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Source / Izvornik: **Collegium Antropologicum, 2010, 34, 99 - 103**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:027249

Coll. Antropol. **34** (2010) 1: 99–103
Original scientific paper

Morphometry of Tumor Cells in Different Grades and Types of Breast Cancer

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A B S T R A C T

The aim of the study was to compare morphometric characteristics of different types and grades of breast cancer. Morphometric analysis was performed using the SFORM software (Vamstec, Zagreb) on the May-Grünwald-Giemsa stained fine needle aspiration cytology (FNAC) breast tissue specimens. The study included 42 patients diagnosed with breast carcinoma by breast smear FNAC at Merkur University Hospital during the 2001–2005 period. Postoperative tumor histopathology and semi-quantitative tumor grading by the method of Elston and Ellis[1] showed invasive ductal carcinoma grade I in 10, invasive ductal carcinoma grade II in 9, invasive ductal carcinoma grade III in 13, and invasive lobular carcinoma in 13 patients, the latter also including a subtype of invasive tubulolobular carcinoma. The following parameters were assessed by use of Statistica 7.1 and χ^2-test: tumor area, circumference, maximal radius, minimal radius, convexity, length, width, elongation, nucleus/cytoplasm ratio, and shape factor. Morphometric analysis yielded statistically significant differences among all study groups ($p<0.001$). Morphometric parameters showed significant individual correlation with tumor type and grade, whereby the area, convexity and circumference were most significant at both nuclear and cellular level.

Key words: *breast cancer, image analysis, morphometry, fine needle aspiration cytology*

Introduction

Invasive breast cancer is the most common cancer in women. It accounts for 22% of all female cancers, which is more than twice the occurrence of cancer in women at any other site. Invasive ductal carcinoma is the most common type of invasive carcinoma of the breast, comprising between 40–75%. This wide range is possibly due to lack of application of strict criteria. Invasive lobular carcinoma represents 5–15% of invasive breast tumors that also includes invasive tubulolobular carcinoma as its subtype[2]. Fine needle aspiration cytology (FNAC) is simple, quick and economic procedure that has important role in the early diagnosis of palpable and non-palpable breast lesions, and it is of great diagnostic value in the preoperative evaluation of breast tumors[3–5]. Frequently, treatment and planning is preoperatively made largely on the basis of the FNAC diagnosis. FNAC reports of breast malignancy should provide not only the diagnosis of malignancy but also the type and grade of carcinoma. Diagnosis of carcinoma may be made easily in the majority of technically satisfactory aspirates, but determining the exact tumor type and grade may be troublesome. Standard morphologic analysis is subjective to some extent and image analysis could be very useful supplement to FNAC. Image morphometry is quantitative method with two major goals in cytopathology: to add objective measurement to diagnostic assessment and to improve diagnostic capabilities[6]. While the main aim of the FNAC is to differentiate between benign and malignant breast disease, further classification could gain new benefits. Morphometric features which describe shape and size could be used for diagnostic decisions for determination of tumor type and grade[7].

Received for publication June 28, 2009

The aim of our study was to compare various morphometric features between different types and grades of breast cancer.

Materials and Methods

Morphometric analysis was performed by means of the SFORM (Vamstec, Zagreb) software on the May-Grünwald-Giemsa (MGG) stained fine needle aspiration specimens of breast cancer. Selected FNAC represented all breast smears from 2001 to 2005 that were malignant on cytological report and that was also postoperatively histologicaly confirmed in other Clinical Hospital in Zagreb. All of the cases were evaluated by computerized interactive morphometry (Figure 1). In the present study system was programmed to rapidly measure cell's and nuclear features and to calculate their form factor, defined as physical size of object as measured by outside dimensions, and nuclear/cytoplasmatic ratio. Nuclei and cell membrane of well preserved cells, without degenerative changes or artifacts and without overlapping between them were outlined on the computer screen using a computer mouse. At the same time a digital database was created in computer memory. Our investigated group consisted of 42 patients that were divided into 4 categories regarding cancer type and grade, as follows:

- 10 patients with invasive ductal carcinoma grade I (IDC I) (Figure 2)
- 9 patients with invasive ductal carcinoma grade II (IDC II) (Figure 3
- 13 patients with invasive ductal carcinoma grade III (IDC III) (Figure 4)
- 10 patients with invasive lobular carcinoma (ILC) (Figure 5).

From each case 100 cells and 100 nuclei were measured, that totally made 4200 cells and 4200 nuclei. The following morphometric features were investigated: area, outline, maximum radius, minimum radius, convex area, breadth, length, nuclear/cytoplasmatic ratio, form factor and elongation. Statistical analysis was performed using statistic 7.1 and χ^2-test.

