carcinogenesis: epidemiology of breast cancer in females. *J Natl Cancer Inst* 1980, **65**, 559–569.
4. Pike MC, Krail MD, Henderson BE, *et al.* Hormonal risk factors, breast tissue, age and the age-incidence of breast cancer. *Nature* 1983, **363**, 767–770.
5. Moore JW, Thomas BS, Wang DY. Endocrine status and the epidemiology and clinical course of breast cancer. *Cancer Surv* 1986, 5, 537–559.
6. Westphal U. Steroid-protein interaction. *Monogr Endocrinol* **4**. Springer, New York, 1971.
7. Siiteri P, Simberg N, Murai J. Estrogens and breast cancer. *Ann NY Acad Sci* 1984, **464**, 100–105.
8. Moore JW, Clark GMG, Bulbrook RD, *et al.* Serum concentrations of total and non-bound estradiol in patients with breast cancer and in normal controls. *Int J Cancer* 1982, **29**, 17–21.
9. Langley MS, Hammond GL, Bardsley A, *et al.* Serum steroid binding proteins and the bioavailability of oestradiol in relation to breast cancer. *J Natl Cancer Inst* 1985, 75, 823–829.
10. Habibollahi F, Fentiman IS. Breast conservation techniques for early breast cancer. *Cancer Treat Rev* 1989, **16**, 177–191.
11. Vermeulen A, Deslypere JP, Paridaens R, *et al.* Aromatase, 17 beta hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in post-menopausal women. *Eur J Cancer Clin Oncol* 1986, **22**, 515–523.
12. Malarkey WB, Schroeder LL, Stevens VC, *et al.* Twenty four hour pre-operative endocrine profiles in women with benign and malignant breast disease. *Cancer Res* 1977, **37**, 4655–4659.
13. Secreto G, Recchione C, Fariselli G, *et al.* High testosterone and low progesterone circulating levels in premenopausal patients with hyperplasia and cancer of the breast. *Cancer Res* 1984, **44**, 841–844.

Eur J Cancer, Vol. 27, No. 1, pp. 44–47, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

Accumulation of Active Androgens in Breast Cyst Fluids

G. Secreto, C. Recchione, P. Ballerini, L. Callegari, A. Cavalleri, A. Attili, G. Fariselli, D. Moglia and I. Del Prato

80 breast cyst fluids (BCF) from 57 patients were divided by K^+/Na^+ ratio: 56 with ratio over 1 (type I) and 24 with ratio less than 1 (type II). Significantly higher amounts of testosterone, dihydrotestosterone and dehydroepiandrosterone sulphate (DHAS) were found in type I than in type II cysts. A positive relation was found between testosterone and dihydrotestosterone in both types. DHAS was significantly correlated with testosterone and dihydrotestosterone in type I cysts only. In 52 patients, blood was sampled after cyst evacuation. Testosterone was significantly higher in blood than in BCF while dihydrotestosterone and androstenedione were significantly higher in BCF. No relation was observed between circulating levels of androgens and their intracystic concentrations. Women bearing type I cysts may be at increased risk of developing cancer. These findings support the hypothesis that androgens play a role in the hormonal aetiology of breast cancer.
Eur J Cancer, Vol. 27, No. 1, pp. 44–47, 1991.

INTRODUCTION

EXAMINATION OF human breast cyst fluid (BCF) reveals two major subgroups of cyst: type I with a high K^+/Na^+ ratio, resembling the intracellular milieu, and large amounts of dehydroepiandrosterone sulphate (DHAS); and type II with low K^+/Na^+ ratio and lower amounts of DHAS. A third population with intermediate K^+ and Na^+ concentrations has also been reported [1–3]. Type I cysts are lined with apocrine epithelium [4, 5] and their ion and hormonal content reflects the activity of the lining cells. Type II cysts are lined with flattened epithelium [4], although a few, less active apocrine cells have been found in their fluids [5]. Apocrine epithelium is under the influence of androgens and is an active site for conversion of weak androgen precursors into more active substances [6, 7]. Accordingly, an androgenic milieu may exist inside breast tissue bearing type I cysts.

