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DISCRIMINANT ANALYSIS OF NUCLEAR IMAGE VARIABLES IN LUNG CARCINOMA

Žaklina Mijović[1], Dragan Mihailović[1], Miloš Kostov[2]

[1]*Medical Faculty, Institute of Pathology, Niš*
[2]*Military Hospital, Department of Pathology, Niš*
E-mail: zami@bankerinter.net

Summary. *Histological classification of lung cancer is subjective and often difficult to reproduce on bronchoscopic biopsies. The aim of this study was to identify which of seven karyometric variables are of diagnostic value in distinguishing major histological types of lung carcinoma. Bronchoscopic mucosal samples from patients with squamous cell carcinoma (n=48), small cell carcinoma (n=41), and adenocarcinoma (n=35) of the lung were retrieved. Specimens were stained with hematoxylin and eosin, and measured by image analyzer. All measured nuclear variables were found to be significantly different between small cell carcinoma and non-small cell carcinoma ($p<0.01$). Using stepwise discriminant function analysis, a correct diagnosis was achieved in 92.7% of non-small cell carcinomas and 90.24% of small cell carcinomas. The total percent of correct classification was 91.93%. The best discriminant variables between these categories of lung carcinoma were nuclear perimeter and area. In conclusion, nuclear image analysis can be used to make distinction between major histological types of lung carcinoma in the biopsy specimens, especially between small cell lung carcinoma and non-small cell lung carcinoma.*

Key words: *Computer-assisted diagnosis, image analysis, lung carcinoma.*

Introduction

With more than 1.1 million deaths annually worldwide, lung cancer is the most frequent and one of the most deadliest cancer types. In men, 85-90% of cases can be attributed to tobacco smoking. The prognosis of lung cancer is still poor, with 5-years survival rates of approximately 10% in most countries (1).

A histological classification should provide guidelines for tumor diagnosis in order to evaluate patient prognosis and therapy. Carcinomas of the lung are classified into four major types: small cell carcinomas, squamous cell carcinomas, adenocarcinomas and large cell carcinomas (1). While the WHO classification for lung carcinomas appears relatively straightforward, it may prove difficult to apply in individual cases. A major reason for this is the histological heterogeneity of lung carcinomas (2). Histological phenotype is the result of multiple differentiation potentialities of individual tumor cells and different cell types can occur in the same tumor. Therefore, it is not surprising that an interobserver variation is present in diagnoses made by expert pathologists (3).

Malignant lung neoplasms are currently clustered in practice into two groups with distinct clinico-pathological features: small cell carcinoma and non-small cell carcinoma. Although small cell carcinoma are usually treated by chemotherapy with or without radiotherapy, the best therapeutic opportunity for patients with non-small cell carcinoma is surgery. The classification of lung neoplasms into these two major groups can be difficult when limited diagnostic material is available. In doubtful cases additional classification methods may be helpful, particularly karyometry. In order to define quantitative differentiating criteria, we performed image analysis of primary carcinoma of the lung in biopsy specimens**.** By quantitative nuclear image analysis the morphology of the nucleus is described by a number of mathematical parameters (4).

The aim of this study was to identify which of seven karyometric variables are of diagnostic value in distinguishing major histological types of lung carcinoma**.**

Material and Methods

At the Institute of Pathology, University of Nis, formalin fixed, paraffin embedded bronchoscopic mucosal samples from patients with squamous cell carcinoma (n=48), patients with small cell carcinoma (n=41), and patients with adenocarcinoma (n=35) of the lung were retrieved from pulmonary pathology archives. Serial histological sections of 4 μm thickness were stained with hematoxylin and eosin in linear staining sistem Leica ST 4040 (Leica Microsystems, Nussloch, Germany). The slides were measured by image analyzer Lucia M 3.51 ab (Laboratory Imaging, Prague, Czeck Republic), using a 40:1 objective (NA=0.65) of FXA microscope (Nikon, Tokyo, Japan). The binary images were manually edited. Seven nuclear variables were

estimated: nuclear area, equivalent diameter, volume of equivalent sphere, perimeter, mean chord, circularity and total optical density (5). In each case a hundred of non-overlapping, non-crushed tumor cell nuclei were measured.

The nuclear area is the number of pixels.

