Breast Cancer

Correlation of Nuclear Morphometry with Clinicopathologic Parameters in Malignant Breast Aspirates

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Abstract

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Background Nuclear size, shape, chromatin pattern, and nucleolar size and number have all been reported to change in breast cancer.

Aim The aim of the study was to quantify nuclear changes on malignant breast aspirates using morphometry and to correlate the morphometric parameters with clinicopathologic features such as cytologic grade, tumor size, lymph node status, mitotic index, and histopathologic grade.

Materials and Methods Forty-five cases of carcinoma breast diagnosed on cytology were included in this study. Cytologic grading was performed as per the Robinson's cytologic grading system. Nuclear morphometry was done on Papanicolaou stained smears. One hundred nonoverlapping cells per case were evaluated. Both geometrical and textural parameters were evaluated.

Results Comparison of cytologic grades with most morphometric features (nuclear area, perimeter, shape, long axis, short axis, intensity, total run length, and TI homogeneity) was highly significant on statistical analysis. Correlation with tumor size yielded significant results for nuclear area, perimeter, long and short axes, and intensity with $p < 0.05$. The study of lymph node status and morphometry showed a highly significant statistical association with all the parameters. Mitotic count was significantly associated with all the geometric parameters and one textural parameter (total run length). On correlation of ductal carcinoma in situ and histopathological Grades 1 to 3 with morphometry, it was found that all the parameters except long–run emphasis were highly significant with $p < 0.001$.

Conclusion Morphometry as a technique holds immense promise in prognostication in breast carcinoma.

Keywords

- breast carcinoma
- cytology
- morphometry
- Robinson's cytologic grading

Introduction

Breast cancer is the second most common cancer among women in India and accounts for 7% of the global burden of breast cancer and one-fifth of all cancers among women in India.[1,2]

Prognosis of breast cancer depends on multiple clinicopathological parameters that include tumor size, lymph node status, estrogen receptor status, tumor histologic grading, and cell proliferation index. These parameters are studied on

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surgically excised specimens. Treatment decisions are taken by the clinicians depending on these factors. Fine-needle aspiration cytology (FNAC) is routinely used as a preoperative diagnostic tool in suspected cases of breast cancer. Information obtained by fine-needle aspiration can be extremely useful in patient management.

This study was planned with the aim to quantify nuclear changes on malignant breast aspirates using morphometry and to correlate the morphometric parameters with clinicopathologic features such as cytologic grade, tumor size, lymph node status, mitotic index, and histopathologic grade.

Materials and Methods

Forty-five cases of carcinoma breast diagnosed on cytology were included in this study. These were categorized as 42 cases of infiltrating duct carcinoma (IDC), not otherwise specified (NOS), and three cases of ductal carcinoma in situ (DCIS) subsequently on histopathology. A concise clinical history, examination, and details of relevant investigations were also obtained. Histopathology material was available in all the 45 cases. Only cases with proven histopathology of DCIS or IDC (NOS) were included in this study. Patients with preoperative radiotherapy or chemotherapy were excluded from this study.

FNAC was performed using standard procedure. Both air-dried and alcohol-fixed smears were stained by Leishman–Giemsa and Papanicolaou (PAP) stains, respectively. Cytologic grading was performed as per the Robinson's cytologic grading system considering six parameters, namely cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin, and chromatin.

Scores of 1 to 3 were assigned for each of the six parameters—cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin, and chromatin, and they were totaled to classify the lesions into Grade 1, score 6 to 11; Grade 2, score 12 to 14; and Grade 3, score 15 to 18.[3]

Nuclear morphometry was done in all the cases on smears stained with PAP stain using the Defense Bioengineering and Electromedical Laboratory Cytoscan indigenously developed by Defense Research and Development Organization, New Delhi, India. One hundred nonoverlapping cells per case were evaluated. Both geometrical and textural parameters were evaluated. Geometrical parameters included nuclear area, perimeter, nuclear shape, long axis, short axis, and intensity. Textural parameters were long-run emphasis (measuring coarseness of nuclear chromatin), total run length (measuring proportion of coarse to fine chromatin), and T1 homogeneity (measuring homogeneity of chromatin distribution).