Correspondence to G. Secreto.
The authors are at the Division of Experimental Oncology "C", Section of Endocrinology, National Cancer Institute, Via Venezian 1, 20133 Milan, Italy.
Revised 18 Oct. 1990; accepted 2 Nov. 1990.

Apocrine metaplasia of breast epithelium is frequently present in breast cancer [8, 9] and in populations at increased risk of breast cancer [10–12]. Dixon *et al.* [12] reported the appearance of cancer in 11 out of 80 patients who had apocrine cysts vs. 1 of 30 who had flattened cysts. These findings fit the original hypothesis from our laboratory pointing to increased androgenic activity in the hormonal aetiology of breast cancer [13].

In this study we have explored the relation between ion and androgen concentrations in BCF. We also correlated BCF contents with circulating levels of testosterone, dihydrotestosterone and androstenedione.

MATERIAL AND METHODS

Biochemical and hormonal examinations were done in 80 BCF specimens drawn by needle aspiration from 57 women with gross cystic breast disease. Simulataneous evacuation of multiple cysts was achieved in 14 patients: 8 with two cysts, 4 with three cysts, 1 with four and 1 with five cysts. The patients were aged 24 to 55 (mean 45.9 [S.D. 5.6]).

49 patients were still menstruating. 5 had their last menstrual cycle 3–9 months before entering the study. The other 3 had

had hysterectomy without oophorectomy; they were aged 50 or under and were considered to be premenopausal. None of the 57 patients had been taking any hormonal drug for at least 6 months. For patients still menstruating the day of the cycle was not taken into account. In 52 patients, 10 ml venous blood was drawn after evacuation of their cysts. Cyst fluids and sera were stored at −30°C.

K^+, Na^+ and Ca^{2+} were measured in intracystic fluid by flame photometer. DHAS was measured by radioimmunoassay (Sclavo kits)in BCF diluted from 1:11 to 1:51. Testosterone, dihydrotestosterone and androstenedione were measured in BCF and in sera after extraction followed by partition chromatography on a Celite column [13]. Commerical kits were purchased from Biomerieux (Charbonnier les Bains). Hormonal measurements in BCF were validated by serial dilution and recovery studies.

Statistical comparisons were done with non-parametric tests (Mann–Whitney U or Wilcoxon matched-pairs signed-ranks tests) because of the non-homogeneity of variances. The Spearman coefficient of correlation was used to analyse associations between pairs of variables.

RESULTS

BCFs were divided into subgroups according to their K^+/Na^+ ratio: 56 cysts (70%) with K^+/Na^+ higher than 1 (type I) and 24 cysts (30%) with K^+/Na^+ lower than 1 (type II). The age of the patients did not differ between the two subgroups (45.84 [6.1] and 45.96 [4.4] in types I and II, respectively).

Table 1. Intracystic concentrations of testosterone, dihydrotestosterone, androstenedione, DHAS and calcium in breast cyst fluids as function of the K^+/Na^+ ratio

	Type I cysts ($K^+/Na^+ > 1$)	Type II cysts ($K^+/Na^+ < 1$)	P
Testosterone (ng/ml)			
No. of cysts	56	23	0.0001
Mean (S.D.)	0.190 (0.113)	0.088 (0.056)	
Median	0.170	0.068	
Range	0.047–0.476	0.031–0.200	
Dihydrotestosterone (ng/ml)			
No. of cysts	55	24	0.0001
Mean (S.D.)	0.429 (0.359)	0.108 (0.069)	
Median	0.389	0.112	
Range	0.035–1.570	0.023–0.289	
Androstenedione (ng/ml)			
No. of cysts	56	24	0.0693
Mean (S.D.)	2.081 (1.385)	1.452 (0.800)	
Median	1.639	1.203	
Range	0.500–6.295	0.120–3.131	
DHAS (μmol/l)			
No. of cysts	52	20	0.0001
Mean (S.D.)	186.7 (118.1)	37.23 (23.05)	
Median	183.5	23.05	
Range	11.2–455.9	3.26–117.7	
Calcium (m eq/l)			
No. of cysts	51	22	0.8853
Mean (S.D.)	8.717 (3.584)	8.032 (2.160)	
Median	8.000	7.950	
Range	4.000–19.40	3.300–13.40	

