Trakia Journal of Sciences, Vol. 23, Suppl. 1, pp 1-6, 2025
Available online at:
http://www.uni-sz.bg
ISSN 1313-3551 (online) **doi:10.15547/tjs.2025.s.01.001**

HISTOMORPHOMETRIC INVESTIGATIONS OF SPONTANEOUS CANINE MAMMARY GLAND TUMOURS

R. Simeonov*, I. Grozeva

Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Histomorphometric analysis was performed on preparations of 18 spontaneous canine mammary epithelial neoplasias (fibroadenoma (n=6), tubulopapillary carcinoma (n=6) and solid carcinoma (n=6). Computer histomorphometric analysis of cell nuclei was performed using a digital microscope and morphometric analysis software (Image Pro Plus® v.4.5 Media Cybernetics, Silver Spring, MD, USA). The studied morphometric parameters were mean nuclear area (MNA, μm^2), mean nuclear perimeter (MNP, μm) and mean nuclear diameter (D mean, μm). The analysis of our results shows that there are reliable statistical differences between benign and malignant mammary neoplasms. In this regard, histomorphometric analysis can be used to differentiate benign from malignant mammary neoplasias in the bitch. On the other hand, however, statistical analysis shows that this study does not allow differentiation between malignant mammary neoplasms.

Keywords: canine mammary gland tumours, quantitative morphology

INTRODUCTION

Mammary neoplasms are the most common tumors in intact female dogs (1-6). According to Brodey et al., (7) and Yager et al., (8), about 95% of them are of epithelial origin, and the remaining 5% are mesenchymal. Studies in Alameda and Contra Costa, California in 1963-1966 showed that mammary tumors accounted for 13.4% of all tumors in dogs and 41.7% of all tumors in intact female dogs (6). The prevalence of mammary neoplasms in bitches in the European Union is significantly higher than in the United States, due to the infrequent performance of ovariohysterectomy as a prophylactic measure to limit the population in this animal species (6). In our country, malignant mammary tumors predominate (Zhelev et al., (9), Tsvetkov, (10), Dinev et al., (11). They are usually multicentric, and more than one tumor type can be observed in one animal (12-13).

There are few reports in the specialized veterinary literature regarding the possibility of using the parameters mean nuclear area, mean nuclear perimeter, and mean nuclear diameter for histomorphometric differentiation of benign from malignant spontaneous mammary epithelial tumors in the bitch.

***Correspondence to:** *Radostin Simeonov, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, tara Zagora, Bulgaria, radostin.simeonov@trakia-uni.bg*

MATERIAL AND METHODS

Computer histomorphometric analysis of cell nuclei was performed using a digital microscope and morphometric analysis software (Image Pro Plus® v.4.5 Media Cybernetics, Silver Spring, MD, USA). Morphometric analysis was performed on histological preparations stained with Hematoxylin/Eosin. The distribution of tumor formations in which we conducted morphometric studies is presented in **Table 1.**

We studied the following morphometric parameters: mean nuclear area (MNA, μm^2), mean nuclear perimeter (MNP, μm) and mean nuclear diameter (D mean, μm). Image Pro Plus® has a statistics program (Statistica 6.0. (StatSoft, Tulsa, OK, USA), which was sufficient for statistical processing of the obtained results (ANOVA, post hoc LSD, level of confidence $p < 0.05$).

***Table 1**. Distribution of tumor formations in which we performed histomorphometric analysis.*

MATERIALS	NUMBER
1. Fibroadenoma	6
3. Tubulopapillary carcinoma	6
4. Solid carcinoma	6

RESULTS

Mean nuclear area

After performing the computer histomorphometric analysis, we obtained the following numerical values: fibroadenoma - 34.49 ± 8.41 μm^{2,} tubulopapillary carcinoma – 43.47 ± 9.03 μm^2 and solid carcinoma - 44.36 ± 12.95 μm^2. The mean nuclear area was the smallest in fibroadenomas and gradually increased in tubulopapillary and solid carcinomas. The results are presented graphically in **Figure 1**. The data from the statistical analysis are reflected in **Table 2**.

