Image DNA Cytometry in FNABs of Libyan Breast Disease

FATHI B. ELMABROUK ABDALLA[1,3], JAMELA MOSTAFA E. BODER[1,4], ABDELBASET BUHMEIDA[1,2,5], ADEM IBRAHIM ELZAGHEID[1,6] and YRJÖ COLLAN[1]

[1]*Department of Pathology,* [2]*Department of Oncology, University of Turku, Turku, Finland;*
[3]*Department of Pathology, African Oncology Institute, Sabratha, Libya;*
[4]*Department of Pathology, Sabratha Teaching Hospital, Sabratha, Libya;*
[5]*Centre of Excellence in Genomic Medicine Research, King Fahad Medical Research Centre, King AbdulAziz University, Jeddah, Saudi Arabia;*
[6]*Department of Pathology, Arab University, Benghazi, Libya*

Abstract. *Background: The sensitivity for identification of malignant cells in conventional fine-needle aspiration biopsy (FNAB) investigation is about 80%. This percentage is dependent on the number of examined cells, type of breast cancer, and experience of the examiner. The aim of our study was to estimate the supporting value of image DNA cytometry of FNAB of the breast, and do so by using different sampling methods. Materials and Methods: This retrospective study was based on 41 cases with an available histological diagnosis: 18 benign lesions and 23 malignant tumours were examined. The smears were submitted to image DNA analysis in a three-step protocol: (i) smears stained with HE method were destained and (ii) then restained with Feulgen staining for DNA and (iii) finally analysed using image cytometry. Results: All non-malignant cases had diploid histogram. However, a few of them had one or two cells of >5c category. Most histologically malignant cases were aneuploid. Only three invasive ductal carcinomas showed diploid histograms. All samples with aneuploid histograms were malignant. Conclusion: The results confirm earlier published data in the Finnish population and indicate that image DNA cytometric analysis of nuclear content is a useful marker for identification of malignant cells in FNAB, especially after free cell sampling. The method can be used to increase the cytological sensitivity and specificity in doubtful breast lesions.*

Correspondence to: Yrjö Collan, Department of Pathology, University of Turku, Kiinamyllynkatu 10, 20520 Turku, Finland. e-mail: yrjo.collan@utu.fi

Key Words: Breast cancer, cytology, fine-needle aspiration biopsy, DNA cytometry, DNA content, benign breast lesions, free cell sampling, cell group sampling.

Breast cancer is one of the most common causes of cancer mortality in women worldwide (1-3). It is responsible of 28-29% of female cancer deaths and 10-18% of all cancer deaths (1, 3). Early diagnosis is important because patients with early stage cancer have better survival than those with advanced disease. Fine-needle aspiration biopsy (FNAB) is minimally invasive and has a specificity greater than 90% and sensitivity of about 80% (4-6). But the method can still potentially be improved by applying DNA cytometry (7, 8). The aim of this work was to study the value of nuclear DNA content in FNAB diagnosis of Libyan cases of female breast cancer. We also wanted to investigate the influence of different sampling methods in the evaluation of the DNA histograms in FNAB of the breast, as assistance in subjective interpretation. In addition, it was our purpose to compare our results on Libyan patients with those of Elzagheid *et al.* (9) on Finnish patients to find the potentially most fruitful ways of applying this methodology in practice. To our knowledge, such comparative study has not been carried out before or reported earlier.

Materials and Methods

All FNAB samples were collected from the files of the Department of Pathology, African Oncology Institute, Sabratha, Libya, biopsied during the period of July 2004-January 2007, from patients suffering from histologically confirmed breast cancer, fibroadenoma, or fibrocystic disease. FNAB samples which were very hypocellular, and/or only contained overlapping cell nuclei or degenerated cells resulting in loss of cellular detail were not included in the study. Apocrine, lobular, mixed variants of neoplasms and all cases without histologically confirmed diagnosis were also excluded. This left 41 samples for this study (Table I).