Table 2. Relation between testosterone, dihydrotestosterone, androstenedione and DHAS in breast cyst fluids

	All cysts			Type I cysts			Type II cysts		
Correlations	No.	r	P	No.	r	P	No.	r	P
Testosterone vs.:									
Dihydrotestosterone	78	0.87	0.000	55	0.86	0.000	23	0.76	0.000
Androstenedione	79	0.23	0.040	56	0.09	0.526	23	0.50	0.015
DHAS	72	0.67	0.000	52	0.62	0.000	20	0.06	0.802
Dihydrotestosterone vs.:									
Androstenedione	79	0.07	0.538	55	0.13	0.354	24	0.38	0.063
DHAS	71	0.69	0.000	51	0.60	0.000	20	0.21	0.383
Androstenedione vs.:									
DHAS	72	0.48	0.000	52	0.49	0.000	20	0.51	0.021

Calcium

Calcium concentrations were similar in the two subgroups of cysts (Table 1). No significant correlation was found between calcium and K^+/Na^+ ratio, age or any of the measured hormones.

Androgens in BCF

DHAS, testosterone and dihydrostestosterone concentrations were significantly higher in type I than in type II cysts. Androstenedione levels were higher, although not statistically different, in a type I than in type II cysts (Table 1).

Overall, a strong positive correlation was found between testosterone and dihydrotestosterone. DHAS was correlated with testosterone, dihydrotestosterone and androstenedione while a weak or no correlation was found between androstenedione and testosterone or dihydrotestosterone, respectively (Table 2). The K^+/Na^+ ratio significantly related to DHAS ($r = 0.70$, $P = 0.0003$), testosterone ($r = 0.56$, $P = 0.0001$) and dihydrotestosterone ($r = 0.61$, $P = 0.0001$). The strong positive correlation between testosterone and dihydrotestosterone was confirmed both in type I and type II cysts, whereas the relation between DHAS and testosterone and between DHAS and dihydrotestosterone was present only in type I cysts. In both types of cyst androstenedione and DHAS were significantly correlated (Table 2).

Androgens in blood

Androgens were measured in the blood of 52 patients. The circulating levels of testosterone, dihydrotestosterone and androstenedione were compared with their intracystic concentrations. In patients with multiple cysts, the average value of each androgen in intra-patient cysts was used for comparison. Testosterone levels were significantly higher in blood than in BCF ($P = 0.0001$) while dihydrotestosterone and androstenedione levels were significantly higher in BCF ($P = 0.013$ and $P = 0.0001$, respectively) (Table 3). For the K^+/Na^+ ratio, circulating androgens did not reflect the differences already observed in type I and type II cysts (Table 4). No direct relation was found between the circulating levels of testosterone, dihydrotestosterone, androstenedione and their intracystic concentrations or the intracystic levels of DHAS. Contrary to what was observed in BCF, androstenedione in the blood was correlated with testosterone ($r = 0.76$, $P = 0.0001$) and dihydrotestosterone ($r = 0.62$, $P = 0.0001$). Testosterone and dihydrotestosterone were also related with each other ($r = 0.54$, $P = 0.0001$). A negative correlation was found between age

Table 3. Circulating levels and intracystic concentrations of testosterone, dihydrotestosterone and androstenedione

	Blood	Cysts	*P*
Testosterone (ng/ml)			
No. of cases	51	51	0.0001
Mean (S.D.)	0.317 (0.101	0.156 (0.096)	
Median	0.307	0.132	
Range	0.117–0.579	0.033–0.476	
Dihydrotestosterone (ng/ml)			
No. of cases	52	52	0.0130
Mean (S.D.)	0.167 (0.057)	0.314 (0.322)	
Median	0.160	0.155	
Range	0.074–0.331	0.023–1.570	
Androstenedione (ng/ml)			
No. of cases	52	52	0.0002
Mean (S.D.)	1.372 (0.522)	2.087 (1.389)	
Median	1.257	1.558	
Range	0.442–2.721	0.120–6.295	

Wilcoxon matched-pairs signed-ranks test.

and testosterone ($r = -0.49, P = 0.0001$), dihydrotestosterone ($r = -0.52$, $P = 0.0001$) and androstenedione ($r = -0.55$, $P = 0.0001$).