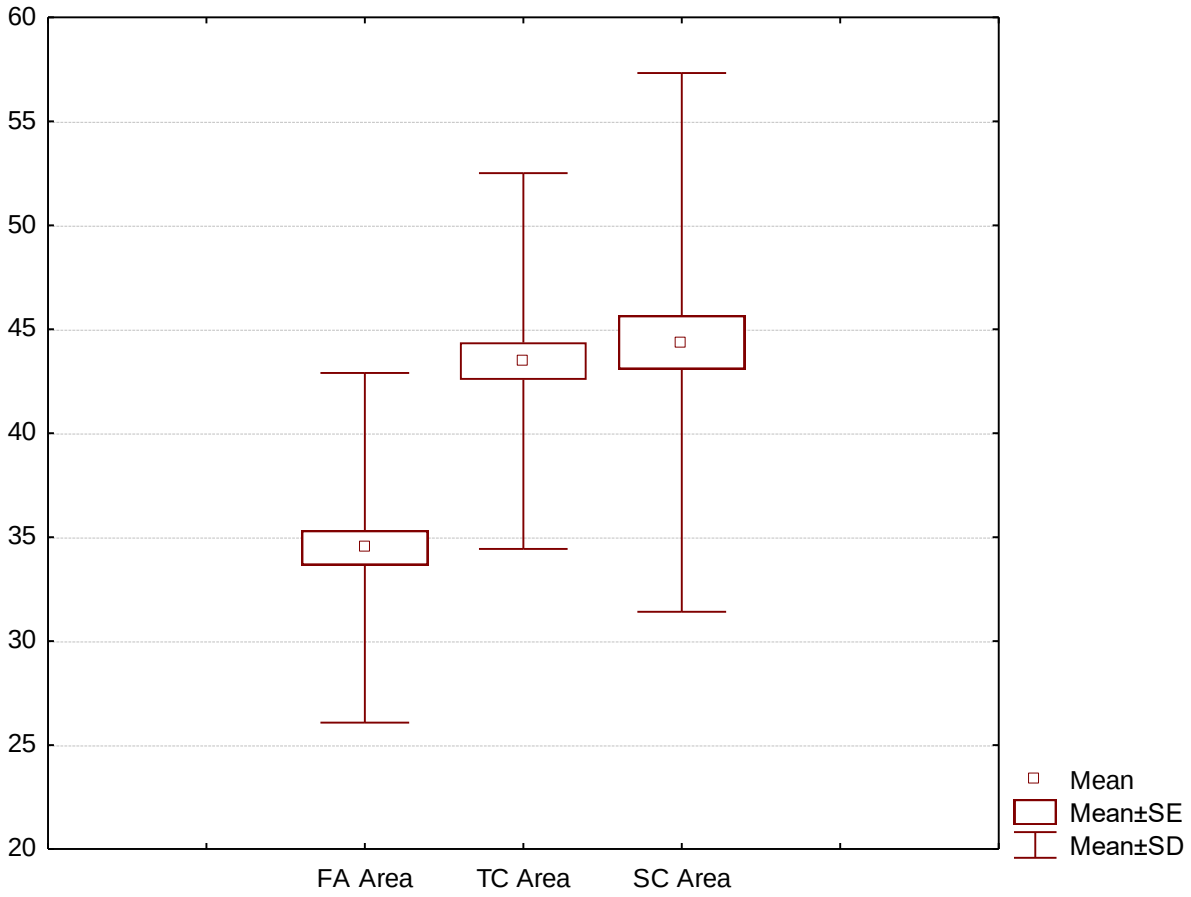

Figure 1. Graphical representation of the mean nuclear area.
FA Area – Fibroadenoma, MNA, μm^2; TC Area – Tubulopapillary carcinoma MNA, μm^2; SC - Area – Solid carcinoma MNA, μm^2

***Table 2**. Reliability of differences between groups regarding the morphometric indicator mean nuclear area ANOVA/LSD test (*p<0.05, **p<0.01, ***p<0.001).*

Groups	FA	TC	SC
FA	-	***	***
TC	***	-	-
SC	***	***	-

Mean nuclear perimeter

The numerical values of this morphometric indicator varied within the following limits: fibroadenoma - 21.25 ± 2.84 μm, tubulopapillary carcinoma - 23.96 ± 2.76 μm, solid carcinoma - 24.62 ± 3.82 μm. The mean nuclear perimeter was the smallest in fibroadenomas, and gradually increased in tubulopapillary and solid carcinomas. The results are presented graphically in **Figure 2**, and the data from the statistical analysis are reflected in **Table 3.**

