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Apocrine Cells in Breast Cyst Fluid and their Relationship to Cyst Type: A Morphometric Study

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Abstract—*The morphology of apocrine cells exfoliated in breast cyst fluid (BCF) was studied in 78 BCF samples obtained from 39 premenopausal patients with gross cystic disease who were bearing two simultaneously aspirated cysts. 57/78 samples showed cell clusters suitable for computer-assisted cytometry. This was performed on 5820 cells using a Leitz Texture Analysis System (TAS). We measured the surface areas of cytoplasm, nucleus and nucleolus; we also calculated the nuclear/cytoplasmic (N/C), nuclear/nucleolar (N/n) ratios and the nuclear roundness factor (RF). Cysts were divided according to the cationic pattern of BCF: Type I, $K^+/Na^+ > 1.5$; Type II, $K^+/Na^+ < 0.66$. The cytometric analysis was made on 47 samples of Type I and 10 samples of Type II. At the light microscope, no difference was apparent between the apocrine cells coming from Type I or Type II cysts. Cytometric measurements showed significant differences for the apocrine cells aspirated from Type I vs. Type II cysts for the mean cytoplasmic area (97.13 ± 24.28 S.D. μ^2 vs. 59.66 ± 14.90 S.D. μ^2, respectively) and the mean nucleolar area (4.35 ± 0.99 S.D. μ^2 vs. 2.75 ± 0.71 S.D. μ^2, respectively). Our data do not allow the inference of apocrine changes in the epithelium lining the cysts simply from the cationic pattern of BCF. The significantly wider cytoplasm and nucleoli of the apocrine cells aspirated from Type I cysts could reflect different functional stages of these particular cells.*

INTRODUCTION

Gross cystic disease of the breast (GCD) is thought to represent an advanced form of the so-called fibrocystic breast disease. The pathological hallmarks of GCD are round or ovoid epithelial cysts of lobular origin [1] that are lined to a variable extent by polygonal cells with ground-glass pale eosinophilic cytoplasm, termed apocrine cells. Although cysts are not premalignant lesions, several studies have indicated that patients with GCD are at higher risk of later developing breast cancer [2].

Breast cyst fluid (BCF), which is easily obtainable from cysts by aspiration, has recently undergone extensive investigation. A growing body of knowledge suggests that this medium has peculiar biochemical features and that defining its composition is of value in identifying subgroups of patients with GCD possibly at different cancer risk [3–5]. In particular, some studies have pointed out that BCF samples may be quite different with regard to their cationic pattern and content of androgen-sulfates, first of dehydroepiandrosterone-sulfate (DHA-S) [6–8]. Accordingly, the subdivision of cysts into two main types has been proposed: Type I (high K^+, low Na^+, high DHA-S), Type II (low K^+, high Na^+, low DHA-S) and a relatively small number of intermediate Type III. Moreover, in a recent publication the cyst type has been correlated with the morphology of the lining epithelium: high K^+ cysts (Type I) were found to be specifically associated with the apocrine epithelium, whereas low K^+ cysts (Type II) were lined by more flattened cells with minimal apocrine changes [9]. This dichotomy has already been questioned [10].

In an attempt to re-evaluate this issue, we have investigated the morphology of the apocrine cells obtainable in BCF samples coming from different cyst types. A quantitative, computer-aided, cytomorphometric approach has been used in order to gain sensitivity with respect to the conventional cytological examination and to obtain objective parameters, if any, of an increased cell susceptibility to cancer transformation.

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MATERIALS AND METHODS

Patients

Between 1983 and 1985, 391 women with well-documented GCD underwent fine-needle aspiration of BCF at the senologic outpatient service of Ravenna Hospital. For the present study we selected 39 patients with a history of two simultaneously aspirated cysts, located in different quadrants of the same or contralateral breast. Thus, additional information was expected on cells coming from different cysts of the same patient.

Patients were premenopausal, aged 32–47 years. Cancer was excluded by thermography, echography, mammography and repeated clinical examination. Aspiration of BCF was always performed during the follicular phase of the menstrual cycle according to the technique of Zajicek [11]. The volume of BCF varied from 1.5 to 30 ml; a total of 78 samples was examined.

Cytology

BCF samples, usually clear yellow-green, were spun down and the sediment gently smeared on slides. The smears were then stained according to Papanicolau's technique and assessed by a cytopathologist (N.G.) and a pathologist (D.B.) with specific training. Further processing involved those smears which showed as a dominant cell type apocrine cells, with large granular eosinophilic or cyanophilic cytoplasm, uniform small nuclei and prominent round nucleoli. Smears with only few or no apocrine cells were discarded; these smears were chiefly composed by foam cells, bare nuclei and/or inflammatory cells.