Feulgen staining. Originally all cases were fixed in 50% ethanol, smeared on glass slides and stained with haematoxylin-eosin (HE). The samples were stained with Feulgen stain according to Gaub's *et al.* method (10). Before staining the samples were washed in xylene for 3-7 days to remove the cover glass and the embedding

Table I. *FNAB samples studied with image DNA analysis. The cytological categories correspond to traditional atypia classes of 2, 3, 4, and 5, respectively. The mean numbers of cells available for measurements are shown after both cell group and free cell sampling methods. The histological evaluation was based on the investigation by experienced pathologists, and confirmed in this study.*

Cytological category	No. of patients	Histological diagnosis		Mean number of measured cells (SD)	
		Carcinoma	Benign (FA, FC)	Cell group sampling	Free cell sampling
Benign and mild atypia (C2)	9	0	9	164.14 (46.6)	183.4 (31.3)
Moderately atypical (C3)	8	3	5	161.25 (68.8)	149.0 (50.1)
Highly suspicious (C4)	6	2	4	117.00 (63.1)	169.3 (37.0)
Definitely malignant (C5)	18	18	0	179.50 (34.1)	202.4 (25.8)
Total	41	23	18	165.11 (50.9)	183.0 (38.9)

FA, Fibroadenoma; FC, fibrocystic disease; SD, standard deviation.

medium. Xylene was removed with sequential immersion in 100%, 95%, 70%, and 50% ethanol. The samples were washed in 1% (0.28M) hydrochloric acid and in 70% ethanol until destained. The samples were washed in distilled water, followed by acid hydrolysis in 5M hydrochloric acid at room temperature (20˚C) for one hour. After washing in distilled water, samples were immersed in Schiff's reagent (stain: pararosaniline) in dark for 2 hours 45 minutes at room temperature (20˚C), rinsed in distilled water, treated for 3×10 minutes in fresh aqueous sodium thiosulphate (180 ml distilled water, 10 ml 1 M HCl, 10 ml 10% $Na_2S_2O_5$), and rinsed for 5 minutes with distilled water. Finally, the samples were dehydrated, treated with xylene and mounted in DPX, then stored in shade.

Image analysis cytometry. The intensity of Feulgen staining was measured using a computer-assisted image analysis cytometric system AHRENS ICM with a Nikon microscope (Eclipse E 400; Japan) designed and produced by Olaf Ahrens (Messtechnische Beratung, Bargteheide/Hamburg, Germany). The field of view from the camera (JAI DSP surveillance (color, American English) CCD camera, CV-S 3200/3300) was stored in image memory at a resolution of 736 by 560 pixels. The image was produced by a plan objective (Nikon; ×40, numerical aperture 0.65) and the measurements were made from that image. Prior to each measurement session, the illumination of the microscope was adjusted according to the method of Köhler (cited in 11). Several histograms were produced twice, and they were found to be very similar (12).

Sampling rules. In our study, 200 nuclei were sampled, if available, from each case for each type of sampling (Table I). Artificially smeared nuclei were excluded. Thirty small lymphocytes, in a few cases also granulocytes, served as internal controls. The DNA values of the lymphocytes were set at 2c, and showed a thin diploid peak. Different sampling strategies were applied. Two methods were used: (i) cell group sampling, and (ii) atypical free cell sampling.

Sampling method 1: cell group sampling. Cells from the cell groups in the sample (cell group defined as more than 2 cells in contact with each other) were selected and the DNA histograms from cell nuclei produced. Free cells were excluded from the analysis at this stage. There was a risk of nuclear overlap within cell groups, but overlapping nuclei were not measured.

Sampling method 2: atypical free cell sampling. Only free cells were measured. Cells were called free when present as single cells, or when two cells were in contact with each other. If there were 3 or more cells in contact, cells were said to form cell groups and not measured for atypical free cell sampling. It was our aim to measure the most atypical free cells.

