mebm
MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

ORIGINAL ARTICLE

PREDICTIVE IMMUNOCYTOCHEMISTRY IN NON-SMALL CELL LUNG CANCER CYTOLOGICAL SAMPLES

Suzana Harabajsa[1,2,3], Vesna Simic[1], Bozica Vrabec Branica[1], Sonja Badovinac[4], Marko Jakopovic[4,5], Silvana Smojver-Jezek[1,3,5]

Abstract: Non-small cell lung cancer (NSCLC) molecular biomarker testing is obligatory for determining therapy. The aim of this study was to compare immunocytochemistry (ICC) results of NSCLC predictive biomarkers between bronchoscopic and non-bronchoscopic type of cytology samples. This study included archive records of 1109 predictive ICC results (ALK, ROS1, and PD-L1). The ICC was done on bronchoscopic, and non-bronchoscopic NSCLC samples prepared as cytological smears and cytospins, using Dako EnVisionTM FLEX detection visualization system. The ALK, ROS1, and PD-L1 distribution between bronchoscopic and non-bronchoscopic samples was analysed. The future perspective of cytology in precision medicine was reconsidered. The obtained positive results of ALK, ROS1, and PD-L1 ICC were in concordance with the previously observed range. There was no statistically significant difference in ALK, ROS1, and PD-L1 ICC distribution between the bronchoscopic and non-bronchoscopic groups of samples (p=0.730). The comparison of PD-L1 expression, and, separately PD-L1 ≥50% expression, between two groups of samples showed no statistically significant difference (p=0.236; p=0.436). Bronchoscopic and non-bronchoscopic samples prepared as cytological smears and cytospins are a suitable, but underutilized resource for ALK, ROS1, and PD-L1 biomarker analysis. The implementation of optimized predictive immunocytochemistry assays to provide rapid and reliable results for limited tumour samples is necessary.

[1] Department of Pathology and Cytology, Division of Pulmonary Cytology Jordanovac, University Hospital Centre Zagreb, Zagreb, Croatia

[2] Department for Biology, Division of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

[3] University of Applied Health Sciences, Zagreb, Croatia

[4] Department for Lung Diseases Jordanovac, University Hospital Centre Zagreb, Zagreb, Croatia

[5] School of Medicine, University of Zagreb, Zagreb, Croatia

Corresponding author:
Silvana Smojver-Jezek
University Hospital Centre Zagreb, Department of Pathology and Cytology, Division of Pulmonary Cytology Jordanovac, Zagreb, Croatia
E-mail: ssmojver@pbf.hr

Submitted: June, 2022
Accepted: July, 2022

Key words: NSCLC, immunocytochemistry, ALK, ROS1, PD-L1

INTRODUCTION

Non-small cell lung cancer (NSCLC) constitutes over 80% of all lung cancer cases.[1, 2] Adenocarcinoma (40%) and squamous cell carcinoma (30%) are the most frequent histologic and cytologic subtypes.[2] Molecular predictive testing is obligatory for therapy decision in NSCLC patients.[1, 2] According to the latest National Comprehensive Cancer Network (NCCN) updated guidelines, routine multigene testing that includes epidermal growth factor receptor mutations (*EGFR*), anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) rearrangements, together with MET proto-oncogene receptor tyrosine kinase (*MET*), rearranged during transfection proto-oncogene (*RET*), neurotrophic tropomyosin-related kinases gene fusion (*NTRK*), Kirsten rat sarcoma viral oncogene (*KRAS*), v-raf murine sarcoma viral oncogene homolog B (*BRAF*) and human epidermal growth factor receptor 2 (HER2) are recommended for all advanced NSCLC with an adenocarcinoma component. Reflex testing for programmed death-ligand 1 protein expression (PD-L1) is recommended in both adenocarcinoma and squamous subtype of NSCLC. EGFR is a receptor tyrosine kinase and often overexpressed in NSCLC. *EGFR* mutations can cause continuing activation of the receptor, leading to uncontrolled cell division and tumour pathogenesis.[3] *EGFR* mutations are more common in adenocarcinomas (10%-12% in Croatia)[4, 5] although they can also appear in squamous cell lung cancers as well, but at a significantly lower rate.[3] *ALK* has been found to fuse with other partners, such as echinoderm microtubule-associated protein-like 4 (EML4) leading to potent malignant transformation in lung cancer. The frequency

