymphoma kinase fusion-positive non-small cell lung carcinoma: implications for optimal clinical testing. *Arch Pathol Lab Med* 2012;136:796–803.
- Provincia M, Carcereny E, Rodríguez-Abreu D, et al. Lung cancer in Spain: information from the thoracic tumors registry (TTR study). *Transl Lung Cancer Res* 2019;8:461–75.
- Thunnissen E, Weynand B, Udovicic-Gagula D, et al. Lung cancer biomarker testing: perspective from Europe. *Transl Lung Cancer Res* 2020;9:887–97.
- Sanders S, Schroeder W, Wright A, et al. The Caris registry: building a biomarker-focused database to advance patient care. *J Clin Oncol* 2012;30:e21152.
- Ong FS, Das K, Wang J, et al. Personalized medicine and pharmacogenetic biomarkers: progress in molecular oncology testing. *Expert Rev Mol Diagn* 2012;12:593–602.