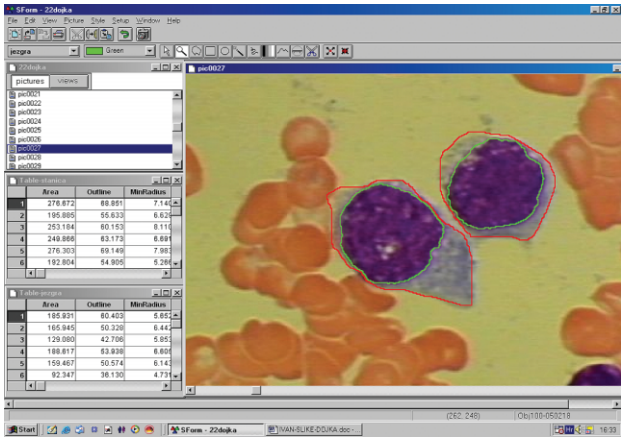

Fig. 1. Computerized interactive morphometry.

Results

For all analyzed types and grades of breast cancer, the following morphometric features showed increased mean values and were statistically significant for the cell and for the nuclei: area, convex area and outline. No significant difference was noted between other investigated features: maximum radius, minimum radius, breadth, length, nuclear/cytoplasmatic ratio, form factor and elongation. In the group of patients with IDC malignant cells showed high variations in shape and size with increasing in mean cell and nuclear area following the progression in tumor grade. Differences were highly statistically significant for mean nuclear and cell area and convex area ($p<0.01$) with increasing mean values of nuclear area in

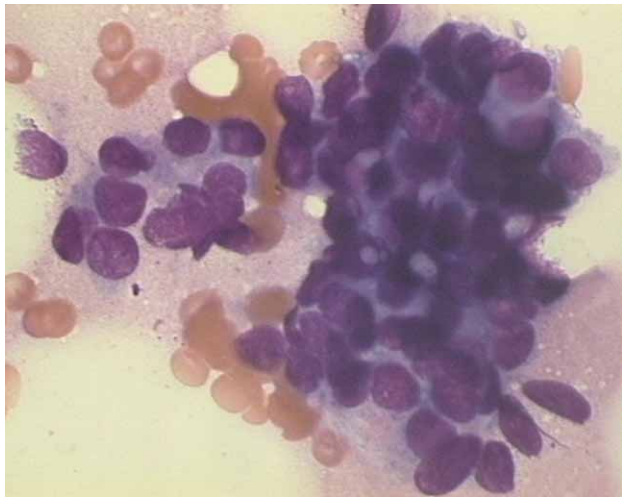

Fig. 2. FNAC smear of the breast. Invasive ductal carcinoma grade I.

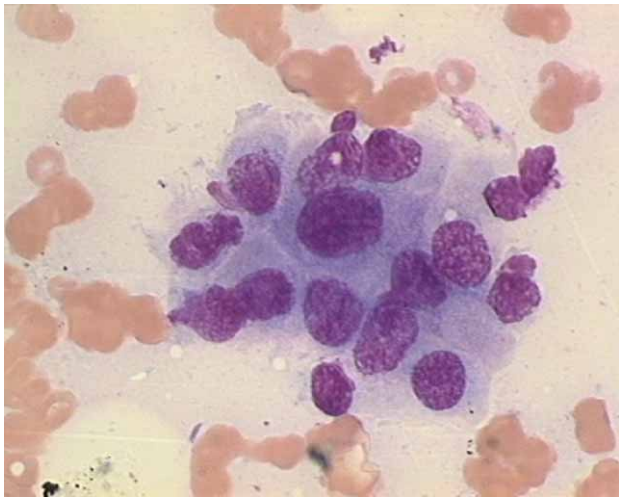

Fig. 3. FNAC smear of the breast. Invasive ductal carcinoma grade II.

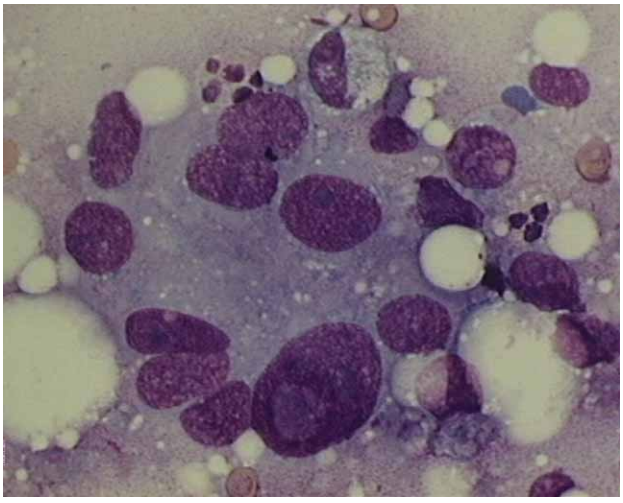

Fig. 4. FNAC smear of the breast. Invasive ductal carcinoma grade III.

