

Evaluation of Clinicopathological Features in Breast Cancer Patients Using Cytonuclear Morphometry

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ABSTRACT

As breast cancer is one of the leading causes of death worldwide, we aim to correlate cytonuclear morphometric parameters with clinicopathological features in order to emphasize their importance to prognostication. Following the pathological processing of tumor specimens, representative areas throughout the tumor mass were selected. These areas have been scanned using an Olympus VS200 slide scanner and analyzed using QuPath v0.4.4. Nine cytonuclear morphometric parameters have been calculated and correlated with clinicopathological features. *P* values were determined through regression analysis and a *p*-value <0.05 was considered significant. Many significant correlations have been obtained between cytonuclear morphometric parameters and clinicopathological features. There have been elaborated mathematical criteria-based algorithms by selecting cut-off values for tubular differentiation score, nuclear pleomorphism score, mitotic rate score, Nottingham score, lymph node status, lymphovascular invasions, perineural invasion, presence of necrosis, presence of in situ carcinoma and presence of microcalcifications. The cytonuclear morphometric parameters show great promise for prognostication in breast cancer patients, as many of them were significantly correlated with clinicopathological features. The values of these parameters have allowed the development of algorithms to predict these features.

Keywords: breast cancer, pathological features, tumor morphometry, digital pathology.

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INTRODUCTION

Morphometry, as a pathology technique, has been gaining more importance in the digital pathology era with the advent of artificial intelligence. Its main advantage lies in the capacity to provide quantitative information in addition to the classical qualitative features of a lesion. By increasing the use of quantitative data in practice, more objective and reproducible parameters and algorithms can be developed (1-6).

It is well-known that cytonuclear features undergo changes during tumorigenesis (7-10). These changes are typically described qualitatively as aggressive features, such as larger nuclei, marked pleomorphism, atypical mitoses and others. Through accumulation of genetic mutations, tumor cells develop specific patterns and features that enable pathologists to generate reports for oncologists. Cytonuclear morphometry allows for the quantification of these cellular changes during tumorigenesis, offering valuable information for prognosis and predicting various clinicopathological features.

Breast cancer remains one of the most common cancers worldwide and its incidence continues to increase, affecting over two million patients annually (11-13). Therefore, new methods for evaluating tumor mass features can be extremely useful for patient management.

Breast cancer morphometry has been extensively utilized in recent decades to obtain additional quantitative information for better tumor mass characterization. Numerous studies have highlighted the significance of breast cancer cell morphometry in pathology practice, and some of the key findings will be presented.

In 1986, Van der Linden, Baak *et al* conducted a study comparing breast cancer cells in primary tumors without lymph node metastases, primary tumors associating metastasis and lymph node metastases using nuclear morphometric parameters. They observed that the nuclei were mostly the same in the primary and metastatic tumors, except for a small subset where the nuclei were more ellipsoidal. Additionally, they noticed significant correlations regarding the cellularity index, mitotic activity index, mean nuclear area, mean nuclear perimeter and the mean nuclear axes length (16).

In 2012, Aggarwal *et al* conducted a study on the morphometric differences between normal and malignant cells in breast tissue. They discovered significantly lower values for mean nuclear area, mean nuclear diameter, mean nuclear perimeter, Feret circle and nucleo-cytoplasmic ratio in benign ductal epithelial cells (17).

In 2022, Kalhan, Garg *et al* carried out a study on the correlations between nuclear morphometric parameters and clinicopathological features in malignant breast aspirates. They identified significant correlations with the cytologic grade (for nuclear area, nuclear perimeter, and axes length), tumor size (for nuclear area, nuclear perimeter, axes length, and intensity), and lymph node status (for nuclear area, nuclear perimeter, axes length, intensity and shape). The researchers concluded that breast cancer cell morphometry holds great potential for practical use and prognostication (10).

