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Commentary

Circulating lung cancer biomarkers: From translational research to clinical practice

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Abstract. Fundamental studies on biomarkers as well as developed assays for their detection can provide valuable information facilitating clinical decisions. For patients with lung cancer, there are established circulating biomarkers such as serum progastrin-releasing peptide (ProGRP), neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCC-Ag), carcinoembryonic antigen (CEA), and cytokeratin-19 fragment (CYFRA21-1). There are also molecular biomarkers for targeted therapy such as epidermal growth factor receptor (EGFR) gene, anaplastic lymphoma kinase (ALK) gene, KRAS gene, and BRAF gene. However, there is still an unmet need for biomarkers that can be used for early detection and predict treatment response and survival. In this review, we describe the lung cancer biomarkers that are currently being used in clinical practice. We also discuss emerging preclinical and clinical studies on new biomarkers such as omics-based biomarkers for their potential clinical use to detect, predict, or monitor subtypes of lung cancer. Additionally, between-method differences in tumor markers warrant further development and improvement of the standardization and harmonization for each assay.

Keywords: Circulating tumor markers, lung cancer diagnostics, small-cell lung carcinoma, non-small cell lung carcinoma, circulating tumor DNA, exosomes

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, with an estimated 238,340 new cases and 127,070 deaths in 2023 in the United States. [1] Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) are the most common subtypes of lung cancer. [1] Although the morbidity and mortality rates of lung cancer vary by region and ethnicity, the overall prognosis of lung cancer is dismal. [2] For patients with lung cancer diagnosed at stage I-II, the overall survival rate is approximately 50%, while for those diagnosed at regional stage, it is approximately 20%, and for distant stage patients, it decreases to 4%. [3, 4] Clinically, the application of low-dose computed

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tomography (LDCT) has improved the detection rate of early-stage lung cancer. [5] However, the limitations of LDCT are its high false-positive rate (FPR), radiation exposure, and high cost. [6, 7] Therefore, establishing an efficient and cost-effective way to detect lung cancer remains a clinical challenge.

While traditional tumor biomarkers continue to play a role in patient management, the role of biomarkers for lung cancer has shown its advantage in molecularly targeted therapy and immune-checkpoint inhibitors for patients with advanced-stage lung cancer but is not satisfactory for early lung cancer screening. Emerging liquid biopsies, including protein or molecular biomarkers, provide an option to improve lung cancer diagnostics. In this paper, we discuss the clinical utility of established tumor markers for lung cancer and new candidates for further discovery. Importantly, standardization of these tumor markers in the clinical laboratory would be able to facilitate their value in clinical practice.

2. Established circulating protein biomarkers and their clinical use

2.1. Circulating protein biomarkers and their correlations with subtypes of lung cancer

While LDCT remains a sensitive screening tool for lung cancer diagnosis, circulating tumor markers specific to lung cancer and its histological type have been evaluated in clinical studies for their sensitivity and specificity (reviewed by Huang et al.). [8] Serum carcinoembryonic antigen (CEA), carbohydrate antigen 15.3 (CA15.3), squamous cell carcinoma–associated antigen (SCC-Ag), cytokeratin-19 fragment (CYFRA 21-1), neuron-specific enolase (NSE), and pro–gastrin-releasing peptide (ProGRP) have shown their value in facilitating patient monitoring. CEA, NSE and ProGRP are characterized as biomarkers for SCLC. CYFRA 21-1 and SCC-Ag are characterized as biomarkers for NSCLC. Furthermore, CEA is sensitive for lung adenocarcinoma (LUAD), while CYFRA 21-1 and SCC-Ag are sensitive for lung squamous cell carcinoma (LUSC). [8] An increase in serum cancer antigen 125 (CA125) is also seen in lung cancer, and its value in the diagnosis and prognosis of lung cancer warrants further study. [9] Interestingly, in a recent report based on a large-scale proteome analysis to identify circulating proteins biomarkers for risk of imminent lung cancer diagnosis demonstrates that 36 proteins including CA125/MUC-16 and CEACAM5/CEA were associated with imminent- but yet-to-be diagnosed lung cancer. [10]