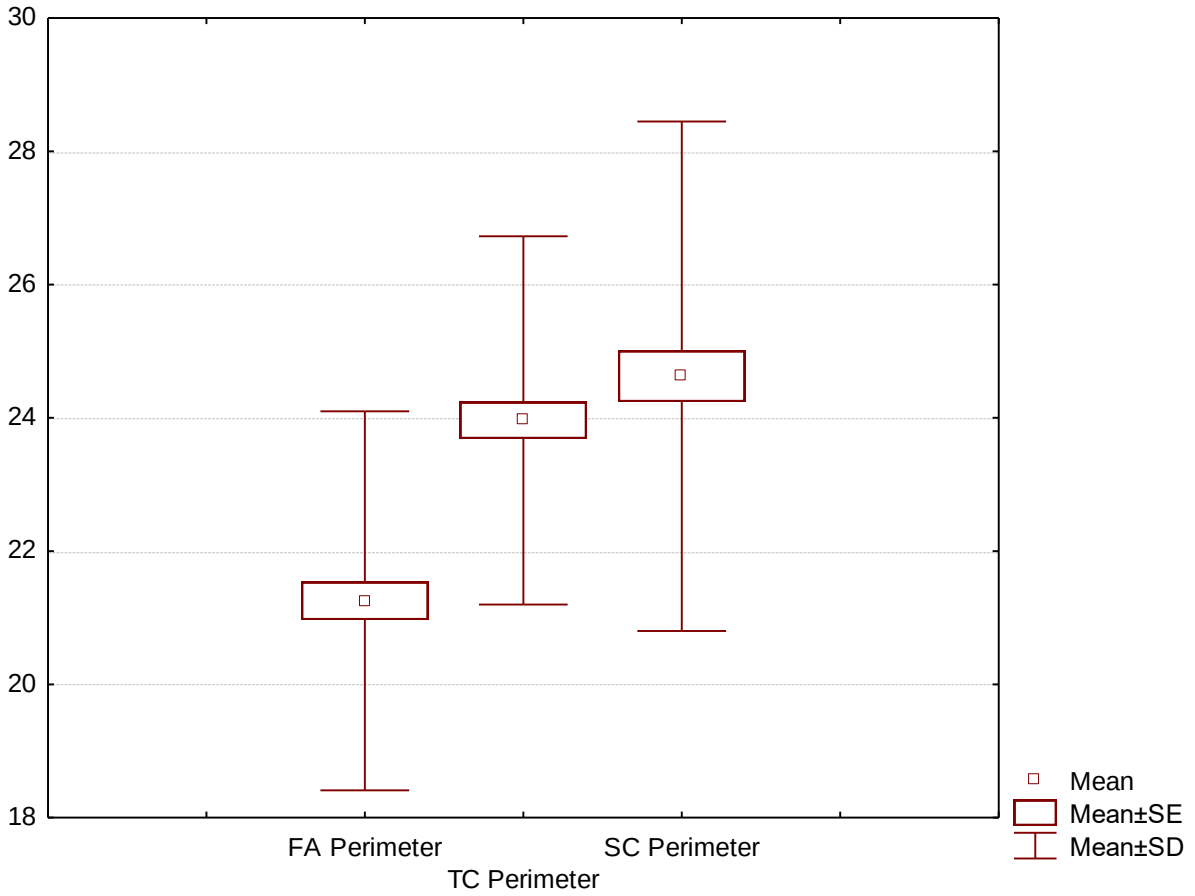

Figure 2. Graphical representation of the mean nuclear perimeter.
FA Perimeter – Fibroadenoma, MNP μm; TC Perimeter – Tubulopapillary carcinoma MNP, μm; SC Perimeter – Solid carcinoma MNP, μm

Table 3. *Reliability of differences between groups regarding the morphometric indicator mean nuclear perimeter. ANOVA/LSD test (*p<0.05, **p<0.01, ***p<0.001).*

Groups	FA	TC	SC
FA	-	***	***
TC	***	-	-
SC	***	***	-

Mean nuclear diameter

The mean numerical values of the morphometric indicator mean nuclear diameter varied within the following limits: fibroadenoma – 6.49 ± 0.84 μm, tubulopapillary carcinoma - 7.28 ± 0.78 μm, solid carcinoma – 7.34 ± 1.05 μm. The mean nuclear diameter was lowest in fibroadenomas, and gradually increased in tubulopapillary and solid carcinomas. The results are presented graphically in **Figure 3**, and the data from the statistical analysis are reflected in **Table 4**.