The equivalent (E_q) diameter is the diameter of a circle having the same area as the corresponding object:

$$E_q\ diameter = \sqrt{4 \cdot area / \pi}\ .$$

Volume E_q sphere is the volume of the ball:

$$Volume\ E_q\ sphere = \frac{\pi}{6} \cdot \mathrm{E_q\,diameter}^3.$$

Perimeter is derived from four projections in the directions 0^0, 45^0, 90^0 and 135^0:

$$Perimeter = \pi\ (Pr_0 + Pr_{45} + Pr_{90} + Pr_{135}) / 4.$$

Mean chord is the mean value of secants in directions 0^0, 45^0, 90^0 and 135^0:

$$Mean\ Chord = 4\ \text{area} / Pr_0 + Pr_{45} + Pr_{90} + Pr_{135.}$$

Circularity is derived a shape measure, calculated from the area and perimeter:

$$Circularity = 4\pi \cdot \text{area}/\ \text{perimeter}^{\,2}.$$

Total optical density value is the sum of individual optical density (O.D.) of each pixel in the area being measured:

$$O.D. = -\log\ (\text{Pixel Gray Value} + 0.5) / 62.5$$

For statistical analysis, stepwise linear discriminant analysis and the Mann-Whitney test were used. Discriminant function analysis was used to determine which karyometric variables discriminate between major histological types of lung carcinoma, with Wilks' lambda for the overall model that will result after removing of respective variable, and partial lambda which is the Wilks' lambda associated with the unique contribution of the respective variable to the discriminatory power of the model.

Results

The values of the nuclear variables from major histological types of primary lung carcinomas that were assessed are listed in Table 1. All measured seven nuclear variables were found to be significantly different between squamous cell carcinoma and small cell carcinoma of the lung ($p<0.01$). The values of nuclear variables (except circularity) of squamous cell carcinoma of the lung were significantly larger than in small cell carcinoma. Similarly, statistically significant differences were found between adenocarcinoma and small cell lung carcinoma ($p<0.01$). No significant differences were found among squamous cell carcinoma and adenocarcinoma of the lung for measured nuclear variables ($p>0.05$), except total optical density. Total nuclear optical density in adenocarinoma was significantly larger than in squamous cell carcinoma of the lung ($p<0.05$) (Table 1).

Since no significant discrimination was possible between the squamous cell carcinoma and adenocarcinoma (Wilks' lambda =0.93, F =3.03, $p>0.05$), the attention was confined to the discrimination between small cell lung carcinoma (Fig. 1) and non-small cell lung carcinoma (Fig 2, squamous cell carcinoma and adenocarcinoma). All measured nuclear variables were found to be significantly different between these two categories of lung carcinoma ($p<0.01$). The values of nuclear variables (except circularity) of non-small cell carcinoma (Table 2) were significantly larger than in small cell lung carcinoma ($p<0.01$). Using stepwise linear discriminant function analysis, the best discriminant variables between these two categories of lung carcinoma were nuclear perimeter and area (Table 3). A correct di-

Table 1. Nuclear variables in squamous cell carcinoma, adenocarcinoma and small cell carcinoma of the lung

Histologic. types of lung carcinoma	Squamous cell carcinoma (n=48)	Adenocarcinoma (n=35)	Small cell carcinoma (n=41)
Variables	X±SD (95% CI)	X±SD (95%CI)	X±SD (95%CI)
Nuclear area (μm^2)	35.17±9.54 (32.39-37.94)	35.70±8.61 (32.74-38.66)	20.12±4.59* (18.67-21.57)
Equivalent diameter (μm)	6.53±0.85 (6.28-6.77)	6.59±0.79 (6.32-6.86)	4.98±0.56* (4.80-5.16)
Volume of equivalent sphere (μm^3)	169.61±73.51 (148.27-190.96)	172.18±62.76 (150.62-193.74)	71.54±24.99* (63.65-79.43)
Perimeter (μm)	21.88±2.74 (21.09-22.68)	22.03±2.70 (21.10-22.96)	16.32±1.83* (15.75-16.90)
Mean chord (μm)	4.82±0.66 (4.63-5.01)	4.88±0.59 (4.68-5.08)	3.74±0.44* (3.60-3.88)
Circularity	0.88±0.03 (0.87-0.89)	0.89±0.02 (0.88-0.89)	0.92±0.03* (0.91-0.93)
Total optical density	325.02±103.67 (294.92-355.12)	376.32±106.26** (339.82-412.82)	250.03±52.07* (233.59-266.46)

X – mean; SD – standard deviation; 95% CI – lower and upper 95% confidence interval.