Interpretation of the histogram. The diploid region was viewed to be situated within the gate of 1.7-2.3c. A small number (<10%) of all cells in the tetraploid region (3.4-4.6c) were not considered to represent abnormality. When the mode of the peak and the peak were completely within the gate of 1.7-2.3c, the peak was defined as diploid. When some of the cells represented by the peak were outside 1.7-2.3c, but within gate 1.5-2.5c, they were called peridiploid. Aneuploid peaks were those with modes outside these defined gates (1.7-2.3c, 1.5-2.5c, 3.4-4.6). Individual cells between 2.3-3.4c (without peak) were classified as proliferative cells and individual cells >5c were classified as aneuploid cells. We considered the peaks as being identical when the mode of the peaks located within the same gate. Non-identical peaks had the mode of the peaks located within different gates. For clinical application we tested the decision rules given by Elzagheid *et al.* (9).

Rule 1: i) Carcinoma can be expected on the basis of image DNA cytometry if a cell group histogram and/or free cell histogram shows tetraploid or aneuploid peak, or if there are more than two >5c cells, or any possible combination of these criteria; ii) Carcinoma cannot be diagnosed on the basis of image DNA cytometry if the cell group histogram and/or free cell histogram shows diploid/peridiploid peaks but no peaks in the tetraploid or aneuploid regions, and two or fewer >5c cells.

Rule 2: i) Carcinoma may be present if cell groups and/or free cells show a tetraploid or an aneuploid peak with two or more than two >5c cells in the histogram; ii) Carcinoma cannot be diagnosed, however, on the basis of the histogram if there is clear evidence of polyploidy in the histogram (diploid, tetraploid, and/or octaploid cells but no aneuploid peaks), and if no >5c cells are present, or if only one such cell is present; iii) Carcinoma cannot be diagnosed if cell groups and/or free cells show diploid/peridiploid histograms but no aneuploid peaks and/or no >5c cells or only one such cell.

Rule 3: i) Carcinoma may be present if cell groups and/or free cells show a tetraploid, or an aneuploid peak, with three or more than three >5c cells in the histogram; ii) Carcinoma cannot be diagnosed, however, if there is clear evidence of polyploidy in the histogram (diploid, tetraploid, and/or octaploid cells but no aneuploid peaks) and if fewer than three >5c cells are present; iii) Carcinoma also cannot be diagnosed if cell groups and/or free cells show diploid/peridiploid histograms but no aneuploid peaks and/or fewer than three >5c cells.

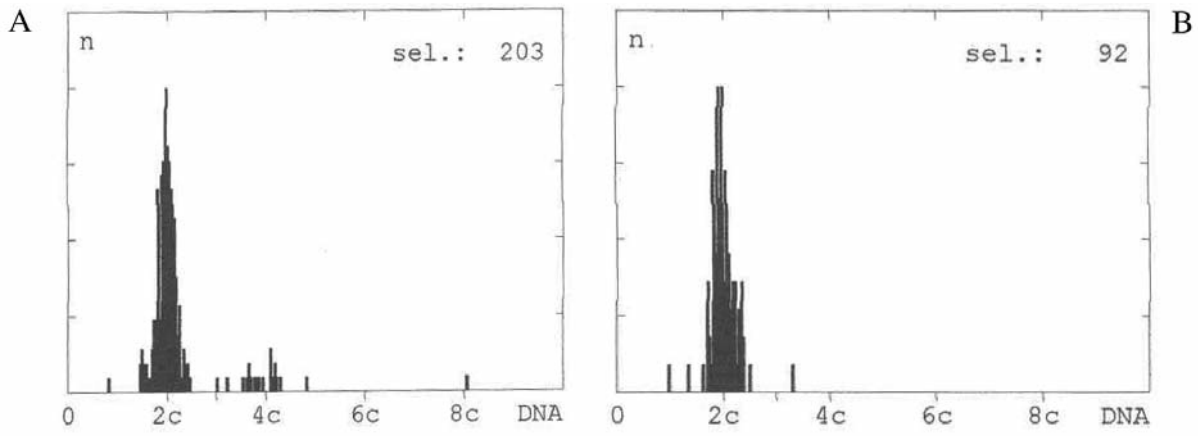

Figure 1. *The diploid histogram in a benign case (fibrocystic disease; A) and a malignant case (B). Note the octaploid cell at 8c, suggesting polyploidy in A.*

Rule 4: i) Carcinoma may be present if cell groups and/or free cells show a tetraploid peak or an aneuploid peak with four or more than four >5c cells in the histogram; ii) Carcinoma cannot be diagnosed, however, if there is clear evidence of polyploidy in the histogram (diploid, tetraploid, and/or octaploid cells, but no aneuploid peaks) and if fewer than four >5c cells are present; iii) Carcinoma also cannot be diagnosed if cell groups and/or free cells show diploid/peridiploid histograms but no aneuploid peaks or less than four >5c cells.

