ORIGINAL ARTICLE

To Evaluate The Usefulness of Cytological Grading of Breast Cancer as a Predictor of Lymph Nodal Metastasis

Subhash Bhardwaj, Farooq Ahmed Wani, Pervez Ahmed Katoch

Abstract
In a 5-year retrospective and 1-year prospective study a total of 110 fine-needle aspiration cytology (FNAC) cases of breast cancer were studied. These were graded according to Robinson's and Mouriquand's grading methods and relationship between grading and lymph node metastasis; cell dissociation parameter of Robinson's grading and lymph node metastasis evaluated. Of the 110 cases graded by Robinson's method, Grade I tumours had lymph node involvement in 3.57% of cases. Grade II and Grade III tumours had lymph node involvement in 6.52% and 47.22% of cases respectively where as when graded by Mouriquand's method, Grade I tumours had lymph node involvement in 3.57% of cases. Grade II and Grade III tumours had lymph node involvement in 7.14% and 42.5% of cases respectively. Although both grading systems showed significant relationship with lymph node involvement but Robinson's grading was a much better predictor of lymph node metastasis. Our study showed that greater cell dissociation was associated with higher incidence of regional lymph node metastasis. A highly significant relationship was observed between cell dissociation and lymph node involvement (p=0.00000). We conclude that cytological grading of breast cancers is a strong predictor of lymph nodal metastasis and greater cell dissociation is associated with higher incidence of regional lymph node metastasis.

Key Words
Breast Cancer, Metastasis, Lymph Nodes

Introduction

Breast cancer is the second most common type of cancer and the second leading cause of cancer related deaths in females. (1) Since a less invasive pre-operative diagnosis is always preferred to open biopsy, fine needle aspiration cytology has been frequently used to decide the benign or malignant nature of particular breast lesions. But many studies have shown that this technique can provide additional information about the intrinsic features of the tumours as well as their prognosis. (2)

Grading of breast carcinoma, while the tumour is still in vivo, would be the most ideal and desirable situation, as it would be helpful in the selection of patients for appropriate therapy. (3) The combined use of clinical, mammographical and cytological findings (Triple Test technique) has been recommended by many authors as it increases the sensitivity and specificity of the final diagnosis. (4, 5, 6)

Greater cell dissociation has been found to be associated with higher incidence of regional lymph node metastasis. (7) The degree of cell dissociation is an indicator of cell cohesion status and to an extent, of the degree of expression of the Cadherin molecules on the cell surface. Loss of cell cohesion appears to facilitate vascular infiltration by tumour cells, which gives rise to an increased incidence of regional lymph node metastasis. (8) High cytological grade has also been found to be associated with nodal metastasis. (9) As we know regional lymph node metastasis can predict the outcome of disease, therefore, cytological grade is a useful tool for predicting the prognosis of the patient.

Robles *et al* (2005) found that 3 out of 6 features of Robinson's grading system (nuclear margins, cell uniformity and cell dissociation) displayed a positive correlation with the presence of metastasis in axillary

lymph nodes. Cancers with more pleomorphism and greater degree of cell dissociation tend to have greater incidence of regional lymph node metastasis. (2) Wallgren and Zajicek (1976) found that the predictive value of size of cell clusters was determined by the poor survival when the smears were dominated by free cells or small clusters, presumably indicating low intercellular cohesiveness. (10)
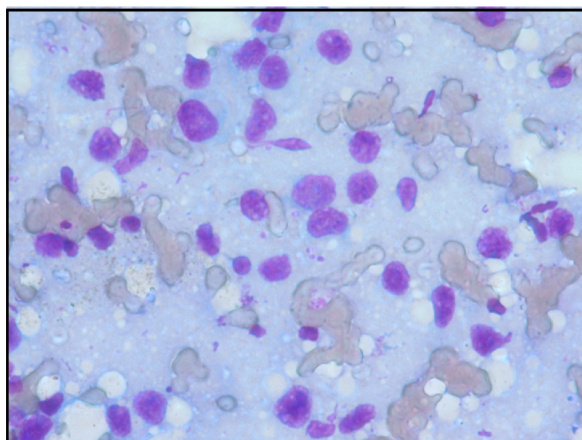
Fan et al proposed cytoprognostic scoring system based on nuclear grade, cellular dyscohesion and bare atypical nuclei. They found that high cytoprognostic score correlated with more positive lymph node metastasis and poor expression of prognostic markers. (11)