In this paper, we aim to correlate the clinicopathological features of breast cancer patients with the main cytonuclear morphometric features. □

PATIENTS, MATERIALS AND METHODS

Patients' description

A total of 29 patients diagnosed with invasive breast carcinoma of no special type along with their clinicopathological features have been selected from the internal database of "Carol Davila" Clinical Nephrology Hospital, Bucharest, Romania, from 2010 to 2020, with no specific selection criteria. Their main features are summarized in Table 1.

Apparatus

An Olympus VS200 slide scanner was used to scan the histopathological Hematoxylin and Eosin-stained slides containing the representative area (14) that was selected from the tumor mass. The scanning occurred at high focusing and an objective of 40x. All slides were identified by numbers and the names were securitized in order to maintain patients' privacy. All virtual slides were uploaded on an external hard disk belonging to the Pathology Laboratory of the hospital. Subsequently, the virtual slides were transferred to another computer for analysis and measurements were performed using QuPath v0.4.4, open-source software (15) as illustrated in Figure 1.

Parameter	Descriptor	Value	Parameter	Descriptor	Value
Age	<50	24.14%	Mitotic rate	1	51.85%
	50-70	41.38%		2	40.74%
	>70	34.48%		3	7.41%
pT	1 (1a, 1b, 1c)	28.57%	Nottingham score	≤5	14.81%
	2	57.14%		6-7	62.96%
	3	3.57%		8-9	22.22%
	4 (4a, 4b)	10.71%	DCIS/LCIS	0	44.83%
pN	0	10.00%		1	55.17%
	1 (1a, 1b, 1c)	45.00%	Necrosis	0	51.72%
	2 (2a, 2b)	45.00%		1	48.28%
Tubular differentiation	1	0.00%	Lymphovascular invasion	0	20.69%
	2	29.63%		1	79.31%
	3	70.37%	Perineural invasion	0	62.07%
Nuclear pleomorphism	1	3.70%		1	37.93%
	2	70.37%	Microcalcifications	0	82.76%
	3	25.93%		1	17.24%

TABLE 1. Clinicopathological features of the selected patients

In the Descriptor column, 0 stands for absence and 1 for presence; pT, pN and pM belong to TNM grading system; DCIS/LCIS=ductal carcinoma *in situ*/lobular carcinoma *in situ*.

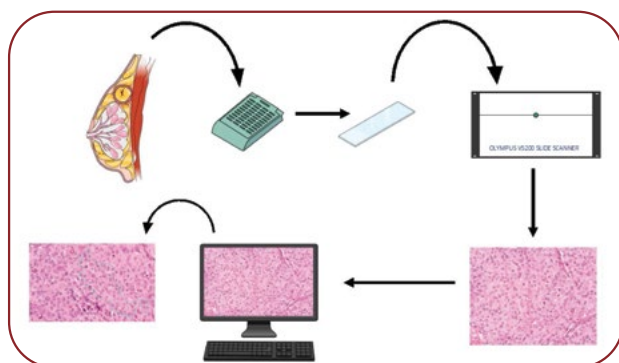


FIGURE 1. Main steps in obtaining the virtual slides used for cytonuclear morphometry – representative slides obtained from the tumor mass were scanned and analyzed using QuPath and Digital Pathology



FIGURE 2. Directly measured parameters in cytonuclear morphometry

Selection and calculus of the cytonuclear morphometric parameters

The morphometric evaluation was both conducted on biopsy and surgical specimens depending on the availability in the pathology department.

Four directly measured parameters, namely the nuclear area, nuclear perimeter, nuclear long

axis length and cell surface, were obtained using QuPath on the representative area of the virtual slide. Other derived parameters, including surface ratio of nucleus to cytoplasm, small axis length, nuclear volume, axes ratio, acyclicity and anellipticity grades were calculated starting from the four directly measures parameters, as shown in Figures 2 and 3 (14).

Formulas for the derived cytonuclear morphometrical parameters are shown in Table 2.