2.2. Protein panels

To date, a panel of protein tumor markers demonstrates a better diagnostic or prognostic value than a single tumor marker. For example, a diagnostic prediction model based on CEA, CYFRA21-1 and NSE can discriminate patients with differentiated lung cancer from healthy controls and benign lung diseases, which are better than that of a single tumor marker. [11] Additionally, the prognostic prediction model also had good performance in predicting overall survival in lung cancer patients. [11] In another study, a combination of serum CEA, CA15.3, SCC-Ag, CYFRA 21-1, NSE, and ProGRP was assessed in a large cohort with the clinical suspicion of lung cancer, which had a sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 88.5, 82, 83.7, and 87.3%, respectively. [12] However, the NPV of this panel in patients with small nodules less than 1 cm was 91.4%, while in those with intermediate-sized nodules (1-3 cm), it was 60.8%. Thus, this panel may add value for lung cancer risk stratification but not for diagnostics. These studies demonstrate the combined value of serum tumor markers. In addition, the role of tumor markers in the differentiation of lung cancer subtypes was also explored. NSE and proGRP had higher expression levels in SCLC

than in other histological types. [12, 13] For lung squamous cell carcinoma (LUSC), CYFRA21-1 shows a high sensitivity, while SCC-Ag shows a high specificity but less sensitivity. [14, 15] In a recent study, a combination of CYFRA21-1, CEA, and human epididymis protein 4 (HE4) had a better performance on NSCLC, while a combination of ProGRP, NSE and HE4 was the best pattern for SCLC. [16] However, these serum biomarkers show less sensitivity for lung cancer diagnostics. On the one hand, the elevated expression level of tumor markers is seen not only in lung cancer but also in other malignancies. However, elevated levels of tumor markers are also seen in healthy people or noncancerous diseases, which is critical and limits their application in early lung cancer screening. [8]

Although the sensitivity of serum tumor markers used in lung cancer screening is generally low, the application of these tumor markers for the prediction of treatment response and prognosis may add value in disease monitoring. [8, 17] Notably, the reduction in serum CYFRA21-1 and NSE levels was associated with the disease control rate in patients with NSCLC who were treated with nivolumab. [18] This finding indicates that more clinical studies are necessary to further evaluate serum tumor markers for the prediction of the treatment response to immunotherapy.

3. Emerging biomarkers for liquid biopsy

Tissue tumor markers can be used for targeted therapy and immunotherapy when tumor tissues are available from biopsy at the time of diagnosis or during the whole treatment period. [19, 20] Examples include molecular tests of EGFR mutations, ALK gene rearrangements, ROS1, BRAF, NTRK, MET and RET for targeted therapies, tumor PD-L1 expression and tumor mutational burden for immune checkpoint inhibitors. For more detailed information, please refer to a recent review by Thai et al. [19] When the tumor tissues are difficult to obtain, circulating tumor DNA (ctDNA) extracted from the blood would be the compliment to tissue biopsy to identify patients who may benefit from targeted therapy or immunotherapy (reviewed by Duffy et al.). [21] The shorter half-life of ctDNA in the circulation made them more sensitive in reflecting tumor recurrence or response to treatments compared to the above established protein tumor markers. [21] Detecting ctDNA minimal residual disease (MRD) has shown value in improving survival outcomes (reviewed by Pellini et al.). [22] An ideal biomarker developed for clinical practice is simple, fast, cost effective, high sensitivity and specificity, and has reproducible detection. There are also limitations of current ctDNA assays due to their relatively long turn-around time and poor standardization. [21] Future large, randomized studies are still necessary to validate the clinical utility of ctDNA in different clinical settings.

Extracellular vesicles (EVs), including exosomes, microvesicles and apoptotic bodies, are membrane-delimited particles that are released from various types of cells. EVs derived from cancer cells carry information from their parental cells to receiving cells and play important roles in modulating the tumor microenvironment and promoting tumor progression; thus, EVs are attractive candidates for liquid biopsy. The cargo contents and corresponding functions of EVs were also studied in lung cancer (reviewed by Li et al.). [23] Accumulating evidence from mechanism-based studies of lung cancer cell-derived EVs has shown that cargo contents, including protein, DNA and various types of RNA, such as mRNA, lncRNA and microRNA, have the potential to serve as biomarkers for tumor diagnosis and prognosis. For example, the combination of serum exosomal PLA2G10 mRNA and protein showed better diagnostic power than CEA, CA125 and NSE for NSCLC in a small cohort study. [24] Three circular RNAs (circRNAs) in serum exosomes (circ_0047921, circ_0056285, and circ_0007761) have good discrimination ability for early-stage NSCLC from healthy controls, chronic obstructive pulmonary disease, or tuberculosis. [25] Plasma exosomal miR-181-5p, miR-30a-3p, miR-30e-3p and miR-361-5p were found to be adenocarcinoma specific, while miR-10b-5p, miR-15b-5p, and miR-320b were SCC-Ag specific. [26] These findings warrant further investigations in a large

