Comparison of cytological categories atypical (C3) and suspected (C4) with histopathological diagnoses of breast lesions

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Summary

Purpose: Fine needle aspiration cytology (FNAC) is a diagnostic method characterized by high sensitivity, specificity and predictive value. In order to obtain uniformed results of FNAC breast changes, the following categories are introduced: C1 (non-representative), C2 (benign), C3 (atypical), C4 (suspected) and C5 (malignant). The purpose of this study was to establish which pathological processes are most frequently diagnosed as C3 and C4 categories, which carry a malignant tumor risk.

Methods: The frequency of all cytological categories was determined in a retrospective analysis which included 1605 patients, all of whom had undergone FNAC of breast lesions, over a period of 5 years (2012-2016). Furthermore, histopathological diagnoses of 212 patients with cytological categories C3 (77) or C4 (135) were compared.

Results: In the sample of 1605 patients, 212 belonged to C3 or C4 cytological category (frequency for C3 4.8%, for C4 8.4%). Also, in the group of patients with cytological categories C3 and C4 there were 208 women. The patients with C3 were younger than C4 patients. There was a statistically significant difference between the number of benign and malignant diagnoses in patients diagnosed with C3 or C4 cytological category (p<0.001). In C3 category, in 57.1% of the cases a benign condition was histopathologically diagnosed, while in C4 category, in 90.4% of the cases malignant tumor was histopathologically diagnosed.

Conclusions: After histopathological analysis, C3 category in FNAC breast lesions is most commonly diagnosed as a fibrocystic breast disease or fibroadenoma, while C4 category is diagnosed as well-differentiated malignant tumor.

Key words: aspiration cytology, breast, histopathological diagnosis

Introduction

According to current recommendations, pathological diagnosis of breast lesions, before any treatment, should be based on Core Needle Biopsy (CNB), or on Fine Needle Aspiration Cytology (FNAC), if CNB is not available [1,2].

Despite CNB introduction, FNAC still plays a significant role in the evaluation of pathological processes in breast, which has been well documented in the literature in the last 20 years [3-5]. The most significant indications for FNAC of breast lesions are evaluation of cystic lesions, diagnosis of recurrent or metastatic disease, confirmation of locally advanced carcinoma and determination of axillary lymph nodes status [1].

Although CNB is the superior method in breast lesions diagnostics, FNAC still has its advantages with regard to CNB. The advantages of FNAC are the promptness of obtaining results, as well as its low cost, which is a significant consideration in the developing countries. FNAC is characterized with solid sensitivity, specificity and predictive value. Major shortcomings of this method are the impossibility of diagnosing *in situ* carcinoma and lesions followed by abundant production of connective tissue [4,6-10].

In the United Kingdom National Health Service Breast Screening Program (UK NHSBSP), that began in 1988, a guideline has been published with regards to the mode of categorizing cell changes that could be seen in cytological samples obtained by needle aspiration. Five categories have been suggested: C1 (unsatisfactory specimen - non-representative), C2 (benign), C3 (atypical - most likely benign), C4 (suspected - most likely malignant), and C5 - (malignant) [11].

In 1996, the American National Cancer Institute (NCI), also suggested 5 categories for cytological diagnostics of breast lesions: benign, atypical, suspected, malignant and unsatisfactory [12].

Patients with C3 and C4 categories, namely, atypical and suspected, which carry the risk of malignant tumor, need to undergo further examination. C1 and C2 categories have to be correlated with the results of clinical and radiological examinations [13].

C3 and C4 categories should not be represented in more than 5% of all analyzed aspirates [4].

Cytological categories C3 and C4 are a controversial area of breast disease cytological diagnostics. A significant number of malignant breast tumors are diagnosed as C3 and C4 categories. Currently, there is no individual morphological criterion that cytological diagnostics of malignant breast tumors could be based on; hence, there is a need for constant evaluation of cytological diagnostics results [14,15].

The purpose of this study was to determine the frequency of C3 and C4 categories in the samples of 1605 patients, processed in the Clinical Centre of Montenegro from 2012 to 2016, as well as to analyze histopathological diagnoses, which are most frequently diagnosed as C3 or C4 category.

Methods

This study included 1605 patients who had undergone FNAC of palpable or impalpable breast lesions in the Clinical Centre of Montenegro over a 5-year period (2012-2016). Medical histories of patients have been used in this paper, as well as protocols for cytological and histopathological analysis of Clinical Centre of Montenegro.

The number of obtained slides for every patient during FNAC ranged from 1 to 5. All slides were air-dried and dyed with May-Grunwald-Giemsa's technique or fixated in alcohol and dyed with haematoxylin-eosin technique.

