

QUANTITATIVE NUCLEAR MORPHOMETRIC FEATURES ASSOCIATED WITH BENIGN AND MALIGNANT BREAST LESIONS

Brian O. Otieno¹, Mercy W. Kamau^{*2}, David K. Mwangi³

¹Department of Pathology, University of Nairobi, Nairobi, Kenya,

²Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya

³Department of Biomedical Sciences, Moi University, Eldoret, Kenya

***Corresponding Author:** *Mercy W. Kamau, mercy@bmsmue.org*

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ABSTRACT

Quantitative evaluation of nuclear morphology may improve the distinction between benign and malignant breast lesions by capturing structural differences in nuclear size, texture, and contour. A cross-sectional secondary-data analysis was conducted using the Breast Cancer Wisconsin (Diagnostic) dataset. Nuclear morphological characteristics derived from breast fine-needle aspiration images were evaluated, including radius, texture, perimeter, area, smoothness, compactness, concavity, concave points, symmetry, and fractal dimension. Descriptive analysis, group comparisons, correlation analysis, and binary logistic regression were used to examine morphological patterns associated with diagnosis. Malignant breast samples showed a consistent pattern of larger nuclear dimensions and greater contour irregularity than benign samples. Radius, perimeter, area, compactness, concavity, and concave points demonstrated clear diagnostic separation, whereas fractal dimension showed limited distinction. Strong correlations were observed among size-related features and among contour-related characteristics, indicating substantial overlap between related morphometric measures. After adjustment for correlated predictors, nuclear radius, texture, smoothness, and concavity remained independently associated with malignant diagnosis. Quantitative nuclear morphology provides useful information for differentiating benign and malignant breast lesions. Features reflecting nuclear enlargement, surface variation, and contour irregularity appear particularly relevant to malignant diagnosis. These findings support the value of integrated morphometric assessment in breast cytology and provide a basis for further validation using histopathological, clinical, and molecular information.

Keyword: Breast lesions; Nuclear morphology; Morphometry; Benign and malignant diagnosis; Fine-needle aspiration cytology

1. Introduction

Breast lesions are a large diversity of benign and malignant conditions, each varying in cellular organization, nuclear morphology, and biological behavior. It is, therefore, crucial to have a correct diagnosis of benign vs. malignant in breast cytology and breast pathology. The morphology of the nucleus is especially significant because malignant transformation is frequently associated with changes in the size, shape, texture, symmetry, and complexity of the nucleus. Such changes can be a key part of a nuclear evaluation - which is an important part of morphological diagnosis - and indicate underlying disturbances in chromatin organization and cellular regulation (Fischer, 2020).

Enlargement, pleomorphism, irregular contours, concavity, and chromatin appearance are common ways of assessing nuclear atypia. It is important to note, however, that there may be some variation in the interpretation of the features between observers, particularly when lesions have bordering or overlapping patterns. Fine-tuning in the nuclear morphological assessment has thus been highlighted to enhance the uniformity of diagnosis in breast disease (Katayama et al., 2022a). This is especially important as atypia can exist on a morphological spectrum from benign to malignant and requires a systematic assessment of several nuclear parameters (Katayama et al., 2022b).

Quantitative nuclear morphometry is an objective means of transforming the microscopic features into measurable quantities. The radius, perimeter, and area indicate the size of the nucleus, while the smoothness, compactness, concavity, number of concave points, symmetry, and fractal dimension offer insight into the regularity of the contour and the complexity of its structure. These measurements are useful for distinguishing benign and malignant cells in the breast in previous studies. Rajbongshi et al. (2018) have shown that morphological characteristics of the images from fine-needle aspiration cytology can be used to show distinct patterns between diagnostic categories. Similarly, Kashyap et al. (2018) reported the association of the nuclear morphometric parameters with the cytomorphological grading, which thus validates the diagnostic significance of quantitative nuclear evaluation.

With the advent of digital pathology, morphometric analysis has gained new strength in the diagnosis of breast lesions. The nuclear characteristics can be extracted from the digitized pathological images and analyzed systematically in a reproducible manner. In the study conducted by Yu et al. (2019), morphological and textural features can be used together to help in the classification of breast pathological images. Automated approaches have also been proposed for nuclear atypia assessment to objectively quantify nuclear size, shape, texture, and irregularity (Das et al., 2020). Similarly, features derived from images have been used in the development of diagnostic support systems for the detection of malignant breast cells in cytological applications (Saba et al., 2019).