Androgens in multiple cysts

14 of the 57 women had undergone simultaneous aspiration of multiple cysts. Similar values of the K^+/Na^+ ratio were found in most of the co-existing cysts. The same type of cyst (ratio above or below 1) was found in 9 patients (64%). Similar concentrations of testosterone (Fig. 1), dihydrotestosterone (Fig. 2) and androstenedione were observed in most of the multiple cysts of each woman, while DHAS showed more scattered values. In type II cysts (open circles in figures) concentrations of both androgens were in the low range of their circulating levels, not exceeding the median value in blood. The highest values of these hormones were found in type I cysts (closed circles in figures). In most of these cysts, concentrations of dihydrostestosterone surpassed the highest value measured in blood.

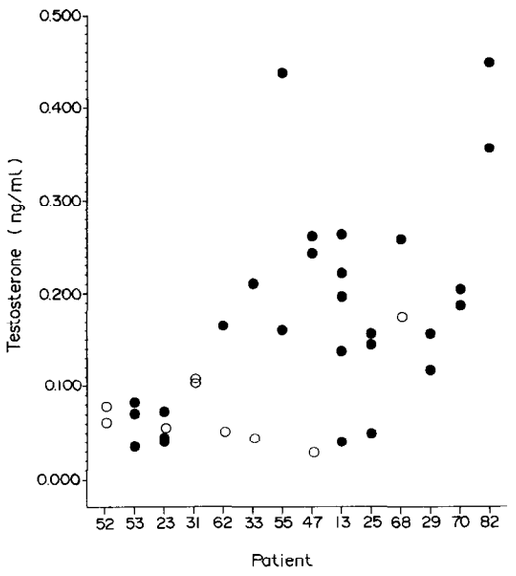

Fig. 1. Levels of testosterone in multiple cysts. In patient 62 testosterone was measured only in 2 of 3 cysts. Full circles = type I cysts (K^+/Na^+ over 1) and open circles = type II cysts (K^+/Na^+ under 1).

DISCUSSION

Mammary cysts develop in the terminal-ductal-lobular-unit (TDLU) of the breast and the metabolic products of the cells

*Table 4. Circulating levels of testosterone, dihydrotestosterone and androstenedione in patients with mammary cysts as function of cyst K^+/Na^+ ratio**

	Type I cysts	Type II cysts	*P*
Testosterone (ng/ml)			
No. of cases	35	16	0.07
Mean (S.D.)	0.301 (0.103)	0.351 (0.087)	
Median	0.288	0.362	
Range	0.117–0.579	0.168–0.486	
Dihydrotestosterone (ng/ml)			
No. of cases	36	16	0.56
Mean (S.D.)	0.164 (0.051)	0.176 (0.069)	
Median	0.160	0.169	
Range	0.086–0.309	0.074–0.331	
Androstenedione (ng/ml)			
No. of cases	36	16	0.47
Mean (S.D.)	1.339 (0.528)	1.446 (0.516)	
Median	1.233	1.469	
Range	0.524–2.472	0.442–2.721	

*Patients bearing multiple cysts were assigned to type I or II group if average intra-patient value of K^+/Na^+ was higher or lower than 1, respectively.

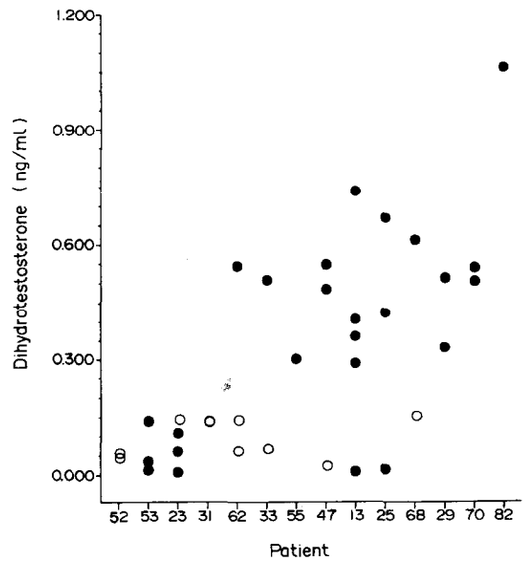

Fig. 2. Levels of dihydrotestosterone in multiple cysts. In patient 55 dihydrotestosterone was measured only in 1 of 2 cysts. In patient 82 same value of 1.090 ng/ml was measured in the 2 cysts. Full cicles = type I and open circles = type II cysts.