Material and Methods

After obtaining clearance from Institutional Ethics Committee, all records regarding diagnosed or highly suspicious breast cancer cases (for the period starting from October 2000) were retrieved from the Cytological section of Department of Pathology. These records were then matched with the database of Breast cancer registered by the department of Radiotherapy and Surgical operation log books. Patients diagnosed at other institutions but receiving treatment in the hospital were excluded from the study. Retrospective study material comprised of 90 cases and prospective study material comprised of 20 new cases. These cases were referred from the department of Surgery or Radiotherapy for confirmation of diagnosis. All the patients referred were subjected to fine needle aspiration cytology and MGG and PAP staining was performed on the smears.

All the relevant clinical information provided in the requisition form was taken into consideration including age as well as lymph node status among other things. Operated breast cancer cases that had their lymph nodes

removed and had recurrence on chest wall also formed part of the study. Grading of breast carcinoma was done according to Robinson's and Mouriquand's method by two independent observers. In case of disagreement between the two, the slides were shown to third observer and his decision was treated as final. In addition, parameters like tumour size, skin involvement, nipple discharge and recurrence of tumour were also evaluated and their relationship with cytograding systems was studied. Robinson's Method takes into account following criteria: Cell dissociation (clusters/single cells), cell size (1-2/3-4>5×RBC size), cell uniformity (monomorphic/ mildly pleomorphic/pleomorphic), nucleoli (indistinct/ noticeable/prominent), nuclear margins (smooth/ folds/ buds or tufts) and nuclear chromatin (vesicular/granular/ clumped or cleaved). Each of the above criteria was given scores 1 - 3 and total sum of scores of all the criteria were used to grade tumours. Based on above mentioned criteria, breast cancers were graded into Grade - I (score 6 - 11), Grade - II (Score 12 - 14) and Grade III (Score 15- 18) respectively. (12)

Mouriquand's Method takes into account the following criteria: - Cellular characters (clustering, 0/isolated cells, 3), nuclear features (anisokaryosis, 2/large cell, 3/budding, 2/naked, 3/hyperchromasia, 2/hypochromasia, 3) nucleoli (blue, 2/red, 3) and number of mitosis (>3/slide=1, >6/ slide=3). Based on these criteria, breast cancers are graded into Grade I (score < 5), Grade II (score 5 - 9) and Grade - III (Score > 10). (13)

Statistical Analysis

The data was analyzed with the help of computer software Epi-Info Version 6.0, CDC Atlanta. The data was cross classified according to grading methods and

Fig. 1 Photomicrograph Showing Grade III Breast Cancer Depicting Dispersed and Pleomorphic Tumour Cells (MGG 200X)

Fig.2 Photomicrograph Shows a Cluster of Cancer Cells in a Background of lymphoid Cells along with Dispersed Tumour Cells (PAP 200X)

Table I. Robinson's Grading
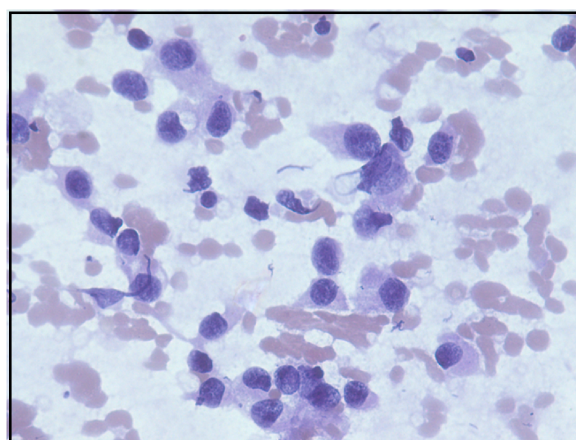
Grade	Total Score	No. of Cases
I	6-11	28(25.45%)
II	12-14	46(41.81%)
III	15-18	36(32.72%)

Table 2. Mouriquand's Grading

Grade	Total Score	No. of Cases
I	<5	28(25.45%)
II	5-9	42(38.18%)
III	=10	40(36.36%)