Biological heterogeneity in breast cancer can also be seen in the morphology of the nucleus. Nuclear structure changes have been studied to understand the relationship between nuclear morphology and the

human epidermal growth factor receptor-positive or negative and important phenotypic differences in tumors (Li et al., 2021). The results suggest that nuclear measurements can give more information than just benign versus malignant discrimination and can show variation in the biology of breast tumors.

Morphological assessment is also crucial for some breast lesions that have similar cytological features. For instance, diagnostic papillary breast lesions can have several cytological features in common and thus may pose a diagnostic challenge. In such cases, systematic evaluation of both the system and the cell parameters has been demonstrated to enhance the morphological interpretation (Jamidi et al., 2021). This favours an integrated approach, where the size, texture, contour and structural complexity of the nuclei are evaluated as a whole, and not separately.

Importantly, several of the nuclear characteristics are related to each other and to the underlying nuclear morphology. Compactness is related to the nuclear size measures of radius and perimeter, and of area, while the other three measures (concavity and concave points) describe various aspects of nuclear contour irregularity. It is important to assess these relationships because high correlations among measurements could mean that they are not giving distinct information. A combination of group difference evaluation, inter-feature correlation, and independent association may then help to identify nuclear attributes best associated with malignant diagnosis.

For these reasons, the present study assesses quantitative nuclear morphological parameters that were extracted from breast fine-needle aspiration samples to determine their ability to separate benign and malignant diagnoses and to identify those nuclear parameters significantly related to malignancy.

Objectives

1. To characterize and compare the major nuclear morphological features of benign and malignant breast samples.
2. To examine relationships among nuclear size, texture, shape, and contour characteristics.
3. To identify nuclear morphological characteristics independently associated with malignant diagnosis.

2. Materials and Methods

2.1 Study Design and Data Source

The design of this study was cross-sectional, descriptive, and secondary data. We used the Breast Cancer Wisconsin (Diagnostic) data from the Kaggle website (UCI,2016). The database includes quantitative nuclear measurements (from digitized images of fine-needle aspirates of breast masses) and cases diagnosed as benign or malignant.

2.2 Study Sample

There were 569 samples of breast masses. All observations were breast lesion samples that had been confirmed as either benign or malignant. All available records with valid diagnostic and nuclear morphological data were taken into consideration.

2.3 Study Variables

Breast diagnosis (benign or malignant) was the main outcome variable. Nuclear morphological features such as radius, texture, perimeter, area, smoothness, compactness, concavity, concave points, symmetry, and fractal dimension were used as independent variables. In the data set, mean, standard error, and worst values were provided for each morphological characteristic.

2.4 Data Preparation

Before analysis, data were examined for missing values, and duplicate data and irrelevant variables were eliminated from the data set. The empty unnamed column and the identification number were omitted since it was not a biological measure. The nuclear morphological variables were left as continuous variables, and the diagnosis was entered as either benign or malignant for statistical analysis.

2.5 Statistical Analysis

Data for the diagnostic groups and nuclear morphological measurements were summarized using descriptive statistics. The results of continuous variables were shown as mean $\pm$ standard deviation or median (interquartile range). The independent-samples t-test or Mann–Whitney U test was used to compare the difference in nuclear characteristics between the benign and malignant groups based on the distribution of the data. Correlation analysis (either Pearson or Spearman) was employed to study the relationships between nuclear features. To determine morphological features associated with the diagnosis of malignancy, binary logistic regression was conducted and reported as odds ratio (OR), 95% confidence interval (CI), and p-value. A p-value < 0.05 was considered to be the threshold of statistical significance.

3. Results

3.1 Diagnostic Distribution and Overall Nuclear Morphology

The final dataset included all available nuclear morphological data for breast samples with no duplicate observations or missing analytical data. Benign and malignant cases were represented, but the main diagnosis group consisted of benign cases. The distribution of the diagnostic categories is shown in Table 1.

