Original Article

Biomarker Potential of Preoperative Tumor Size in Determination of the Lymphovascular Invasion in Squamous Cell Lung Cancer and Lung Adenocarcinoma

Kemal Grbic, Aida Mujakovic[1], Orhan Lepara[2], Zahid Lepara[3], Edin Begic[4,5], Ferid Krupic[6]

Clinical Center University of Sarajevo, Clinic for Thoracic Surgery, [1]Department of Internal Medicine, Division of Pulmonary Diseases, General Hospital "Prim. Dr. Abdulah Nakas," [2]Department of Human Physiology, Faculty of Medicine, University of Sarajevo, [3]Clinical Center University of Sarajevo, Urology Clinic, [4]Department of Cardiology, General Hospital "Prim.Dr. Abdulah Nakas", [5]Department of Pharmacology, Sarajevo Medical School, Sarajevo School of Science and Technology, Sarajevo, Bosnia and Herzegovina, [6]Department of Anesthesiology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Abstract

Introduction: The invasion of blood and lymph vessels with tumor tissue represents a negative prognostic factor of the disease course in patients with non-small cell lung cancer. **Aim:** The aim of the study was to determine the marker value of a preoperatively determined size of pulmonary squamous cell carcinoma and adenocarcinoma and its impact on lymphovascular invasion (LVI) in resected lung tissue. **Materials and Methods:** The conducted observational cross-sectional study included 322 patients with a complete resection of confirmed squamous cell lung carcinoma and lung adenocarcinoma. Preoperative size and type of tumor were determined by a preoperative chest computed tomography scan and cytological/histological analysis of obtained samples, while LVI status was determined by pathohistological analysis of resected tumor lung tissue. Receiver operating characteristic (ROC) curve analysis was performed to assess whether tumor size could serve as a reliable marker for LVI. $P < 0.05$ was considered statically significant. **Results:** A statistically significant difference in the frequency of tumor size ($P = 0.580$) along with LVI ($P = 0.656$) was not established between the patients with squamous cell lung cancer and lung adenocarcinoma. A ratio between the size of lung adenocarcinoma and LVI status ($P < 0.001$) was determined as statistically significant, while such a difference was not established in squamous cell lung cancer ($P = 0.052$). The ROC analysis revealed that tumor size >39 mm in patients with lung adenocarcinoma has obtained a sensitivity of 70.8% and a specificity of 60.9% to differentiate patients with a LVI (areas under the curve [AUC] = 0.70; 95% CI 0.60–0.79; $P < 0.001$). A tumor size >4.6 cm in patients with squamous cell lung cancer obtained a sensitivity of 56.5% and a specificity of 60.3% to differentiate patients with a LVI (AUC = 0.59; 95% CI 0.50–0.67; $P = 0.043$). **Conclusion:** The preoperative size of lung adenocarcinoma could be an acceptable marker of LVI presence in resected lung tissue, while in the squamous cell lung cancer, a potential biomarker role of the preoperative size of the tumor was inadequate.

Keywords: *Lymphovascular invasion, nonsmall cell lung cancer, preoperative tumor size*

Introduction

Non-small cell lung cancer (NSCLC) has become a commonly diagnosed primary lung neoplasm, representing a frequent cause of mortality among malignant neoplasms in general.[1,2] Despite the fact that the new pathohistological classification implied an outstanding revision in categorization, diagnostic and therapeutic approach in tumors of glandular origin, nomenclature of the two most frequent lung tumors remained unchanged – squamous cell lung cancer and lung adenocarcinoma.[3]

Squamous cell lung cancers are tumors located in larger airways, featuring early signs and clinical symptoms onset, as well as a later metastasis appearance and statistical decrease in the frequency of occurrence. They are predominant in smokers, males, and the older population.[1,4,5] Lung adenocarcinomas usually appear in the periphery of the lung, with late symptom onset and diagnostic finding, and with a long-lasting increase in incidence. They often appear in nonsmokers, the younger population, females, and patients with a positive family history.[1,4,6]

The presence of lymphovascular invasion (LVI) in a tumor is related to a more frequent occurrence of local and distant metastasis, as well as an expected worse course of disease and prognosis in comparison to patients who did not

How to cite this article: Grbic K, Mujakovic A, Lepara O, Lepara Z, Begic E, Krupic F. Biomarker potential of preoperative tumor size in determination of the lymphovascular invasion in squamous cell lung cancer and lung adenocarcinoma. Int J App Basic Med Res 2021;11:95-9.