lining the cysts accumulate in the cystic fluid. Thus, BCF composition is thought to reflect, in a magnified manner, the activity of TDLU epithelium. TDLU is also the site where breast cancer originates. Although mammary cysts cannot be considered precancerous lesions *per se*, an increased risk of developing a cancer later has been reported in women with gross cystic breast disease, thus indicating that the same endogenous milieu in which breast cysts grow also favours cancer development. The greatest interest in the study of BCF composition lies, therefore, in the opportunity of acquiring a new insight into the environment where breast cancer arises.

The existence of apocrine epithelium lining the cyst walls [4] together with large amounts of DHAS in BCF [2, 3] are well documented and point to the presence of an androgenic milieu inside breast-bearing cysts, at least those of type I.

Our data confirm DHAS accumulation in BCF and show significantly higher amounts of the active androgens dihydrotestosterone and androstenedione BCF than in blood. In a comparison of the two subgroups of cysts, testosterone, dihydrotestosterone and DHAS were significantly higher in type I than in type II cysts. Differences between the two subgroups are possibly due to the different activity of the lining epithelia. Correlation analyses of androgens in BCF further suggested that different metabolic pathways are being followed in type I and in type II cysts.

Belanger *et al.* [14] have reported the intracystic concentrations of 18 non-conjugated and conjugated steroids, including those measured in our study. As far as testosterone, dihydrotestosterone, androstenedione and DHAS are concerned, our findings fit with theirs. Belanger *et al.* suggested that DHAS is converted into potent androgens by the breast tissue of patients with type I cysts; this implies an hyperandrogenic milieu in these patients' breasts. Women with type I cysts are at increased risk of breast cancer [8–12] and our data and those of Belanger *et al.* indicate that androgens play a role in the neoplastic change of breast epithelium. Other studies support this view since, in breast fluids obtained by nipple aspiration, testosterone levels were significantly higher in breast cancer patients than in healthy controls [15].

The mechanisms by which androgens may influence neoplastic growth are unknown. These steroids could act directly through binding to androgen receptors or indirectly after conversion into oestrogens. Reports of large amounts of epidermal growth factor (EGF) in BCFs, mainly in those from type I cysts [16–18] offer a new interpretation of the possible relation between occurrence of apocrine cysts, androgens and risk of subsequent breast cancer. Growth factors are believed to have an important role in the autocrine or paracrine control of breast carcinogenesis [19]. Evidence that androgens stimulate EGF synthesis has been found in the submaxillary salivary glands of rats [20, 21] and it has been suggested that EGF measured in human BCF is produced under androgen modulation inside the apocrine cells lining the cyst wall [6, 22, 23].