Results

DNA cytometry showed a diploid peak in most cases, which were classified as definitely benign in clinical cytological investigation (n=9). One sample, however, also showed one cell in the >5c category. In this sample, nuclei were located at diploid, tetraploid, and octaploid regions, suggesting polyploidy (Figure 1A). Among moderately atypical samples (n=8), three samples were diagnosed later as carcinoma. DNA cytometry showed aneuploidy in one of the latter and a tetraploid peak with >5c cells in another. The third case had a peridiploid peak. Among six highly suspect samples, two were diagnosed later as carcinoma, and both had diploid histograms (Figure 1B). This finding was probably associated with diploid carcinoma. Among 18 definitely malignant samples, 17 were suggestive of carcinoma on the basis of their DNA histograms, which showed aneuploid or tetraploid peaks and/or >5c cells. One sample generated a diploid histogram, and this case obviously was associated with a diploid carcinoma (Table II, Table III). The results showed that DNA cytometry was able to support a diagnosis of carcinoma and to improve sensitivity, especially in moderately atypical cases. However, the method was less powerful in improving sensitivity for detecting carcinoma among highly suspect cases. In addition, DNA cytometry confirmed the cytological diagnosis among the bulk of definitely benign (C2) and malignant cases (C5). Among highly suspicious and definitely malignant samples, DNA cytometry supported the presence of carcinoma in 75% of samples, when the interpretation was based on cell groups. From histograms of free cells, the diagnosis of carcinoma was supported in 85% of samples. Among all samples (from definitely benign to definitely malignant), cell group sampling had a sensitivity of detecting carcinoma of 73.9%. Free-cell analysis increased sensitivity to 82.6.0%. These results show clearly that sampling methods can influence the ability of DNA cytometry to detect malignant lesions (Figure 2). In some cases, nuclear overlapping in cell groups made it impossible to produce adequate histograms, i.e. a sufficient number of nonoverlapping nuclei were not available (Figure 3). Our results were considered representative if above 45 cells were available for the histogram.

Interobserver variation in interpretation of the histogram. Among benign cases, the interpretation of the histogram by two observers was equal in all histograms in all cases. Malignant cases showed identical histogram interpretation in 19/20 histograms after cell group sampling (Spearman correlation 0.99, Kappa statistic=0.93) and 20/23 histograms from free cell sampling (Spearman correlation 0.75, Kappa statistic=0.75). The differences were usually associated with cases with features suggesting both aneuploid and tetraploid grouping. The two sampling methods showed identical histogram peaks in 31/35 comparisons.

Differences in histograms between sampling methods. In addition to the differences in the histogram peaks, the free cell samples showed more aneuploid cells (>5c) than did the cell group samples. In general, we found that cell group histograms were less atypical than free cell histograms.

Table II. *Classification of histograms in cell group sampling of the 41 Feulgen-stained samples. Six cases had too few cells or overlaps for an interpretable histogram (3 of these benign, 3 malignant), which left 35 interpretable cases.*

Histogram interpretation	Histological diagnosis	C2	C3	C4	C5	All histograms
Diploid or peridiploid peak with < 2 cells in the >5c category	M	0	1	1	1	3
	B	7	5	3	0	15
Diploid or peridiploid peak with 2 cells in the >5c category	M	0	0	0	0	0
	B	0	0	0	0	0
Diploid or peridiploid peak with >2 cells in the >5c category	M	0	0	0	2	2
	B	0	0	0	0	1
Tetraploid peak with >2 cells in the >5c category	M	0	1	0	4	5
	B	0	0	0	0	0
Aneuploid peak with/without >5c cells	M	0	1	0	9	10
	B	0	0	0	0	0
	All M	0	3	1	16	20
	All B	7	5	3	0	15
	Not interpretable					6
	Total	7	8	4	16	41

M, Malignant (histology); B, benign (histology); C2-C5, traditional cytological atypia classes.