Microscopic analysis was done under microscopes Nikon Eclipse E600 and Olympus BX41.

The analyzed cytological samples were classified according to NHSBSP into the following categories:

C1 – inadequate or unsatisfactory sample
C2 – cells of benign cytological characteristics
C3 – mild atypia in some cells, most likely benign
C4 – suspected for malignancy
C5 – cells with malignant cytological characteristics [11].

The frequencies of all cytological categories in the sample were analyzed in the study.

In patients with C3 (n=77) and C4 (n=135) cytological categories, the distribution of histopathological diagnoses was analyzed after the breast surgical operation.

Statistics

Data was shown by descriptive statistical methods (absolute and relative frequencies). The diagnostic value of cytological diagnosis was assessed by comparing the frequencies of benign and malignant histopathological diagnosis in the C3 and C4 categories by chi-square test. All statistical analyses were performed using the IBM SPSS software, version 20.0. A p value <0.05 was considered statistically significant.

Results

The frequency of cytological categories in the overall samples of examined patients is shown in Table 1. The most frequently diagnosed cytological categories in our study were benign (49.2%), malignant (27.9%), while there were 9.7% unsatisfactory samples. Cytological category C3 was diagnosed in 4.8%, while C4 was diagnosed in 8.4% of the cases.

Out of 212 patients (with C3 and C4 cytological categories), there were 4 men and 208 women. The age of patients is shown in Table 2.

Table 1. Distribution of patients according to cytological category

Cytological category	Number of patients (%)
C1	156 (9.7)
C2	790 (49.2)
C3	77 (4.8)
C4	135 (8.4)
C5	447 (27.9)
Total	1605 (100)

In the group of patients with C3 category, most of them were aged 30 to 49 years (55.8%), while in the C4 group of patients the majority of them belonged to the 50 to 69 years age group (54.1%).

Histopathological diagnoses of the patients who were preoperatively diagnosed with C3 category are shown in Table 3.

In the majority of patients (44; 57.1%) preoperatively diagnosed with C3 category, a benign pathological process was confirmed. The most frequent histopathological diagnoses were fibrocystic breast disease (n=18), fibradenoma (n=15) (Figure 1), and adenosis (n=7).

When we analyzed malignant breast lesions, which were diagnosed as cytological category C3, the most frequently diagnosed were well differentiated malignant tumors (well differentiated ductal invasive carcinoma, lobular carcinoma, tubular carcinoma, papillary carcinoma, mucinous carcinoma).

Histopathological diagnoses of patients who were preoperatively diagnosed with C4 category are shown in Table 4. The vast majority of these pa-

Table 2. Distribution of patients according to age, in reference to cytological categories C3 and C4

Age (years)	C3 category	C4 category
10-19	1	0
20-29	0	1
30-39	19	9
40-49	24	30
50-59	12	37
60-69	12	36
70-79	8	16
80-89	0	4
No data	1	2