Statistical calculus

Regression analysis calculus was used to determine the p values. A p-value <0.05 was considered statistically significant. The parameters correlated with a clinicopathological feature were used to determine cut-off values and establish algorithms for the prediction of the selected characteristics. Parameters that showed slight correlations with specific clinicopathological features were also used to enhance the algorithm. □

RESULTS

Applying regression analysis, all the correlations that were considered significant (p-value < 0.05) or with a p-value close to 0.05 are shown in Table 3.

For each of the mentioned clinicopathological features, cut-off values were selected to differentiate between possible results. Combining these cut-off values, criteria-based algorithms were developed, as outlined below.

Although there was no significant correlation between the tubular differentiation score (tdf)

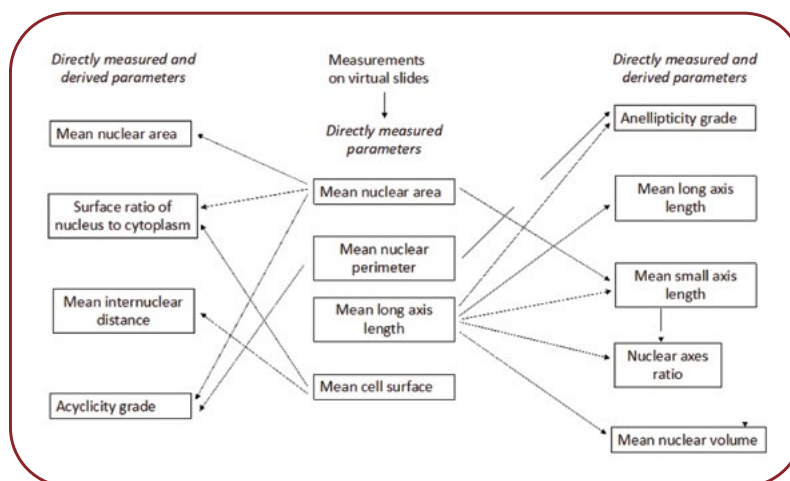


FIGURE 3. Directly measured and derived parameters used for cytonuclear morphometry

TABLE 2. Main derived parameters with their formulas and associated hypotheses

Cytonuclear morphometrical derived parameter	Calculus formulae	Hypotheses and explanations
Small axis length (r)	$r = \frac{A_{nc}}{\pi \cdot R}$	The small axis length is calculated according to the assumption that the nucleus is elliptic.
Surface ratio of nucleus to cytoplasm (Ratio_1)	$Ratio_1 = \frac{A_{nc}}{S_{cell} - A_{nc}}$	The surface ratio can be calculated from directly measured parameters.
Nuclear axes ratio (Ratio_2)	$Ratio_2 = \frac{R}{r}$	This ratio evaluates the elongation of the nucleus from circular to ovoid.
Acyclicity grade (AcG)	$r_c = \sqrt{\frac{A_{cell}}{\pi}}$ $P_c = 2 \cdot \pi \cdot r_c$ $AcG = \frac{P_{nc}}{P_c}$	Acyclicity grade reflects the deviation of the nuclei from the round form and it is calculated as the ratio between nuclear perimeter and the perimeter of a circle with the same area.
Anellipticity grade (AnG)	$P_e = 2 \cdot \pi \cdot \sqrt{\frac{R^2 + r^2}{2}}$ $AnG = \frac{P_{nc}}{P_e}$	Anellipticity grade evaluates how folded an elliptic nucleus is and it is calculated as the ratio between the nuclear perimeter and the perimeter of an ellipse with the same length of the two axes.
Mean internuclear distance	$d = \sqrt{S_{cell}}$	Each nucleus is considered the center of a square and the mean area of a square is equal to the mean area of a cell. Therefore, the mean internuclear distance is equal to the distance between the centers of two adjacent squares.
Mean nuclear volume	$V = \frac{4}{3} \cdot \pi \cdot R \cdot r^2$	The nucleus is considered an ellipsoid with the third axis equal to the small axis of the nucleus.