of *ALK* rearrangements in patients with NSCLC is reported to be 4% to 7%.[3, 5, 6] The *ROS1* gene encodes a receptor tyrosine kinase and is located on chromosome 6q22.1. Chromosomal rearrangements involving the *ROS1* gene have been identified in 1% to 2% of patients with NSCLC.[3, 7, 8] The PD-L1 ligand binds to the PD-1 receptor on activated T-cells and this connection results in suppression of the immune system. It is believed that PD-L1 expression allows cancer cells to avoid the immune response.[3, 9, 10] The *MET* mutations that result in the loss of exon 14 (*METex14*) lead to dysregulation and inappropriate signaling.[2, 3] The presence of *RET* rearrangements results in dysregulation and inappropriate signalling through RET kinase domain. The most common RET fusion partners *KIF5B*, *NCOA4*, and *CCDC6* are found in 1% to 2% of NSCLC.[2, 3] NTRK1/2/3 are tyrosine kinase receptors that are rarely rearranged in NSCLC, resulting in dysregulation and inappropriate signalling. Point mutations in *NTRK1/2/3* are generally non-activating and rarely identified in NSCLC.[2, 3] The mutations of *KRAS* are commonly seen at codon 12 and are prognostic of poor survival when compared with NSCLC patients without *KRAS* mutations.[2, 3, 5] *BRAF* mutations as a result of a change in amino acid position 600 (p.V600E) can also be seen in NSCLC and interfere with responsiveness to therapy.[2, 3] *HER2* mutations have been identified as oncogenic drivers and are found in 1–2% of lung adenocarcinomas. The most common *HER2* mutations in NSCLC occur in exon 20 followed by the *S310F* mutation in the extracellular domain. Therapeutic agents targeting these specific genetic changes enable increased overall survival in NSCLC patients.[10-12] NSCLC predictive biomarkers can be analysed by different testing methodologies. These days, the next generation sequencing method (NGS) is in the focus for NSCLC multigene testing, but not all types of alterations are detected by individual RNA-based NGS assays.[2, 13] The Real-time polymerase chain reaction method (PCR) is usually used for targeting specific mutations.[2] The immunohistochemistry (IHC) on formalin-fixed and paraffin-embedded (FFPE) NSCLC small biopsies and cell blocks is preferred because of some validation advantages over immunocytochemistry (ICC) on smears and cytospins.[13] All testing methods require the optimal quantity of malignant cells in testing samples, and the preservation of quality DNA and RNA in case of NGS and PCR.[14, 15] In many cases, cytological samples are the only available testing materials because surgery or a small biopsy at a given time is not possible due to the advanced stage of the disease and/or the poor status of the patient.[13] Precision medicine based on targeted molecular and immunotherapy presents a great challenge in obtaining the sufficient and adequate samples for many ongoing DNA- and RNA-based testing platforms.[2] ALK, ROS1, and PD-L1 ICC on NSCLC samples prepared as smears and cytospins have been the common practice in clinical cytopathological laboratories due to sample availability, minor invasiveness, and cost-effectiveness.[7, 12, 13] Previously published research confirms non-cell block cytology samples to be suitable for predictive ICC, but proper optimization and rigorous quality control for high-quality staining must be considered.[13, 14]

The aim of this study was to compare ICC results of NSCLC predictive biomarkers between cytological samples obtained with bronchoscopy and other types of various non-bronchoscopic samples prepared as smears and cytospins, and to discuss the future perspective of cytology in precision medicine.

MATERIAL AND METHODS

This retrospective study included archival records of 1109 ICC results of ALK, ROS1, and PD-L1 expression in NSCLC cytological samples routinely processed at the Division of Pulmonary Cytology, Department for Pathology and Cytology, University Hospital Centre Zagreb over a one-year period. The study was approved by the Ethics committee of the University Hospital Centre Zagreb.