Submitted: 04-Aug-2020
Revised: 21-Jan-2021
Accepted: 24-Feb-2021
Published: 08-Apr-2021

Address for correspondence:
Dr. Edin Begic, Department of Cardiology, General Hospital "Prim.Dr. Abdulah Nakas", Sarajevo 71000, Bosnia and Herzegovina.
E-mail: edinbegic90@gmail.com

Access this article online

Website: www.ijabmr.org

DOI: 10.4103/ijabmr.IJABMR_500_20

Quick Response Code:

experience this pathohistological characteristic.[7-9] From the current research, it can be concluded that an increase in tumor size leads to an increase in LVI in resected lung tissue.[10-12] However, the preoperative tumor size according to which we can expect a presence of LVI in resected tissue samples with a certain level of certainty is not clearly defined.

Aim

The aim of this research is to determine the precise measurements and to evaluate the marker role of the preoperatively determined size of the tumor in the evaluation of LVI presence in resected tissue of squamous cell lung cancer and lung adenocarcinoma.

Materials and Methods

Study design and participants

The research was conducted as a cross-sectional study, which included 322 operatively treated patients due to previously diagnosed NSCLC at the Clinic for Thoracic Surgery, Clinical Center University of Sarajevo, from January 2018 to December 2019. Patients were divided into two groups: one group with lung adenocarcinoma and the other with squamous cell lung cancer, regardless of the size of resected tumor. The study precisely included patients with a preoperatively determined size of tumor as well as a cytological/histological classification of tumor, while the patients with mixed and transitional forms of malignancy were excluded. Due to the category of the study, informed consent of patients included in the research was not requested.

Methods

Preoperative diagnostic preparation and patient selection for the operative procedure were conducted at the Interdisciplinary Clinic for Pulmonary Diseases, Clinical Center University of Sarajevo. The standard diagnostic protocol consisted of an endoscopic examination of the tracheobronchial tree, tissue sampling for the cytological/histological diagnosis, a chest computed tomography (CT) scan, tumor operability/resectability evaluation, and a cardiologic and lung function status evaluation for the planned level of resection.

Preoperative bronchoscopy was performed by a previous application of inhalatory/local anesthetic agent with available flexible bronchoscopes of different types (Manufacturer Olympus). While the bronchoscopy was performed, tissue and liquid samples for cytological and/or histological analysis were obtained. The tissue biopsies implied sampling of endoluminal tumor infiltrates, as well as transtracheal and transbronchial needle biopsies. In tumors of the periphery, transthoracic needle biopsies with CT or ultrasound guidance were obtained. The obtained samples after a routine procedural checkup were transferred for further analysis to a thoracic pathologist.

The precisely determined preoperative tumor size was performed at the Clinic for Radiology, Clinical Center University of Sarajevo, during chest CT scanning (from the neck to the inferior kidney poles, in slices [ranging: 0.625 mm]). For diagnostic purposes, a 64-multisliceCT scan (Marke GE LightSpeed VCT; General Electric Company, Fairfield, Connecticut, USA) was used. Native and contrast series (Ultravist 370, Schering, Germany) were performed as well as lung parenchyma module with a 3D reconstruction. Scans were analyzed by a thoracic radiologist, and beyond other parameters, tumor dimensions were determined in three planes (anteroposterior, laterolateral, and craniocaudal). The analysis was performed according to the maximal measure expressed in millimeters (mm).

Resection procedures were performed at the Clinic for Thoracic Surgery, Clinical Center University of Sarajevo. Standard thoracotomies with an anterolateral or posterolateral approach were performed under general anesthesia with the use of Carlens endotracheal tube, having patients situated in a decubital position. A thoracoscopic exploration of the pleural cavity was performed on every patient prior to the procedure. Resection of lung parenchyma together with bronchial and vascular structure sutures was performed with differently shaped and sized staplers originating from numerous manufacturers, or double proximal transfixational ligatures were placed. Resected lung tissue samples were transferred to a thoracic pathologist for further analysis.