Table III. *Classification of DNA histograms in free cell sampling of the 41 Feulgen- stained samples.*

Histogram interpretation	Histological diagnosis	C2	C3	C4	C5	All histograms
Diploid or peridiploid peak with < 2 cells in the >5c category	M	0	1	2	1	4
	B	8	3	4	0	15
Diploid or peridiploid peak with 2 cells in the >5c category	M	0	0	0	0	0
	B	1	2	0	0	3
Diploid or peridiploid peak with >2 cells in the >5c category	M	0	0	0	0	0
	B	0	0	0	0	0
Tetraploid peak with >2 cells in the >5c category	M	0	1	0	7	8
	B	0	0	0	0	0
Aneuploid peak with/without >5c cells	M	0	1	0	10	11
	B	0	0	0	0	0
	All M	0	3	2	18	23
	All B	9	5	4	0	18
	Total	9	8	6	18	41

M, Malignant (histology); B, benign (histology); C2-C5, traditional cytological atypia classes.

Discussion

Sensitivity and specificity. The cytological analysis of FNAB is a successful application for preliminary diagnosis of malignant diseases and this is true of many cancer types (4-6). However, for some patients with breast cancer, the identification of malignant cells was difficult (specificity is high but sensitivity is variable 70-90%) (5, 6, 17). About 50-70% of suspicious cytology samples (class 3 and 4) were benign in surgical biopsy according to Teague *et al*. (5). In our study, 64.3% of suspicious samples were benign. In general, FNAB diagnosis in experienced hands reaches the specificity of about 90% (4, 5, 13). The factors contributing to low sensitivity are the presence, in some cases, of few malignant cells and the difficulty of distinguishing low- grade malignant cases from reactive and/or benign epithelial lesions. Further techniques have been proposed by some authors to increase the sensitivity of the FNAB diagnosis (14). Cytometric quantification of nuclear DNA content by static cytometry is one of these techniques (15-17). Our results showed that image DNA cytometry can have very high specificity (100%) in free cell sampling and in cell group sampling (93%). Sensitivity in