Sample preparation and cellularity evaluation

Bronchoscopic samples obtained during bronchoscopy comprise bronchial washing, bronchial brushing, transbronchial fine needle aspiration (transbronchial FNA), and endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA), while non-bronchoscopic cytological samples were pleural effusions, FNA of peripheral lymph nodes and skin nodules, and transthoracic FNA and/or biopsies. Non-liquid samples were directly applied as a thin layer onto cytological slides, while liquid samples (bronchial washings and pleural effusions) were transferred to a cytological laboratory for further processing by centrifuge (Thermo Fisher Scientific Medifuge™; 600 g; 5 minutes) and cytocentrifuge (Hettich rotofix 32 A; 275 g; 5 minutes). Initial slides were air-dried for two hours and stained by May Grünwald Giemsa (MGG) staining, while others were left unstained and were subjected to deep-freezing (-20ºC) for further ICC analysis.[16] Cytomorphological analysis of MGG stained NSCLC smears and cytospins was the starting point for the assessment of sample cellularity. NSCLC cytological smears and cytospins with numerous single malignant cells and/or clusters of malignant cells were considered adequate for predictive molecular ICC (Figure 1a).

Immunocytochemical and cytomorphological analysis

Cytological smears and cytospins of NSCLC samples were stained by Immunocytochemistry Autostainer using Dako EnVision™ FLEX detection visualization system. Cell Signaling anti-ALK Clone D5F3, Cell