Morphometric analysis

Computer-assisted cytometry was accomplished on well-characterized flat, non-overlapping clusters of apocrine cells using a Leitz Texture Analysis System (TAS). The TAS device consists of hardware modules, connected to a digital PDP-11 computer which allows discrimination and measurement according to the theory of mathematical morphology [12]. The system is connected to a Leitz Ortophan microscope by a Bosch Plumbicon-tube camera in such a way that optical images can directly be stored, analysed and measured by TAS. The PDP-11 computer associated with the TAS allows users to make programs which establish the steps of image transformation, measurement of a number of parameters, and statistical processing of data from cell populations. Programs are written in a special language (TASIC) which enables the user to call quickly and with a single statement the function of any TAS module. The contour of each cell is traced on the TAS monitor by a light pen (Fig. 1); the surface areas of cytoplasm, nucleus and nucleolus are measured together with the length of the nuclear perimeter. The computer then calculates the nuclear/cytoplasmic (N/C), the nuclear/nucleolar (N/n) ratios and the nuclear roundness factor (RF). This last parameter, calculated by the formula perimeter^2/area $\times 4\pi$ is defined as the degree to which the nucleus in cross-section approximates a perfect circle [13].

We measured a total number of 5820 cells for 57 cysts, with a mean number of 102 cells/cyst.

Biochemistry

K^+ and Na^+ were measured by flame photometry and DHA-S was measured by radioimmunoassay as reported elsewhere [8]. Classification of the cyst type was done according to the following criteria: Type I, $K^+/Na^+ > 1.5$; Type II, $K^+/Na^+ < 0.66$; Type III, $K^+/Na^+ > 0.66 < 1.5$.

Statistical analysis

DHA-S levels did not show an acceptable Gaussian distribution and were logarithmically transformed. Correlations between cytoplasmic area, nuclear area, N/C ratio, nucleolar area on the one side and log DHA–S, K^+/Na^+ on the other side were assessed using the Pearson's coefficient of correlation. Morphocytometric parameters obtained in cells belonging to Type I cysts were compared to the corresponding parameters of cells belonging to Type II cysts by Student's t test. Levels of statistical significance were set at $P < 0.05$.

RESULTS

Subdivision of our 78 BCF specimens according to the cationic type yielded the following percentage distribution: Type I, 71.8% (no. 56); Type II, 23% (no. 18); Type III, 5.2% (no. 4). The cyst type was concordant in the two cysts aspirated from different quadrants of the same or contralateral breast in 32 out of 39 patients (82%). With regard to DHA-S concentrations, we confirmed our previously reported results [8]: the range was between 1 and 408 μmol/l, with a median of 98 μmol/l.

57/78 samples had cell clusters which met the cytological criteria for being submitted to the morphometric analysis. Forty-seven samples were of Type I (84% of fluids classified in this subset); 10 samples were of Type II (56% of fluids classified in this subset). None of the samples classified as Type III showed clusters of apocrine cells suitable for the cytometric approach. Interestingly, the minimal expression of apocrine cells in about 27% of our BCF specimens was often matched by the presence of foam cells and signs of prominant cytolysis.

In the light microscope, no difference was apparent between the apocrine cells aspirated from different cysts in the same patient nor from Type I vs. Type II cysts, apart from a trend for Type I cells to shed in wider clusters (Fig. 2). This feature,

Fig. 1. Example of TAS light pen identification of cytoplasmic, nuclear and nucleolar boundaries in a cluster of BCF apocrine cells.