Table.3 Lypmh Node Involvement & Robinson's Grading

Grade	Total No. of cases	Lymph Node Involvement		
		Palpable & involved	Palpable not involved	Not palpable
I	28(25.45%)	1(3.57%)	3 (10.71%)	24 (85.71%)
II	46(41.81%)	3 (6.52%)	6 (13.04%)	37 (80.43%)
III	36(32.72%)	17 (47.22%)	1 (2.77%)	18 (50%)
Total	110 (100%)	21 (19.09%)	10 (9.09%)	79 (71.81%)

$X^2(4)$ 28.32 $p = 0.0000$ *Highly Significant*

Table.4 Lypmh node involvement & Mouriquand's Grading

Grade	Total No. of cases	Lymph Node Involvement		
		Palpable & involved	Palpable not involved	Not palpable
I	28(25.45%)	1 (3.57%)	3 (10.71%)	24 (85.71%)
II	42(38.18%)	3 (7.14%)	6 (14.28%)	31 (73.80%)
III	40(36.36%)	17 (42.5%)	1 (2.5%)	22 (55%)
Total	110 (100%)	21 (19.09%)	10 (9.09%)	79 (71.81%)

$X^2(4)$ 23.48 $p = 0.0001$ *Highly Significant*

lymph node involvement and presented as percentages. The difference in proportions was evaluated using Chi Squared test. A p-value (two tailed) of less than .05 was considered as statistically significant.

Results

Of the 110 cases graded according to Robinson's method, 28 (25.45%) cases were grade I, 46 (41.81%) cases were grade II and 36 (32.72%) cases were grade III tumours whereas using Mouriquand's method, 28 (25.45%) cases were grade I [scores < 5], 42 (38.18%) cases were grade II [scores 5-9], 40 (36.36%) cases were grade III [scores >10].

Of the 28 cases graded as grade I by Robinson's method, 1 (3.57%) case had lymph node involvement. Grade II tumours had lymph node involvement in 3(6.52%) of cases. Grade III tumours had a maximum lymph node involvement in 17(47.22%) cases (*Table 1, Figure 1&2*).

Relationship between lymph node involvement and Robinson's grade was found to be highly significant in our study (p=0.0000).

Out of 28 cases reported as grade I by Mouriquand's method, only 1 (3.57%) case had lymph node involvement. Grade II tumours had lymph node involvement in 3 (7.14%) cases. Grade III tumours had lymph node involvement in 17 (42.5%) cases (Table 2). Relationship between lymph node involvement and Mouriquand's grading was highly significant in our study (p=0.0001). In Robinson's grading, out of 21 cases showing lymph node involvement, 15 (71.42%) cases showed cell dissociation score of 3 followed by 5 (23.80%) cases showing cell dissociation score of 2 and 1 (4.76%)

case showing cell dissociation score of 1 (*Table 3*). Highly significant relationship was found between cell dissociation scoring of Robinson's method and lymph node involvement (p=0.00000). Relationship between the cytograding methods and tumour size, skin involvement, nipple discharge and recurrence of tumour was found to be statistically insignificant in our study.

Discussion

A highly significant relationship was observed between lymph node involvement and Robinson's grading (p=0.0000) and between lymph node involvement and Mouriquand's grading (p=0.0001). These results are similar to earlier studies done by Mouriquand's *et al* (13), Taniguchi *et al* (9) and Robles *et al* (2). Robles *et al* found that 88% of grade III tumours in their study had lymph nodal metastasis. (2) We found that 47.22% of grade III tumours had lymph node involvement. The lower incidence of nodal metastasis as compared to study

by Robles *et al* (2) seems to be due to inclusion of recurrent breast cancer cases who already had undergone radical surgery and had their lymph nodes removed. Furthermore, greater cell dissociation was associated with higher incidence of regional lymph node metastasis. Out of 21 (19.09%) cases showing lymph node involvement, 15 (71.42%) showed a cell dissociation score of 3 when Robinson's grading was applied (*Table-3*). A highly significant relationship was observed between cell dissociation and lymph node involvement (p=0.00000). Our findings were in accordance with previous studies conducted by Mouriquand *et al* (13), Layfield *et al* (14), and Robles *et al* (2). Robles *et al* (2) found that 86% of their patients with nodal metastasis had cell dissociation score 3. Mouriquand and Pasquier found that 66% of their patients with grade III carcinoma had local recurrence of disease, metastasis or death within one year of follow up. Layfield found that 57.14% of their patients with individual cell pattern had