cohort validation study. To establish clinically useful biomarkers based on EVs, more reproducible and less labor- or equipment-intensive methods for EV isolation and characterization need to be developed. To that end, nanodevices and microfluidics-based isolation or detection methods for EVs are currently an active field of research, and promising results have been reported (reviewed by Li et al.). [27]

4. Implementation for early-stage lung cancer diagnostics

Early-stage lung cancer diagnosis remains a major challenge. In a recent meta-analysis, the diagnostic performances of ctDNA (Sensitivity 0.50, specificity 0.98, AUC = 0.64), DNA methylation (Sensitivity 0.72, specificity 0.82, AUC = 0.84) and CTC (Sensitivity 0.68, specificity 0.85, AUC = 0.82) in the stage I disease were calculated. [28] Additionally, the concentrations of circulating cell-free DNA (cfDNA) were lower in stage I NSCLC compared to stages II and III (10.28 ng/ml vs. 12.72 ng/mal, 12.34 ng/ml, respectively). [28] As the authors concluded, the diagnostic accuracy would be limited by the lower concentration of cfDNA in plasma and influenced by clonal hematopoiesis of undetermined potential during aging leading to a fasle-postive ctDNA detection rates.

Recent studies highlight that metabolomics in cooperation with artificial intelligence detectors has the potential for the early detection of lung cancer (reviewed by Mariën et al.). [29] With the advent of single-cell RNA sequencing (scRNA-seq), early lung cancer was featured with dysregulation of lipid metabolism in a study, and further detection on plasma untargeted lipidomics identified nine lipid-based models that reached more than 90.00% sensitivity and 92.00% specificity in two validation cohorts. [30] In addition, a study on salivary metabolomics identified differential metabolites that can distinguish early lung cancer patients from healthy controls with a sensitivity of 97.2% and a specificity of 92%. [31] Importantly, the power of protein- or metabolite-based disease classifiers has the potential for rapid translation into clinical practice.

5. Achieving consensus for result comparison by standardization of tumor markers

Achieving a qualified analytical result with comparable analyte measurements is the main goal of clinical laboratories. [32] However, most tumor markers being used are non-FDA or other regulatory agency approved (are indeed physician-prescribed laboratory-developed tests (LDTs)). Non-FDA approved LDT is referred to as an *in vitro* diagnostic device (IVD) that has risen quickly in recent years. Moreover, the results are varied from lab to lab and even FDA-approved LDT conducted in a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory are not consistent and comparable. The use of different standards, calibrators, reagents, antibodies (even targeting different epitopes), etc., is likely attributed to the variation in the results. In this context, standardization of tumor markers by improving internal quality control (IQC) processes and participating in external quality assessment (EQA) programs or proficiency testing (PT) are important variations to improve the inconsistency of results across different instruments or lab procedures. [33, 34]

For serum tumor markers, the lack of international standards remains an obstacle to achieving harmonization when the method changes or different laboratory measurement procedures are used. [35, 36] Among those established serum tumor markers for lung cancer, there is an established WHO International Standard (IS) and International Reference Reagents (IRR) for CEA but not the others. [36] In a study of the EQA assessment of eight tumor markers (AFP, CEA, t-PSA, CA125, CA153, CA199) in China, there was an increasing trend of improved testing performance with an overall decrease in the robust coefficient of variability (CV) from 2006 to 2013. [33] However, the EQA of CEA did not show further improvement from 2010 to 2013. [33] Thus, to avoid bias, continuous test performance in

the same laboratory would be helpful to monitor patients. Making efforts to standardize or harmonize tumor markers requires multiple stakeholders, such as governmental agencies, regulatory agencies, the IVD industry, labs, and professional organizations, to be involved and engaged. [37]

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Author contributions

Q.M. contributed to conceiving the concept and supervision. X.Q. wrote the first draft of the manuscript. X.Q. and Q.M revised the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

References

[1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17-48. doi: 10.3322/caac.21763.

