



Original Paper

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## Quantitative classification of breast fine needle aspirates using the AxioHOME system

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### ABSTRACT

Fine needle aspiration cytology is useful in the pre-operative assessment of patients with breast lumps. Lesions are reported as benign, suspicious or malignant. The number of suspicious categories is high in inexperienced hands thus limiting this useful diagnostic tool. The aim was to evaluate quantitative methods of classifying breast fine needle aspirates using the Highly Optimized Microscope Environment system. May-Grumwald Giemsa-stained archived slides were retrieved and smear quality assessed. Fifty epithelial cells corresponding to the cytological grading on each slide were measured using the system's general morphometry program. Generated data was exported to Microsoft Excel for analysis. A significant difference in the mean nuclear area, mean nuclear perimeter, mean largest nuclear diameter was found between the slides graded as benign, suspicious malignant and malignant. C2 & C4, D 1,  $P < 0.004$ . C4 & C5, Area  $P < 0.001$ . The means of the number count of nucleoli was able to distinguish between FNAC Benign and Malignant category C2/C5,  $P = 0.00440$  and suspicious benign and malignant C3/C5  $P = 0.00486$ . Morphometry could be useful in situations where experienced cytopathologists are unavailable especially when this program can also measure the degree of dispersion between cells.

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**Keywords:** Morphometric image analysis, aspiration cytology, breast.

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### INTRODUCTION

Breast cancer is the leading malignancy accounting for 16% and 11% of all cancer deaths in developed and developing countries respectively with large differences in mortality among countries world wide. (Pisani, 1993; Kestlelot and Zang, 2006).

In 2003, 582,000 people in Africa presented with cancer – 299,000 men and 283,000 women. Current trends as seen on several cancer registries in Ibadan, Calabar, Zaria, and Enugu show increasing relative frequency of cancer of the breast at 25.3% (Parkin et al., 2003). As these are only hospital figures, the real incidence is likely to be higher due to under reporting with many women dying at home undiagnosed.

Most breast cancers are detected as painless lumps either by the patient or the doctor during a routine physical examination (Kelsey and Ross, 1993). Clinical suspicions of malignancy can be investigated by surgical removal of the lump, i. e surgical biopsy followed by histological examination, by use of large core needle biopsy and by fine needle aspiration cytology. Surgical biopsy is an invasive procedure that requires the use of theatre space and a variable waiting period for processing the sample, but it provides all the required information for a definitive diagnosis, tumour grading and is the gold standard upon which treatment is based. It is however expensive. Large core needle biopsy can be performed in the clinic but it requires

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local anaesthetic and an expensive instrument – the biopsy needle. It however also provides some architectural detail and is processed in the same way as a surgical biopsy sample. Fine needle aspiration of breast lumps can be performed as an out patient procedure with the use of fine needles (Size 23 G or less). It is relatively painless and requires no anaesthetic.

The sensitivity of fine needle aspiration cytology (FNAC) in the diagnosis of breast cancer is 90 -95% in most series (Zarbo et al., 1991). Although the technique is simple, it requires lot of training and practice to acquire and maintain the skill to do it properly. Some women with breast complaints may have benign lesions. The challenge has been to have clearly defined criteria for correctly identifying cases of malignancy while excluding benign processes.

A multidisciplinary approach to the diagnosis of breast lesions seeks to do this. In the “*Triple Approach*” a combination of clinical examination, mammography /ultrasound and fine needle aspiration are used to evaluate breast lumps (Gray 1996). Definitive treatment can be based on the cytological diagnosis when all three agree i.e. concordance between clinical examination, mammographic and ultrasound assessment. Breast fine needle aspirates are graded cytologically as Benign (C2), Suspicious Benign (C3), Suspicious Malignant (C4), and Malignant (C5) with corresponding categories by clinical examination and by ultrasound.