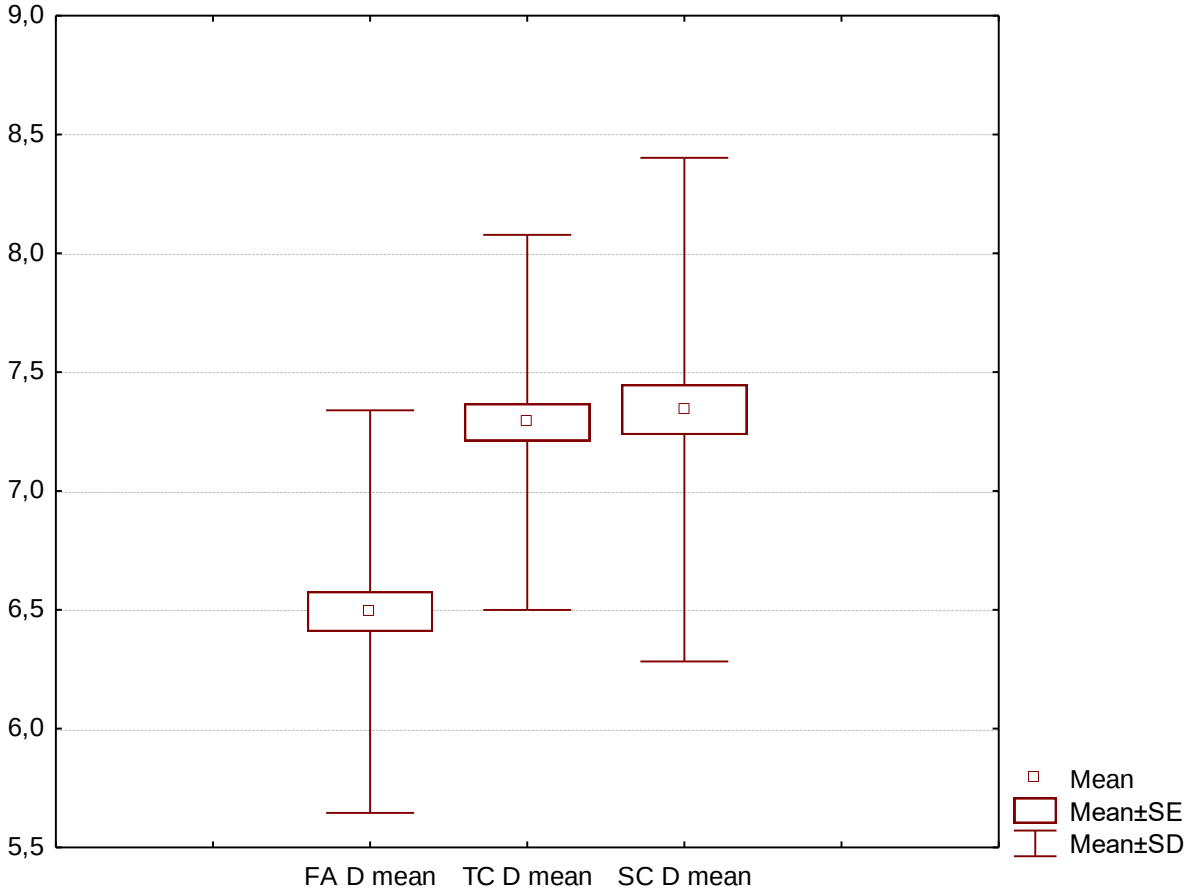

Figure 3. Graphical representation of the mean nuclear diameter of the nuclei. FA D mean – Fibroadenoma, D mean μm; TC Perimeter – Tubulopapillary carcinoma D mean, μm; SC D mean – Solid carcinoma D mean, μm

Table 4. *Reliability of differences between groups regarding the morphometric indicator mean nuclear diameter. ANOVA/LSD test (*p<0.05, **p<0.01, ***p<0.001).*

