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Cations and Dehydroepiandrosterone-Sulfate in Cyst Fluid of Pre- and Menopausal Patients with Gross Cystic Disease of the Breast. Evidence for the Existence of Subpopulations of Cysts

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Abstract—*Cations* (K^+ *and* Na^+) *content was evaluated in* 444 *breast cyst fluid (BCF) specimens, aspirated from* 391 *patients with gross cystic disease of the breast (GCD), a benign form admittedly at major risk of cancer. In* 306/444 *BCF, dehydroepiandrosterone-sulfate (DHA-S) content was also evaluated. A positive correlation* ($P < 0.001$) *was observed between log* K^+ *vs. log DHA-S whereas a negative correlation was found between log* Na^+ *and log DHA-S* ($P < 0.001$).

Cysts were subdivided in three types according to their cationic concentration: most were of type I ($K^+/Na^+ > 1.5$) *and type* II ($K^+/Na^+ < 0.66$) *whereas only* 10% *was of the type* III *(intermediate). No statistical difference in subtype distribution was apparent when considering patients aspirated in the follicular vs. luteal phase of the menstrual cycle; on the contrary, a significant difference* ($P < 0.001$) *was found between menstruating vs. menopausal patients (type* I = 54.8% *vs.* 32.2%; *type* II = 34.5% *vs.* 58.1%, *respectively). Ninety-four BCF samples were aspirated simultaneously in* 41 *patients bearing multiple cysts: the same cationic subtype was present in* 29/41 *patients.*

Our data confirm and extend previous observations, and provide conclusive evidence that breast macrocysts can be divided on the basis of their electrolyte composition into different types. Accordingly, the composition of BCF should always be assessed for prospective studies on GCD and breast cancer risk.

INTRODUCTION

Gross cystic disease of the breast (GCD) is a peculiar form of fibrocystic breast disease which occurs predominantly between the age of 35 and 50 yr. Based on a large number of studies the development of cysts results in a 2–4-fold greater risk of developing carcinoma of the breast even though the site of the cyst is exceptionally the site of formation [1,2]. Biochemical examination of human breast cyst fluid (BCF), which is easily obtainable by fine needle aspiration, may be a useful tool to investigate the pathophysiology of the disease. Accordingly, in the last decade several reports have appeared that pointed to particular hormonal and electrolyte patterns of BCF, consistently different from blood plasma [3–5]. We measured several hormones in a large number of BCF samples and provided evidence that both thyroid and steroid hormones are present in this medium mostly in the free, unbound form due to the very low concentration of specific binding proteins [6,7]. It was found that very large amounts of androgen sulfates are often associated in BCF with high K^+, low Cl^- concentrations similar to that of the intracellular compartment [8,9]. Moreover, Dixon *et al.* [10] have recently suggested that intracystic dehydroepiandrosterone-sulfate (DHA-S) levels parallel the degree of apocrine metaplasia within the cells lining the cysts. The aim of this study was to investigate in a large

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number of cysts, cations (K^+ and Na^+) and DHA-S content and their possible correlations.

MATERIALS AND METHODS

Sodium (Na^+) and potassium (K^+) were measured in 444 BCF specimens obtained by fine needle aspiration, according to Zajicek [11], from 391 patients bearing well documented GCD, aged 27–54 yr. In all patients cancer was excluded by thermography, echography, mammography and repeated clinical examinations. In order to gain homogeneity in terms of hormonal status we initially performed the aspiration during the early or mid-follicular phase of the menstrual cycle (352 specimens). Then, we extended our study to 54 BCF samples coming from patients in the luteal phase (plasma progesterone levels > 5 ng/ml). We also examined 38 BCF specimens aspirated from menopausal women. In all patients plasma levels of 17-beta estradiol, progesterone and gonadotropins were measured in concomitant blood samples.

Ninety-four BCF specimens were aspirated on the same day from 41 patients bearing multiple cysts in different quadrants of the same or contralateral breast. Repeated samples from the same patients aspirated in different periods were not included. Volume of BCF samples varied from 0.5 to 40 ml. Initial processing of the fluid until storing at −20° C was described elsewhere [6]. Because of the small volume of BCF aspirated in some patients and of additional biochemical evaluation we did not perform DHA-S in all samples, but only in 306/444.

Intracystic DHA-S was analysed using procedures originally devised for the analysis of blood plasma. Applicability of the chosen procedures to the analysis of BCF was validated both by serial dilution and recovery studies. Estimates of intracystic Na^+ and K^+ concentrations were performed by flame photometry (Eppendorf, FCM 6341). DHA-S was measured by a radioimmunoassay procedure using a commercially available kit (Medical System, Los Angeles, U.S.A.). The lowest measurable level was 0.05 μmol/l; intra-assay variability was 10%, and inter-assay variability was 12%.