A_{nc} =nuclear area; R =long axis length; P_{nc} =nuclear perimeter; S_{cell} =cell surface; r_c =radius of the circle in the assumption of round cell; P_c =the perimeter of the cell in the assumption of a round cell; P_e =perimeter of cell in the assumption of an elliptic cell; V =volume.

and each individual parameter, it was observed that the tdf score could be predicted using mean internuclear distance with a cut-off of 12.8 μm , long axis length with a cut-off of 10 μm and mean nuclear volume with a cut-off of 265 μm^3 as 73.68% of the patient with tdf 3 had at least two parameters' levels higher than the cut-off levels, while 62.5% of the patients with tdf 1 or tdf 2 associated at most one parameter's level higher than the cut-off.

There were significant correlations between the nuclear pleomorphism score (P) and average nuclear area, N/C ratio, mean internuclear distance, mean nuclear volume and lengths of long and small axis. Additionally, the nuclear pleo-

morphism score can be predicted using average nuclear area, surface ratio of nucleus to cytoplasm (N/C), mean internuclear distance and mean nuclear volume. Specifically, 71.43% of P3 patients associated with at least three out of four parameters: average nuclear area >95 μm^2 , N/C >0.6, mean internuclear distance >12.8 μm and mean nuclear volume >600 μm^3 , compared to only 5% of patients with P1 or P2.

Mitotic rate score (M) can be predicted using average nuclear area, small axis length and anellipticity grade. A higher score is associated with average nuclear area >100 μm^2 , small axis length >10 μm and anellipticity grade <1.125. Specifically, all M3 patients met at least two of

TABLE 3. P-values for the selected parameters

Feature	Parameter	p-value
Tubular differentiation	Average nuclear area (μm^2)	0.127
	Long axis length (μm)	0.088
	Mean nuclear volume (μm^3)	0.169
Nuclear pleomorphism	Average nuclear area (μm^2)	0.005
	N/C ratio*	0.050
	Mean internuclear distance (μm)	0.011
	Long axis length (μm)	0.002
	Small axis length (μm)	0.014
	Mean nuclear volume (μm^3)	0.009
Mitotic rate	Average nuclear area (μm^2)	0.013
	Acyclicity grade	0.054
	Mean internuclear distance (μm)	0.086
	Long axis length (μm)	0.012
	Small axis length (μm)	0.009
	Mean nuclear volume (μm^3)	0.016
Lymph nodes status	Average nuclear area (μm^2)	0.070
	Long axis length (μm)	0.072
	Small axis length (μm)	0.070
	Mean nuclear volume (μm^3)	0.097
	Acyclicity grade	0.002
	Mean internuclear distance (μm)	0.008
Lymphovascular invasion	Long axis length (μm)	0.054
	Small axis length (μm)	0.051
	Mean nuclear volume (μm^3)	0.034
	Anellipticity grade	0.002
	Average nuclear area (μm^2)	0.054
	Axis ratio of long nuclear axis to small nuclear axis	0.034
Perineural invasion	Mean internuclear distance (μm)	0.072
	Long axis length (μm)	0.088
	Small axis length (μm)	0.029
	Mean nuclear volume (μm^3)	0.073
	Anellipticity grade	0.194
	Mean nuclear volume (μm^3)	0.242
DCIS/LCIS	Acyclicity grade	0.139
	Anellipticity grade	0.156
Microcalcification	Acyclicity grade	0.228
	Anellipticity grade	0.155
Nottingham score	Acyclicity grade	0.043
	Mean internuclear distance (μm)	0.042
	Long axis length (μm)	0.001
	Small axis length (μm)	0.002
	Mean nuclear volume (μm^3)	0.002

N/C ratio=the ratio between nuclear and cytoplasm areas;

DCIS/LCIS=ductal carcinoma *in situ*/lobular carcinoma *in situ*.

the three criteria, while all M1 patients met at most one criterion.

