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ORIGINAL ARTICLE

Cytological grading of breast carcinoma with histological correlation

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Summary

Purpose: *A grading system based on cytology would be helpful in the selection of patients for appropriate therapy. The aim of this study was to devise such a system for grading breast carcinoma based on cytological features alone.*

Patients and methods: *Diagnostic fine needle aspiration (FNA) smears taken from 100 patients with invasive breast carcinoma were studied without knowledge of the subsequent grade and type of the tumors. The technique of aspiration employed a 10 ml syringe and 23 gauge needle. The aspirates were spread onto slides and half of the smears were rapidly air-dried and stained by May-Grunwald-Giemsa, while the rest were alcohol-fixed and stained by Papanicolaou technique. The features assessed were: nuclear pleomorphism, nucleoli, mitoses, nuclear/cytoplasmic ratio, apoptosis, necrosis, cell clustering, cellularity and tubular formation. Cytological features were compared to the histological grade of breast carcinomas following excision, and the results were analyzed by the x^2 test (the significance level was set to $p<0.05$), as well as by the correlation coefficients (ri). Multivariate analysis was carried out by multiple correlation coefficients (Rij) for each pair of significant parameters.*

Results: *Significant association between worsening cytological features and increasing histological grade were found with nuclear pleomorphism, nucleoli, mitoses, apoptosis, cellularity and tubular formation. A scoring system based on these 6 parameters enabled the classification of tumors into low and high cytological grades which showed a close correlation with histological grade with 81% concordance. The best multiple correlations were found for the following pairs of cytological parameters: mitoses-apoptosis (0.603), mitoses-tubular formation (0.572), apoptosis-nuclear pleomorphism (0.550) and mitoses-nuclear pleomorphism (0.545).*

Conclusion: *On the basis of this study we conclude that the proposed system of grading breast carcinoma is possible from FNA cytology and it shows a good correlation with histological grade.*

Key words: breast carcinoma, fine needle aspiration cytology, grading

Introduction

FNA cytology of the breast has become widely accepted as a means of diagnosing breast cancer. Moreover, it may be possible to process and report aspirated samples more quickly, allowing assessment of the adequacy of the sample and diagnosis at the time of the initial outpatient visit. The triple approach to the diagnosis of breast carcinoma, involving clinical examination, FNA cytology and imaging (mammography and ultrasound), has an accepted place in the assessment of patients presenting with symptomatic and screen-detected breast disease [1]. Histological assessment of the type and grade of a tumor is an important prognostic indicator [1,2]. With the current trend toward less aggressive diagnostic methods, however, definitive treatment may be carried out without prior biopsy and histological examination. More re-

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cently, cytological features seen in smears after FNA have added valuable additional information about the prognosis of the disease [3,4].

The aim of this study was to assess the feasibility of a grading system for breast carcinoma based on cytological features of malignant cells in FNA cytology. Such a system could then be used to contribute prognostic information at an early stage in the assessment of the patient.

Patients and methods

Diagnostic FNA smears taken from 100 consecutive patients with breast carcinoma from the files of the Institute of Pathology, Nis, were studied. Those patients had undergone FNA prior to excision biopsy or mastectomy. All lesions included were invasive breast carcinomas diagnosed cytologically and confirmed histologically. According to histopathological features, invasive breast carcinomas included in this study were of two histological types: 89 infiltrating ductal and 11 infiltrating ductal-lobular carcinomas. The FNA smears were studied without knowledge of the subsequent grade and type of tumors. The technique of aspiration employed a 10 ml syringe and 23 gauge needle. The aspirates were spread onto slides and half of the smears were rapidly air-dried and stained by May-Grunwald-Giemsa, while the rest were alcohol-fixed and stained by Papanicolaou technique. There were usually at least two slides from each patient. Therefore, we compared the results of the cytological grading with the histological grade of the tumors, independently assessed according to the modified Bloom and Richardson system described by Elston and Ellis [2]. The length of follow-up of these cases was short; consequently adequate survival and recurrence information was not available. Each of the features studied was scored separately as shown in Table 1.