We tested the distribution of the values for each of the variables by evaluating coefficients for the asymmetry (skewness) and the peakedness (kurtosis) [12]. Intracystic concentrations of the three examined variables did not show a gaussian distribution. Logarithmic transformation, however, resulted in acceptable normalization of the data. For determination of the degree of correlation between different variables in BCF, we used linear regression analyses (concentrations were logarithmically transformed). Levels of statistical significance were set at $P < 0.05$.

The subdivision of cysts in subgroups according to the K^+/Na^+ ratio was statistically validated using the Bartlett's test [13], and the difference in the distribution of the subgroups in pre- vs. menopausal patients was assessed by the chi-squared test.

RESULTS

In the present series of BCF samples, very ample interindividual variations were apparent both for cations and for DHA-S intracystic levels. However, a highly significant positive correlation ($P < 0.001$) was observed between log K^+ vs. log DHA-S and between K^+/Na^+ ratio vs. log DHA-S as well as a significant negative correlation ($P < 0.001$) was found between log Na^+ vs. log DHA-S (306 specimens) as represented in Fig. 1.

With regard to Na^+ and K^+ concentrations we could subdivide the cysts into three different types considering the K^+/Na^+ ratio: type 1 ($K^+/Na^+ > 1.5$), type 2 ($K^+/Na^+ < 0.66$), type 3 ($K^+/Na^+ > 0.66 < 1.5$). Table 1 shows the median concentration and the range of the examined variables in the three types of cysts aspirated during the follicular and the luteal phase of the menstrual cycle and from menopausal women. In the type 1 subgroup there was only one case with DHA-S level lower than 20 μmol/l; on the contrary, in the type 2 subgroup only two cases had K^+ values higher than 100 mmol/l and DHA-S levels higher than 200 μmol/l. These observations suggest the homogeneity of the two opposite subgroups which was also statistically validated by the Bartlett's test ($P < 0.001$).

A non-negligible number of patients (41 cases) who bore multiple cysts (94 BCF samples) were aspirated on the same day in different quadrants of the same or contralateral breast (Table 2). The cationic subtype was the same in different cysts of 29/41 women. Patients bearing at least one type 1 cyst were the great majority (34/41); cysts were homogeneous or inhomogeneous independently of the side.

When considering the subgroups distribution of cysts aspirated during the follicular and the luteal phase of the menstrual cycle we did not observe any significant difference. In the menstruating patients the majority of cysts was attributable to the subgroup with K^+/Na^+ ratio > 1.5. On the other hand, in BCF specimens aspirated from menopausal patients the distribution was different with the prevalence of cysts characterized by K^+/Na^+ ratio < 0.66 (Table 3).

DISCUSSION

The present study, performed in a large number of BCF samples, provides conclusive evidence that breast macrocysts can be divided into different