The success of needle aspiration cytology relies heavily on the ability of the cytologist to identify and characterize cytological changes in the prepared aspirate. This presents a number of problems. Diagnosis is based on visual criteria which are subjective and can be misleading in certain circumstances. The number of visual clues which need to be assessed and the number of options available may limit consistent reproducibility. Cytological classification as C2-C5 in the triple approach is thus subjective with a margin of error. Significant improvement in accuracy occurs with experience (Zarbo et al., 1991). Lesions in the suspicious category require further evaluation by surgical or trucut biopsy. This is not only more expensive but worrying for the patient.

Various quantitative methods have been developed for the measurement of

microscopic features in histology and cytology. Most of these are tedious and time consuming to perform and are not employed in routine diagnosis (Beck, 1987; Baak, 1987; Marchevsky and Silage, 1986; Collan et al., 1987; Ellis, 1996). Reliable objective criteria using a simple, readily applicable method can increase the accuracy, efficiency and thus the cost effectiveness of the diagnosis of breast lesions. Objective criteria are not only useful in interlaboratory comparisons but will encourage the widespread use of cytomorphometry to resolve diagnostic problems in breast fine needle aspiration cytology by reducing the error margin during the early learning period before much experience is gained. This is important in our environment where we do not have sufficiently trained manpower in the field.

The objective of this study was to determine if the number of specimens assigned morphologically to the suspicious category C3/C4 in breast fine needle aspirates can be reduced by employing a quantitative approach using image analysis.

## MATERIALS AND METHODS

This was a retrospective study of archived breast fine needle aspirates diagnosed in the Cytology Department of St Mary’s Hospital London.

A search of computer files for breast aspirates graded cytologically as C2, C3, C4 and C5 seen within a two year period (1995/96) was made. The air-dried slides stained by May Grumwald Giemsa according to standard protocol were retrieved from the stored slides and the quality of smears assessed. Only well-stained slides without cracks, containing epithelial cell clusters which were used to determine the cytological grading were selected. 10 slides were selected for each category.

Fifty ductal epithelial cells on areas of minimum nuclear overlap were circled for analysis on each slide using the General Morphometry Programme on the Highly Optimised Microscope Environment (The AxioHOME System) (Brugal et al, 1992). This is a computerized microscope designed to assist pathologists and cytotechnologists in routine clinical tasks. The prototype of the system is an IBM Compatible computer and light microscope in which a built-in high

resolution computer display image is superimposed on the optical image of the specimen. By using the mouse-driven cursor to click on dialogue boxes and icons, the user can interact with the computer without looking up from the microscope. The image which the HOME system generates is a real optical image. This enables the pathologist to be on familiar ground and the use of the system can take place as part of routine diagnostic practice.

Before each morphometry session, a stage and objective calibration was carried out using the HOME calibration slide. Measurements were made under oil immersion using the small pixel pen on the tool menu to draw around the nuclear margins of the cells and the small mark size to tag the measured cells.

### Statistical analysis

The data generated for each slide was saved and exported in D Base format to Microsoft Excel where descriptive and comparative statistical analysis was made among the categories by means of the Student's t-test using the Microsoft Excel Data Analysis Tool Pak.

The number count of nucleoli on each slide was performed and means determined for each FNAC category. The Student's t-test was also performed among the categories

using the means of the number count of nucleoli for each category.

### RESULTS

Forty cases of breast five needle aspirates (FNA) were studied. The slides were from female patients aged 18–85. The mean age was 50.

Table 1 shows the result of the measurement of nuclear features in each FNA Category presented as means and standard error of the mean. Variables 3-6 describe nuclear size i.e. Area, Perimeter, D1, D2 and variables 7-12 describe nuclear shape i.e. D1/D2, form factor, circularity, ellipticity, regularity, convex hull.

Table 2 is the result of the comparison of the size variables using the Student's t-test. The probabilities giving 9 degrees of freedom are the figures on brackets. The mean largest nuclear diameter (D1) distinguished breast FNA Category C5 from the benign C2 and suspicious categories C2/C4 (Figure 1).