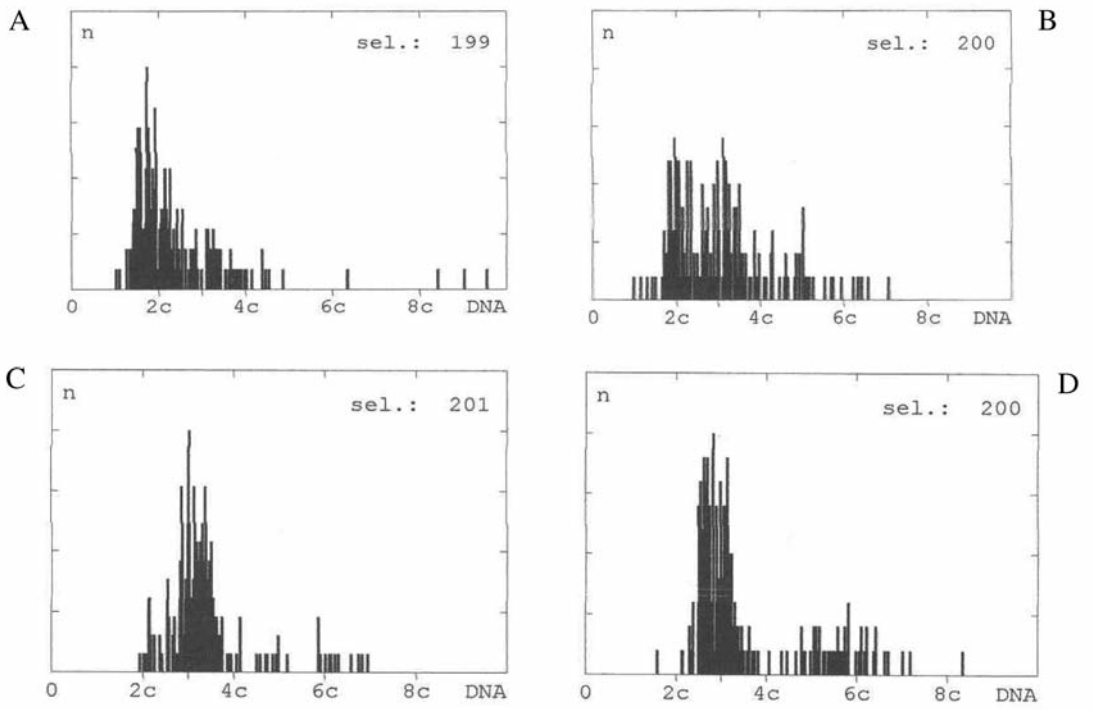

Figure 2. *Examples of the use of different sampling methods in image DNA cytometry of two malignant aneuploid fine needle aspiration biopsies. A and B are from one and the same lesion, and C and D from another lesion, respectively. A and C: cell group sampling. B and D: free cell sampling. Note that free cell sampling may give wider separation of DNA values.*

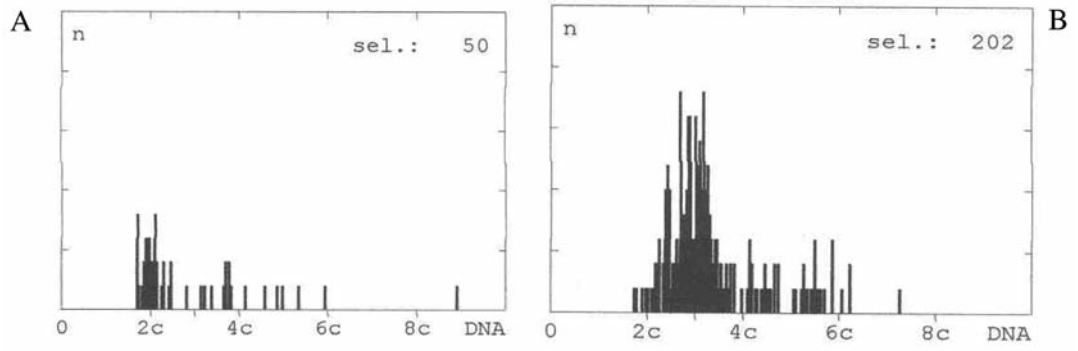

Figure 3. *A: The cell group sampling method in fine-needle aspiration biopsy shows only few cells. The reason is that cells groups show nuclear overlapping and these nuclei were excluded from analysis. B: Free cell sampling of the same sample: more cells are presented because there is no nuclear overlapping.*

detecting malignancy in our DNA study was 82.6%. This seems to suggest that image DNA cytometry may improve the sensitivity and specificity of the cytological diagnosis and help in distinguishing between malignant and benign lesions.

Value of image DNA cytometry in FNAB in a Libyan female population. One of our aims was to determine the value of image DNA cytometry in FNAB of Libyan breast lesions. Our results showed that all benign FNAB samples were diploid. Three out of 23 malignant cases also were diploid; all other available malignant cases had abnormal histograms. These results indicate a clear diagnostic value of the non-diploid histogram. Neither fibroadenoma nor fibrocystic changes gave aneuploid or tetraploid histogram. Comparison of the study of Elzagheid *et al*. and ours (free cell sampling sensitivities: 78.1% and 82.6%, respectively) leads to a conclusion that the abnormal histogram is a marker for malignancy. However, the presence of a diploid histogram cannot exclude malignancy (Figure 3B). The