nodal metastasis. (14) Schiller et al in their study failed to show cellular dyscohesion in FNA specimens as predictive of lymph node metastasis, however, the scoring method for determining the degree of cellular dyscohesion was reproducible between two independent observers. (15) No statistically significant relationship was found between cytograding methods and tumour size, skin involvement, nipple discharge and recurrence of tumour, consistent with the studies done by Layfield *et al* (14) and Taniguchi *et al* (9).

Conclusion

Cytological grading of breast cancers is a strong predictor of lymph nodal metastasis; higher grade cancers were associated with higher incidence of lymph node metastasis. Furthermore greater cell dissociation was associated with higher incidence of regional lymph node metastasis.

References

1. Lester SC. The breast. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran, Pathologic basis of disease. 7th ed. Philadelphia: WB Saunders Company Elsevier Inc; 2004. pp. 1129-31.

2. Robles Frias A, González-Cámpora R, Martinez- Parra D, *et al*. Robinson cytologic grading of invasive ductal breast carcinoma. Correlation with histologic grading and regional lymph node metastasis. *Acta Cytol* 2005; 4: 149-53.

3. Khan MZ, Haleem A, Hassani HA, Kfoury H. Cytopathological grading, as a predictor of histopathological grade, in ductal carcinoma (NOS) of breast, on air-dried Diff-Quick smears. *Diagn Cytopathol* 2003; 29: 185-93.

4. Chaiwun B, Sukhamwang N, Lekawanvijit S, *et al*. Atypical and suspicious categories in fine needle aspiration cytology of the breast : histological and mammographical correlation and clinical significance. *Singapore Med J* 2005; 46 : 706-9

5. Choi Y D, Choi Y H, Lee J H, Nam JH, Juhng SW, Choi C. Analysis of fine needle aspiration cytology of the breast. A review of 1,297 cases and correlation with histologic diagnoses. *Acta Cytol* 2004; 48: 801-6.

6. Salami N, Hirschowitz SL, Nieberg RK, Apple SK. Triple Test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. *Acta Cytol* 1999; 43: 339-43.

7. Yu GH, Cajulis RS, De Frias DVS. Tumour cell (dys) cohesion as a prognostic factor in aspirate smears of breast carcinoma. *Am J Clin Pathol* 1998; 109: 315 -19

8. Yoshida R, Kimura N, Harada Y, Ohuchi N. The loss of E-cadherin, alpha- and beta - catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. *Int J Oncol* 2001; 18: 513-20

9. Taniguchi E, Yang Q, Tang W. Cytologic grading of invasive breast carcinoma.Correlation with clinicopathologic variables and predictive value of nodal metastasis. *Acta Cytol* 2000 ; 44 : 587-91.

10. Wallgren A and Zajicek J. The prognostic value of the aspiration biopsy smear in mammary carcinoma. *Acta Cytol* 1976; 20: 479-85.

11. Fan F, Namiq AL, Tawfiq OW, Thomas PA. Proposed prognostic score for breast carcinoma on fine needle aspiration based on nuclear grade, cellular dyscohesion and bare atypical nuclei. *Diagn Cytopathol* 2006; 34: 542-6.

12. Robinson IA, Mckee G, Nicholson A, *et al*. Prognostic value of cytological grading of fine needle aspirates from breast carcinomas. *Lancet* 1994; 343: 947- 9.

13. Mouriquand J, Pasquier D. Fine needle aspiration of breast carcinoma. A preliminary cytoprognostic study. *Acta Cytol* 1980; 24: 153-9.

14. Layfield LJ, Robert ME, Cramer H, Guiliano A. Aspiration biopsy smear pattern as a predictor of biologic behaviour in adenocarcinoma of breast. *Acta Cytol* 1992; 36: 208-11

15. Schiller AB, Tadros TS, Birdsong GG, Grossl N. Cellular dyscohesion in fine needle aspiration of breast carcinoma. Prognostic indicator for axillary lymph node metastasis? *Am J Clin Pathol* 2001; 115: 219-23.