The lymph node status was evaluated via pN value from TNM grading system. Although there was no significant correlation between the lymph node status and each individual parameter, the lengths of the long and short axis were the most representative for a predictive algorithm. By setting as criteria long axis length $<12 \mu\text{m}$ and short axis length $<10 \mu\text{m}$, it was observed that 94.44%

of patients with metastatic lymph nodes met at least one of the two criteria, while all those without lymph node metastases met none of the established criteria.

Lymphovascular invasion showed statistically significant correlations with the average nuclear area, the acyclicity grade, the mean internuclear distance, the mean nuclear volume and the anellipticity grade. Additionally, the above-mentioned invasion can be predicted using an algorithm that is based on the mean internuclear distance, mean nuclear volume and anellipticity grade. The selected cut-off values are $14.5 \mu\text{m}$, $460 \mu\text{m}^3$ and 1.12. It was observed that invasion in lymph and blood vessels was associated with lower mean internuclear distances, lower nuclear volumes and higher anellipticity grades as 86.96% of patients with this pathological feature met at least one of the three criteria ($<14.5 \mu\text{m}$, $<460 \mu\text{m}^3$ and >1.12), while 83.33% of those without this pathological feature met none of the established criteria.

Regarding perineural invasion, its presence was correlated with the small axis length and the ratio of long nuclear axis to small nuclear axis. It was observed that 72.72% of patients with invasion and only 16.66% of those without invasion had at least two out of the following parameters: average nuclear area $<60 \mu\text{m}^2$, small axis length $<8 \mu\text{m}$ and anellipticity grade >1.125 .

The Nottingham score strongly correlated with the average nuclear area, acyclicity grade, mean internuclear distance, long axis length, small axis length, mean nuclear volume and anellipticity grade. Additionally, the Nottingham score can be predicted by an algorithm including four morphometric parameters. The four criteria are average nuclear area $>95 \mu\text{m}^2$, acyclicity grade <1.14 , mean internuclear distance $>12.8 \mu\text{m}$ and long axis length $>12.25 \mu\text{m}$. Specifically, 83.33% of patients with a Nottingham score of 3 met at least three of the four criteria, while all those with a Nottingham score of 1 met none of the established criteria.

For the presence of necrosis, DCIS/LCIS and microcalcifications, no significant correlations were observed, but algorithms to predict the presence or absence have been established empirically.

The presence of necrosis was more frequent (64.28% vs. 26.66%) when two of the following were present: N/C >0.6 , mean nuclear volume

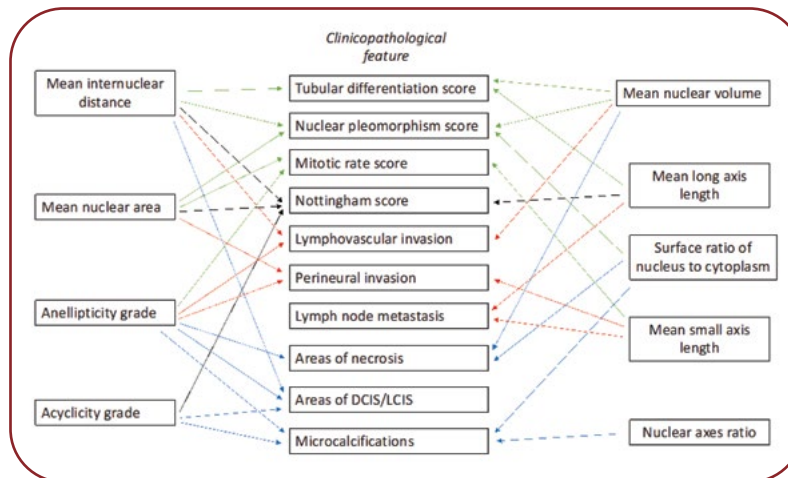


FIGURE 4. Correlations between cytonuclear morphometric parameters and clinicopathological features, with colors being used just to make the selected correlations easier to visualise

$<300 \mu\text{m}^3$ and anellipticity grade >1.1166 . DCIS/LCIS were more frequently present (62.5% vs. 30.77%) when at least two out of the three criteria, including acyclicity grade ≥ 1.16 , mean internuclear distance $\leq 12.85 \mu\text{m}$ and anellipticity grade >1.1133 , were met.