results suggest a false-negative rate of 15%-20% (in our study 17.4%). If the clinician knows that there is a risk of false negatives, he will act accordingly and subject the patient to further investigations and follow-up. However, the situation with false positives is different: if DNA cytometry only is used as the basis for treatment decision, occasionally patients with a benign lesion will be subjected to cancer treatment, which is unnecessarily radical. Elzaghied *et al*. had false-positive cases of about 20% among their benign samples. Although we did not have false positives, when we used the interpretation rule 3, we think that the possibility of false positives must be taken into consideration. Hence, DNA diagnosis alone can only be preliminary, but is helpful for further management of the patient. We compared the finding in these two studies (Elzagheid *et al*. and ours) and got an average sensitivity, specificity and efficiency of 80%, 87% and 83%, respectively, when the results of the two studies were combined after applying rule 2. This means that through DNA alone, 83% of patients were correctly diagnosed. However, we prefer using of rule 3 which is the best rule to minimise the false positive interpretation (Table I and II). We can also state that African (Libyan) breast cancers in DNA cytometry of FNAB in principle behaved as European (Finnish) breast cancers.

Cause of diploid histograms in malignant cases. In our study, three malignant cases were diploid, and one had a peridiploid histogram in free cell sampling. Some carcinomas may present such slight chromosomal variations that abnormalities are difficult to detect with the analysis of nuclear DNA content. Such cases can represent diploid carcinoma (18, 19). Furthermore, some FNABs may not be representative of the actual lesion, with few malignant cells mixed with a large number of benign cells (5, 20, 21). In our study, all but one of the cytologically definitely malignant cases had a non-diploid histogram. It is known that only a small proportion of malignant tumours are diploid (18, 19).

The problem of cost-effectiveness. An image analysis examination costs as much as or slightly more than a core needle biopsy. Therefore it is evident that DNA cytometry can be used to help to support the FNAB cytology or core needle biopsy investigation. FNAB cytology and core needle biopsy in experienced hands are good methods to distinguish between benign and malignant lesions. In cases of histologically proven or cytologically obvious malignancy, DNA cytometry does not seem to be necessary. But it should be remembered that both needle biopsy and core biopsy also have false-positive and false-negative results. Moreover, while DNA cytometry is potentially fast, core biopsy in some cases may lead to delay in the final diagnosis and treatment.

Sampling methods. Our paper confirms the earlier findings by Elzagheid *et al*. (9), and Buhmeida *et al*. on prostatic cancer (22) that sampling methods can influence the ability of DNA cytometry to detect malignant lesions. Table II shows that histograms based on atypical free cells reached a sensitivity of 82.6%, and histograms based on cell group sampling of 73.9%.

Reproducibility. In addition to improving sensitivity of FNAB cytology, the DNA cytology method provides more objective and reproducible results with observer agreement in free cell sampling, cell group sampling, and in comprehensive (both samplings applied) classification. Observer agreements are 90.2%, 97.1% and 93.4%, and kappa statistic 0.85, 0.96 and 0.90, respectively. We encourage the primary application of DNA cytometry in support of FNAB of breast lesions.

In conclusion, these results indicate that cytometric analysis of nuclear DNA content is a useful means for the identification of malignant cells in FNAB cytology, particularly after sampling of atypical free cells. The method can increase the cytological sensitivity in doubtful lesions, both after FNAB analysis and core biopsy analysis.

Acknowledgements

The authors acknowledge Sinikka Kollanus for her help in Feulgen staining. We also wish to thank the Libyan Medical Specialization Board, and Libyan Health Ministry for stipends to Dr. Abdalla to visit the Department of Pathology, University of Turku, Finland, for applying DNA cytometry in this research. Financial support of the University of Turku is also acknowledged in the laboratory expenses, and laboratory assistance.