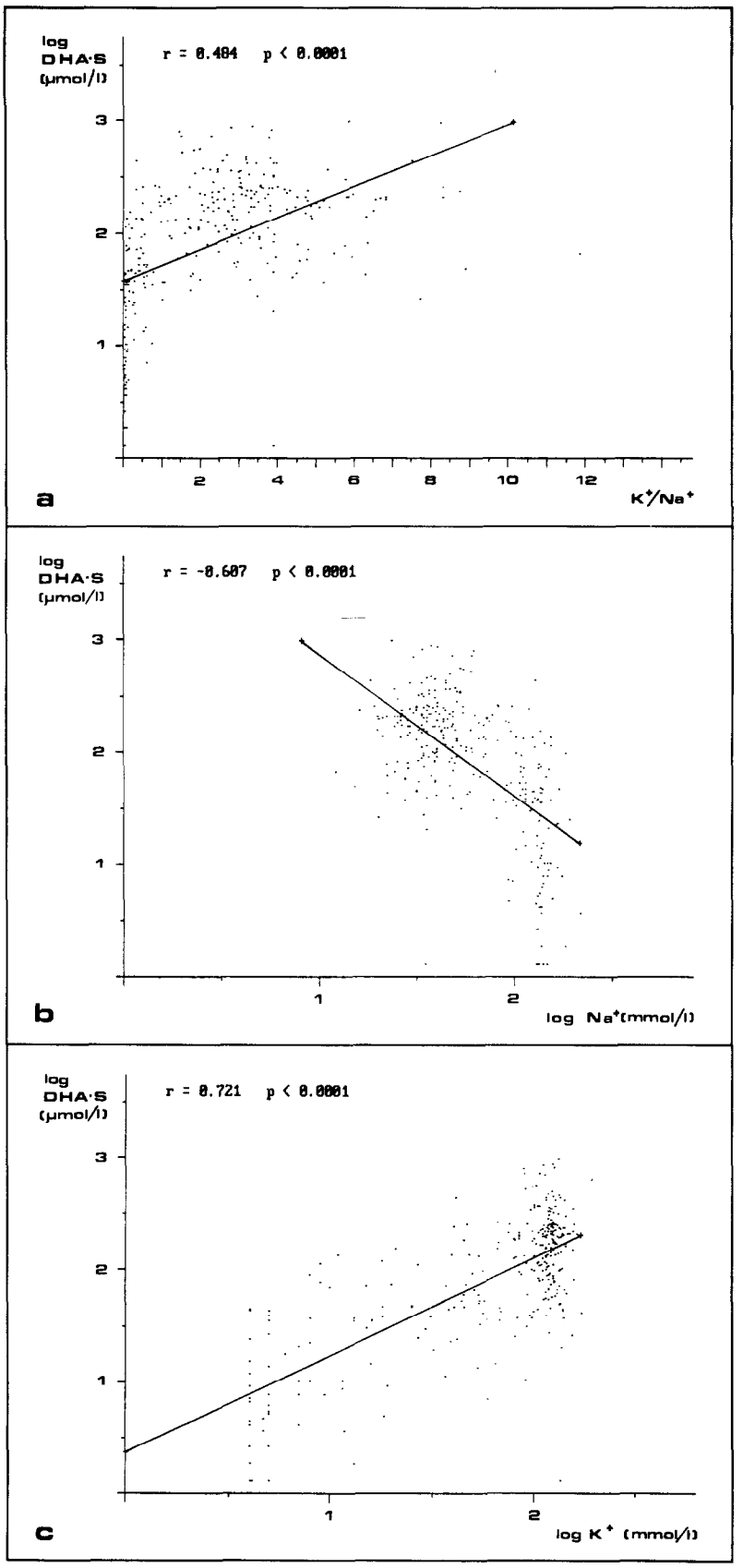

Fig. 1. Correlation between intracystic log DHA-S and intracystic K^+/Na^+ (a), *log* Na^+ (b) *and log* K^+ (c) *in* 305 *BCF specimens.*

Table 1. Median values and range of intracystic K^+, Na^+ and DHA-S according to the three subgroups of cysts

			No.	Median	Range
	F		196	119	80.5–184.1
	L	K^+ (mmol/l)	32	119.5	82–156
	M		12	117	91–140
	F		196	36	8.9–72
$K^+/Na^+>1.5$	L	Na^+ (mmol/l)	32	34.5	12–52
	M		12	38.5	21–54
	F		139	177.4	1.3–986.25
	L	DHA-S (μmol/l)	31	164.7	37.6–730.2
	M		10	181.6	68.5–467.2
	F		118	12.55	3.2–107
	L	K^+ (mmol/l)	19	16	4–54
	M		23	21	4–64
	F		118	133.5	50–273
$K^+/Na^+<0.66$	L	Na^+ (mmol/l)	19	132	93–162
	M		23	135	96–190
	F		65	27	1.3–436.3
	L	DHA-S (μmol/l)	16	31.5	1.3–186.8
	M		20	38.6	1.8–255.5
	F		38	80.7	52–118
	L	K^+ (mmol/l)	3	79	52–84
	M		3	82	76–84
	F		38	73.9	47.8–122
$K^+/Na^+>0.66<1.5$	L	Na^+ (mmol/l)	3	69	62–81
	M		3	77	72–82
	F		20	143.6	10.6–799
	L	DHA-S (μmol/l)	2		127–214.5
	M		3	202.3	38–259.4

F, L: cysts aspirated in follicular and luteal phase, respectively.
M: cysts aspirated in menopause.

types according to their electrolyte composition.

Our data confirm and extend previous observations [14–16]; in addition, they point to the occurrence of highly significant correlations between K^+ and Na^+ and DHA-S content in BCF (positive and negative correlation, respectively).

After reviewing data coming from present and previous series we propose here to classify the cysts according to cut-off values for electrolytes which are slightly different with respect to those proposed by Bradlow *et al.* [8] and Miller *et al.* [15]. We think it more appropriate to define cysts as "type 1" or "type 2" when the ratio between K^+ and Na^+ or vice versa is more than 1.5. Besides easier applicability to routine screening, another advantage of these cut-off values can be seen in the reduction of the number of cysts with intermediate pattern, which allows better focusing on the two opposite groups which correspond to the initial definition by Bradlow *et al.* [8] of "high K^+" and "high Na^+". In fact, when we recalculated our data on the basis of the cut-off values proposed by Bradlow we were not able to classify a certain number of cases; when applying the values proposed by Miller we found a higher number of intermediate type of cysts. Moreover, differences between menstruating and menopausal patients were not apparent.