[2] Lu T, Yang X, Huang Y, Zhao M, Li M, Ma K, Yin J, Zhan C, Wang Q. Trends in the incidence, treatment, and survival of patients with lung cancer in the last four decades. Cancer Manag Res. 2019;11:943-53. Epub 20190121. doi: 10.2147/cmar.S187317.

[3] Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271-89. Epub 20160602. doi: 10.3322/caac.21349.

[4] Merritt RE, Abdel-Rasoul M, D'Souza DM, Kneuertz PJ. Racial Disparities in Overall Survival and Surgical Treatment for Early Stage Lung Cancer by Facility Type. Clin Lung Cancer. 2021;22(5):e691-e8. Epub 20210122. doi: 10.1016/j.cllc.2021.01.007.

[5] van Klaveren RJ, Oudkerk M, Prokop M, Scholten ET, Nackaerts K, Vernhout R, van Iersel CA, van den Bergh KA, van 't Westeinde S, van der Aalst C, Thunnissen E, Xu DM, Wang Y, Zhao Y, Gietema HA, de Hoop BJ, Groen HJ, de Bock GH, van Ooijen P, Weenink C, Verschakelen J, Lammers JW, Timens W, Willebrand D, Vink A, Mali W, de Koning HJ. Management of lung nodules detected by volume CT scanning. N Engl J Med. 2009;361(23):2221-9. doi: 10.1056/NEJMoa0906085.

[6] Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, Brawley OW, Wender RC. Cancer screening in the United States, 2017: A review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin. 2017;67(2):100-21. Epub 20170207. doi: 10.3322/caac.21392.

[7] Oudkerk M, Liu S, Heuvelmans MA, Walter JE, Field JK. Lung cancer LDCT screening and mortality reduction - evidence, pitfalls and future perspectives. Nat Rev Clin Oncol. 2021;18(3):135-51. Epub 20201012. doi: 10.1038/s41571-020-00432-6.

[8] Huang H, Yang Y, Zhu Y, Chen H, Yang Y, Zhang L, Li W. Blood protein biomarkers in lung cancer. Cancer Lett. 2022;551:215886. Epub 20220819. doi: 10.1016/j.canlet.2022.215886.

[9] Saad HM, Tourky GF, Al-Kuraishy HM, Al-Gareeb AI, Khattab AM, Elmasry SA, Alsayegh AA, Hakami ZH, Alsulimani A, Sabatier JM, Eid MW, Shaheen HM, Mohammed AA, Batiha GE, De Waard M. The Potential Role of MUC16 (CA125) Biomarker in Lung Cancer: A Magic Biomarker but with Adversity. Diagnostics (Basel). 2022;12(12). Epub 20221129. doi: 10.3390/diagnostics12122985.

[10] The blood proteome of imminent lung cancer diagnosis. Nat Commun. 2023;14(1):3042. Epub 20230601. doi: 10.1038/s41467-023-37979-8.