Groups	FA	TC	SC
FA	-	***	***
TC	***	-	-
SC	***	***	-

DISCUSSION

In the study of the morphometric parameter mean nuclear area, we found statistically significant differences in all neoplasms (p<0.001), with the exception of the differences between tubulopapillary and solid carcinoma. In veterinary oncology, to date, there have only cytomorphometric studies on the indicator mean nuclear area in spontaneous mammary neoplasms (14). In it, the researchers found statistically significant differences in all studied mammary tumours. Similar studies have been performed on pathohistological preparations, and the conclusion that the authors make is that there are statistically significant differences between benign and malignant mammary tumors in the bitch as a whole (15-16). In addition, Ciurea et al., (15) in their study indicated that nuclear morphometric indicators increase gradually in relation to the degree of malignancy of the neoplasms. The mean nuclear area has been studied for many years in human medicine. In this regard, the studies of our and foreign authors have proven categorically that it can be used for histomorphometric differentiation of benign from malignant mammary gland tumors in women (17-22). This is fully confirmed by the results of our study.

In the study of the morphometric indicator of the mean nuclear perimeter, we found statistically significant differences between all studied neoplasias, with the exception of the differences between tubulopapillary and solid

carcinoma. In human medicine, it has been definitively established that this indicator can be used for histomorphometric differentiation of benign from malignant mammary tumors (17, 22, 18, 20-22). Similar results were obtained in the study of Simeonov (14), but on cytological preparations of mammary neoplasms stained with Hemacolor®. The analysis of the results of the indicator mean nuclear diameter are analogous. Moreover, a number of researchers in human medicine use the mean numerical values of nuclear diameters (mean, minimum and maximum) for subtyping mammary epithelial tumours (24, 19, 15, 20, 22).

In conclusion, the results of our study show that the mean numerical values of the parameters related to the area, perimeter and diameter of the nuclei can be used for histomomorphometric differentiation of benign from malignant mammary neoplasms, i.e. they have diagnostic significance. At the same time, however, they cannot be used for subtyping malignant mammary tumours in the bitch. In benign mammary neoplasias, the cell populations are generally homogeneous and nuclei of approximately the same size predominate. Conversely, in malignant mammary neoplasms, the cell populations are generally heterogeneous and the nuclei are of different sizes. Thus, we fully confirm the results of studies by other authors working on the problem in human medicine (17, 18, 19, 21-22).

Computer morphometry can be used for both cytological and histological purposes. In cytology, due to the arrangement of the nuclei in one plane, the manipulation is easier to perform. In histology, the nuclei of the cells easily overlap, and this requires their manual differentiation. Another advantage of using morphometry in cytology is that the method can be used preoperatively. In our opinion, performing morphometric studies on cytoplasmic structures, as well as determining the nucleus/cytoplasm ratio, is difficult to implement due to the easy rupture of the cytoplasm of cells. In our opinion, the methods for fixing the cellular material play an extremely important role in morphometric analysis. For example, a number of publications indicate different values in morphometric studies of the same cytological and histological preparations. This is explained by the influence of different fixatives on the shape of the cells during the histological processing of the materials (23-25). According to Elzagheid and Collan (26), the problem is also open in cytology. The lack of standardization of the procedure leads in practice to differences in the numerical values of the same morphometric indicators in the same neoplastic cells, fixed and stained by different methods. Furthermore, each fixative modifies the shape of the cells to some extent (23-25). In this regard, modern researchers in this field require the introduction of a uniform procedure for fixing cells before performing morphometric studies.