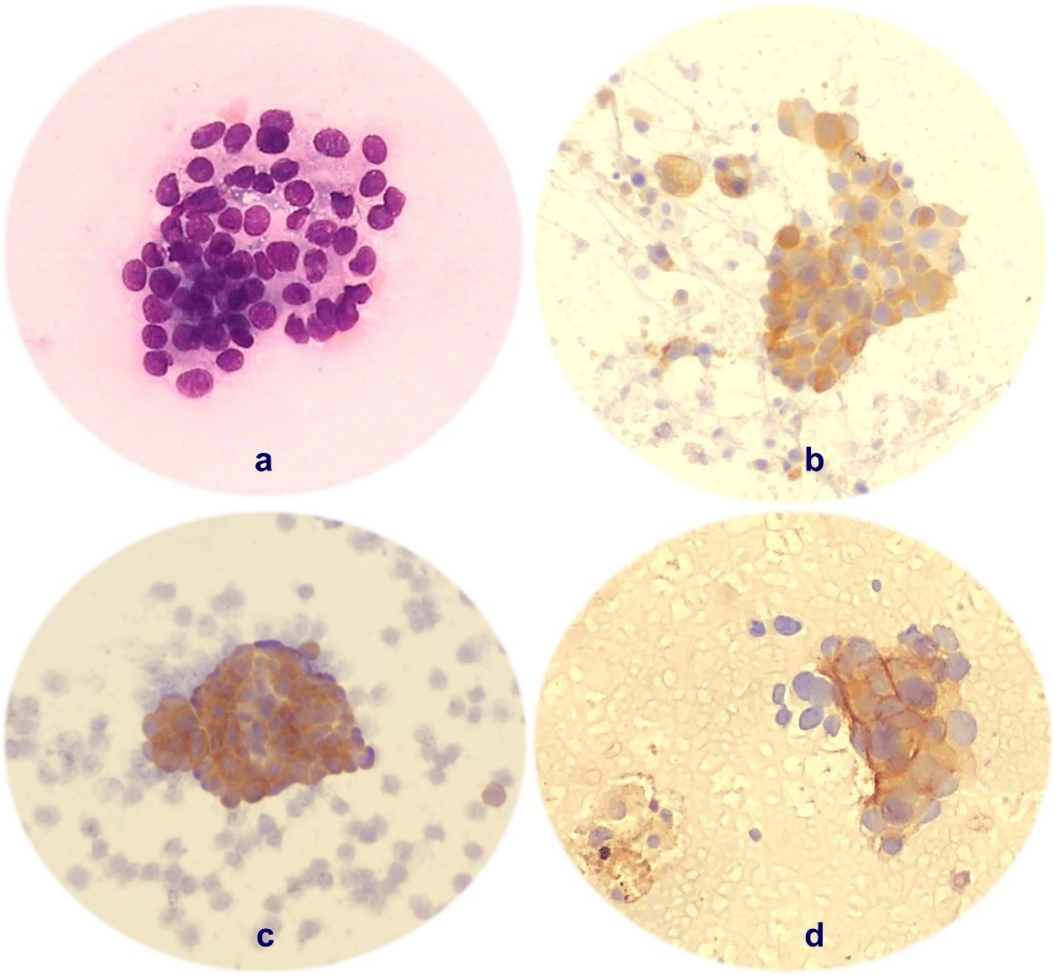

Figure 1. Non-small cell lung cancer a) Cluster of adenocarcinoma cells, MGG, magnification 200x; b) Cell Signaling anti-ALK Clone D5F3, cytoplasmic positivity, ICC, magnification 200x; c) Cell Signaling anti-ROS1 Clone D4D6, cytoplasmic positivity, ICC, magnification 200x; d) Dako anti-PD-L1 Clone 22C3, membranous positivity, ICC, magnification 200x.
Abbreviations: ALK, anaplastic lymphoma kinase; ROS, c-ros oncogene 1; PD-L1; programmed death-ligand 1 protein; MGG, May Grünwald Giemsa; ICC, immunocytochemistry- Dako EnVision™ FLEX detection visualization system.

Signaling anti-ROS1 Clone D4D6, and Dako anti-PD-L1 Clone 22C3 monoclonal antibodies were applied for 35 minutes, after 10-minute fixation in a refrigerated acetone/methanol mixture and after the release of cancer cell epitope by heat. Dako EnVision FLEX+ Mouse (LINKER) was used to amplify the signal of primary ALK, ROS1, and PD-L1 mouse antibodies. Dako Lily's Hematoxylin was used as counterstaining. Positive controls (appendix for ALK, HCC-78 cell line for ROS1, and placenta imprint for PD-L1) were used for each group of ALK, ROS1, and PD-L1 ICC staining.[2, 6, 9]

Internal and external quality control were performed on FFPE cell blocks and histology slides with corresponding Ventana antibodies and staining systems.[6, 9] The ALK, ROS1, and PD-L1 stained slides were glass-covered and subjected to microscopic analysis by two cytologists. The immunoreactivity in NSCLC cytological smears and cytospins was microscopically evaluated at low and medium (200x and 400x) magnification. The ALK and ROS1 overexpression was presented as typically cytoplasmic staining (Figure 1b, c), ranging from weak to strong. ROS1-positive smears and cytospins were retested and confirmed positive by fluorescence in situ hybridization method (FISH), according to recommendations.[6, 15] The NSCLC smears and cytospins with a minimum of 100 cells with membranous staining were considered as PD-L1-positive (Figure 1d). PD-L1 protein expression was scored using Tumor Proportion Score (TPS) with a positive cut-off of ≥1%.[9] Nuclear or cytoplasmic staining is considered non-specific for PD-L1.[9]

Statistical analysis

The evaluation of association between categorical variables was performed using Chi-Square Test (STATISTICA10, STATA17). The significance level was set at p-value <0.05.

RESULTS

Among 1109 ICC results, there were 440 (39.7%) ALK, 111 (10.0%) ROS1 and 558 (50.3%) PD-L1 staining. The majority of the ALK (71.4%), ROS1 (67.6%), and PD-L1 (70.3%) ICC results were from bronchoscopic samples (n=781; 70.4%), while a significantly smaller number of molecular predictive ALK (28.6%), ROS1 (32.4%), and PD-L1 (29.7%) ICC results were in various non-bronchoscopic samples (n=328; 29.6%) (Figure 2). A comparison of ALK, ROS1, and PD-L1 ICC distribution between the bronchoscopic and non-bronchoscopic groups of samples showed no statistically significant difference (p=0.730).