It was proposed to correlate cytological grade, tumor size, lymph node status, mitotic count, histological grade versus nuclear area, perimeter, nuclear shape, long axis, short axis, intensity, long-run emphasis, total run length, and T1 homogeneity and see if the association was statistically significant.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software for Windows, version 17.0 (SPSS, Chicago, Illinois, United States). Continuous variables were presented as mean ± standard deviation (SD) and compared using the two-tailed, independent sample *t*-test, and one-way analysis of variance test. Tests were performed at significance level 0.05, that is, $p < 0.05$ was taken to indicate a statistically significant difference.

Results

The study included 45 cases of carcinoma breast diagnosed and graded on cytology. There were 44 female and 1 male patients. The mean age at diagnosis across the groups was 49.8 ± 13.2 years and that in the DCIS group was 35.3 ± 1.5 years; IDC (NOS) with negative nodes was 50.3 ± 13.5 and IDC (NOS) with positive nodes was 51.2 ± 12.9 years.

Histopathology was available in all the 45 cases.

There were nine cases in cytologic Grade 1, 26 in Grade 2, and 10 cases in cytologic Grade 3 (►**Figs. 1–3**. Correlation of nuclear morphometry with cytologic grades was performed. It was found that nuclear area, perimeter, shape, long axis, short axis, intensity, total run length, and T1 homogeneity were highly significant on statistical analysis (►**Table 1**).

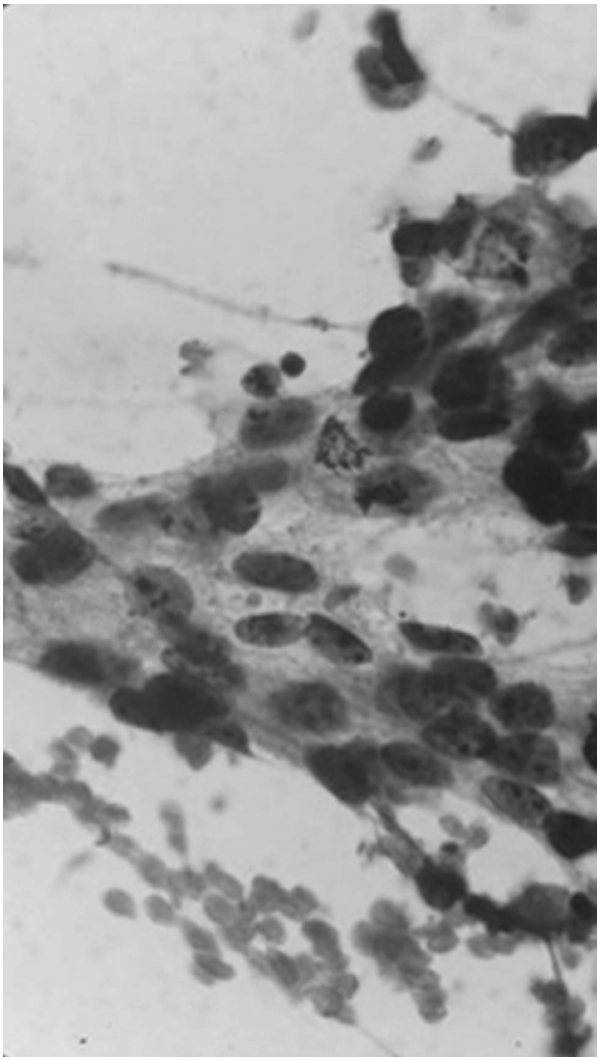

Fig. 1 Cytological Grade 1 showing cell clustering and atypical mitoses (Papanicolaou, x400).