Total scores were calculated by the sum of the scores for the individual features. The cytological scores for the individual features were compared with the histological grade of the tumors and the results were analyzed by the chi-square test (the significance level was set to $p < 0.05$), as well as by the correlation coefficients (r_i). Multivariate analysis was carried out by multiple correlation coefficients (R_{ij}) for each pair of significant parameters. Discriminant analysis was performed to assess which combination of cytological features showed the best correlation with histological grade.

Table 1. Features evaluated and scoring

Feature	*Score*
Nuclear pleomorphism	
mild	1
moderate	2
severe	3
Multiple easily visible nucleoli	
absent	0
present	1
Mitoses	
absent	0
a few	1
many	2
Nuclear (N) /cytoplasmic (C) ratio (cytoplasmic volume)	
N/C>1 (scanty)	1
N/C<1 (plentiful)	2
Apoptosis	
absent	0
present	1
Confluent necrosis	
absent	0
present	1
Cell clustering (cohesion)	
tight clusters of epithelial cells	1
mostly clusters but some areas show loss of cohesion	2
few clusters (dispersed epithelial cells)	3
Cellularity	
mild	1
moderate	2
severe	3
Tubular formation	
great majority	1
moderate amounts	2
absent	3

Results

The relations of 9 various individual cytological features to histological grades of breast carcinomas are shown in Table 2. When malignant cells were examined within 6 distinct parameters (nuclear pleomorphism, presence of multiple nucleoli, mitoses, apoptosis, cellularity and tubular formation), we observed an apparent correlation between their cytological features and different histological grades. The corresponding figures in Table 2 showed that worsening cytological features demonstrated increasing histological grade. The cytological scores for these 6 individual parameters were lower in the majority of breast carcinoma cases, belonging to histological grades I and II. Although histological grade III carcinomas mostly showed higher cytological scores, we observed that a cytological score of 3 for cellularity existed in only 8 cases. However, this result also showed a significant histology-cytology association ($p < 0.05$).

Table 2. Cytological scores for 9 individual cytological parameters (nuclear pleomorphism, nucleoli, mitoses, nuclear (N) / cytoplasmic (C) ratio, apoptosis, confluent necrosis, cell clustering, cellularity, and tubular formation) compared with histological grade, with statistical parameters (χ^2, p, and correlation coefficient r)

		Histological grade				*Statistical parameters*	
		I	*II*	*III*	*Total*	*χ^2, p*	*correlation coefficient*
Nuclear pleomorphism	1	6	8	3	17	χ^2=23.16	r_1=0.444
	2	6	18	24	48	p <0.001	
	3	2	4	29	35		
Nucleoli	0	11	29	28	68	χ^2=20.37	r_2=0.342
	1	3	1	28	32	p <0.0001	
Mitoses	0	5	8	2	15	χ^2=32.16	r_3=0.527
	1	8	19	20	47	p <0.00001	
	2	1	3	34	38		
N/C ratio	1	10	24	48	82	χ^2=1.66	r_4=–0.128
	2	4	6	8	18	p >0.05	
Apoptosis	0	14	27	26	67	χ^2=24.79	r_5=0.474
	1	0	3	30	33	p <0.00001	
Confluent necrosis	0	14	21	45	80	χ^2=5.38	r_6=0.09
	1	0	9	11	20	p >0.05	
Cell clustering	1	2	5	14	21	χ^2=3.54	r_7=–0.142
	2	7	20	32	59	p >0.05	
	3	5	5	10	20		
Cellularity	1	10	18	23	51	χ^2=9.71	r_8=0.284
	2	4	12	25	41	p <0.05	
	3	0	0	8	8		
Tubular formation	1	2	8	0	10	χ^2=26.02	r_9=0.424
	2	12	20	36	68	p <0.0001	
	3	0	2	20	22		
Total		14	30	56	100		

No significant association (p >0.05) was found with nuclear/cytoplasmic ratio, confluent necrosis and cell clustering. The correlation coefficients, r_i (i=1,..,9), showed that the best correlations with histological grades were found for mitoses, apoptosis, nuclear pleomorphism and tubular formation (r_3=0.527, r_5=0.474, r_1=0.444, and r_9=0.424, respectively).