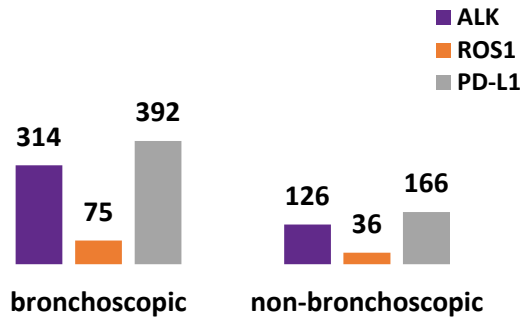

Figure 2. Non-small cell lung cancer predictive immunocytochemistry in bronchoscopic and non-bronchoscopic cytological samples.
Abbreviations: ALK, anaplastic lymphoma kinase; ROS, c-ros oncogene 1; PD-L1; programmed death-ligand 1 protein

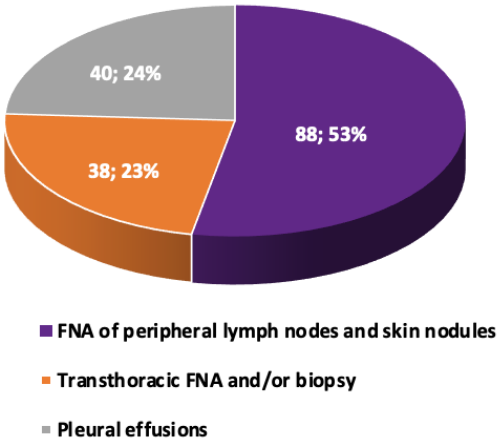

Figure 3. Programmed death-ligand 1 protein (PD-L1) immunocytochemistry in various non-bronchoscopic cytological samples of non-small cell lung cancer.
Abbreviations: FNA, fine needle aspiration

Positive ALK (3.3%) and ROS1 (1.2%) numbers were in concordance with the previously observed range, but insufficient for statistical analysis. Among 558 PD-L1 ICC scored results, 392 (70.3%) samples were obtained by bronchoscopy, while 166 (29.7%) belonged to the non-bronchoscopic group of various samples (Figures 2 and 3).
PD-L1 ICC scored negative in 281 (50.4%) samples and positive in 277 (49.6%) (Figure 4). A comparison of PD-L1 expression between the bronchoscopic and non-bronchoscopic groups of samples showed no statistically significant difference (p=0.236). Out of all PD-L1 positive smears, 128 (46.2%) were PD-L1 ≥50% positive (Figure 4). Comparison of only PD-L1 ≥50% positive expression between the bronchoscopic and non-bronchoscopic groups of samples also showed no statistically significant difference (p=0.436).

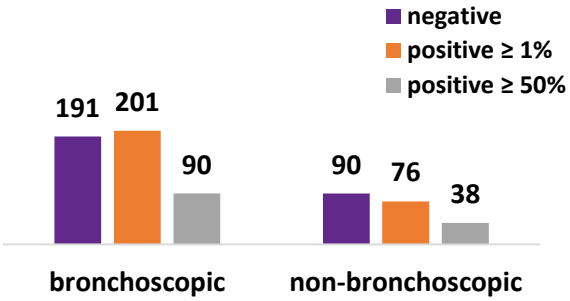

Figure 4. Programmed death-ligand 1 protein (PD-L1) immunocytochemistry scores in bronchoscopic and non-bronchoscopic cytological samples of non-small cell lung cancer. Non-small cell lung cancer cytological smears and cytospins in the absence of immunocytochemical reactivity and with the protein expression under 1% were considered PD-L1 negative.