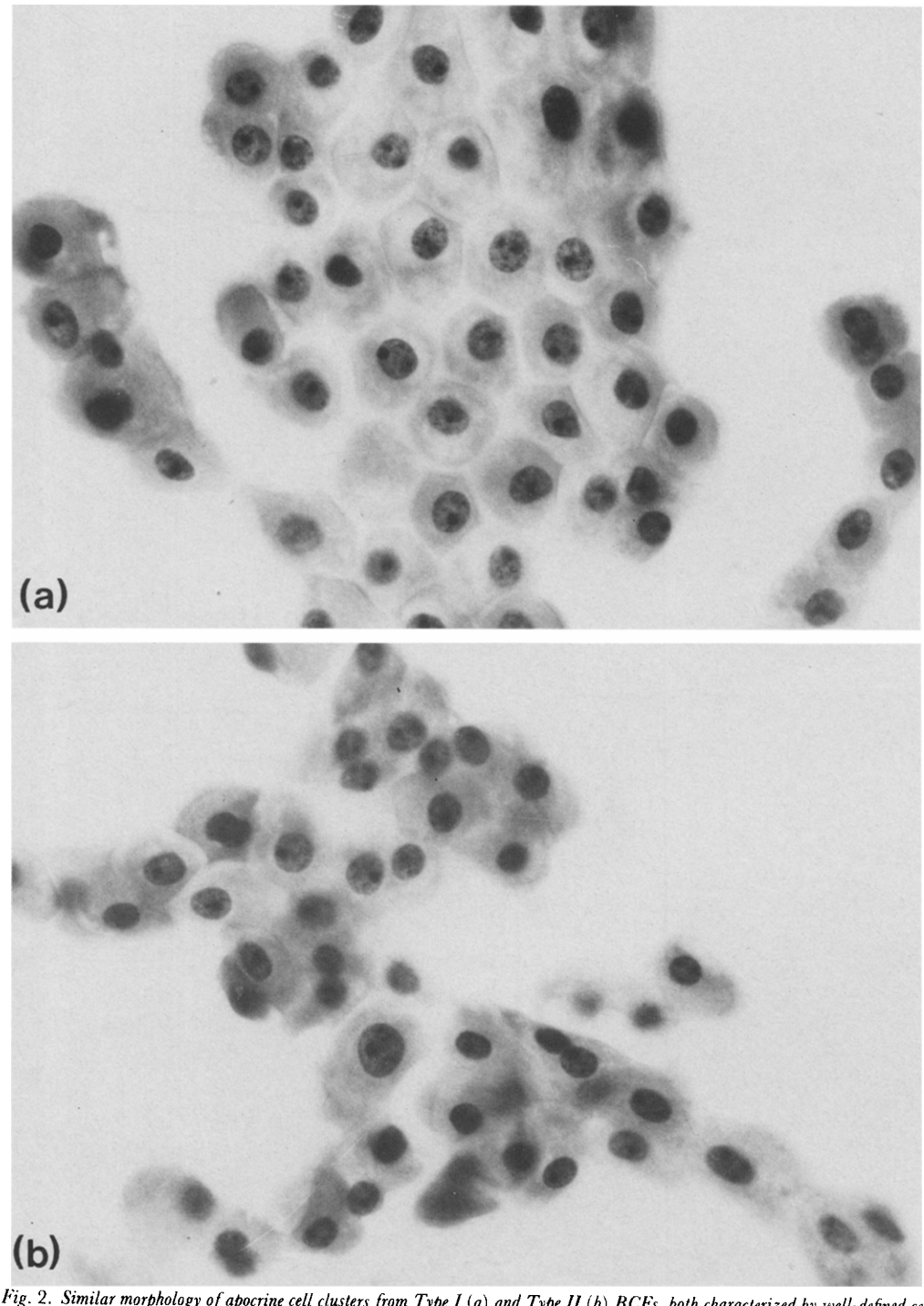

Fig. 2. Similar morphology of apocrine cell clusters from Type I (a) and Type II (b) BCFs, both characterized by well-defined cell boundaries, round nuclea and prominent nucleola. (Papanicolau staining, 40 ×, original magnification).

Table 1. *Morphometric results in Type I cysts* (47 *samples*) *and Type II cysts* (10 *samples*)

	Type I Mean ± S.D.	Type II Mean ± S.D.	*P*
Cytoplasmic area (μm^2)	97.13 ± 24.28	59.66 ± 14.90	<0.05
Nuclear area (μm^2)	42.65 ± 10.41	41.88 ± 10.53	N.S.
Nucleolar area (μm^2)	4.35 ± 0.99	2.75 ± 0.71	<0.05
RF	0.9704 ± 0.0437	0.9920 ± 0.0422	N.S.

however, could be anecdotal being dependent on the technical handling. The apocrine cells never displayed atypical features; when binucleation was seen, the cell was excluded from the morphometric analysis.

Table 1 shows the results of the cytometric measurements. Statistically significant differences were apparent for the cytoplasmic and nucleolar areas when the apocrine cells aspirated from Type I cysts were compared to the apocrine cells aspirated from Type II cysts. Searching for correlations between the morphometric parameters and the K^+/Na^+ ratio and log DHA-S yielded statistically significant values only for the N/C ratio which was inversely correlated with both variables ($P < 0.05$).