Table 3. Histopathological diagnoses of patients preoperatively diagnosed as C3

Histopathological diagnosis	Number of patients
Fibrocystic breast disease	18
Adenosis	7
Gynecomastia	1
Fibroadenoma	15
Papilloma	3
Ductal carcinoma *in situ*	3
Ductal invasive carcinoma	12
Lobular invasive carcinoma	10
Tubular carcinoma	3
Papillary carcinoma	2
Mucinous carcinoma	3
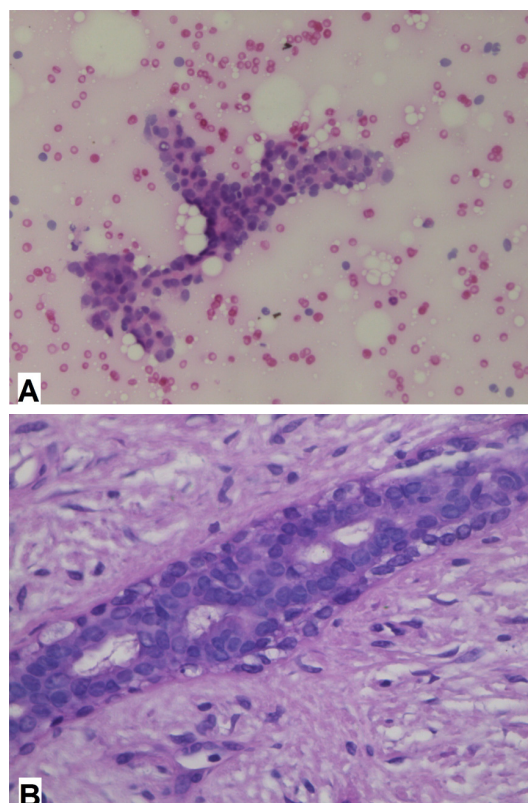
Figure 1. A: C3, haematoxylin-eosin, ×20; **B:** Subsequent histopathological examination revealed features compatible with fibroadenoma (haematoxylin-eosin ×40).
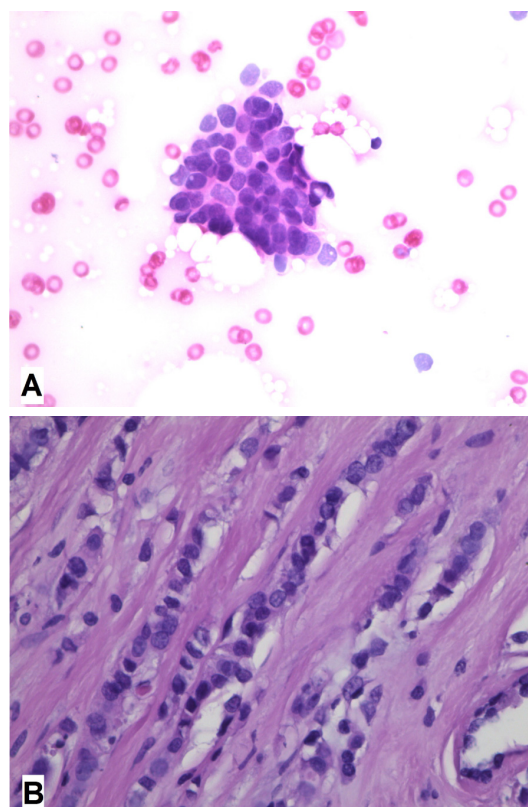
Figure 2. A: C4, haematoxylin-eosin, ×20; **B:** Subsequent histopathological examination revealed features compatible with invasive lobular carcinoma (haematoxylin-eosin ×40).

tients (90.4%) had a histopathologically confirmed malignant tumor. Invasive ductal carcinoma was diagnosed in 73 patients (54.1%), lobular invasive carcinoma in 27 (20.0%) (Figure 2) and mucinous carcinoma in 10 patients (7.4%).

The most frequently diagnosed benign processes were fibrocystic breast disease, adenosis and mastitis.

The distribution of patients diagnosed with cytological categories C3 or C4 with regard to histopathological category (benign or malignant) is shown in Table 5.

The results of statistical analysis indicate that there was a statistically significant difference (p<0.001) in the number of benign and malignant histopathological diagnoses in patients who were preoperatively classified as C3 and C4, with higher frequency of malignant tumors in C4 category (90.4%) compared to C3 category (42.9%). In most of the patients preoperatively diagnosed with C3 category, benign pathological conditions were confirmed, while in C4 category, most patients had breast cancer.

Table 4. Histopathological diagnoses of patients preoperatively diagnosed as C4

Histopathological diagnosis	Number of patients
Fibrocystic breast disease	5
Adenosis	2
Mastitis	2
Abscess	1
Fibroadenoma	1
Adenoma	1
Papilloma	1
Ductal carcinoma *in situ*	5
Ductal invasive carcinoma	73
Lobular invasive carcinoma	27
Mucinous carcinoma	10
Papillary carcinoma	3
Secretory carcinoma	1
Medullary carcinoma	2
Non Hodgkin lymphoma	1

Table 5. Distribution of patients according to cytological category (C3 or C4) and histopathological diagnosis (benign – malignant)

Cytological category	Benign	Malignant
C3	44	33
C4	13	122

x^2=56.310, p<0.001

Discussion

In 2012 it was estimated that in 40 European countries breast carcinoma incidence was 94.2/100 000, while the mortality rate was 23.1/100 000 inhabitants [16].

The incidence has been increasing since the introduction of the screening programs. Breast carcinoma is still the leading cause of mortality in females [1,17,18].

Diagnosis of breast carcinoma in women is based on two methods: FNAC or CNB. Both methods have similar specificity [2].

In many countries, FNAC is applied in screening programs for the diagnosis of breast lesions, as well as in the group of patients not included in the screening programs, but displaying symptoms and signs of pathological processes in the breast. Most European countries use the same reporting system: C1, C2, C3, C4 and C5 [19].

FNAC is simple to perform, requires little time for technical preparations and has low cost. This method also allows to obtain results a few hrs after delivering the samples. Importantly, this method is followed by minimal complications. Determination of steroid receptors status is possible on cytological samples, status of HER2 receptors, molecular subtype, as well as the application of immunohistochemical dyeing with the aim of giving final diagnosis of the abnormal process [18,20-23].

A total of 1605 samples obtained by FNAC method was analyzed in this study. C1 category was found in 9.7% of the cases, C2 in 49.2%, C3 in 4.8%, C4 in 8.4% and C5 in 27.6% of the cases.