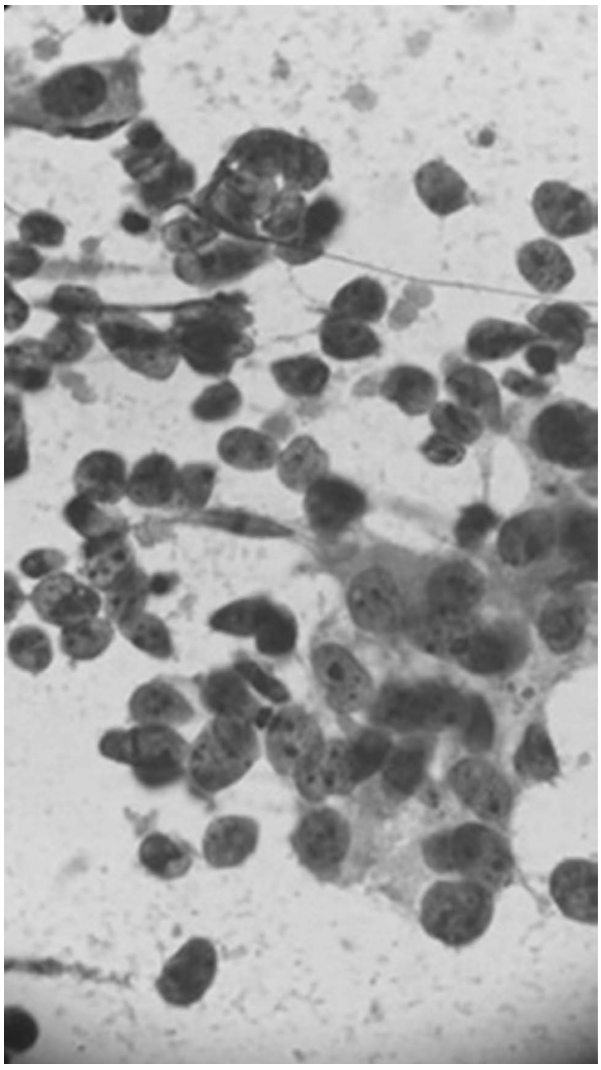

Fig. 2 Cytological Grade 2 showing mixture of moderately pleomorphic single cells and clusters (Papanicolaou, x400).

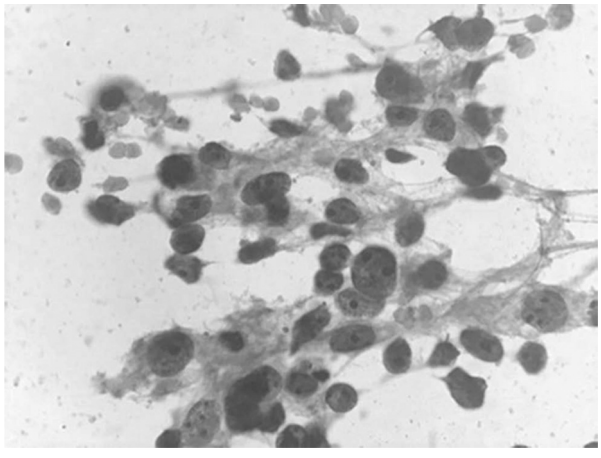

Fig. 3 Cytological Grade 3 showing cell size >5 red blood cell and prominent nucleoli with coarsely granular chromatin (Papanicolaou, x400).

Majority of our cases had a tumor size <5 cm ($n = 38$, 84.4%). There were seven cases with tumor size >5 cm. Comparison of morphometric features with tumor size yielded significant results for nuclear area, perimeter, long and short axes, and intensity with $p < 0.05$ for these parameters (►Table 2).

There were 30 cases (66.7%) with positive lymph nodes. Evaluation of lymph node status (positive/negative) versus morphometry showed a highly significant association between all the geometric as well as textural parameters, that is, nuclear area, perimeter, shape, long and short axes, intensity, long-run emphasis, total run length, and Tl homogeneity (►Table 3). The three cases of DCIS were grouped in the node–negative category since none of them had positive lymph nodes. Mitotic count was, on the other hand, significantly associated with geometric parameters such as nuclear area, perimeter, shape, long axis, short axis, and intensity. Among the textural parameters, only total run length, signifying proportion of coarse to fine chromatin, was found to be significantly associated with mitotic count on statistical analysis. Majority of our cases were in the <6 mitosis categories ($n = 33$, 73.3%) (►Table 4).

The histopathological examination of 42 cases showed IDC (NOS) and three cases (6.7%) of DCIS. Among the 42 cases of IDC (NOS), there were 6, 27, and 9 cases in Grades 1, 2, and 3, comprising 13.3, 60, and 20%, respectively. On correlation of DCIS and histopathological Grades 1 to 3 with morphometry, it was found that all the parameters except long–run emphasis, that is, nuclear area, perimeter, shape, long and short axes, intensity, total run length, and Tl homogeneity were highly significant on statistical analysis with all having $p < 0.001$ (►Table 5).