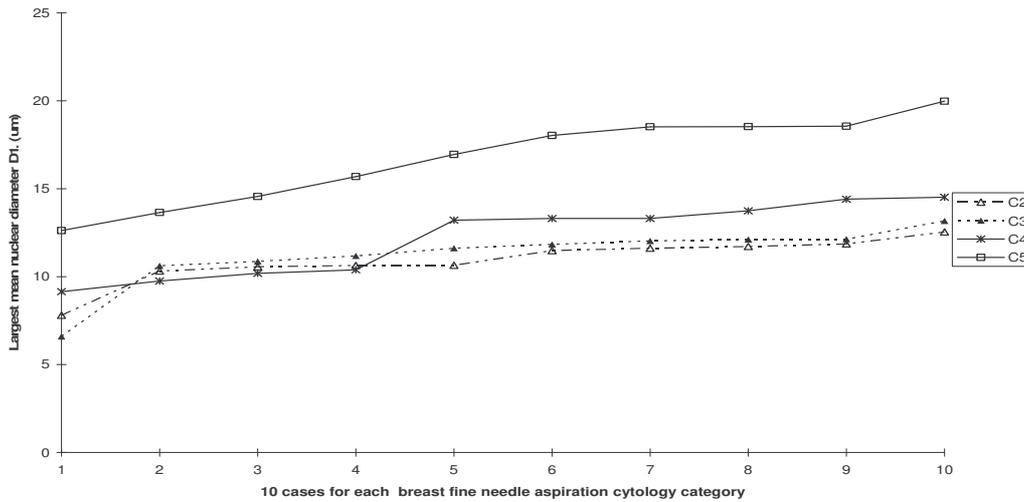
The mean nuclear area showed some discrimination between the malignant and suspicious categories but not so much between the benign and suspicious categories (Figure 2). Table 3 shows the means of the number count of nucleoli for each FNA Category. Table 4 shows the result of the student t-test of the means of the number count of nucleoli between the categories.

**Table 1:** Result of the measurement of nuclear features in each fine needle aspirate category.

FNAC Category	C2	C3	C4	C5
1. Number of cases (50 epithelial cell nuclei in each case)	10	10	10	10
2. Mean age of cases (n = 10 for each category)	41	44.3	55.6	59.4
<b>Variables</b>				
3. Area ( $\mu\text{m}^2$ )	70.29±5.148	74.27±7.648	83.46±9.832	164.3±20.82
4. Perimeter ( $\mu\text{m}$ )	36.66±1.580	37.38±2.137	39.09±2.169	52.69±3.704
5. D1	10.95±0.459	11.17±0.627	11.94±0.675	16.94±0.936
6. D2	8.22±0.376	8.33±0.526	8.76±0.559	12.52±0.772
7. D1/D2	1.35±0.019	1.36±0.017	1.38±0.021	1.37±0.037
8. Formfactor	1.56±0.26	1.58±0.026	1.54±0.031	1.47±0.033
9. Circularity	0.93±0.077	0.88±0.006	0.84±0.008	0.84±0.011
10. Ellipticity	1.29 ±0.58	1.36±0.017	1.38±0.021	1.37±0.037
11. Regularity	7.84±0.249	7.76±0.345	7.01±0.294	7.63±0.439
12. Convexhull	0.76±0.001	0.77±0.003	0.76±0.002	0.76±0.005

Tabulated values are Means ± Standard Error of Mean.

Key: Fine needle aspiration cytology category C2= Benign, C3 = Suspicious Benign, C4 = Suspicious Malignant, C5 = Malignant. Variables 3-6 describe nuclear size and variables 7-12 describe nuclear shape. D1 = Largest Nuclear Diameter, D2 = Nuclear Diameter.



**Figure 1:** The largest nuclear diameter, D1, for breast fine needle aspiration diagnostic categories. The mean largest nuclear diameter distinguished breast FNA malignant category, C5 from the benign C2 and suspicious categories C3/C4.