The pathohistological analysis of the resected lung tissue specimen was performed at the Department of Clinical Cytology and Pathology, Clinical Center University of Sarajevo. After primary sectioning of the specimen and 24 h of fixation in 10% buffered formalin, the resected lung tissue specimen was cut into slices 4–5 mm thick. Then, samples of the tumor were put into cassettes for a further automatic tissue processing in the tissue processor "Logos One Milestone" according to the manufacturer's protocol. Further on, samples were embedded into paraffin blocks, cut with a microtome into 3–5 μm section slices, and stained with the standard H and E (HE) method. Microscopic examination of stained slides determined the type of tumor according to the current classification of lung tumors 4th ed.ition.[3] The unequivocal presence of LVI is referred to as LVI+ or absent as LVI negative (LVI−).

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software. The distribution of variables was tested by the Shapiro–Wilk test. In the analysis of the dependence between categorical variables, the Chi-square test was performed. Depending on the distribution of variables, a comparison between the groups was performed using the Mann–Whitney U-test. To determine the optimal cutoff values of potential biomarkers for differentiation between

LVI+ and LVI–, receiver operating characteristic (ROC) curves and their corresponding areas under the curve (AUC) were used. Statistical significance was set at $P < 0.05$.

Results

In the group of patients with lung adenocarcinoma ($n = 140$), 91 (65%) of them were males, while 49 (35.0%) were females. In the group of patients with squamous cell lung cancer ($n = 182$), 160 (87.9%) were males, while 22 (12.1%) were females. The established difference in gender distribution between groups was statistically significant ($P < 0.001$). Age, tumor size, and presence of LVI displayed no statistically significant difference between the groups [Table 1].

In patients with lung adenocarcinoma and the presence of LVI, the median tumor size was 50.0 mm (35.0–75.0) and was statistically significant compared to the size of tumor in patients with the same type of tumor without LVI, amounting to 35.0 mm (20.25–50.0); ($P < 0.001$). The median size of squamous cell lung cancer in patients with LVI present in the tumor was 50.0 mm (35.0–65.0) and a statistically significant difference was not established compared to the same tumor size without LVI 40.0 mm (30.0–60.0); ($P = 0.052$). The tumor size did not differ with statistical significance between patients with lung adenocarcinoma and squamous cell lung cancer stratified according to LVI status ($P = 0.065$; $P = 0.633$); [Figure 1].

In the detection of patients presenting LVI in lung adenocarcinoma, the borderline preoperative tumor size accounted for >39 mm, sensitivity amounted to 70.8%, specificity: 60.9%, positive predictive value: 87.8%, and negative predictive value: 76.3%. AUC for the size of tumor amounted to 0.70; $P < 0.001$ [Figure 2].

In squamous cell lung cancer having presence of LVI in the tumor and borderline values of >46 mm, sensitivity amounted to 56.5%, specificity: 60.3%, positive predictive value: 52.5%, and negative predictive value: 81.5%. AUC for the tumor size amounted to 0.59; $P = 0.043$ [Figure 3].

Discussion

In the observed group of patients in this study, an increased incidence of squamous lung cancer compared to lung adenocarcinoma (57% vs. 43%) does not correlate with most of the statistical reports, which define the predominant representation of lung adenocarcinoma in the NSCLC group.[1,2,13] According to the last report from the American Cancer Society, lung adenocarcinoma accounts for 45%, squamous cell lung cancer for 30%, while other NSCLC makes up the rest.[1] Statistical data from German-speaking countries denote an increase of lung adenocarcinoma amounting to 40%–50%, while the remainder represents squamous cell lung cancer and other primary lung tumors.[2] In some parts of Japan, lung adenocarcinoma accounts for 60% among patients suffering from NSCLC.[13]