REFERENCES

1. Dorn, C., Taylor D. and Schneider, R., Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda Country. *J Natl Cancer Instite*, 40: 307-318, 1968.
2. Bostock, D., Canine and feline mammary neoplasms. *Br Vet J*, 142: 506-515, 1986.
3. Moe, L., Population-based incidence of mammary tumors in some dog breeds. *J Reprod Fertil*, 57: 439-443, 2001.
4. Morris, J and Dobson, J., Mammary gland. In: Morris J. Dobson J (eds), *Small Animal Oncology* Iowa State University Press, 184-192, 2001.
5. Misdorp, B., Else R., Hellmen E. and Lipscomb T., Histological classification of mammary tumors of the dog and cat. WHO International Histological Classification of Tumors in Domestic Animals. 2nd series, Vol VII. Washington DC: Armed Forces Institute of Pathology, American Registry of Pathology, 2001.
6. Sorenmo, K., Canine mammary gland tumors. *Vet Clin Small Anim*, 33: 573-596, 2003.
7. Brodey, R., Goldschmidt M. and Roszel, J., Canine mammary gland neoplasms. *J Am Anim Hosp Assoc*, 19: 61-90, 1983.
8. Yager, J., Scott, D. and Misdorp, W., The skin and appedages. In: Jubb K, Kennedi P. and Palmer, N (eds). *Pathology of Domestic Animals*. ed 4. Academic Press, San Diego, 1993.
9. Zhelev, V., Dzhurov, A. and Angelov, A., Studies on the spread and localization of tumors in domestic animals and birds. Scientific works of the Institute of Veterinary Medicine, volume XVI, 1966.
10. Tsvetkov, Y. Pathomorphological studies and some epizootological features of tumors in dogs. Dissertation, Sofia, 1998

11. Dinev, I., Dimov D., Parvanov P., Georgiev P. and Simeonova, G., Incidence of canine neoplasms-a retrospective histopathological study. I. Mammary neoplasms in the bitch. *BJVM*, 5(3): 195-204, 2002.
12. Allen, S., Prasse K. and Mahaffey, E., Cytologic differentiation of benign from malignant canine mammary tumors. *Vet Pathol*, 23: 649-655, 1986.
13. Allison, M., Bain P. and Latimer, K., Canine mammary carcinoma. http://www.vet.uga.edu/vpp/clerk/mccarthy/, 2003.
14. Simeonov. R., Cytological and computer cytomorphological studies in spontaneous mammary neoplasias in bitches. Dissertation, 2006.
15. Ciurea, D., Wilkins R., Shalev M., Liu Z., Barba B. and Gil, J., Use of computerezed interactive morphometry in the diagnosis of mammary adenoma and adenocarcinoma in dogs. *Am J Vet Res*, 53: 300-303, 1992
16. Juntes, P. and Pogacnik, M., Morphometric analysis of AgNOR in tubular and papillary parts of canine mammary gland tumors. *Anal Quant Cytol Histol*, 22: 185-192, 2000.
17. Dobreva, A., Cytological diagnostics of pre-cancerous diseases and mammary gland cancer. Dissertation, Sofia, 1980.
18. Denchev, D., Quantitative morphology and diagnostics of pre-tumor and tumor processes in the mammary gland. Dissertation. Sofia, 1988.
19. Dey, P., Ghoshal S. and Pattari, S., Nuclear image morphometry and cytologic grade of breast carcinoma. *Anal Quant Cytol Histol*, 22: 483-485, 2000.
20. Tahlan, A., Nijhawan R. and Joshi, J., Grading of ductal breast carcinoma by cytomorphology and image morphometry with histologic correlation. *Anal Quant Cytol Histol*, 22: 193-198, 2000.
21. Tan, P., Goh B., Chiang G. and Bay, B., Correlation of nuclear morphometry with pathologic parameters in ductal carcinoma in situ in the breast. *Mod Pathol*, 14: 937-941, 2001.
22. Rajesh, L., Dey P. and Joshi, K., Automated image morphometry of lobular breast carcinoma. *Anal Quant Cytol Histol*, 24: 81-84, 2002.
23. Fiorella, R., Kragel P., Shariff A. and Dubey, S., Fine-needle aspiration of well differentiated small-cell duct carcinoma of the breast. *Diagn Cytopathol*, 16: 226-229, 1997.
24. Ladecarl, M., The influence of tissue processing on quantitative histopathology in breast cancer. *J Microscop*, 174: 93-100, 1994.
25. De Vico, G., Sfacteria A., Maiolino P. and Mazzullo, G., Comparison of nuclear morpfometric parameters in cytologic smears and histologic section of spontaneous canine tumors. *Vet Clin Pathol*, 31: 16-18, 2002.
26. Elzagheid, A. and Collan, Y,. Fine-needle aspiration biopsy of the breast. Value of nuclear morphometry after different sampling methods. Anal Quan Cytol Histol, 25: 43-80, 2003