**Table 2:** Student's t-test analysis of the fine needle aspiration cytology categories.

Category	Area	Perimeter	D1
C2 and C3	0.8256 (P=0.2164)	0.6067 (p=0.02498)	0.3914 (p=0.2465)
C2 and C4	2.941 (p=0.008)	3.029 (p=0.007)	3.318 (p=0.004)
C3 and C4	2.1800 (p= 0.28)	1.8371 (p= 0.496)	2.4212 (p= 0.019)
C4 and C5	4.2912 (p= 0.001)	3.9421 (p= 0.002)	4.9661 (p= 0.0005)

Key: Fine needle aspiration cytology category C2= Benign, C3 = Suspicious Benign, C4 = Suspicious Malignant, C5 = Malignant. D1 = Largest Nuclear Diameter.

**Table 3:** Means of number count of nucleoli for each fine needle aspiration cytology category.

Category	Mean	Variance
C2	4.6	16.044
C3	5.8	48.431
C4	13.2	136.75
C5	21.4	182.335

Key: Fine needle aspiration cytology categories- C2= Benign, C3 = Suspicious Benign, C4 = Suspicious Malignant, C5 = Malignant.

There was a significant difference between the mean number of nucleoli of C2/C5, P = 0.00440 and C3/C5, P= 0.00486.

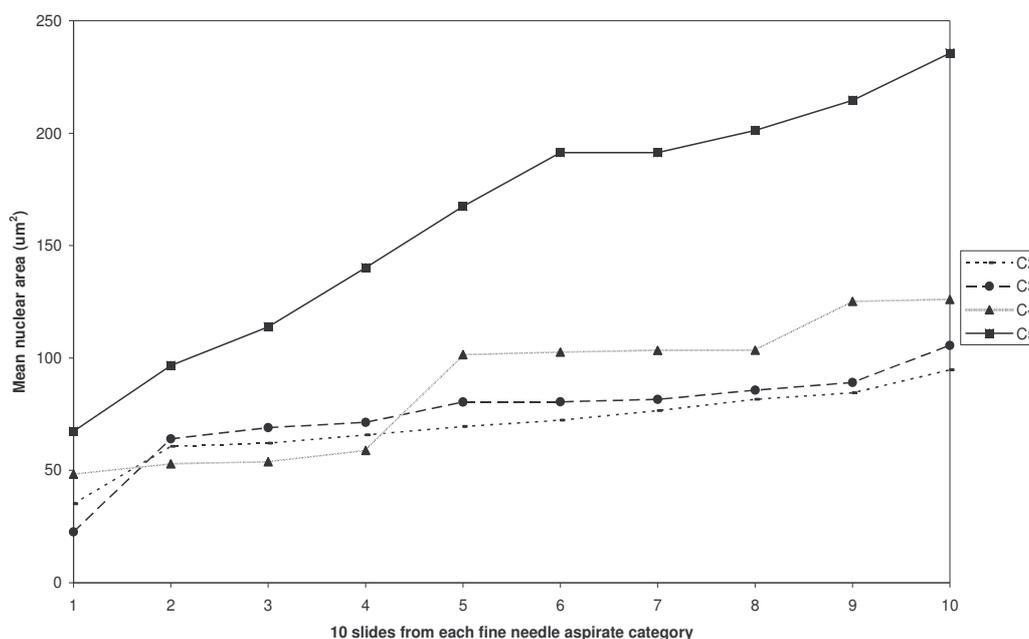
The means of the number count of nucleoli was able to distinguish between FNAC Benign and Malignant category C2/C5,

P = 0.00440 and suspicious benign and malignant C3/C5 P = 0.00486. There was no significant difference in the number count of nucleoli between the suspicious benign and suspicious malignant categories.

**Table 4:** Result of Student's t-test of the means of number count of nucleoli between the breast fine needle aspiration cytology categories.