DISCUSSION

The successful application of personalized therapy depends on the identification of tumour-specific molecular changes, usually in a very small and limited sample.[2, 17] The ideal samples for molecular testing would be freshly obtained tumour tissues followed by immediate snap freezing.[11, 13] These samples are described as ideal even for multigene RNA-based NGS testing due to the optimal cellularity but usually available in a small number of cases after surgical extraction and for research purposes only.[13] FFPE small biopsy tissues and cell blocks are considered as preferred samples for lung cancer molecular biomarker testing over cytology smears and cytospins despite the latest published guidelines.[2, 15-19, 20] NSCLC biomarkers should be tested in any available sample (FFPE small biopsies and/or FFPE cell blocks and/or cytological smears and cytospins) by molecular biomarker assays that are able to detect molecular alterations in samples with as little as 20% cancer cells.[20] The required

percentage of cancer cells can be easily found in a majority of NSCLC smears and cytospins.[4, 5, 16, 18] Although approximately 51% of cytology laboratories in the United States and 75% in Europe reported using non-cell block samples for diagnostic ICC21, their use has been in decline when predictive ICC is considered.[13, 22] Following the current references, the predictive ICC on non-cell block cytology samples is limited to ALK, ROS1, PD-L1, and forthcoming NTRK biomarker with the obligation of establishing validated in-house ICC methods and following strict rules to avoid the influence of preanalytical factors that may lead to false negative or positive results.[2, 13, 20, 22] To provide the best treatment option to lung cancer patients with no possibility of obtaining a small biopsy but with a positive cytology sample[23-25], beside the *EGFR* mutation testing, ALK, ROS1, and PD-L1 ICC is reflexively done under support of the Croatian Health Insurance Fund. In our institution, predictive ICC testing is mainly done on cytological samples obtained with bronchoscopy and prepared as smears and cytospins. Various types of non-bronchoscopic samples, if they contain the optimal number of NSCLC cells, are also tested by the same ICC procedure. Methods for obtaining and preparing the cytological sample were used according to valid recommendations.[2, 6, 9] Additionally, many years of experience with ICC testing and interpretation have enabled us to overcome potential analytical challenges and have led to the results presented in our study. There were no statistically significant differences in ALK, ROS1, and PD-L1 ICC results between samples obtained during bronchoscopy and non-bronchoscopic samples prepared as non-cell blocks in our study. The PD-L1 scoring results were also shown to be independent of cytology sample type. Sometimes the NSCLC in cytological smears and cytospins is presented with the three-dimensionality of cell clusters and overlapping, therefore PD-L1 membranous staining may appear as cytoplasmic staining. Also, non-specific staining of background cells such as histiocytes and inflammatory cells can lead to overestimation of PD-L1 TPS.[2, 9, 13, 15, 22] Despite the usual challenges, our results and experience confirm that cytological samples are suitable for predictive molecular ICC. ICC can be performed on all types of cytological samples if they contain the optimal number of tumour cells.[13, 19, 21, 23, 26, 27] The main reason why cytology remains an underutilized resource for predictive molecular ICC in NSCLC patients is not the insufficient quality of non-cell block samples but the challenging translation of biomarker assays validated for FFPE tissue to cytological samples.[22, 28] Multigene single assay testing is considered sample saving and less time consuming due to the possibility of analysing numerus biomarkers in only one run.[2, 20, 22] However, obtaining the biopsy sample of optimal quantity and quality for multigene single assay testing is of great challenge in everyday clinical practice. Therefore, implementing predictive ICC assays in routine cytology practice could provide a rapid and cost-effective alternative to multigene testing in case of limited tumour samples. [2, 13, 22, 26, 29]

CONCLUSION

Bronchoscopic and non-bronchoscopic samples prepared as cytological smears and cytospins are suitable for ALK, ROS1, and PD-L1 biomarker analysis by immunocytochemistry. The increasing number of emerging molecular biomarkers available for NSCLC patients demands the optimization, validation and clinical implementation of immunocytochemistry assays to provide rapid and reliable results for limited tumour samples.

