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Quantitative analysis of fine needle aspiration biopsy samples

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Background. *The fine needle aspiration biopsy (FNAB) is one of the methods used in tumour evaluation. Since a certain number of tumour cells are needed for a complete diagnostic algorithm, we wanted to test how many cells remain in the needle and syringe after routine stains have been made and which factors influence this number. The remaining cells are used in ancillary diagnostic procedures.*

Materials and methods. *One hundred fifty two FNAB samples of tumours of the breast, thyroid and lymph nodes were included in our study. We counted the cells which were left in the needle and the syringe after the standard smears had been made. Buerker-Tuerk's chamber was used for this purpose.*

Results. *The number of cells depended on the organ from which the cells had been aspirated, on the type of tumour and, in the case of breast cancer, also on the level of experience of the FNAB performer. The percentage of samples with too few cells for all modern diagnostic methods (<5x105) is lowest in FNAB of lymph nodes (4.9%), followed by breast (16.7%) and thyroid (18%).*

Conclusions. *We concluded that FNAB in the majority of cases grants a sufficient number of cells for the standard microscopic evaluation and also ancillary diagnostic procedures.*

Key words: neoplasms – pathology; biopsy, needle; cytodiagnosis; cell count

Introduction

The fine needle aspiration biopsy (FNAB) is a quick, simple, safe, painless and inexpensive method. It is of the utmost importance in the preoperative diagnostics of tumours.[1] The diagnostic reliability of the method is good. It enables us to classify tumours as malignant or benignant in almost 100% cases and to further specify the type of the tumour in 80-98% of cases.[2] Serious side effects (pneumothorax, severe bleeding, infection, pain, vomiting etc.) are rare.[3] In the process of aspiration the cells are seldom extensively damaged since the small diameter of the needle enables it to push aside the tissue rather than tearing it.[1,4]

New, highly specialized methods of treatment require the specification of the tumour lesion to the highest extent. Any additional information about the morphology and cell structure, which determines the prognosis and helps to choose an appropriate treat-

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ment, is a welcome addition to the standard morphologic analysis. Such analyses are used for example in determining the hormonal receptor status in breast cancer.[5] The number of cells in the sample is, however, limited and determines how many additional diagnostic procedures can be made, besides the standard two smears.[6]

The purpose of our study was two-fold. First of all, to determine the number of cells that remain in the needle and syringe after FNAB had been performed and the standard two smears had been made and secondly, to establish eventual impact of tumour characteristics and performer's previous experience on the results. For our study, we chose tumours of the breast, thyroid and lymph nodes as the number of ancillary cytological methods is the greatest in these types of lesions.

Materials and methods

Data from 152 samples (54 tumours of the breast, 33 of the thyroid and 65 of the lymph nodes) were included in our study. We analysed the cells which were left in the needle and syringe after FNAB and the standard two smears have been made. We rinsed the needle and syringe with a so called »rinsing solution«, composed of 4.5% of bovine serum albumin and 0.45% EDTA in phosphate buffer with 100 I.U. of penicillin in 100 ml of the solution. We processed the sample according to the Buerker-Tuerk's protocol for cell counts; this was done in Buerker-Tuerk's chamber. We counted the cells by using a 100x magnification of the standard light microscope. Cells in four squares of the chamber were counted and the average number was calculated.

Next, we calculated the number of remaining cells. The equation used for this purpose was:

$$x = c \times V$$

where x stands for the total number of cells, c for density and V for volume of the remainder of the sample with the volume of the rinsing fluid included.

The density of the cells was calculated by using the following equation:

$$c = n \times 20 \times 10^4$$

where n stands for the number of counted cells. We had to multiply this number by 20 as the dilution ratio of the cell suspension to Buerker Tuerk's solution had been 10 μl: 190 μl. The volume of the chamber is 104 ml, hence the last multiplicator.[7]

Eight cytologists performed the biopsies. They were divided in two groups, based on their previous experience. In the first group there were three cytologists with more than five years of experience each. The rest, with less than one year of experience each, were in group number two. A two-sided t-test was used to calculate the level of the statistical significance of the between-group comparison.

Results

The percentage of samples with 500.000 cells in the syringe after the two standard smears were made was 95% in lymph node biopsy, 82% in breast cancer biopsy and 81% in thyroid cancer biopsy.