Discussion

Alterations in nuclear structure are the morphologic hallmark of cancer diagnosis. Nuclear size, shape, chromatin pattern, and nucleolar size and number have all been reported to change in breast cancer. Attempts to quantify nuclear alterations to establish grading systems, predict prognosis, and/or set guidelines for therapy have met with varied success.[4]

This study was planned to evaluate the role of nuclear morphometry vis-a-vis various clinicopathologic parameters in breast cancer. The aim was to evaluate each of these criteria and also to explore the move from subjectivity to quantified objectivity in breast cancer diagnosis and prognostication.

Diagnostic cutoff values for mean nuclear area (MNA) have been proposed to distinguish between benign and malignant breast lesions. Imprint cytology has been combined with morphometric analysis and has yielded superior results compared with those obtained by imprint cytology and frozen section in breast cancer diagnosis.

With morphometric analysis, it has been reported that there was a significant difference between the means of nuclear area, nuclear perimeter, and nuclear diameter between benign and malignant tissues. Feret circle, a measure of ellipticity, was not significant. These parameters have been advocated to be used intraoperatively in imprint smears to distinguish benign from malignant and suspicious lesions.[5,6]

In this study, geometrical (nuclear area, perimeter, nuclear shape, long axis, short axis, and intensity) and textural (long-

Table 1 Correlation of cytologic grades with nuclear morphometry grades

Morphometric parameter	Cytology Grade 1 ($n=9$)	Cytology Grade 2 ($n=26$)	Cytology Grade 3 ($n=10$)	*p*-Value (one–way ANOVA test)
Nuclear area	78.21 ± 12.40	93.12 ± 13.85	115.89 ± 19.03	<0.001[a]
Perimeter	31.08 ± 4.24	36.81 ± 3.77	39.33 ± 5.32	<0.001[a]
Shape	1.05 ± 0.01	1.09 ± 0.04	1.10 ± 0.03	0.005[a]
Long axis	11.94 ± 0.97	13.70 ± 1.26	14.82 ± 1.19	<0.001[a]
Short axis	8.24 ± 0.81	9.33 ± 1.12	10.20 ± 0.95	0.001[a]
Intensity	100.12 ± 19.03	119.03 ± 17.23	131.61 ± 18.04	0.002[a]
Long-run emphasis	1.19 ± 0.08	1.20 ± 0.08	1.19 ± 0.05	0.906
Total run length	3433.29 ± 808.54	4140.06 ± 1065.69	5171.36 ± 982.90	0.002[a]
Tl homogeneity	0.006 ± 0.0004	0.0008 ± 0.001	0.007 ± 0.0003	<0.001[a]

Abbreviation: ANOVA, analysis of variance.
[a]Statistically significant.

Table 2 Correlation of morphometric parameters with tumor size

Morphometric parameter	Tumor size <5 cm ($n=38$)	Tumor size ³5 cm ($n=7$)	*p*-Value (independent sample *t*–test)
Nuclear area	91.92 ± 18.48	112.99 ± 12.99	0.006[a]
Perimeter	35.38 ± 4.95	40.82 ± 1.69	0.006[a]
Shape	1.08 ± 0.04	1.11 ± 0.03	0.076
Long axis	13.39 ± 1.53	14.75 ± 0.59	0.025[a]
Short axis	9.12 ± 1.20	10.31 ± 0.46	0.013[a]
Intensity	114.76 ± 20.19	135.85 ± 7.04	0.009[a]
Long–run emphasis	1.19 ± 0.08	1.20 ± 0.08	0.782
Total run length	4095.59 ± 1160.11	4946.06 ± 710.60	0.068
Tl homogeneity	0.0071 ± 0.0012	0.0078 ± 0.0015	0.252

[a]Statistically significant.