[11] Yuan J, Sun Y, Wang K, Wang Z, Li D, Fan M, Bu X, Chen J, Wu Z, Geng H, Wu J, Xu Y, Chen M, Ren H. Development and validation of reassigned CEA, CYFRA21-1 and NSE-based models for lung cancer diagnosis and prognosis prediction. BMC Cancer. 2022;22(1):686. Epub 20220622. doi: 10.1186/s12885-022-09728-5.
[12] Molina R, Marrades RM, Augé JM, Escudero JM, Viñolas N, Reguart N, Ramirez J, Filella X, Molins L, Agustí A. Assessment of a Combined Panel of Six Serum Tumor Markers for Lung Cancer. Am J Respir Crit Care Med. 2016;193(4):427-37. doi: 10.1164/rccm.201404-0603OC.
[13] Korse CM, Holdenrieder S, Zhi XY, Zhang X, Qiu L, Geistanger A, Lisy MR, Wehnl B, van den Broek D, Escudero JM, Standop J, Hu M, Molina R. Multicenter evaluation of a new progastrin-releasing peptide (ProGRP) immunoassay across Europe and China. Clin Chim Acta. 2015;438:388-95. Epub 20140928. doi: 10.1016/j.cca.2014.09.015.
[14] Wu H, Wang Q, Liu Q, Zhang Q, Huang Q, Yu Z. The Serum Tumor Markers in Combination for Clinical Diagnosis of Lung Cancer. Clin Lab. 2020;66(3). doi: 10.7754/Clin.Lab.2019.190533.
[15] Yang Q, Zhang P, Wu R, Lu K, Zhou H. Identifying the Best Marker Combination in CEA, CA125, CY211, NSE, and SCC for Lung Cancer Screening by Combining ROC Curve and Logistic Regression Analyses: Is It Feasible? Dis Markers. 2018;2018:2082840. Epub 20181001. doi: 10.1155/2018/2082840.
[16] Li M, Zhang Y, Jiang L, Li Y, Li G, Zhou J, Yang C, Li X, Qu W, Chen Y, Chen Q, Wang S, Xing J, Huang H. New insights into the diagnostic characteristics and clinical application of serum biomarkers for lung cancer, and human epididymis protein 4 as a new biomarker? Neoplasma. 2022;69(3):729-40. Epub 20220426. doi: 10.4149/neo_2022_220207N144.
[17] Holdenrieder S, Wehnl B, Hettwer K, Simon K, Uhlig S, Dayyani F. Carcinoembryonic antigen and cytokeratin-19 fragments for assessment of therapy response in non-small cell lung cancer: a systematic review and meta-analysis. Br J Cancer. 2017;116(8):1037-45. Epub 20170309. doi: 10.1038/bjc.2017.45.
[18] Dal Bello MG, Filiberti RA, Alama A, Orengo AM, Mussap M, Coco S, Vanni I, Boccardo S, Rijavec E, Genova C, Biello F, Barletta G, Rossi G, Tagliamento M, Maggioni C, Grossi F. The role of CEA, CYFRA21-1 and NSE in monitoring tumor response to Nivolumab in advanced non-small cell lung cancer (NSCLC) patients. J Transl Med. 2019;17(1):74. Epub 20190308. doi: 10.1186/s12967-019-1828-0.
[19] Melichar B. Biomarkers in the management of lung cancer: changing the practice of thoracic oncology. Clin Chem Lab Med. 2023;61(5):906-20. Epub 20221116. doi: 10.1515/cclm-2022-1108.
[20] Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. Lancet. 2021;398(10299):535-54. Epub 20210721. doi: 10.1016/s0140-6736(21)00312-3.
[21] Duffy MJ, Crown J. Circulating Tumor DNA as a Biomarker for Monitoring Patients with Solid Cancers: Comparison with Standard Protein Biomarkers. Clin Chem. 2022;68(11):1381-90. doi: 10.1093/clinchem/hvac121.
[22] Pellini B, Chaudhuri AA. Circulating Tumor DNA Minimal Residual Disease Detection of Non-Small-Cell Lung Cancer Treated With Curative Intent. J Clin Oncol. 2022;40(6):567-75. Epub 20220105. doi: 10.1200/jco.21.01929.
[23] Li MY, Liu LZ, Dong M. Progress on pivotal role and application of exosome in lung cancer carcinogenesis, diagnosis, therapy and prognosis. Mol Cancer. 2021;20(1):22. Epub 20210127. doi: 10.1186/s12943-021-01312-y.
[24] Chen Y, Ma X, Lou C, Zhou C, Zhao X, Li N, Tian H, Meng X. PLA2G10 incorporated in exosomes could be diagnostic and prognostic biomarker for non-small cell lung cancer. Clin Chim Acta. 2022;530:55-65. Epub 20220226. doi: 10.1016/j.cca.2022.02.016.
[25] Xian J, Su W, Liu L, Rao B, Lin M, Feng Y, Qiu F, Chen J, Zhou Q, Zhao Z, Lu J, Yang L. Identification of Three Circular RNA Cargoes in Serum Exosomes as Diagnostic Biomarkers of Non-Small-Cell Lung Cancer in the Chinese Population. J Mol Diagn. 2020;22(8):1096-108. Epub 20200612. doi: 10.1016/j.jmoldx.2020.05.011.
[26] Jin X, Chen Y, Chen H, Fei S, Chen D, Cai X, Liu L, Lin B, Su H, Zhao L, Su M, Pan H, Shen L, Xie D, Xie C. Evaluation of Tumor-Derived Exosomal miRNA as Potential Diagnostic Biomarkers for Early-Stage Non-Small Cell Lung Cancer Using Next-Generation Sequencing. Clin Cancer Res. 2017;23(17):5311-9. Epub 20170612. doi: 10.1158/1078-0432.Ccr-17-0577.
[27] Li L, Zhang L, Montgomery KC, Jiang L, Lyon CJ, Hu TY. Advanced technologies for molecular diagnosis of cancer: State of pre-clinical tumor-derived exosome liquid biopsies. Mater Today Bio. 2023;18:100538. Epub 20221229. doi: 10.1016/j.mtbio.2022.100538.
[28] Shen H, Jin Y, Zhao H, Wu M, Zhang K, Wei Z, Wang X, Wang Z, Li Y, Yang F, Wang J, Chen K. Potential clinical utility of liquid biopsy in early-stage non-small cell lung cancer. BMC Med. 2022;20(1):480. Epub 20221214. doi: 10.1186/s12916-022-02681-x.
[29] Mariën H, Derveaux E, Vanhove K, Adriaensens P, Thomeer M, Mesotten L. Changes in Metabolism as a Diagnostic Tool for Lung Cancer: Systematic Review. Metabolites. 2022;12(6). Epub 20220614. doi: 10.3390/metabo12060545.
[30] Wang G, Qiu M, Xing X, Zhou J, Yao H, Li M, Yin R, Hou Y, Li Y, Pan S, Huang Y, Yang F, Bai F, Nie H, Di S, Guo L, Meng Z, Wang J, Yin Y. Lung cancer scRNA-seq and lipidomics reveal aberrant lipid metabolism for early-stage diagnosis. Sci Transl Med. 2022;14(630):eabk2756. Epub 20220202. doi: 10.1126/scitranslmed.abk2756.