1. Bradlow HL, Skidmore FD, Schwartz MK, Fleisher M. Cation levels in human breast cyst fluid. *Clin Oncol* 1981, 7, 388–390.
2. Miller WR, Dixon JM, Scott WN, Forrest APM. Classification of human breast cysts according to elettrolyte and androgen conjugate composition. *Clin Oncol* 1983, **9**, 227–232.
3. Dogliotti L, Orlandi F, Torta M, *et al.* Cations and dehydroepiandrosterone-sulphate in cyst fluid from pre and menopausal patients with gross cystic disease of the breast. Evidence for the existence of subpopulation of cysts. *Eur J Cancer Clin Oncol* 1986, **22**, 1301–1307.
4. Dixon JM, Miller WR, Scott WN, Forrest APM. The morfologic basis of human breast cyst populations. *Br J Surg* 1983, **70**, 604–606.
5. Beccati D, Grilli N, Schincaglia P, *et al.* Apocrine cells in breast cyst fluid and their relationship to cyst type: a morphometric study. *Eur J Cancer Clin Oncol* 1988, **24**, 597–602.
6. Wales NA, Ebling FJ. The control of apocrine glands of rabbit by steroid hormones. *J Endocrinol* 1971, **51**, 763–770.
7. Labows JN, Petri G, Hoelze E, Leyden J, Klingman A. Steroid analysis of human apocrine secretion. *Steroids* 1979, **34**, 249–258.
8. Wellings SR, Alpers CE. Apocrine cystic metaplasia: subgross pathology and prevalence in cancer. *Hum Pathol* 1987, **18**, 381–386.
9. Mazoujian G, Pinkus GS, Davis S, Haagensen DE. Immunochemistry of a gross cystic disease fluid protein (GCDFP-15) of the breast. A marker of apocrine epithelium and breast carcinomas with apocrine features. *Am J Pathol* 1983, **110**, 105–112.
10. Wellings SR, Jensen HM, Marcum RG. An atlas of subgross pathology of the human breast with reference to possible precancerous lesions. *J Natl Cancer Inst* 1975, **55**, 231–273.
11. Schuerch C, Rosen PP, Hirota T, *et al.* A pathologic study of benign breast disease in Tokyo and New York. *Cancer* 1982, **50**, 1899–1903.
12. Dixon JM, Lumdsen AB, Miller WR. The relationship of cyst type to risk factors for breast cancer and the subsequent development of breast cancer in patients with breast cystic disease. *Eur J Cancer Clin Oncol* 1985, **21**, 1047–1050.
13. Secreto G, Toniolo P, Pisani P, *et al.* Androgens and breast cancer in premenopausal women. *Cancer Res* 1989, **49**, 471–476.
14. Belanger A, Caron S, Labrie F, Naldoni C, Dogliotti L, Angeli A. Levels of eighteen non-conjugated and conjugated steroids in human breast cyst fluid: relationships with cyst type. *Eur J Cancer* 1990, **26**, 277–281.
15. Hill P, Garbaczewski L, Winder EL. Testosterone in breast fluid. *Lancet* 1983, **i**, 761.
16. Boccardo F, Valenti G, Zanardi S, *et al.* Epidermal growth factor in breast cyst fluid: relationship with intracystic cation and androgen conjugate content. *Cancer Res* 1988, **48**, 5860–5863.
17. Hamed H, Wang DY, Moore JW, Clark GMG, Fentiman IS. Growth factor and electrolyte concentration in human breast cyst fluid. *Eur J Cancer* 1990, **26**, 479–480.
18. Lai LC, Dunkley SA, Reed MJ, Ghilchik MW, Shaikh NA, James VHT. Epidermal growth factor and oestradiol in human breast cyst fluid. *Eur J Cancer* 1990, **26**, 481–484.
19. Lippman ME, Dickson RB, Bates CH, *et al.* Autocrine and paracrine growth regulation of human breast cancer. *Breast Cancer Res Treat* 1986, 7, 59–70.
20. Barthe PL, Bullock LP, Mowszowicz I, Bardin CW, Orth DN. Submaxillary gland epidermal growth factor: a sensitive index of biologic androgen activity. *Endocrinology* 1974, **95**, 1019–1025.
21. Perheentupa J, Lakshmanan J, Hoath SB, Fisher DA. Hormonal modulation of mouse plasma concentration of epidermal growth factor. *Acta Endocrinol* 1984, **107**, 571–576.
22. Collette J, Hendrick JC, Jaspar JM, Franchimont P. Presence of alfa-lactalbumin, epidermal growth factor, epithelial membrane antigen and gross cystic disease fluid protein (15,000 daltons) in breast cyst fluid. *Cancer Res* 1986, **46**, 3728–3733.
23. Lai LC, Ghilchik MW, Shaikh NA, Reed MJ, James VHT. Relationship between epidermal growth and dehydroepiandrosterone and its sulphate in breast cyst fluid. *Br J Cancer* 1989, **60**, 320–323.

Acknowledgements—This work was supported in part by grant 88/01127/44 Progetto Finalizzato Oncologia from the Italian National Research Council, Rome.