*$p<0.01$ – small cell carcinoma versus other two types, **$p<0.05$ – squamous cell carcinoma versus adenocarcinoma

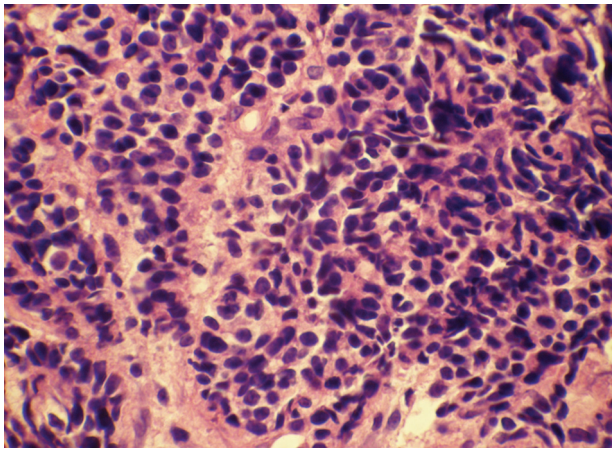

Fig 1. Small cell carcinoma of the lung (HE, obj.x40).

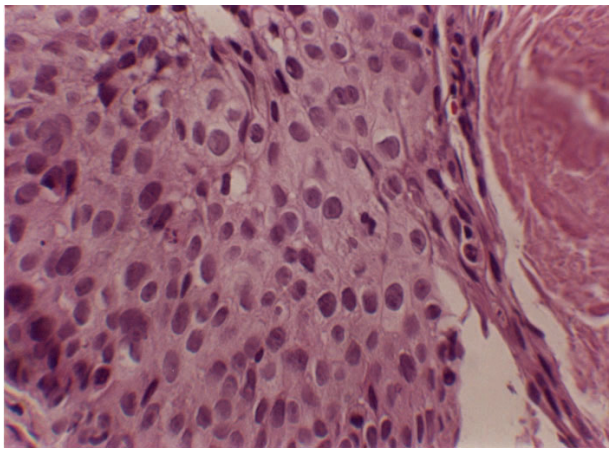

Fig 2. Non-small cell carcinoma of the lung (HE, obj.x40).

agnosis was achieved in 92.7% of non-small cell carcinomas and 90.24% of small cell carcinomas. The total percent of correct classification was 91.93% (Table 4).

Table 2. Nuclear variables in non-small cell and small cell carcinoma of the lung

	Non-small cell carcinoma of the lung (n=83)	Small cell carcinoma (n=41)
Variables	X±SD (95% CI)	X±SD (95%CI)
Nuclear area (μm^2)	35.39±9.11 (33.40-37.38)	20.12±4.59* (18.67-21.57)
Eq diameter (μm)	6.56±0.82 (6.38-6.74)	4.98±0.56* (4.80-5.16)
Volume Eq sphere (μm^3)	170.69±68.79 (155.67-185.71)	71.54±24.99* (63.65-79.43)
Perimeter (μm)	21.95±2.71 (21.35-22.54)	16.32±1.83* (15.75-16.90)
Mean chord (μm)	4.85±0.63 (4.71-4.98)	3.74±0.44* (3.60-3.88)
Circularity	0.89±0.03 (0.88-0.89)	0.92±0.03* (0.91-0.93)
Total optical density	346.66±107.20 (323.25-370.06)	250.03±52.07* (233.59-266.46)

*p<0.01 – small cell carcinoma versus non-small cell carcinoma

Six cases of non-small cell carcinoma were misclassified in a small cell group by discriminant analysis. Two of them were a small cell variant of squamous cell carcinomas and four were adenocarcinomas. Four small cell carcinomas were misclassified in a non-small cell group, but after histological revision they were combined small cell carcinomas (small cell carcinoma with squamous cell carcinoma).