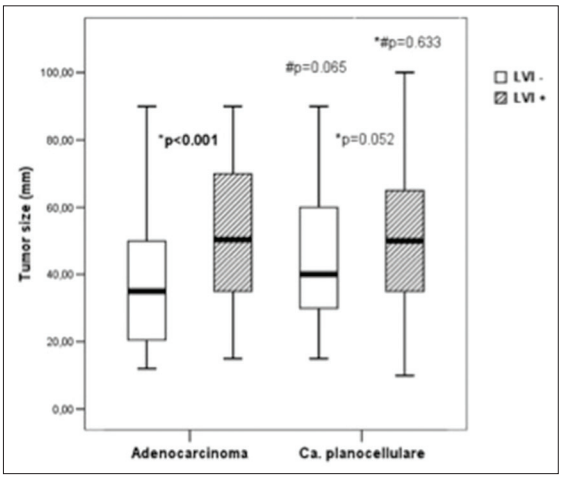

Figure 1: Comparison of tumor size and LVI status. *Compared to tumor size of the same patient group; #Compared to squamous cell lung cancer and lung adenocarcinoma without the presence of LVI; *#Compared to squamous cell lung cancer and lung adenocarcinoma with the presence of LVI

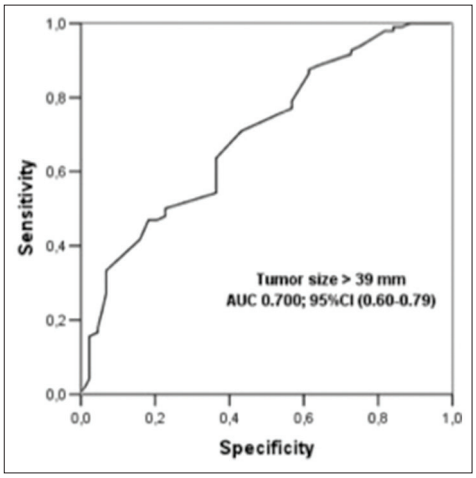

Figure 2: ROC curve of tumor size as a potential marker of lymphovascular invasion appearance in lung adenocarcinoma. ROC: Receiver Operating Characteristics, AUC: Area under the curve, CI: Confidence interval

Table 1: Baseline characteristics of observed variables

Variable	Group: Lung adenocarcinoma (n=140)	Group: Squamous cell lung cancer (n=182)	P
Gender (male/female), n (%)	91 (65.0)/49 (35.0)	160 (87.9)/22 (12.1)	<0.001
Age (years)	63.0 (59.0–66.0)	63.0 (60.0–67.0)	0.064
Tumor size (mm)	45.0 (30–61.5)	50.0 (32.0–60.0)	0.580
LVI present, n (%)	96 (68.6)	129 (70.9)	0.656

Results are displayed as absolute numbers (n), as well as percentage values (%). LVI: Lymphovascular invasion

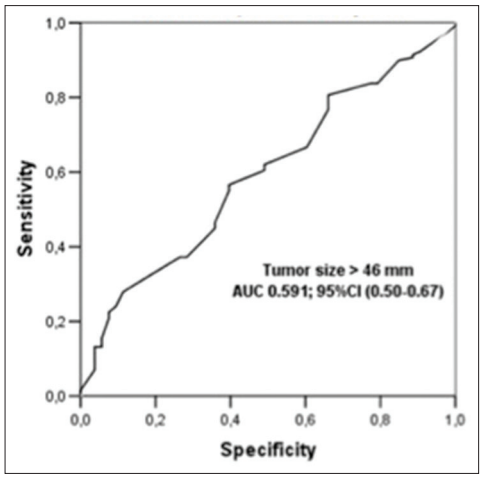

Figure 3: ROC curve of tumor size as a potential marker of lymphovascular invasion in squamous cell lung cancer. ROC: Receiver operating characteristics, AUC: Area under the curve, CI: Confidence interval

In the group of patients suffering from lung adenocarcinoma, males were more represented accounting for 65% and the average age being 63 years, even though current data reveals that with this type of lung tumor, females younger in age are more affected.[1,2,6] Age and gender structure of patients suffering from squamous lung cancer correlates with the demographic data of patients from other resources.[2,4,6] The incidence of LVI in resected lung adenocarcinomas of our patients is 68.6% and is slightly decreased in comparison to the report accounting for current lung cancer classification where this pathohistological finding appears in 70%–90% of cases.[3] However, there are studies with a decreased incidence of LVI in resected samples, taking into consideration all sizes of resected tumors.[4,7,10]