Table 1. Distribution of breast samples according to diagnosis

Diagnosis	Frequency (n)	Percentage (%)
Benign	357	62.74
Malignant	212	37.26
Total	569	100.00

As shown in **Table 1**, both diagnostic categories were sufficiently represented for comparative assessment, with benign lesions accounting for the larger proportion of the study population.

3.2 Morphological Characteristics of Benign and Malignant Breast Samples

There were differences in the morphology of the samples taken from the benign and malignant breast. Nuclear dimension, irregularity of the surface and contour abnormality were more prominent in malignant lesions. Some differences were most marked in those features which show nuclear size, compactness, nuclear concavity and concave points. However, fractal dimension had only relatively small differences between the two diagnostic groups. The summary of the main morphological features is presented in Table 2.

Table 2. Principal nuclear morphological characteristics according to diagnosis

Nuclear characteristic	Benign, Mean $\pm$ SD	Malignant, Mean $\pm$ SD
Radius	12.15 $\pm$ 1.78	17.46 $\pm$ 3.20
Texture	17.91 $\pm$ 4.00	21.60 $\pm$ 3.78
Perimeter	78.08 $\pm$ 11.81	115.37 $\pm$ 21.85
Area	462.79 $\pm$ 134.29	978.38 $\pm$ 367.94
Smoothness	0.092 $\pm$ 0.013	0.103 $\pm$ 0.013
Compactness	0.080 $\pm$ 0.034	0.145 $\pm$ 0.054
Concavity	0.046 $\pm$ 0.043	0.161 $\pm$ 0.075
Concave points	0.026 $\pm$ 0.016	0.088 $\pm$ 0.034
Symmetry	0.174 $\pm$ 0.025	0.193 $\pm$ 0.028
Fractal dimension	0.063 $\pm$ 0.007	0.063 $\pm$ 0.008

As indicated in the pattern in Tab.2, nuclear enlargement and increased contour irregularity were the main features of the malignant lesions. The same patterns were also seen for the size- and shape-related measurements when compared to the worst values, with malignant samples having higher values for these measurements.

3.3 Distribution of Selected Nuclear Morphological Features

The representative size and shape characteristics of the nuclei are shown in the distribution in Figure 1. Overall nuclear size was represented by the radius and the area, and the nuclear contour irregularity was represented by the concave points and the concavity.