For the presence of microcalcification, a two-step algorithm is proposed. The first step involves evaluating the following four criteria: $N/C \geq 0.54$, axes ratio ≥ 1.26 , acyclicity grade ≥ 1.13 and anellipticity grade ≥ 1.1155 . The second step consists of checking whether the long axis length is ≤ 9.35 . Patients who meet all four criteria from the first step or three out of the four criteria from the first step and the criterion from the second step are more likely to have microcalcification. Using this algorithm, all patients with microcalcifications and 79.17% of those without microcalcifications have been correctly identified.

All the above-mentioned correlations can be seen in Figure 4. □

DISCUSSIONS

Even though some of the evaluated parameters can be more easily determined on the slide by the pathologist, a possible use of the developed algorithms consists in automatized determination of the pathological features using simple cytonuclear morphometrical measurements, and not complex patterns recognition such as mitotic figures.

Our study serves as a valuable complement to previous research, not only establishing numerous significant correlations but also offering

practical algorithms to facilitate interpretation. This dual contribution enhances the understanding and applicability of breast cancer cell morphometry in clinical practice.

The novelty in our research paper consists in the development of algorithms for a large series of pathological features. The majority of the follow-up papers report significant correlations and mostly show some cut-off values, but do not develop algorithms or structures to predict pathological characteristics.

In a period of transition from the classical qualitative analysis to the modern quantitative one with the aid of artificial intelligence, the understanding of mathematical algorithms in order to use coding and calculus software is indispensable. □

CONCLUSIONS

In the era of digital pathology, the significance of morphometry in pathology practice is continuously increasing. The advantages of morphometry, such as higher accuracy, reproducibility and provision of more quantitative and less qualitative data, make it an increasingly important tool in the field. The shift towards digital pathology enhances the potential and efficiency of morphometric analysis.

The integration of artificial intelligence has indeed revolutionized the field. Mathematical and criteria-based algorithms can be efficiently solved through coding, leading to increased time efficiency and accuracy. These advancements not only benefit the pathologist in their analysis but also provide valuable support to oncologists in

the comprehensive management of patients. There is a great synergy between technology and healthcare as artificial intelligence is used for a better personalization of patient pathology and the valuable data can be used in further research for a more personalized management.

In our study we have observed that the mean internuclear distance, mean nuclear volume, lengths of small and long axis were strongly correlated with the presence of aggressive risk factors, including lymphovascular invasion, lymph node metastasis, nuclear pleomorphism and perineural invasion. By measuring the nuclear area, nuclear perimeter, cell surface and nuclear long axis length on virtual slides, all essential morphometric parameters can be obtained. Additionally, these parameters can then be utilized in the defined algorithms for predicting clinicopathological features with high probability. Morphometric and criteria-based algorithms have been developed and detailed to obtain a highly accurate prediction of the Nottingham score, in-

cluding its individual elements, as well as lymphovascular and perineural invasions, lymph node metastases and presence of necrotic areas, DCIS/LCIS areas and microcalcifications. Our comprehensive approach enhances the predictive capabilities in pathology practice. ▣

Institutional review board: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "Carol Davila" Clinical Nephrology Hospital, Bucharest, Romania (approval number: 40, approval date: 4 August 2023).

Informed consent: Informed consent was obtained from all subjects involved in the present study.

Data availability: The data reported in this article are available upon reasonable request from the corresponding author.

Conflicts of interest: none declared.

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