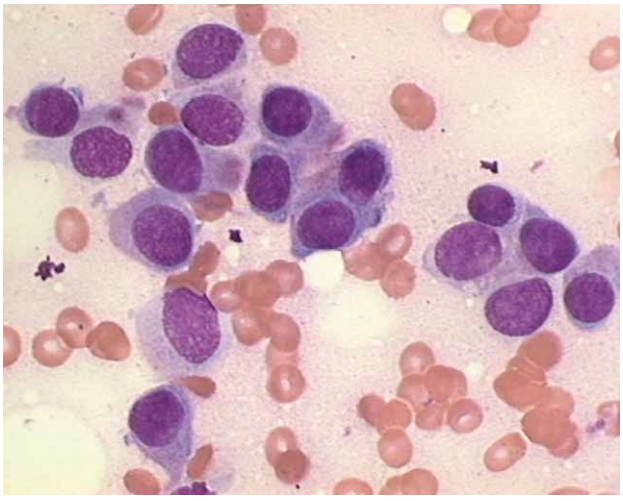

Fig. 5. FNAC smear of the breast. Invasive lobular carcinoma.

tumors with grade progression from grade I to grade III. The same variables (area and convex area) were also most useful for distinction between ILC and all three grades of IDC ($p<0.001$). Nuclear and cell features of ILC were greater then those of IDC I and much lower then those of IDC II and IDC III. The detailed results with mean values and standard deviation (SD) of the most useful morphometric features in our study (area, convex area and outline) for the cell and for the nuclei are shown in Tables 1 and 3 and p values are shown in Tables 2 and 4.

Discussion and Conclusion

Standard morphologic analysis is to some extent subjective method and morphometric analysis, that is quantitative method with objective and reproducible results, could be very useful supplement to FNAC. Most of our traditional knowledge of diagnostic cytology exists in conceptual form of descriptive terms and only a modest amount of such information is available in numeric form. Exact measurement could provide points of references, basic descriptive measures of value and variation, as a basis for checking reproducibility. Nowdays, demands for higher productivity and higher accuracy in cytopathology are increasing and it is clear, from abundant research on that subject, that, quantitative measurement of patterns and features of cells in cytological specimens could be very helpful in routine cytopathology practice. Image morphometric analysis has already been applied for diagnosis and grading of IDC[8–10] but only a small number of studies compared different types and grades of breast cancer using morphometric features as diagnostic variables. Yu et al. compared the morphometric features of IDC and ILC and found that 7 features, of 21 analyzed, were significant: area, defined as the number of points within a nuclear image; kurtosis, a reflection of the »flatness« of the area histogram; long run length emphasis, a measure of coarse texture; perimeter and bending energy, a measure of object complexity and diameter 1, a measure of nuclear width and diameter 2, a measure of nuclear length[11]. Compared to that study, Rajesh et al. investigated a large number of ILC (19 cases) and IDC (30 cases) and found that similar morphometric features (area, convex area, perimeter, convex perimeter, diameter and roundness) were helpful in distinguish ILC from IDC[12]. However, in all studies mentioned above ILC was compared to IDC regardless to its grade. It is well known that cytological differentiation between ILC and IDC grade I could be very difficult. According to our results,

TABLE 1
CELL MORPHOMETRIC FINDINGS IN DIFFERENT TYPES AND GRADES OF BREAST CANCER

Type and grade of tumor	Area (μm²) $\overline{X}$±SD	Outline (μm) $\overline{X}$±SD	Convex area (μm²) $\overline{X}$±SD
IDC I	163.8397±25.29	51.2083±3.83	172.4295±25.48
IDC II	184.2420±53.33	54.2363±8.64	194.5791±56.33
IDC III	232.9815±66.61	60.4498±9.62	245.9972±69.76
ILC	166.4137±37.46	53.2546±5.52	186.3856±31.20

IDC I – invasive ductal carcinoma grade I, IDC II – invasive ductal carcinoma grade II, IDC III – invasive ductal carcinoma grade III, ILC – invasive lobular carcinoma