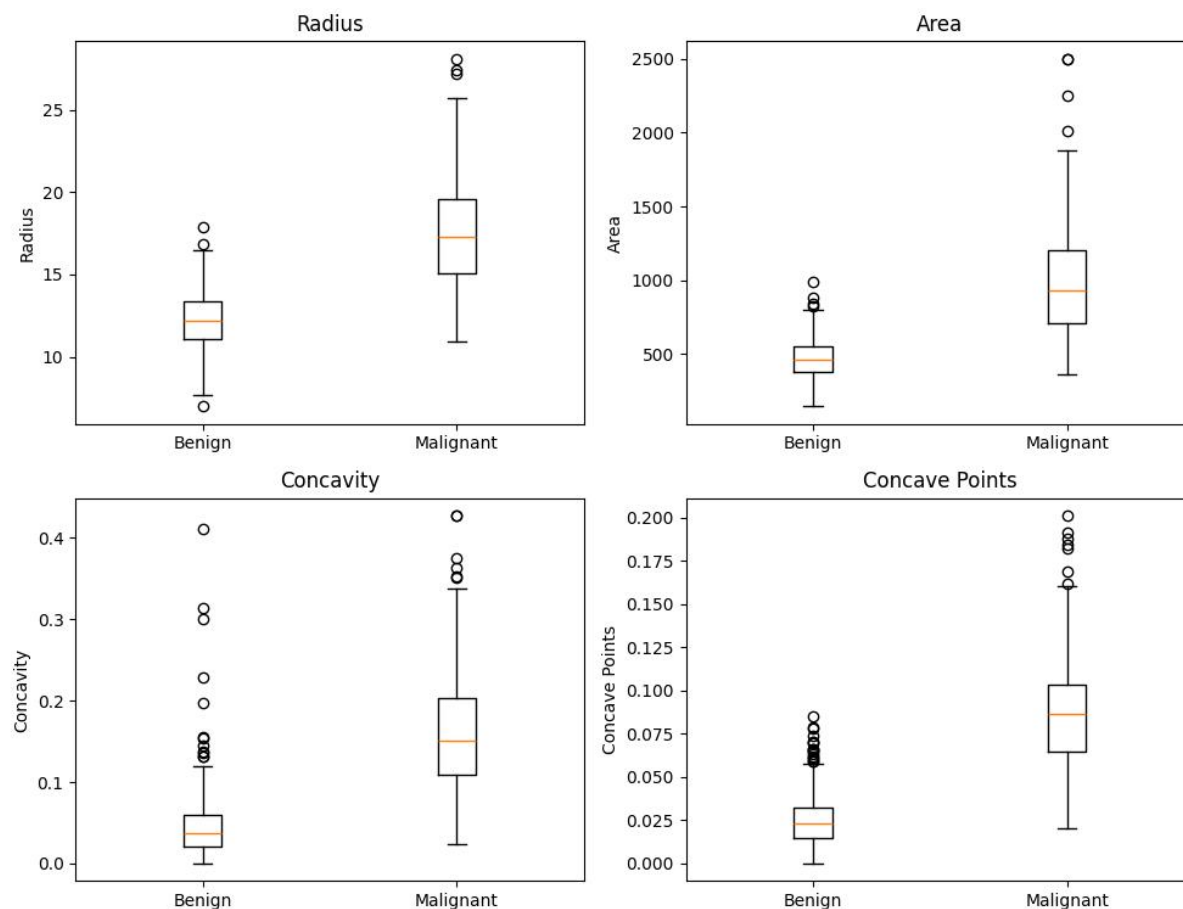


Figure 1. Selected nuclear morphological characteristics by benign and malignant diagnosis

The box and whisker plots revealed a uniform trend of an increase in the distribution of the selected characteristics in the malignant samples. Clear separation between the diagnostic groups was observed in nuclear size measures, and a marked difference in nuclear contour morphology was observed in the concavity-related measures.

3.4 Relationships Among Nuclear Morphological Characteristics

Correlation analysis showed that some of the nuclear features correlated with closely related structural dimensions. Compactness, concavity and concave points had the greatest relationships within a set, as did measures of nuclear size. Table 3 displays some relationships that were selected for presentation.

Table 3. Selected Spearman correlations among nuclear morphological characteristics

Variable pair	Spearman r_s
Radius – Perimeter	0.998
Radius – Area	1.000
Perimeter – Area	0.997
Radius – Concave points	0.760
Perimeter – Concave points	0.789
Area – Concave points	0.755
Smoothness – Compactness	0.679
Compactness – Concavity	0.897
Compactness – Concave points	0.848
Concavity – Concave points	0.927

As shown in Table 3, very similar size variables gave a very similar morphologic information. Compactness of the nucleus and the indentation of the contours were also strongly correlated with each other through shape variables.