The results of this study have demonstrated that in the group of patients with squamous cell lung cancer, a statistically significant difference in tumor size between LVI+ to LVI− status was not determined, while in patients with lung adenocarcinoma, a statistically significant difference was determined. In this group of patients, the tumor size was increased in LVI+ patients correlating with other research which determined the presence of a positive correlation between tumor size and LVI status in lung adenocarcinoma.[12,14,15]

In the resected tumor lung tissue after lobectomy, 122 patients suffering from lung adenocarcinoma sized up to 1 cm, the study of *Igai et al*. determined the presence of LVI in only 9% of patients.[12] In a 10-year follow-up of 229 operatively treated patients with lung adenocarcionoma sized up to 2 cm, Funai *et al.* determined the presence of LVI in only 15% of resected lung tumors.[14] In the study of Higgins *et al.*, out of 734 operatively treated patients with lung adenocarcinoma sized up to 3 cm, LVI was confirmed in 22% of patients.[15]

The statistical analysis of variables in this research defined that the preoperative size of lung adenocarcinoma with the borderline value of 39 mm can be an acceptable marker for LVI presence in a tumor (AUC = 0.7). Due to inadequate patient selection (AUC = 0.6) in those with squamous cell lung cancer, the preoperative tumor size represents an inadequate marker of LVI presence in resected tumor lung tissue. In the available literature, we did not find studies analyzing the marker role of the preoperative tumor size on LVI status by ROC curve application. However, the studies displayed a significant predictive role of preoperative NSCLC size on the appearance of nodal metastases, free relapse period, and overall survival.[16-18]

The application of other statistical tests in the research proved that the CT scan determined the value of lung adenocarcinoma has a significant prognostic role in the appearance of LVI and the course of the disease.[19-21] Suh *et al.* followed 988 operatively treated patients during 44.7 months, and through the use of Cox model, it was shown that CT scan characteristics of the tumor, including size, have prognostic values on LVI status and relapse of the disease.[19] Similar results were presented in the study of Lee *et al.* who followed 275 operatively treated patients with lung adenocarcinoma.[20] The group of Corean authors used the Kaplan–Meier curve in 723 operatively treated patients to present the predictive role of lung adenocarcinoma size in LVI appearance, free progression disease period, and patients' overall survival.[21]

Conclusion

From the provided information above, it is concluded that the preoperative size of squamous cell lung cancer is unacceptable and inadequate as a marker, while the dimension of lung adenocarcinoma amounting to 39 mm is an acceptable marker of LVI+ status in resected tumor lung tissue. In clinical practice, the listed value could provide help in determining the treatment modality and could present the parameter of expected intraoperative finding and postoperative course of the disease.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. American Cancer Society/Cancer Fact & Figures/Lung Cancer 2019. Available from: http://www.cancer.org/acs/groups/cid/documents/webcontent/003115-pdf.pdf. [Last accessed on 2020 Apr 26].
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
3. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin HM, Beasley MB, *et al.* The 2015 World Healt Organization Classification of lung tumors impact of genetic, clinical and

radiologic advances since the 2004 classification. J Thorac Oncol 2015;10:1243-60.