REFERENCES

1. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Petersen I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M, Yankelewitz D.. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. J Thorac Oncol. 2011;6:244-285.
2. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, Colasacco C, Dacic S, Hirsch FR, Kerr K, Kwiatkowski DJ, Ladanyi M, Nowak JA, Sholl L, Temple-Smolkin R, Solomon B, Souter LH, Thunnissen E, Tsao MS, Ventura CB, Wynes MW, Yatabe Y. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment with Targeted Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med. 2018;142(3):321-346.
3. Šutić M, Vukić A, Baranašić J, Försti A, Džubur F, Samaržija M, Jakopović M, Brčić L, Knežević J. Diagnostic, Predictive, and Prognostic Biomarkers in Non-Small Cell Lung Cancer (NSCLC) Management. J Pers Med. 2021;11(11):1102.
4. Mohar B, Smojver Ježek S, Rajković Molek K, Štemberger C, Kurpis M, Kupanovac Ž, Samaržija M, Jonjić N, Grahovac B. Detection of an EGFR mutation in cytological specimens of lung adenocarcinoma. Cytopathology. 2016;6:444-451.
5. Brcic L, Jakopovic M, Misic M, Seiwerth F, Kern I, Smojver-Jezek S, Quehenberger F, Samarzija M, Seiwerth S. Analysis of the frequency of EGFR, KRAS and ALK mutations in patients with lung adenocarcinoma in Croatia. Diag Pathol. 2016;11:90.
6. International association for the study of lung cancer. IASLC atlas of ALK and ROS1 testing in lung cancer second edition. Colorado, Editorial Rx Press; 2016.
7. Lin JJ, Shaw AT. Recent Advances in Targeting ROS1 in Lung Cancer. J Thorac Oncol. 2017;12(11):1611-1625.
8. Bubendorf L, Büttner R, Al-Dayel F, Dietel M, Elmberger G, Kerr K, López-Ríos F, Marchetti A, Öz B, Pauwels P, Penault-Llorca F, Rossi G, Ryška A, Thunnissen E. Testing for ROS1 in non-small cell lung cancer: a review with recommendations. Virchows Arch. 2016;469(5):489-503.