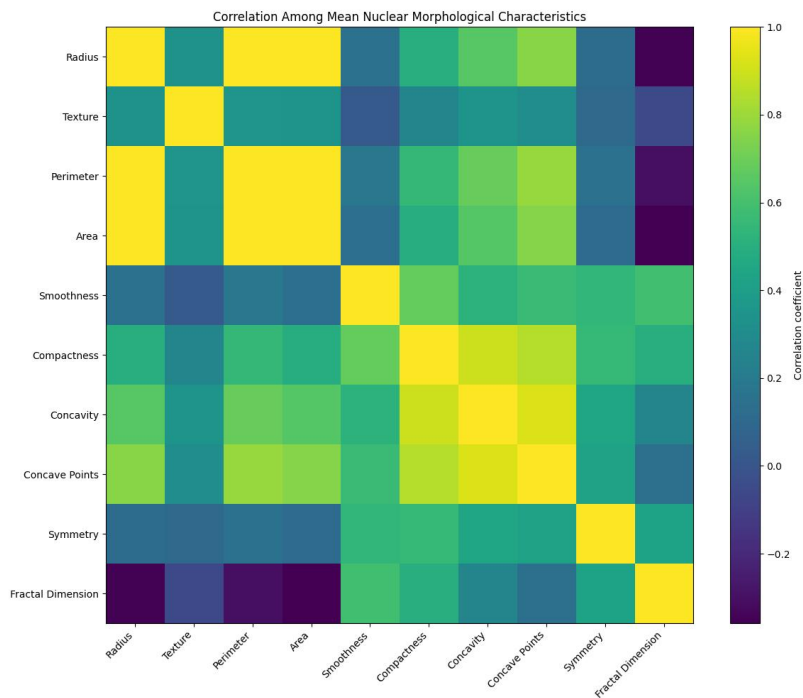


Figure 2. Spearman correlation heatmap of nuclear morphological characteristics

The correlation heatmap also showed two major morphological pattern groups, a size group (radius, perimeter, area) and a contour group (compactness, concavity, concave points). There were also strong relationships between the corresponding mean and worst-value measures, suggesting consistency between the various representations of the same underlying nuclear characteristics.

3.5 Morphological Characteristics Associated With Malignant Diagnosis

The regression analysis revealed that, after controlling for the correlation between the strongly correlated variables, several morphological features significantly contributed independently to the distinction between malignant and benign diagnosis. To avoid high levels of redundancy in size and contour variables, these were dropped prior to the final model, with the remaining variables reflecting complementary aspects of nuclear form. The results from the multivariable analyses are summarized in Table 4.

Table 4. Multivariable association of nuclear morphological characteristics with malignant diagnosis

Nuclear characteristic	Adjusted OR	95% CI	p-value
Radius mean	70.43	15.25–325.26	<0.001
Texture mean	5.25	3.09–8.90	<0.001
Smoothness mean	5.74	2.81–11.75	<0.001
Compactness mean	0.66	0.19–2.35	0.524

Concavity mean	8.34	3.25–21.39	<0.001
Symmetry mean	1.58	0.87–2.85	0.131
Fractal dimension mean	0.59	0.19–1.81	0.353

OR, odds ratio; CI, confidence interval. Odds ratios correspond to a one-standard-deviation increase in each continuous predictor.

Nuclear radius, texture, smoothness and concavity remained independent of malignant diagnosis as indicated in Table 4. However, compactness, symmetry and fractal dimension did not maintain independent associations following simultaneous adjustment.

4. Discussion

In the present study, clear morphological differences were observed between the benign and malignant samples of the breast, and the malignant breast samples were found to exhibit greater nuclear size and nuclear irregularity than the benign samples; there was greater alteration in several shape-related parameters. The distributions of radius, area, concavity, and concave points were particularly distinguishable between the two diagnostic groups, and multivariable analysis revealed that the variables of radius, texture, smoothness, and concavity were independent characteristics that were associated with malignant diagnosis. These results confirm the hypothesis that nuclear morphology is related to significant structural alterations associated with malignant transformation, and could be a valuable quantitative parameter for distinguishing between breast lesions.

The enlargement of the nucleus in the malignant samples observed here is in line with the pathological knowledge that breast carcinomas are heterogeneous as far as their histology is concerned, but often present with different extents of nuclear enlargement and atypia. In line with the multiple classifications of breast carcinoma, Cserni (2020) pointed out the need for considering the histological characteristics in selecting the type of the tumor, as the present result indicates that quantitative nuclear dimensions can provide a certain degree of morphological diversity. Enlargement of the nucleus can also be seen in abnormal forms of breast cancer. For instance, Ohashi et al. (2018) reported unique cytologic and pathological characteristics of breast carcinoma associated with osteoclast-like giant cells, which showed that nuclear and cellular variation are associated with certain malignant phenotypes.

The greater the concavity and the more concave points, the greater the distortion of the boundary of the nucleus is in the malignant lesion. This irregularity is of biologic interest because the cells of malignant tumors often lose the regular outline and uniform structure that is characteristic of benign epithelial cells. Pronounced atypical features have also been recorded in aggressive breast cancer phenotypes by cytomorphological studies. Kala et al. (2019) reported that triple-negative breast cancer is characterized as a very heterogeneous and morphologically atypical cancer. Talu et al. (2018) demonstrated, however,

that primary breast carcinomas with neuroendocrine (NE) characteristics can have variable growth patterns and unique pathological features. The observations confirm that the irregularity of the nuclear architecture is a part of the malignant morphology, but to different extents in different subtypes of breast cancer.