4. Regina G, Pedro L, Rebeca C, Canabate M, Dolores TM. Ten years of lung cancer in a single center: Gender, histology, stage and survival. J Cancer Metastasis Treat 2015;1:201-7.
5. Oncopedia, Lungenkarzinom, Nicht-Kleinzellig (NSCLC). Stand; October 2019. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/lungenkarzinom-nicht-kleinzellig-nsclc/@@guideline/html/index.html. [Last accessed on 2020 May 10].
6. Lortet-Tieulent J, Soerjomataram I, Ferlay J, Rutherford M, Weiderpass E, Bray F. International trends in lung cancer incidence by histological subtype: Adenocarcinoma stabilizing in men but still increasing in women. Lung Cancer 2014;84:13-22.
7. Okiror L, Harling L, Toufektzian L, King J, Routledge T, Harrison-Phipps K, *et al*. Prognostic factors including lymphovascular invasion on survival for resected non-small cell lung cancer. J Thorac Cardiovasc Surg 2018;156:785-93.
8. Sung SY, Kwak YK, Lee SW, Jo IY, Park JK, Kim KS, *et al*. Lymphovascular invasion increases the risk of nodal and distant recurrence in node-negative stage I-IIA non-small-cell lung cancer. Oncology 2018;95:156-62.
9. Mollberg NM, Bennette C, Howell E, Backhus L, Devine B, Ferguson MK. Lymphovascular invasion as a prognostic indicator in stage I non-small cell lung cancer: A systematic review and meta-analysis. Ann Thorac Surg 2014;97:965-71.
10. Grbić K, Mehić B. Characteristics of lymphovascular metastatic spread in lung adenocarcinoma according to the primary cancer location. Med Glas (Zenica) 2020;17:66-72.
11. Wang S, Zhang B, Qian J, Qiao R, Xu J, Zhang L, *et al*. Proposal on incorporating lymphovascular invasion as a T-descriptor for stage I lung cancer. Lung Cancer 2018;125:245-52.
12. Igai H, Matsuura N, Tarumi S, Chang SS, Misaki N, Ishikawa S, *et al*. Prognostic factors in patients after lobectomy for p-T1aN0M0 adenocarcinoma. Eur J Cardiothorac Surg 2012;41:603-6.
13. Forman D, Bray F, Brewster DH, Gombe Mbalawa C, Kohler B, Pineros M, Steliarova-Foucher E, Swaminathan R, Ferlay J. Cancer Incidence in Five Continents. Vol 10. Lyon: International Agency for Research on Cancer (IARC); 2014. Available from: https://ci5.iarc.fr/CI5I-X/old/vol10/CI5vol10.pdf. [Last accessed on 2020 May 24].
14. Funai K, Sugimura H, Morita T, Shundo Y, Shimizu K, Shiiya N. Lymphatic vessel invasion is a significant prognostic indicator in stage IA lung adenocarcinoma. Ann Surg Oncol 2011;18:2968-72.
15. Higgins KA, Chino JP, Ready N, D'Amico TA, Berry MF, Sporn T, *et al*. Lymphovascular invasion in non-small-cell lung cancer: Implications for staging and adjuvant therapy. J Thorac Oncol 2012;7:1141-7.
16. Seok Y, Yang HC, Kim TJ, Lee KW, Kim K, Jheon S, *et al*. Frequency of lymph node metastasis according to the size of tumors in resected pulmonary adenocarcinoma with a size of 30 mm or smaller. J Thorac Oncol 2014;9:818-24.
17. Kim H, Goo JM, Kim YT, Park CM. Clinical T category of non-small cell lung cancers: Prognostic performance of unidimensional versus bidimensional measurements at CT. Radiology 2019;290:807-13.
18. Zhang J, Gold KA, Lin HY, Swisher SG, Xing Y, Lee JJ, *et al*. Relationship between tumor size and survival in non-small-cell lung cancer (NSCLC): An analysis of the surveillance, epidemiology, and end results (SEER) registry. J Thorac Oncol 2015;10:682-90.
19. Suh YJ, Lee HJ, Kim YT, Kang CH, Park IK, Jeon YK, *et al*. Added prognostic value of CT characteristics and IASLC/ATS/ERS histologic subtype in surgically resected lung adenocarcinomas. Lung Cancer 2018;120:130-6.
20. Koo HJ, Xu H, Choi CM, Song JS, Kim HR, Lee JB, *et al*. Preoperative CT predicting recurrence of surgically resected adenocarcinoma of the lung. Medicine (Baltimore) 2016;95:e2513.
21. Lee HJ, Lee SW, Lee KS, Jeong JY, Choi JY, Kwon OJ, *et al*. Role of CT and PET inaging in predicting tumor recurrence and survival in patients with lung adenocarcinoma. J Thorac Oncol 2015;10:1785-94.