9. International association for the study of lung cancer. IASLC atlas of PD-L1 immunohistochemistry testing in lung cancer. Colorado, Editorial Rx Press; 2017.
10. Pawelczyk K, Piotrowska A, Ciesielska U, Jablonska K, Gletzel-Plucinska N, Grzegrzolka J, Podhorska-Okolow M, Dziegiel P, Nowinska K. Role of PD-L1 Expression in Non-Small Cell Lung Cancer and Their Prognostic Significance according to Clinicopathological Factors and Diagnostic Markers. Int J Mol Sci. 2019;20(4):824.
11. Wistuba II. Molecular testing of non-small cell lung carcinoma biopsy and cytology specimens. Am Soc Clin Oncol Educ Book. 2012;459-464.
12. Hofman P, Rouleau E, Sabourin JC, Denis M, Deleuze JF, Barlesi F, Laurent-Puig P. Predictive molecular pathology in non-small cell lung cancer in France: The past, the present and the perspectives. Cancer Cytopathol. 2020;128:601-610.
13. Jain D, Nambirajan A, Borczuk A, Chen G, Minami Y, Moreira AL, Motoi N, Papotti M, Rekhtman N, Russell PA, Savic Prince S, Yatabe Y, Bubendorf L; IASLC Pathology Committee. Immunocytochemistry for predictive biomarker testing in lung cancer cytology. Cancer Cytopathol. 2019;127(5):325-339.
14. Dejmek A, Zendehrokh N, Tomaszewska M, Edsjö A. Preparation of DNA from cytological material: effects of fixation, staining, and mounting medium on DNA yield and quality. Cancer Cytopathol. 2013;121(7):344-353.
15. Roy-Chowdhuri S, Aisner DL, Allen TC, Beasley MB, Borczuk A, Cagle PT, Capelozzi V, Dacic S, da Cunha Santos G, Hariri LP, Kerr KM, Lantuejoul S, Mino-Kenudson M, Moreira A, Raparia K, Rekhtman N, Sholl L, Thunnissen E, Tsao MS, Vivero M, Yatabe Y. Biomarker Testing in Lung Carcinoma Cytology Specimens: A Perspective from Members of the Pulmonary Pathology Society. Arch Pathol Lab Med. 2016;140(11):1267-1272.
16. Harabajsa S, Begić V, Gršković P, Šimić V, Vrabec Branica B, Badovinac S, Smojver-Ježek S, Korać P. Influence of deep-freezing and MGG staining on DNA and RNA quality in different types of lung adenocarcinoma cytological smears. Diagn Cytopathol. 2021;49(12):1244-1250
17. Bellevicine C, Malapelle U, Vigliar E, Pisapia P, Vita G, Troncone G. How to prepare cytological samples for molecular testing. J Clin Pathol. 2017;70(10):819-826.
18. Roh MH. The utilization of cytologic and small biopsy samples for ancillary molecular testing. Mod Pathol. 2019;32(1):77-85.
19. Elizheva NP, Rechsteiner MP, Wong CE, Zhong Q, Rössle M, Bode B, Moch H, Soltermann A, Wild PJ, Tischler V. Cytology smears as excellent starting material for next-generation sequencing-based molecular testing of patients with adenocarcinoma of the lung. Cancer Cytopathol. 2017;125(1):30-40.
20. Kalemkerian GP, Narula N, Kennedy EB, Biermann WA, Donington J, Leighl NB, Lew M, Pantelas J, Ramalingam SS, Reck M, Saqi A, Simoff M, Singh N, Sundaram B. Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. J Clin Oncol. 2018;36(9):911-919.
21. Srebotnik Kirbiš I, Rodrigues Roque R, Bongiovanni M, Strojan Fležar M, Cochand-Priollet B. Immunocytochemistry practices in European cytopathology laboratories-Review of European Federation of Cytology Societies (EFCS) online survey results with best practice recommendations. Cancer Cytopathol. 2020;128(10):757-766.
22. Roy-Chowdhuri S. Immunocytochemistry of cytology specimens for predictive biomarkers in lung cancer. Transl Lung Cancer Res. 2020;9(3):898-905.
23. Rosenblum F, Hutchinson LM, Garver J, Woda B, Cosar E, Kurian EM. Cytology specimens offer an effective alternative to formalin-fixed tissue as demonstrated by novel automated detection for ALK break-apart FISH testing and immunohistochemistry in lung adenocarcinoma. Cancer Cytopathol. 2014;122(11):810-821.
24. Piskorz AM, Ennis D, Macintyre G, Goranova TE, Eldridge M, Segui-Gracia N, Valganon M, Hoyle A, Orange C, Moore L, Jimenez-Linan M, Millan D, McNeish IA, Brenton JD. Methanol-based fixation is superior to buffered formalin for next-generation sequencing of DNA from clinical cancer samples. Ann Oncol. 2016;27(3):532-539.
25. Furuya N, Matsumoto S, Kakinuma K, Morikawa K, Inoue T, Saji H, Goto K, Mineshita M. Suitability of transbronchial brushing cytology specimens for next-generation sequencing in peripheral lung cancer. Cancer Sci. 2021;112(1):380-387.
26. Tejerina E, Garca Tobar L, Echeveste JI, de Andrea CE, Vigliar E, Lozano MD. PD-L1 in Cytological Samples: A Review and a Practical Approach. Front Med (Lausanne). 2021;8:668612.
27. Skoog L, Tani E. Immunocytochemistry: an indispensable technique in routine cytology. Cytopathology. 2011;22(4):215-229.
28. Sorber L, Claes B, Zwaenepoel K, Van Dorst B, De Winne K, Fransen E, Wener R, Lapperre T, Lardon F, Pauwels P. Evaluation of Cytologic Sample Preparations for Compatibility with Nucleic Acid Analysis. Am J Clin Pathol. 2022;157(2):293-304.
29. VanderLaan PA. Non-small cell lung cancer predictive biomarker testing via immunocytochemistry: Ways of future past?. Cancer Cytopathol. 2019;127(5):278-280.