Multivariate analysis by multiple correlation coefficients for significant parameters is presented in Table 3. For each pair of significant parameters, it was found that multivariant correlation coefficient was slightly higher than the highest individual correlation coefficient out of the examined pair. The best multiple correlations were found for the following pairs of cytological parameters: mitoses-apoptosis (0.603), mitoses-tubular formation (0.572), apoptosis-nuclear pleomorphism (0.550) and mitoses-nuclear pleomorphism (0.545).

Table 3. Multivariate analysis by multiple correlation coefficients (R_{ij}) for each pair of significant parameters

	R_{ij}	*Nuclear pleomorphism*	*Nucleoli*	*Mitosis*	*Apoptosis*	*Cellularity*	*Tubular formation*
		0.444	0.342	0.527	0.474	0.284	0.424
Nucl.pleomorphism	0.444	–	0.470	0.545	0.550	0.473	0.508
Nucleoli	0.342	0.470	–	0.527	0.504	0.395	0.458
Mitosis	0.527	0.545	0.527	–	0.603	0.548	0.572
Apoptosis	0.474	0.550	0.504	0.603	–	0.503	0.534
Cellularity	0.284	0.473	0.395	0.548	0.503	–	0.442
Tubul. formation	0.424	0.508	0.458	0.572	0.534	0.442	–

Table 4. Proposed scoring system based on 6 statistical parameters in FNA

Feature	*Score*
Nuclear pleomorphism	
mild	1
moderate	2
severe	3
Multiple easily visible nucleoli	
absent	0
present	1
Mitoses	
absent	0
a few	1
many	2
Apoptosis	
absent	0
present	1
Cellularity	
mild	1
moderate	2
severe	3
Tubular formation	
great majority	1
moderate amounts	2
absent	3

Our results indicate that a cytological grading system for breast carcinoma may be feasible and practical. The proposed scoring system is shown in Table 4.

Discriminant analysis confirmed these findings, showing that a combination of the cytological scores (obtained by summarizing of individual scores for nuclear pleomorphism, presence of multiple easily visible nucleoli, mitoses, apoptosis, cellularity and tubular formation) gave the best correlation with histological grade (Table 5). In this study group the cytological grading system [grade 1 (score 3-6); grade 2 (score 7-9); and grade 3 (score 10-13)] showed a 65% concordance with histological grade, with contingency coefficient C=0.66. The differences observed were mainly due to a lack of discrimination between histological grades I and II. By combining histological grades I and II, and reducing the cytological grades to two groups [low grade (score less or equal to 9) and high grade (score greater or equal to 10)], a highly significant association was found between the grading systems, with 81% concordance and contingency coefficient C=0.74 (Table 6).

Table 5. Cytological grade (obtained by summarizing statistically significant parameters in FNA cytology) compared with histological grade

Cytological grade	*Histological grade*			*Total*
	I	*II*	*III*	
1 (3-6)	9	12	5	26
2 (7-9)	4	11	6	21
3 (10-13)	1	7	45	53
Total	14	30	56	100

χ^2=41.58, p <10^{-7}, C=0.66, 65% of cases correctly classified

Table 6. Cytological grade (low -L and high -H) compared with histological grade (grades I and II are joined)

Cytological grade	*Histological grade*		*Total*
	I+II	*III*	
L (3-9)	36	11	47
H (10-13)	8	45	53
Total	44	56	100

χ^2=38.24, p <10^{-9}, C=0.74, 81% of cases correctly classified

Discussion

The widely used system of histological grading of breast carcinomas, modified by Elston and Ellis, relies on the assessment of 3 features: tubular formation, nuclear pleomorphism and mitotic rate [2]. The histological grade is obtained by analysis of these features, each of which is given a score of 1-3. The use of a numerical system is not meant to ascribe mathematical accuracy to grading. Rather than giving a subjective view of the degree of differentiation, the numerical system makes the pathologist assess each factor separately and think carefully about its relative value independently.