[31] Jiang X, Chen X, Chen Z, Yu J, Lou H, Wu J. High-Throughput Salivary Metabolite Profiling on an Ultralow Noise Tip-Enhanced Laser Desorption Ionization Mass Spectrometry Platform for Noninvasive Diagnosis of Early Lung Cancer. J Proteome Res. 2021;20(9):4346-56. Epub 20210803. doi: 10.1021/acs.jproteome.1c00310.

[32] Plebani M, Gillery P, Greaves RF, Lackner KJ, Lippi G, Melichar B, Payne DA, Schlattmann P. Rethinking internal quality control: the time is now. Clin Chem Lab Med. 2022;60(9):1316-7. Epub 20220629. doi: 10.1515/cclm-2022-0587.

[33] Xiao Y, Zhang C, Zhao H, Wang W, He F, Zhong K, Yuan S, Wang Z. Status of External Quality Assessment on Tumor Markers in China. Clin Lab. 2015;61(10):1383-90. doi: 10.7754/clin.lab.2015.150309.

[34] Dufraing K, Fenizia F, Torlakovic E, Wolstenholme N, Deans ZC, Rouleau E, Vyberg M, Parry S, Schuuring E, Dequeker EMC. Biomarker testing in oncology - Requirements for organizing external quality assessment programs to improve the performance of laboratory testing: revision of an expert opinion paper on behalf of IQNPath ABSL. Virchows Arch. 2021;478(3):553-65. Epub 20201013. doi: 10.1007/s00428-020-02928-z.

[35] Greg Miller W, Myers GL, Lou Gantzer M, Kahn SE, Schönbrunner ER, Thienpont LM, Bunk DM, Christenson RH, Eckfeldt JH, Lo SF, Nübling CM, Sturgeon CM. Roadmap for harmonization of clinical laboratory measurement procedures. Clin Chem. 2011;57(8):1108-17. Epub 20110615. doi: 10.1373/clinchem.2011.164012.

[36] Sturgeon C. Standardization of tumor markers - priorities identified through external quality assessment. Scand J Clin Lab Invest Suppl. 2016;245:S94-9. doi: 10.1080/00365513.2016.1210334.

[37] Armbruster D, Donnelly J. Harmonization of Clinical Laboratory Test Results: The Role of the IVD Industry. Ejifcc. 2016;27(1):37-47. Epub 20160209.