The correlations between intracystic cations and DHA-S are of interest when considering that the cysts containing high androgen conjugate concentrations reportedly present a major degree of apocrine metaplasia of the epithelial cells lining the cysts [10]. In fact, it seems likely that accumulation in BCF of K^+, an intracellular cation, depends on the peculiar way of secretion of apocrine cells. Indirect evidence for a link between androgens and apocrine secretion comes from a well established androgenic control of extramammary apocrine cells (axillary sweat glands, anogenital sweat glands, cerumen producing glands of the external auditory meatus, eyelid Moll's gland) [17]. In the present state of knowledge it seems necessary to better investigate the mechanisms by which androgen conjugates accumulate in breast cysts, before drawing any conclusion on the sequence DHA-S → apocrine secretion → cations in BCF. However, it is per-

Table 2. Cation patterns in multiple cysts

Patient	Cyst 1	Cyst 2	Cyst 3	Cyst 4	Cyst 5	Cyst 6
1	^{R}Na	^{R}Na				
2	^{L}Na	^{R}Na	^{R}K			
3	^{R}K	^{R}Na				
4	^{R}Na	^{L}Na				
5	^{L}K	^{L}K				
6	^{R}Na	^{R}Na				
7	^{L}K	^{L}Na				
8	^{R}K	^{L}K				
9	^{L}K	^{L}Na				
10	^{R}K	^{L}K				
11	^{R}K	^{L}K				
12	^{L}K	^{R}Na				
13	^{R}K	^{L}K				
14	^{R}Na	^{R}Na				
15	^{R}Na	^{R}K				
16	^{R}Na	^{L}Mix				
17	^{L}K	^{L}K	^{R}K			
18	^{L}K	^{L}Na	^{R}K			
19	^{R}Na	^{L}K				
20	^{R}K	^{L}K				
21	^{L}K	^{L}K				
22	^{R}K	^{L}K				
23	^{R}K	^{R}K	^{L}Mix			
24	^{L}K	^{L}K				
25	^{R}K	^{L}K				
26	^{L}Na	^{L}K				
27	^{R}K	^{R}K	^{L}K	^{L}K		
28	^{R}K	^{L}K				
29	^{R}K	^{L}K				
30	^{L}K	^{L}K				
31	^{R}K	^{L}K				
32	^{L}K	^{L}K	^{L}K			
33	^{R}K	^{L}K				
34	^{R}K	^{L}K				
35	^{R}K	^{L}K				
36	^{L}K	^{R}K	^{R}K			
37	^{R}K	^{R}K				
38	^{R}K	^{L}K				
39	^{L}Na	^{L}Na	^{L}Na	^{L}Na	^{R}Na	^{R}Na
40	^{R}Na	^{L}K				
41	^{R}Na	^{L}Na				

K = $K^+/Na^+ > 1.5$; ^{L} = left;
Na = $K^+/Na^+ < 0.66$; ^{R} = right;
Mix = $K^+/Na^+ > 0.66 < 1.5$

Table 3. Differences in cation patterns of BCF samples aspirated from patients in the follicular and luteal phase of the menstrual cycle and from menopausal patients

	Follicular Phase 305 Cases	Luteal Phase 43 Cases	Menopause 31 Cases
I. $K^+/Na^+ > 1.5$	54.4%	58.1%	32.2%
II. $K^+/Na^+ < 0.66$	34.4%	34.9%	58.1%
III. $K^+/Na^+ > 0.66 < 1.5$	11.2%	7.0%	9.7%

% Distribution (chi-squared test).
Follicular vs. luteal: n.s.
Follicular vs. menopause: $P < 0.001$.
Luteal vs. menopause: $P < 0.001$.

tinent to state that interest in this area of research is rised by accumulating evidence that apocrine changes occur more frequently in populations at higher risk of breast cancer [18–20]. We did not observe any significant difference in the subtype distribution of BCF samples aspirated in the follicular or the luteal phase. On the other hand, data obtained in menopausal patients suggest that the accumulation of DHA-S and K^+ occur less frequently in these subjects with respect to menstruating women. This finding could be viewed as compatible with a decreased frequency of apocrine metaplasia. However, our data though statistically significant have to be regarded cautiously in the light of the different number of patients belonging to the groups considered.

In a number of cases we were able to analyse multiple cysts aspirated on the same day; a good consistency of the cationic pattern was apparent. Another interesting finding was relevant to the high frequency of patients who beared at least one type 1 cyst; our data are in agreement with those of Dixon *et al.* [21], who suggested that multiple cysts are associated with a higher incidence of apocrine metaplasia and a greater frequency of developing further cysts.

The cationic homogeneity both of homolateral and contralateral cysts supports the view that composition of BCF is a consequence of mechanisms involving the displastic mammary tissue in a general way and does not reflect simply local events.

The significance of electrolyte and hormonal accumulation in BCF still remains to be elucidated with regard to breast cancer risk. However, the existence of different subpopulations of cysts and the availability of sorting markers lead us to conclude that patients with GCD need to be classified according to the composition of BCF prior to any program of follow-up.

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