Table 3 Correlation of morphometric parameters with lymph node status

Morphometric parameter	Lymph node status negative ($n=15$)	Lymph node status positive ($n=30$)	*p*-Value (independent sample *t*–test)
Nuclear area	76.65 ± 12.46	104.47 ± 14.81	<0.001[a]
Perimeter	30.49 ± 3.29	39.10 ± 2.67	<0.001[a]
Shape	1.05 ± 0.02	1.11 ± 0.03	<0.001[a]
Long axis	11.92 ± 0.98	14.44 ± 0.89	<0.001[a]
Short axis	8.06 ± 0.84	9.92 ± 0.79	<0.001[a]
Intensity	98.19 ± 12.18	127.97 ± 15.60	<0.001[a]
Long-run emphasis	1.16 ± 0.05	1.21 ± 0.08	0.019[a]
Total run length	3260.32 ± 818.74	4711.66 ± 958.68	<0.001[a]
Tl homogeneity	0.0064 ± 0.0008	0.008 ± 0.001	0.003[a]

[a]Statistically significant.

run emphasis for coarseness of nuclear chromatin, total run length measuring proportion of coarse to fine chromatin, and Tl homogeneity indicating homogeneity of chromatin distribution) parameters were compared with clinicopathologic ones including cytological grade, tumor size, lymph node status, mitotic count, and histological grade, and this association was subjected to statistical analysis.

The value of automated quantitative three-dimensional nuclear morphometry as an objective tool to enable the development of sensitive and specific nuclear grade

Table 4 Correlation of morphometric parameters with mitotic rate

Morphometric parameter	Mitosis <6 ($n = 33$)	Mitosis ³6 ($n = 12$)	*p*-Value (independent sample *t*–test)
Nuclear area	87.86 ± 14.30	115.39 ± 16.69	<0.001[a]
Perimeter	34.72 ± 4.58	40.36 ± 3.68	<0.001[a]
Shape	1.08 ± 0.03	1.12 ± 0.028	0.002[a]
Long axis	13.11 ± 1.36	14.93 ± 1.04	<0.001[a]
Short axis	8.96 ± 1.12	10.24 ± 0.86	<0.001[a]
Intensity	111.89 ± 18.49	134.96 ± 14.69	0.003[a]
Long–run emphasis	1.19 ± 0.08	1.21 ± 0.07	0.482
Total run length	3849.13 ± 995.71	5269.46 ± 832.49	<0.001[a]
Tl homogeneity	0.0073 ± 0.001	0.0071 ± 0.0012	0.510

[a]Statistically significant.

Table 5 Correlation of morphometric parameters with histopathological grading

Morphometric parameter	DCIS ($n = 3$)	Histopathology Grade 1 ($n = 6$)	Histopathology Grade 2 ($n = 27$)	Histopathology Grade 3 ($n = 9$)	*p*-Value (one–way ANOVA test)
Nuclear area	85.63 ± 5.10	69.81 ± 8.08	93.08 ± 12.78	118.9 ± 14.63	<0.001[a]
Perimeter	32.37 ± 2.15	29.58 ± 4.36	35.35 ± 3.94	40.19 ± 4.31	<0.001[a]
Shape	1.04 ± 0.005	1.05 ± 0.01	1.09 ± 0.03	1.11 ± 0.03	<0.001[a]
Long axis	12.02 ± 0.59	11.64 ± 1.01	13.66 ± 1.18	15.12 ± 0.70	<0.001[a]
Short axis	8.63 ± 0.54	7.72 ± 0.72	9.31 ± 1.03	10.54 ± 0.43	<0.001[a]
Intensity	92.64 ± 6.21	100.62 ± 20.80	117.65 ± 17.25	135.33 ± 10.95	<0.001[a]
Long-run emphasis	1.17 ± 0.05	1.20 ± 0.09	1.20 ± 0.09	1.19 ± 0.05	0.934
Total run length	4043.24 ± 209.25	2836.74 ± 496.43	4136.50 ± 996.54	5446.82 ± 752.46	<0.001[a]
Tl homogeneity	0.006 ± 0.0006	0.006 ± 0.000	0.008 ± 0.001	0.007 ± 0.001	<0.001[a]

Abbreviations: ANOVA, analysis of variance; DCIS, ductal carcinoma in situ.
[a]Statistically significant.

classification in breast cancer diagnosis has been documented. Abnormal cell nuclei have been found to have more nucleoli, markedly higher density, and clumpier chromatin organization compared with normal ones.[7]

In the present study, cytologic grades were correlated with nuclear morphometry. Barring long-run emphasis, all the other parameters, namely nuclear area, perimeter, shape, long axis, short axis, intensity, total run length, and Tl homogeneity, were highly significant on statistical analysis with $p < 0.05$.