The cytological features of malignant cells in FNA smears also could be analyzed with reference to the appropriate scores. Previous studies have used a variety of cytological features to grade breast carcinoma aspirates [4-15]. In most of them there have been formal measurements of nuclear size (either diameter or area) using image analysis systems [5-8], or ocular micrometers [10,15]. Such methods are not widely available and can be time-consuming and therefore difficult to apply in a busy department. Nuclear size also varies considerably with factors such as fixation methods, speed of air drying and staining.

Nuclear pleomorphism was found useful in few earlier studies [4,9,11,12]. In this category an assessment is made of the variability of both size and shape of the cancer cells nuclei. We found the assessment of nuclear pleomorphism straightforward, and noted a good correlation with histological grade of breast

carcinoma.

The presence of nucleoli in breast aspirates is used as a marker of malignancy and 3 earlier studies [4,9,11] used this feature in their grading systems. Thomas et al. [10], however, found no correlation between the presence of nucleoli and histological grade. The reason for this discrepancy is not clear, unless it can be attributed to differences in fixation and staining methods used in the different studies. An increase in the size and number of nucleoli is a well known characteristic of cells engaged in growth and synthesis. This sign indicates poor prognosis in the present cytological classification [4,11].

Mitosis is also one of the main features considered in the histological grading of breast carcinomas [2]. In practice it is often impossible to distinguish hyperchromatic nuclei from those which are simply pyknotic. It is therefore recommended that hyperchromatic nuclei are ignored. We found that the evaluation of mitotic figures showed high correlation with histological grade, which is in accordance with other findings [9,13,15].

Primary morphologic criteria for the identification of apoptosis in breast aspirates was the presence of pyknosis and karyorrhexis characterized by cell shrinkage, nuclear condensation, fragmentation, apoptotic bodies and lack of inflammatory components. Apoptotic cell death can be distinguished from necrotic cell death which is a pathologic form of cell death resulting from acute cellular injury typified by rapid cell swelling and lysis [11]. Apoptosis on FNA smear specimens occurred only in breast carcinomas with grade II or III, but not grade I, suggesting that it may correlate with the proliferative status [16]. By considering the apoptotic rates, the sensitivity of cytological grading significantly rose in relation to histological grade [14].

In addition, cellularity and tubular formation also showed good correlation with histological grade of breast carcinoma and this coincides with previous findings [9,13].

Four earlier studies [4,9,10,15] considered the degree of cell clustering and two found it useful [9, 10]. On the contrary, we found that this was difficult to assess and that it showed no correlation with histological grade. We observed great variation in the degree of clustering in individual slides. Most clusters accumulated at the edges of the smears, with fewer clusters in the centers. It would appear that clustering depends to some degree on the method of spreading the smears; this may explain the lack of correlation that we observed between this feature and histological grade.

Both univariate and multivariate analysis confirmed that the most important cytological parameters, which showed real and significant correlation with histological grades, were mitoses, apoptosis, nuclear pleomorphism and tubular formation.

Further studies on a larger number of patients are required to determine whether only those four most important cytological parameters are enough to comprise the cytological grading system. In that case, multivariate analysis could be used for determining of distinct ponders (importance in grading system) for each parameter; this would be the subject of further investigation.

The use of scoring system based on 6 features gives a better correlation with histological grade than any one feature alone and still is simple to apply in routine practice. The cytological grading system in our study showed a 65% correlation with histological grade, which is better than the 57.1% obtained by Howell et al. [13]. Although we were unable to distinguish reliably between histological grades I and II, the division of the tumors into 2 groups (low grade and high grade) may be a useful division for clinical purposes. Using the proposed cytological grade, correct classification of cases between low and high cytological grades was achieved in 81%, which is better than the 79.73% obtained by Skrbinc et al. [17].

On the basis of this study we conclude that the proposed system of grading breast carcinoma is possible from FNA cytology and it shows a good correlation with histological grade.

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