When examining the cytometric data calculated on clusters aspirated fom different cysts in the same patients, it was found that in about 50% of patients (no. 19) there were intriguing differences of the mean values for the cytoplasmic, nuclear and nucleolar sizes. These differences were not associated with the side (ipsi- or contralateral samples) or the cyst type (alike or different). The variability was much lower for the roundness factor which in our cell population ranged from 1.058 to 0.890. Finally, no correlation was found between cytometric parameters and number of cells in the clusters counted.

DISCUSSION

In the broad spectrum of fibrocystic changes, the so-called apocrine cysts have been suggested to be a marker for increased risk of breast cancer [1, 2], even if the association of apocrine cells with hyperplastic focal lesions is not generally acknowledged [14]. A peculiar composition of BCF (high K^+, high levels of androgen-sulfates) reportedly complements the apocrine morphology of the epithelium lining the cysts. On this basis, it was proposed that measuring cations and/or DHA-S in the fluid could identify the apocrine changes, hence those patients need to be followed up with more attention.

In the present study, the morphometric approach was selected to investigate a large number of cells with well-recognized apocrine features obtained from biochemically different BCFs. In such a way we have obtained objective and reproducible quantitation of some cell characteristics and accurate analysis of subtle cytological variances.

We found that smears excluded from the cytometric evaluation due to the paucity of apocrine cells were unequally distributed when considering the cationic type of cysts. Whereas only 16% of BCFs coming from the cysts classified as Type I were excluded, the percentage rose to 44% of BCFs coming from the cysts of Type II, and to 100% of BCFs coming from those of Type III. This observation is compatible with the view that apocrine cysts are more frequent in the subgroup identified as Type I. This could be due to an inherent diversity of the biochemical environment which could account for the extent of the apocrine changes, as suggested by Dixon *et al.* [9]; alternatively, one could suggest that specific arrangements of the epithelium lining the cysts (papillarity) favour exfoliation, or that the presence of apocrine cells in BCF reflects loss of cellular cohesiveness and possibly a more rapid cell turnover.

When present, the apocrine cells were not different at the conventional microscope examination in the fluids aspirated from Type I or Type II cysts. Also, the absolute number of cells or clusters did not discriminate the cationic type of our cysts. Taken together, our data do not allow the inference of apocrine changes in the epithelium lining the cysts simply from the cationic pattern of BCF. Accordingly, the term apocrine cysts should be used only after morphological evaluation.

By means of computer-assisted cytometry we could demonstrate that the cells aspirated from Type I cysts had significantly wider cytoplasm and nucleoli than those aspirated from Type II. In addition, a negative correlation was found between both the levels of DHA-S and the K^+/Na^+ ratio and the N/C ratio. The question could be raised whether the differences in TAS-computed parameters reflect different functional stages of the apocrine cells in Type I vs. Type II cysts, possibly related to hormonal effects, in turn depending on the intensity of steroid exposure and/or metabolization. Prominent nucleoli and wide cytoplasm are generally found in secretory epithelial cells when post-transcriptional protein synthesis is actually or potentially active [15]. Functional exhaustion or late steps of the cellular life are conceivably charac-

terized by the volume reduction of these organelles.

Type II BCF reflects the normal ionic concentration outside a mammalian cell, as regulated by the $Na^+:K^+$−ATPase-dependent pump. Conversely, Type I BCF could be associated with defects of the $Na^+:K^+$ pump which eventually produce cell swelling or loss. In this sense, our data could be viewed as compatible with the concept that the cationic composition of BCF, hence the type of cyst, is a mirror of the cellular activity of the lining epithelium. If this were true, the so-called apocrine or flattened cysts could be subsequent, albeit functionally different, stages of a single type.

Apocrine cells in BCF cannot be viewed as being prone to malignant proliferation. In fact, no morphological sign of preneoplastic significance was found in our population of about 6000 cells. In this regard, calculation of RF deserves value. Round nuclei with regular outline are usually devoid of malignant significance [13]; the RF values near to 1 found unanimously in our cells corroborate the absence of atypia at light microscope examination.

To conclude, we think that both biochemistry and cell morphology of BCF may provide information on the activity of the epithelium lining the cysts, hence on a whole-organ promoting status towards associated proliferative lesions, in which probably lies the relationship between GCD and cancer risk [16]. Further work is needed to ascertain the role of the apocrine cells in the mechanisms accounting for the composition of BCF.

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