Nuclear and histologic grade, lymph node status, tumor size, mitotic activity index, cellularity index, and mean and SD of nuclear area have been reported to be the most important single predictors of prognosis in breast carcinoma. Morphometry significantly adds to the prognosis prediction of lymph node status and tumor size. MNA has been reported to be significantly higher in tumors of the postmenopausal than premenopausal, in LN+ than LN–patients, and in tumors over 3 cm than smaller ones. Significant differences between different clinical stages, histological grades, and histological types of tumors have been reported. Significant correlations have been reported between MNA and histological grade, standard mitotic index, and tumor size.[8–10]

In the present study, the comparison of tumor size with morphometry yielded significant results only for geometric parameters, that is, nuclear area, perimeter, long and short axes, and intensity with $p < 0.05$. All the geometric and textural parameters, that is, nuclear area, perimeter, shape, long and short axes, intensity, long-run emphasis, total run length, and Tl homogeneity were, on the other hand, found to be significantly associated with lymph node status (positive/negative).

We found mitotic count to have a statistically significant association with all the geometric parameters including nuclear area, perimeter, shape, long axis, short axis, and intensity, with only one textural parameter, that is, total run length, signifying proportion of coarse to fine chromatin being significantly associated. This is majorly in concurrence with the published literature.

The histopathological examination showed IDC (NOS) in 42 cases and DCIS in 3 cases (6.7%) in our study. Among the 42 cases of IDC (NOS), there were 6, 27, and 9 cases in histopathological Grades 1, 2, and 3, comprising 13.3, 60, and 20%, respectively. On correlation of DCIS and histopathological Grades 1 to 3 with morphometry, it was found that all the geometric and most of the textural parameters except long-run emphasis, that is, nuclear area, perimeter, shape, long and short axes, intensity,

total run length, and Tl homogeneity were highly significant on statistical analysis with all having $p < 0.001$.

Conclusion

Based on this study, one can summarize that morphometry as a technique holds immense promise in prognostication in breast carcinoma. It carries the added advantage of being objective and thus is free of individual biases. More studies need to be conducted to further evaluate the strength of this association and its utility in clinical practice.

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Nil.

Conflicts of Interest
There are no conflicts of interest.

References

1 Rajaraman P, Anderson BO, Basu P, et al. Recommendations for screening and early detection of common cancers in India. Lancet Oncol 2015;16(07):e352–e361
2 Kumar P, Bolshette NB, Jamdade VS, et al. Breast cancer status in India: an overview. Biomed Prev Nutr 2013;3:177–183
3 Robinson IA, McKee G, Nicholson A, et al. Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. Lancet 1994;343(8903)947–949
4 Pienta KJ, Coffey DS. Correlation of nuclear morphometry with progression of breast cancer. Cancer 1991;68(09):2012–2016
5 Abdalla F, Boder J, Buhmeida A, Hashmi H, Elzagheid A, Collan Y. Nuclear morphometry in FNABs of breast disease in Libyans. Anticancer Res 2008;28(6B)3985–3989
6 Aggarwal G, Singh S, Marwah S, et al. Morphometric analysis in breast lesions. A rapid conjunct to intraoperative imprint smears. Middle East J Cancer 2012;3:1–8
7 Prvulović I, Kardum-Skelin I, Sustercić D, Jakić-Razumović J, Manojlović S Morphometry of tumor cells in different grades and types of breast cancer. Coll Antropol 2010;34 (01):99–103
8 Tahlan A, Nijhawan R, Joshi K. Grading of ductal breast carcinoma by cytomorphology and image morphometry with histologic correlation. Anal Quant Cytol Histol 2000;22(03):193–198
9 Baak JP, Van Dop H, Kurver PH, Hermans J. The value of morphometry to classic prognosticators in breast cancer. Cancer 1985;56 (02):374–382
10 Ikpatt OF, Kuopio T, Collan Y. Nuclear morphometry in African breast cancer. Image Anal Stereol 2011;21:145