Discussion

Although the lung is an organ in which many histological types of malignant epithelial tumor can develop, about 95% of cancers occurring there are of four major histological types: squamous cell carcinoma, small cell carcinoma, adenocarcinoma and large cell carcinoma. The distinction between these four major types of lung carcinoma on routine bronchoscopic biopsies sometimes presents diagnostic problem (2, 6). Materials obtained under fiberoptic bronchoscopic guidance are often very small and are sometimes crushed, particularly in the case of small cell carcinoma. Also, in small tissue fragments, differentiated tumor features (such as a glandular structure or keratinization) may not be presented. Histological diagnosis of large cell carcinoma is made after the exclusion of squamous cell carcinoma, small cell carcinoma, adenocarcinoma, and other lung cancers of a

Table 3. Discriminant function analysis of non-small cell carcinoma (n=83) and small cell carcinoma (n=41)

Variables	Wilks Lambda	Partial Lambda	F-remove (1,121)	p
Perimeter (μm)	0.54	0.65	65.67	p< 0.01
Area (μm^2)	0.46	0.77	36.13	p< 0.01

Table 4. Classification matrix of non-small cell carcinoma (n=83) and small cell lung carcinoma (n=41)

Histopathologic classification	Number of cases	Percent of correct computer classification	Non-small cell carcinoma	Small cell carcinoma
Non-small cell carcinoma	83	92.7	77	6
Small cell carcinoma	41	90.24	4	37
Total	124	91.93	81	43

specific type (especially main groups like large cell neuroendocrine carcinoma and carcinoids) (1). Because diagnosis of large cell carcinoma based on small tissue fragments may not agree with the one based on tissue from resected lung tumor, this major type of lung carcinoma was excluded from the measurement procedure. As tumor differentiation is often variable within a given tumor, a diagnosis of large cell lung carcinoma requires examination of the entire tumor to rule out areas of squamous or glandular differentiation (7).

Histological classification of lung cancer is subjective and often difficult to reproduce. Image analysis permits pathologists to obtain quantitative measurements on histological specimens, so that visual impressions can be augmented by quantitative morphometry (8). For correct results, standardization of staining prior to analysis is very important. In our study, a staining machine was used. Image analysis is cheaper than immunohistochemistry and therefore meaningful in routine diagnosis. In order to obtain additional differentiating criteria for major histological types of lung carcinoma, in the present study, we estimated seven quantitative nuclear image features. We identified significant differences with regard to nuclear size, shape and optical density between squamous cell carcinoma and small cell carcinoma of the lung. The mean values of all measured nuclear variables (except circularity) of squamous cell carcinoma were significantly larger than in small cell carcinoma of the lung. Our findings are in agreement with other studies (3, 9, 10).

No significant differences were found among squamous cell carcinoma and adenocarcinoma of the lung for the measured nuclear variables except total optical density. Total optical density in adenocarinoma was significantly larger than in squamous cell carcinoma of the lung.

The results of this study show significant differences for all measured seven nuclear variables between small cell and non-small cell carcinomas in biopsy specimens**.** The best discriminant variables between these two categories of lung carcinoma were nuclear perimeter and area, reflecting nuclear size. In a study of Kavantzas and colleagues (11) statistically significant differences were found among small cell and non-small cell lung carcinoma in nuclear size (minor axis), similarly to our results. The total percent of correct classification was 91.93%, in agreement with result of other reports (3, 12). Nuclear texture feature analysis has repeatedly been used to discriminate between malignant tumors of the lung. Nuclear texture features describe DNA distribution patterns in cell nuclei, and can be reliably measured by high resolution image cytometry, both in tissue sections as well as in single cell preparations such as smears from fine needle aspirates or cell suspension preparations. By applying the classification rule that described the granularity of the nuclear chromatin (defined by four different parameters), Schmid and colleagues (12) correctly discriminated small cell and non-small cell lung carcinoma in 93%. In the present study, the nuclear texture features were not estimated, but the overall percent of correct classification of the small cell and non-small cell lung carcinoma was very similar.

Small cell variant of squamous cell carcinomas, which were misclassified in our study, were poorly differentiated variants of squamous cell carcinomas with small tumour cells that retained cellular characteristics of a non-small cell carcinoma such as coarse chromatin, nucleoli, distinct cell borders and showed focal squamous differentiation, without areas of necrosis. Also, adenocarcinomas that were incorrectly classified were adenocarcinomas with small tumor cells as an effect of biopsy crushing. Combined small cell carcinomas, which were misclassified, were a mixture of squamous cell carcinoma and small cell carcinoma.

Conclusion

Quantitative nuclear image analysis can be used to make a distinction between major histological types of lung carcinoma in the biopsy specimens, especially between categories small cell lung carcinoma and non-small cell carcinoma. This quantitative measurement should be used in clinical practice as an ancillary technique. Using discriminant function analysis, nuclear perimeter and area appeared to have a major potential for the therapeutically and prognostically important differentiating small cell carcinomas from non- small cell carcinomas of the lung.