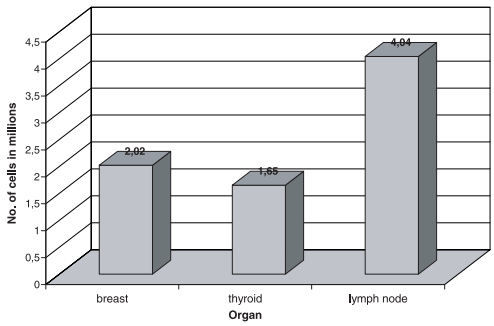

Figure 1. Average number of cells regarding the target organ on which FNAB was performed.

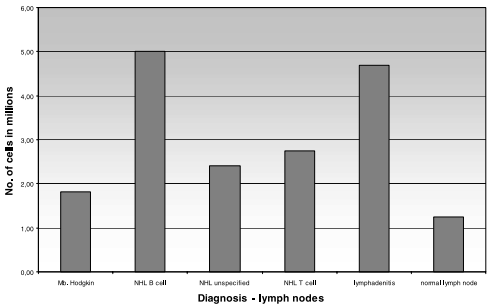

Figure 2. Average cell number according to the type of lymph node pathology.

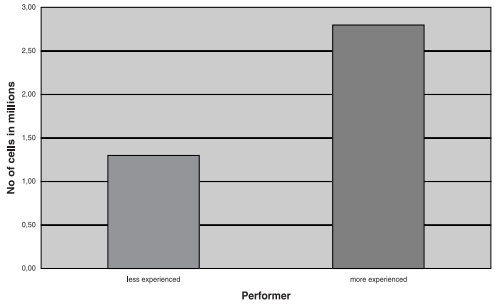

Figure 3. Average number of cells regarding the experience of the FNAB performer in breast samples.

Average number of cells regarding the target organ

We found that the average number of cells was the highest in samples acquired from lymph nodes, followed by breast samples. The average number was the lowest for thyroid samples. The difference was statistically significant when comparing averages of lymph node to breast ($p = 0.0003$) and lymph node to thyroid samples ($p = 0.00006$). The difference between breast and thyroid samples was not statistically significant (Figure 1).

Average number of cells regarding the type and size of the tumour

There was a statistically significant difference when comparing different types of tumours. For example, in invasive ductal carcinoma of the breast the number of cells was significantly higher than in invasive lobular carcinoma ($p = 0.01$). The results for different lymph node tumours were similar (p values ranging from 0.0002 to 0.05) while there was no statistically significant difference in different types of tumours of the thyroid (Figure 2).

In all three organs there was no significant difference in the number of acquired cells regarding the tumour size.

Average number of cells regarding the performer of FNAB

The only statistically significant difference in the number of cells between younger and older performers was present in FNAB samples of the breast ($p = 0.03$), while samples of the thyroid and lymph node did not show any significant difference (Figure 3).

Discussion

The percentage of samples containing enough cells to perform ancillary diagnostic methods (more than 500.000 cells in the syringe after the two standard smears)[1] was different according to the organ from which the sample was taken. Most cells were present in lymph nodes samples, on average 4 millions, followed by breast samples with 2 million on average and thyroid samples with 1.65 million. This result is not surprising if we consider that the tissues have a different structure.

We expected to get more cells from bigger tumours, but this was not the case in our study. The possible explanation for this fact could be that in bigger tumours, there is more tumour regression and necrosis which lowers the number of aspirated cells.

Different types of tumours have a different structure and the number of cells obtained from them was different. Cells of malignant lymphoma are connected by fragile nests of stroma and surrounded by a gentle capsule.8 This explains why we can obtain a large number of cells with ABTI by applying only a low pressure to the needle.

Even though the absolute average number

of aspirated cells was lower in all three organs in the second, younger group of cytologists, we found that the only statistically significant difference was present in FNAB of the breast. While there is some difference between the two groups it is not so important and proves that FNAB is easy to learn and to perform. All the FNAB-s in our study were done in the same centre and it would be interesting to compare different centres.

In most of the cases FNAB provides enough cells for basic and advanced diagnostic procedures. The number of necessary cells is especially high in breast cancer, because of the number of available diagnostic tests. In the future we expect more new and accurate diagnostic procedures that will enable us to make a clearer picture of the nature of the tumour and will thus lead to better treatment decisions.

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