TABLE 2
STATISTICALLY SIGNIFICANT QUANTITATIVE CELL MORPHOMETRIC VARIABLES BETWEEN STUDIED GROUPS

	AREA	CONVEX AREA	OUTLINE
IDC I / IDC II	$p<0.00000$	$p<0.00000$	$p<0.0431$
IDC I / IDC III	$p<0.00000$	$p<0.00000$	$p<0.00005$
IDC II / IDC III	$p<0.0253$	$p<0.00000$	$p<0.00000$
IDC I / ILC	$p<0.00000$	$p<0.00000$	$p<0.0191$
IDC II / ILC	$p<0.00000$	$p<0.00000$	$p<0.000015$
IDC III / ILC	$p<0.00000$	$p<0.00000$	$p<0.0243$

IDC I – invasive ductal carcinoma grade I, IDC II – invasive ductal carcinoma grade II, IDC III – invasive ductal carcinoma grade III, ILC – invasive lobular carcinoma

TABLE 3
NUCLEAR MORPHOMETRIC FINDINGS IN DIFFERENT TYPES AND GRADES OF BREAST CANCER

Type and grade of tumor	Area (μm^2) $\overline{X}$±SD	Outline (μm) $\overline{X}$±SD	Convex area (μm^2) $\overline{X}$±SD
IDC I	85.4926±12.49	36.4892±3.20	88.4191±13.15
IDC II	89.0447±34.24	36.0676±7.22	91.5290±34.93
IDC III	116.9542±32.73	41.6016±6.16	120.3853±33.66
ILC	87.2724±21.25	39.2048±6.15	90.6378±31.78

IDC I – invasive ductal carcinoma grade I, IDC II – invasive ductal carcinoma grade II, IDC III – invasive ductal carcinoma grade III, ILC – invasive lobular carcinoma

applied morphometrical analysis could be very helpful in this area, especially with measuring of nuclear features. However, we analysed all subtypes of ILC as one group because of small number of cases. ILC group in our study, including all subtypes of ILC, showed higher nuclear and cell pleomorphism, making the distinction of ILC and IDC I more successful.

In our study, calculated values of mean cell and nuclear area and convex area in IDC increased with progression of grade, and differences were highly statistically significant ($p<0.01$). Mean cell and nuclear outline also increased with grade progression ($p<0.05$).

In our study out of all investigated features the following three were the most useful ones: area, convex area and outline, and they were significant for the cell and for the nuclei comparing all three grades of IDC between themselves.

The differences in the observed values of different morphometric features among different publications may be due to applications of different morphometric methods. However, a strictly standardized and uniform measuring technique, with regular calibration of the computerized morphometric equipment could enhance reproducibility.

In addition to the previously mentioned studies, Elzagheid and Collan investigated only nuclear morphometry because outlining of the nuclear borders was easily performed making nuclear measurements reliable and reproducible, while cell membrane outlining was more difficult due to overlapping of cells in groups[13]. In our study

TABLE 4
STATISTICALLY SIGNIFICANT QUANTITATIVE NUCLEAR MORPHOMETRIC VARIABLES BETWEEN STUDIED GROUPS

	AREA	CONVEX AREA	OUTLINE
IDC I / IDC II	$p<0.00000$	$p<0.00000$	$p<0.0289$
IDC I / IDC III	$p<0.00000$	$p<0.00000$	$p<0.0206$
IDC II / IDC III	$p<0.00000$	$p<0.00000$	$p<0.0004$
IDC I / ILC	$p<0.00000$	$p<0.00000$	$p<0.256$
IDC II / ILC	$p<0.00000$	$p<0.00000$	$p<0.00088$
IDC III / ILC	$p<0.00000$	$p<0.00000$	$p<0.487$

IDC I – invasive ductal carcinoma grade I, IDC II – invasive ductal carcinoma grade II, IDC III – invasive ductal carcinoma grade III, ILC – invasive lobular carcinoma

we analyzed only single cells, without overlapping, making both cytoplasmatic and nuclear features available for investigation.

When performed as a part of routine everyday work morphometric analysis is more laborious and time consuming than the traditional cytological analysis. However, if we improve the method, and investigate only the most valuable morphometric features, this method would not be too time-consuming for cytopathologists, especially if they were adequately trained. The morphometric analysis could help in standardizing grading of breast cancer between different laboratories and an addition to quality control[14].

Development of a quantitative morphometrical method requires continued efforts. Because of our encouraging results, we started a larger, prospective study involving DNA image analysis with the aim to improve cytological grading and image morphometry.