Paired t-test	Probability	Comment
C2/C3	0.6674	Not significant
C2/C4	0.1426	Not Significant
C2/C5	0.00440	Significant
C3/C4	0.2470	Not significant
C3/C5	0.00486	Significant
C4/C5	0.2684	Not significant

Key: Fine needle aspiration cytology categories: C2= Benign, C3 = Suspicious Benign, C4 = Suspicious Malignant, C5 = Malignant.



**Figure 2:** Mean nuclear areas for breast fine needle aspiration diagnostic category. The mean nuclear area showed some discrimination between the malignant and suspicious categories but not so much between the benign and suspicious categories.

**DISCUSSION**

At 95% confidence level, the means of the variables obtained by morphometric image analysis using the HOME System for breast FNA diagnostic categories are as presented in table 2. There was little difference in the means of the shape descriptors such as formfactor circularity, convexity, regularity and convexhull between the FNA categories. The shape variables cannot therefore be used to distinguish one FNA category from the other.

The difference in the largest nuclear diameter, D1, mean nuclear perimeter and the mean nuclear area between FNA Benign, C2,

and the suspicious benign C3 categories was not significant. There was however a significant difference in these size variables between C2 & C4 (Table 3). The t- test for the mean nuclear area was  $t = 2.941$ ,  $P < 0.01$  for the mean nuclear perimeter,  $t = 3.3029$ ,  $P < 0.01$ , and for the mean largest nuclear diameter D1,  $t$  was  $3.313$ ,  $P < 0.01$ . D1 also demonstrated a significant difference between C3/C4 and C4/C5.

However the distinction between the two suspicious categories C3/C4 was not as marked as that between C2/C4 and C4/C5. The mean largest nuclear diameter D1, showed the least standard error of the mean

and the highest significant level. It is therefore a more reliable and useful criterion than the other size descriptors for this purpose. When combined with the mean of the number count of nucleoli, D1 can be used to distinguish the suspicious benign from malignant categories.

The term "image analysis" is reserved for a special discipline in pathology that aims to obtain diagnostically important information in an objective and reproducible manner by measuring and counting. The introduction of computers has been a major influence on the evolution of image analysis (Meijer et al., 1997). Although studies have shown that nuclear and cellular measurements can be beneficial in the objective diagnosis of breast cytology, the tissue type, choice of texture and methods used to carry out these measurements in previous studies of morphometric analysis of breast lesions have varied widely. Most of these are time consuming and cannot be employed on routine practice. It is therefore not surprising to find a wide range of nuclear sizes quoted in literature for morphometry of breast carcinoma lesions (Walgren et al., 1976; Yu et al., 1995; Wolgerg et al., 1993; Wittekind and Schutte, 1987; Hamilton et al., 1994). Recent studies have also attempted to derive cytoprognostic scores based on nuclear features and nuclear morphometry for breast and prostate cancer respectively (Fan et al., 2006; Buhmeida et al., 2006). This is the first report of image morphometry on breast fine needle aspirates using the AxioHOME system.

This study has described quantitative features which can be applied in the discrimination of malignant from benign, suspicious benign categories using the mean nuclear area and the mean largest nuclear diameter. The discrimination between the suspicious categories is not that high. Cases with mean nuclear diameter of 16.94  $\mu$ m, standard error of mean  $\pm$  0.94  $\mu$ m can within 95% confidence limits be said to be malignant. These measurements can easily be carried out using the immediate morphometry program on the HOME System on routine practice.

An extension of this study would be to use the HOME System to ascertain the number of cells on a slide, the degree of cellular dissociation and nuclear pleomorphism and to use this to define

morphometric parameters for cytological grading. This will not only have prognostic implications but can be used to follow up patients on adjuvant therapy for breast cancer. Other applications can be carrying out the same measurement on breast FNA Slides prepared by liquid based technology. The system can also be developed to be used in forming part of Grid Technology for information sharing, training, expert opinion, and quality assurance for the remote practitioner by telepathology (Brugal et al., 1992; Gotler et al., 2006).

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