References

1 Veronesi U, Boyle P, Goldhirsch A, Orecchia R and Viale G: Breast cancer. Lancet *14*: 1727-1741, 2005.
2 Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin *55*: 74-108, 2005.
3 Fisher B: Malignancies of the breast. *In*: Practical Oncology. First edition. Cameron RB (editor). Norwalk, Appleton & Lange, pp. 417-434, 1994.
4 Rosen PP: Breast Pathology. Philadelphia, Lippicot-Raven, pp. 817-835, 1997.
5 Teague MW, Wolberg WH, Street WN, Mangasarian OL, Lambrement S and Page DL: Indeterminate fine-needle aspiration of the breast. Image analysis-assisted diagnosis. Cancer *81*: 129-135, 1997.
6 Alatise OI, Lawal OO, Olasode OO and Adesunkanmi ARK: Breast fine-needle aspiration cytology in a Nigerian tertiary hospital. Afr J Surg *12*: 126-132, 2007.
7 Auer GU, Caspersson TO and Wallgren AS: DNA content and survival in mammary carcinoma. Anal Quant Cytol *2*: 161-165, 1980.
8 Sarker SK: Diagnostic use of mean nuclear area and nuclear DNA content in preoperative breast cancer cytology. Anal Quant Cytol Histol *25*: 81-84, 2003.

9 Elzagheid A, Kuopio T and Collan Y: Implementation of DNA cytometric measurements in fine-needle aspiration biopsy diagnostics of breast disease. Cancer (Cancer Cytopathology) *102*: 380-388, 2004.
10 Gaub J, Auer G and Zetterberg A: Quantitative cytochemical aspects of a combined Feulgen-naphthol yellow S staining procedure for the simultaneous determination of nuclear and cytoplasmic proteins and DNA in mammalian cells. Exp Cell Res *92*: 323-332, 1975.
11 Bohnhoff GL: Setting up proper illumination on your microscope. Am J Med Technol *45*: 650-651, 1979.
12 Buhmeida A, Algars A, Ristamaki R, Collan Y, Syrjanen K and Pyrhonen S: DNA image cytometry is a useful adjunct tool in the prediction of disease outcome in patients with stage II and stage III colorectal cancer. Oncology *70*: 427-437, 2006.
13 Sun W, Li A, Abreo F, Turbat-Herrera E and Grafton WD: Comparison of fine-needle aspiration cytology and core biopsy for diagnosis of breast cancer. Diagn Cytopathol *6*: 421-425, 2001.
14 Abdalla F, Boder J, Buhmeida A, Hashmi H, Elzagheid A and Collan Y: Nuclear morphometry in FNABs of breast disease in Libyans. Anticancer Res *28*: 3985-3990, 2008.
15 Böcking A, Giroud F and Reith A: Consensus report of the European Society for Analytical Cellular Pathology task force on standardization of diagnostic DNA image cytometry. Anal Quant Cytol Histol *17*: 1-7, 1995.
16 Böcking A, Chatelain R, Biesterfeld S, Noll E, Biesterfeld D, Wohltmann D and Goecke C: DNA grading of malignancy in breast cancer. Prognostic validity, reproducibility and comparison with other classifications. Anal Quant Cytol Histol *11*: 73-80, 1989.
17 Ruiz A, Almenar S, Callaghan RC and Llombart-Bosch A: Benign, preinvasive and invasive ductal breast lesions. A comparative study with quantitative techniques: morphometry, image and flow cytometry. Pathol Res Pract *195*: 741-746, 1999.
18 Ludwig AS, Okagaki T, Richart RM and Lattes R: Nuclear DNA content of lobular carcinoma in situ of the breast. Cancer *31*: 1553-1560, 1973.
19 Ruiz A, Almenar-Medina S, Callaghan RC, Calderon J and Llombart-Bosch A: Radial scar versus tubular carcinoma of the breast. A comparative study with quantitative techniques (morphometry, image and flow cytometry). Pathol Res Pract *191*: 547-554, 1995.
20 Zuk JA, Maudsley G and Zakhour HD: Rapid reporting on fine-needle aspiration of breast lumps in outpatients. J Clin Pathol *42*: 906-911, 1989.
21 Grosby JH: The role of fine-needle aspiration biopsy in the diagnosis and management of palpable masses. Journal of the Medical Association of Georgia *1*: 33-36, 1996.
22 Buhmeida A, Backman H and Collan Y: DNA cytometry in diagnostic cytology of the prostate gland. Anticancer Res *22*: 2397-2402, 2002.

Received July 1, 2009
Revised November 19, 2009
Accepted November 25, 2009