REFERENCES

1. ELSTON CW, ELLIS IO, Histopathology, 19 (1991) 403. — 2. ELLIS IO, SCHNITT SJ, SASTRE-GARAU X, BUSSOLATI G, TAVASSOLI FA, EUSEBI V, PETERSE JL, MUKAI K, TABAR L, JACQUEMIER J, CORNELISSE CJ, SASCO AJ, KAAKS R, PISANI P, GOLDGAR DE, DEVILEE P, CLETON-JANSEN MJ, BØRRESEN--DALE AL, VAN'T VEER L, SAPINO A, Invasive breast carcinoma. In: JAFFE ES, HARRIS NL, STEIN H, VARDIMAN JW, (Eds) World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Breast and Female Genital Organs (IARC Press, Lyon, 2003). — 3. TAYE AA, GEMECHU T, Ethiop Med J, 36 (1998) 219. — 4. SCOPA CD, KOUKOURAS D, SPILIOTIS J, HARKOFTAKIS J, KOURELEAS S, KYRIAKOPOULOU D, TZORACOLEFTHERAKIS E, Cancer Detect Prev, 20 (1996) 620. — 5. SREENIVAS M, KUMAR GH, REDDY SJ, BHASKARAN CS, Indian J Pathol Microbiol, 32 (1989) 133. — 6. MARCHEVSKY AM, BARTELS PH, Image analysis a primer for pathologists (Raven Press, New York, 1994). — 7. CASTREN JP, KUOPIO T, NURMI MJ, COLLAN YU, J Urol, 154 (1995) 1302. — 8. DAWSON AE, AUSTIN RE JR, WEINBEREG DS, Am J Clin Pathol, 95 (1991) 29. — 9. THALAN A, NIJHAWAN R, JOSHI K, Analyt Quant Cytol Histol, 22 (2001) 193. — 10. DEY P, GHOSHAL S, PATTARI SK, Analyt Quant Cytol Histol, 22 (2000) 483. — 11. YU GH, SNEIGE N, KIDD LD, JOHNSTON DA, KATZ RL, Analyt Quant Cytol Histol, 17 (1995) 88. — 12. RAJESH L, DEY P, JOSHI K, Analyt Quant Cytol Histol, 24 (2002) 81. — 13. ELZAGHEID A, COLLAN Y, Analyt Quant Cytol Histol, 25 (2003) 73. — 14. KRONQVIST P, KUOPIO T, JALAVA P, COLLAN Y, Br J Cancer, 87 (2002) 1257.

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MORFOMETRIJA TUMORSKIH STANICA KOD RAZLIČITIH GRADUSA I TIPOVA KARCINOMA DOJKE

SAŽETAK

Cilj istraživanja bio je usporediti morfometrijske karakteristike karcinoma dojke različitog subtipa i stupnja (gradusa) diferenciranosti. Morfometrijska analiza provedena je na citološkim razmazima punktata dojke obojenim po metodi May-Grünwald-Giemsa (MGG) programom SFORM (Vamstec, Zagreb). Istraživanjem su obuhvaćene 42 bolesnice kod kojih je dijagnoza karcinoma dojke postavljena na uzorcima dobivenim citološkom punkcijom dojke u Kliničkoj bolnici Merkur u razdoblju od 2001. do 2005. godine. Postoperativno je učinjena patohistološka verifikacija te određivanje gradusa tumora semi-kvantitativnom metodom prema Elstonu i Ellisu[1]. Kod 10 bolesnica radilo se o invazivnom duktalnom karcinomu gradusa I (IDC I), kod 9 bolesnica o invazivnom duktalnom karcinomu gradusa II (IDC II), kod 13 bolesnica o invazivnom duktalnom karcinomu gradusa III (IDC III), a kod 10 bolesnica postavljena je dijagnoza invazivnog lobularnog karcinoma (ILC) u koji je uključen i invazivni tubulo-lobularni karcinom (TLC) kao njegov podtip. Istraživani su slijedeći parametri: površina, opseg, maksimalni radijus, minimalni radijus, konveksitet, dužina, širina, izduženost, omjer jezgra/citoplazma i faktor oblika. Korištena je statistika 7,1, χ^2-test. Morfometrijska je analiza u ovom istraživanju pokazala statistički značajne razlike, ($p<0,001$), između svih ispitivanih grupa. Morfometrijski su parametri, primjenjeni pojedinačno, bili značajno povezani sa tipom i gradusom tumora, a najznačajniji su parametri, i za jezgru i za stanicu, bili: površina, konveksitet i opseg.