The present study also verified that the radius had strong relationships with the perimeter and the area, as well as the compactness, concavity, and concave points. Several of these nuclear variables correspond to overlapping structural domains and are not, therefore, completely independent of biological properties. Overlap is essential for the interpretation of breast lesions with unusual morphology. In the case of triple-negative breast carcinomas with low malignant potential, Cima et al. (2022) noted that diagnostic complexity and careful morphological evaluation should be performed to prevent misdiagnosis. Similarly, Rakha et al. (2022) highlighted the need for a structured morphological approach to spindle cell lesions of the breast, as there may be some morphological similarities between benign and malignant entities. The high correlation among the features found in this dataset thus calls for the consideration of representative morphological variables instead of analyzing each of the measurements separately.

In addition to the benign breast disease distinction, the difference between benign and malignant morphology should also be taken into account. Although architectural and cytological alterations may be noted in benign lesions, these alterations are not often of the magnitude seen when combined with enlargement and disruption of the nuclear contour in malignant disease. In their study, Stachs et al. (2019) provided an overview of benign breast disorders and the diversity of benign breast diseases in terms of clinical and pathological features, highlighting the importance of distinguishing from malignant breast disease. Similarly, Sanati (2019) discussed the morphological and molecular characteristics of ductal carcinoma in situ, and explained that neoplastic progression could be accompanied by increasingly abnormal characteristics of the cells and nuclei. This continuum was reflected in the present results, in which structural deviation from benign morphology was found to be more pronounced in malignant samples.

Variations in the appearance of the nucleus may also be a reflection of the variety of rare breast tumors. Tevatia et al. (2021) reported that primary breast tumors with the presence of a mesenchymal morphology could be variable in histological findings, and Abreu et al. (2020) showed that granulomatous cell tumours of the breast may pose a diagnostic challenge due to a similar appearance on imaging and histological examination with malignant tumours. The examples in these studies show why the use of any single morphological feature is inadequate. Instead, a combination of size, contour, texture and structural complexity is more informative, a finding that is consistent with the present study's multivariable findings.

The results also have relevance for digital pathology and computer-assisted diagnosis. Feng et al. (2018) indicated the possibility of classifying breast cancer cells using nuclear information extracted from histopathology images, and Baker et al. (2018) indicated the feasibility of automated detection of benign

and malignant breast histopathology images. The present study contributes to approaches like these by providing information on nuclear features that are of special value for diagnostic separation. Morphological characteristics must be used in conjunction with existing pathological evaluation, however. Sun et al. (2023) highlighted that immunohistochemistry remains a valuable tool for diagnosis and classification of breast tumors even when morphologic features were absent.

The overall findings suggest that nuclear enlargement, altered surface, and irregular contours of the nucleus are coordinated in the case of malignant breast lesions. The radius, texture, smoothness, and concavity are independent, implying that the size of the nucleus and structural complexity both play roles in distinguishing it diagnostically. One of the advantages of the study is the simultaneous assessment of several quantitative nuclear parameters, but the limitations of a secondary database and the lack of detailed histological subtyping, receptor, and clinical data constrain broader biological interpretation. Future studies should assess whether these morphological patterns are stable within pertinent breast tumor categories and include morphometry with histopathological or molecular markers.

5. Conclusion

The results of this study confirmed that the differences in nuclear size and shape between benign and malignant breast lesions are significant, and that objective morphometric parameters of these features can be quantified. The malignant samples showed a larger nuclear size of the nucleus, stronger contours, and changes in the structural features than the benign samples. However, a number of related measures were highly intercorrelated, and several measures had significant differences between the two diagnostic groups, with the largest differences occurring for radius, perimeter, area, concavity, and concave points. The multivariable analysis also demonstrated that nuclear radius, texture, smoothness, and concavity remained independently associated with malignant diagnosis despite the fact that these features were correlated. These results help validate quantitative nuclear morphology as a diagnostic tool for assessing breast lesions. Evaluation of complementary characteristics of size, texture, and contour should be used in addition to a single morphological parameter, and may give a more informative representation of malignant nuclear change. The results also reveal the need to identify and remove these redundancies among the closely related morphometric variables in the construction of analytical or diagnostic models. The study has a secondary data design and is restrained by the lack of clinical and detailed histological subtype and molecular data, but the results add to the current use of nuclear morphometry to validate further in breast pathology. Future studies need to combine quantitative nuclear features with histopathological and molecular markers in various breast tumour subtypes.

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