Arul et al., while analyzing 523 samples, found C1 category in 2.7% of the cases, C2 in 67.3%, C3 in 5.2%, C4 in 7.8% and C5 in 17.0% of the cases [20].

The occurrence of cytological categories C3 and C4 in our study (C3 in 4.8% and C4 in 8.4% of the cases), did not display any significant difference when compared with the results of other relevant studies.

Arul et al., in a sample of 728 cases, found C3 in 4% of the cases, while the C4 category was diagnosed in 9.3% of the cases [24].

In the study published by Madubogwu et al., in a sample of 180 patients, C3 category occurred in 4.5% of the cases and C4 category in 3.6% [25].

In a sample of 7727 breast aspirations Kanhousk et al. found that 6% of the cases were classified as atypical and suspected, i.e., there were 225 cases of C3 and 162 cases of C4 among the lot [15].

Analyzing 14935 cytological samples Deb et al. reported that 3.7% of the cases were C3 and 3.9% C4 [26].

The results of other authors determining the occurrence of C3 and C4 categories on samples that included fewer patients, showed a percent occurrence ranging from 3 to 17% [4,12].

In our study, in the group of patients with C3 category, the most frequent histopathological diagnoses were fibrocystic breast disease (23.4%) and fibroadenoma (19.5%), while in the group of patients diagnosed with C4 category, histopathological analysis showed malignant tumors in 122 patients (90.4%).

In a sample of 523 patients (C3 category in 21 patients, C4 category in 62 patients), Arul et al. reported that after correlating cytological category C3 with histopathological diagnosis, benign pathological lesions most frequently occurred (81.8% of the cases), while in the group with cytological category C4, most frequently a malignant tumor was diagnosed (95.2% of the cases) [20].

The same group of authors, in a sample of 728 patients (C3 in 28 patients and C4 in 65 patients), concluded that the group of patients with C3 category involved most frequently a benign process (64.3% of the cases), while in the group with C4 category, most frequently a malignant tumor was diagnosed (86.2% of the cases). In both groups of patients, when benign lesions were analyzed, most frequently diagnosed were fibroadenoma, fibrocystic disease with atypia, while in the case of malignant tumors, invasive ductal carcinoma and lobular invasive carcinoma were prevaling. Analyzing their results, the authors concluded that there was a statistically significant difference in the number of benign and malignant histopathological diagnoses comparing to cytological categories C3 and C4 [24].

The results of other authors also indicate that, when cytological category C3 is analyzed, most frequently it reveals a benign process, and the percentage varies from 52 to 84%, depending on the study. Most frequently encountered are fibrocystic breast disease, fibroadenoma, radial scar, papilloma, gynecomastia, changes related to lactation and epithelial ductal hyperplasia. They also stated that in the group of malignant tumors diagnosed as category C3, most frequently there were well differentiated ductal carcinoma and special types of carcinoma [9,12-15,26-28].

The analyses of studies that correlated cytological category C4 with histopathological diagnoses indicated that most frequently there existed a malignant tumor, with percentages ranging from 71 to 83%. The most frequently diagnosed malignant tumors were well differentiated carcinoma or special types of carcinoma. The most frequent benign lesions diagnosed as cytological category C4 were fibroadenoma, fibrocystic disease and papilloma [12,15,26,27].

Chaiwun et al. recommended microscopic analysis of cytological preparations immediately after sampling, in order to reduce the number of cases diagnosed as C3 and C4 due to technical difficulties, such as inadequate drying of sample smear, blood contamination or a small number of malignant cells [27].

Cytological diagnostic categories define the probability of a malignant tumor in the breast. While analyzing cytological criteria (cellularity, cohesion loss, presence of myoepithelial cells, nucleus enlargement, prominent nucleoli etc.), it should be borne in mind that there is a certain overlapping of morphological characteristics between a part of benign pathological processes and malignant tumors, especially in cases of fibroadenoma, fibrocystic disease, adenosis, proliferative breast lesions, fatty necrotic breast tissue and well differentiated carcinoma, which require later histopathological revision. Diagnostic categories C3 and C4 (atypical and suspected) indicate the existence of changes in cell morphology, but it is not possible to define with certainty which pathological process is involved. Despite the existence of these "grey areas" in cytological diagnostics of breast diseases, their everyday application has proven their clinical significance [11,12,29,30].

Our study has shown that after histopathological analysis, C3 category in FNAC breast lesions is most commonly diagnosed as a fibrocystic breast disease or fibroadenoma, while C4 category is most commonly diagnosed as well differentiated malignant tumor.

Conflict of interests

The authors declare no conflict of interests.

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