References

1. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. World Health Organization Classification of Tumours. Pathology end Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Press, Lyon, 2004.
2. Colby T, Koss M, Travis W. Tumors of the Lower Respiratory Tract. Armed Forces Institute of Pathology,Washington, 1995.
3. Thunnissen F, Diegenbach P, Hattum A et al. Further evaluation of quantitative nuclear image features for classification of lung carcinomas. Path Res Pract 1992; 188: 531-535.
4. Thunnissen FB, Diegenbach PC, Dingemans KP, Hattum AH, Houthoff HJ, Baak JP. The effect of sampling on quantitative nuclear image features in histological sections of lung carcinomas. Anal Quant Cytol Histol 1988; 10: 355-359.
5. Lucia. User' s guide: Laboratory Imaging, 1996.
6. Shimosato Y. Pulmonary neoplasms. In: Sternberg S (ed), Diagnostic surgical pathology. Lippincott-Raven, Philadelphia, 1994: 1045-1093.
7. Beasley MB, Brambilla E, Travis WD. The 2004 World Health Organization classification of lung tumors. Semin Roentgenol 2005; 40: 90-97.
8. Berman JJ, Moore GW. Image analysis software for the detection of preneoplastic and early neoplastic lesions. Cancer Lett 1994; 77: 103-109.
9. Maubon I, Cottier M, Pasteur X, Morcelet M, Marsan C, Laurent JL. Bronchial carcinoma and image analysis. Differentiation between poorly differentiated epidermoid carcinoma and small cell carcinoma. Anal Quant Cytol Histol 1987; 9: 499-504.

10. Gottlieb C, Kini S, Feingold M. Cytomorphometric analysis of small cell neoplasms of the lung from specimens obtained via bronchoscopy. Anal Quant Cytol Histol 1992; 14: 410-416.
11. Kavantzas N, Lazaris AC, Chatzigianni E, Davaris PS. The nuclear morphometry by image analysis in the histopathological diagnosis of lung cancer. J Exp Clin Cancer Res 2000; 19: 201-206.
12. Schmid K, Angerstein N, Geleff S, Gschwendtner A. Quantitative nuclear texture features analysis confirms WHO classification 2004 for lung carcinomas. Mod Pathol 2006; 19: 453-459.

DISKRIMINANTNA ANALIZA NUKLEARNIH IMIDŽ VARIJABLI KARCINOMA PLUĆA

Žaklina Mijović[1], Dragan Mihailović[1], Miloš Kostov[2]

[1]Medicicinski fakultet, Institut za patologiju, Niš
[2]Vojna bolnica, Odeljenje za patologiju, Niš
E-mail: zami@bankerinter.net

Kratak sadržaj: *Histološka klasifikacija karcinoma pluća je subjektivna i često teško reproducibilna na bronhoskopskim biopsijama. Cilj rada bio je da se utvrdi koja od sedam kariometrijskih varijabli ima dijagnostički značaj u razdvajanju glavnih histoloških tipova karcinoma pluća. Izdvojene su bronhoskopske mukozne biopsije pacijenata sa planocelularnim karcinomom (n=48), mikrocelularnim karcinomom (n=41) i adenokarcinomom pluća (n=35). Uzorci su bojeni hematoksilinom i eozinom i mereni imidž analizatorom. Za sve merene nuklearne varijable nađene su signifikantne razlike između sitnoćelijskog i ne-sitnoćelijskog karcinoma pluća (p<0.01). "Stepwise" discriminantnom funkcionom analizom je korektna dijagnoza postignuta u 92,7% nesitnoćelijskih karcinoma i 90,24% sitnoćelijskih karcinoma. Ukupni procenat korektne klasifikacije je 91,93%. Najbolje diskriminantne varijable između ovih kategorija karcinoma pluća su nuklearni perimetar i areal. U zaključku, nuklearna imidž analiza se može koristiti za razlikovanje glavnih histoloških tipova karcinoma pluća na biopsijskim uzorcima, naročito sitnoćelijskog i ne-sitnoćelijskog karcinoma.*

Ključne reči: *Kompjuterski asistirana dijagnoza, imidž analiza, karcinom pluća*