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Original Paper

A Non-invasive Test for the Pre-cancerous Breast

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This paper describes a non-invasive, self-measured procedure by which the precancerous breast can be distinguished from the normal breast. The method involves wearing a specially designed thermometric brassiere for 90 min each evening at home through one menstrual cycle. Profiles of progesterone through the cycle, obtained from daily saliva sampling, and determination of the steroid content by radiommunoassay, are made to allow the status and calendar date timing of the luteal phase to be established. Thus, cycles can be synchronised across subjects. In this study, two types of breast were compared: 50 normal breasts and 41 age-matched precancerous breasts. Differences between the groups were striking in terms of amplitude, phasing and average temperature during the luteal heat cycle. When these parameters and others were used as predictors in a linear discrimination and/or neural net analysis, a sensitivity and specificity of >90% was achieved.

Key words: breast cancer, precancer, menstrual cycle
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INTRODUCTION

TRADITIONALLY, in breast cancer practice, the disease has been detected by palpation and confirmed by histopathology. Currently, X-ray mammography may detect impalpable lesions, and this is encouraging for a necessary improvement in the mortality statistics. Not all age groups are likely to benefit, however, because the X-radiographs have relatively poor definition in women of child-bearing age due to the density of the (normal) breast parenchyma. The United Kingdom health authorities do not recommend mammography as a screening method in those under 50 years of age [1]. The numerical importance of this younger group is emphasised in our own U.K. teaching hospital, where this age group represents as much as 36% of the clinically presenting breast cancer [2]. The approach outlined in this paper specifically targets the detection of precancer in this group. Because the method is novel, an explanation of the origins and principles of the method will be given.

Histopathological examiniation of breast tissue blocks from the margins of cancer mastectomies suggested that cancer does not grow in normal breast tissue, but rather in tissue with a high prevalence of epithelial hyperplasia, especially in premenopausal disease. A systematic comparison of the breast tissue from 500 consecutive mastectomies and 800 age-matched (autopsy derived) 'control' breasts has confirmed the observation [3]; and, while the type of hyperplasia has not been documented, most pathologists would agree that *per se* it carries an increased risk of carcinoma [4]. A similar transition occurs in the prostate [5]. Complementary to the above, Jensen and colleagues [6] introduced the term "cancer-associated" breast tissue to describe the residual ipsilateral and contralateral breast tissue because they were so impressed (as histopathologists) with the prevalence of precancerous epithelial lesions. The increased risk of a (second) primary has been documented as 6-fold [7, 8], which in practice means a 50% carcinoma rate in the survivors, in the 16 years following surgery for the primary carcinoma [7].

It follows that, if carcinogenesis does not, as a rule, occur in normal mammary tissue, then diagnostic emphasis may move from the detection of a small lump to the detection of an abnormality in the breast tissue, in general. Herein, we suggest that such tissue exhibits an abnormal luteal heat cycle [9] which can be measured non-invasively by a thermometric brassiere [9].

This report is concerned with the evaluation of luteal heat cycle parameters (amplitude, phase and average) from 50 control breasts (25 women) and 41 cancer-associated breasts (31 subjects) from (generally) parous women of similar average age (39 years). The only known difference between the breasts of the two groups of women was that the latter were cancer-associated and, therefore, precancerous.

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PATIENTS AND METHODS

General

The method for measuring the luteal heat cycle of the breast has been described elsewhere [9]. In essence, the subject self-measured the surface temperature of the breast for 90 min each evening, at home, through one menstrual cycle. The

measurements were made with a special brassiere incorporating temperature sensors and solid state thermometric electronics and semiconductor memory in a marsupial pouch between the cups. Some occasional domestic supervision and all downloading of the data were carried out by a nursing sister who co-ordinated the field trial. Overclothing and room temperature were controlled (20–24°C). Oral temperature was recorded at the start of each session using two maximally recording clinical thermometers, one being documented by the subject and the other being kept for the visiting nurse to read. The nurse's reading was the one adopted unless there was a discrepancy, in which case the higher reading of the two was used. The oral temperature was then subtracted from the breast surface temperature to exclude *inter alia*, the basal body temperature rhythm effect on the breast temperature. Saliva samples were collected and stored in appropriate containers in the domestic freezer until the end of the menstrual cycle. The progesterone concentration in the saliva was determined [10] to confirm the status and calendar date timing of the luteal phase. Twelve per cent of the 2730 (potential) breast temperature data points are missing due to subject non-compliance or instrument malfunction. These missing data points were extrapolated by linear interpolations in the graphed points of Figures 1 and 2; in fact, most occurred in the follicular rather than the luteal phase of the cycle.

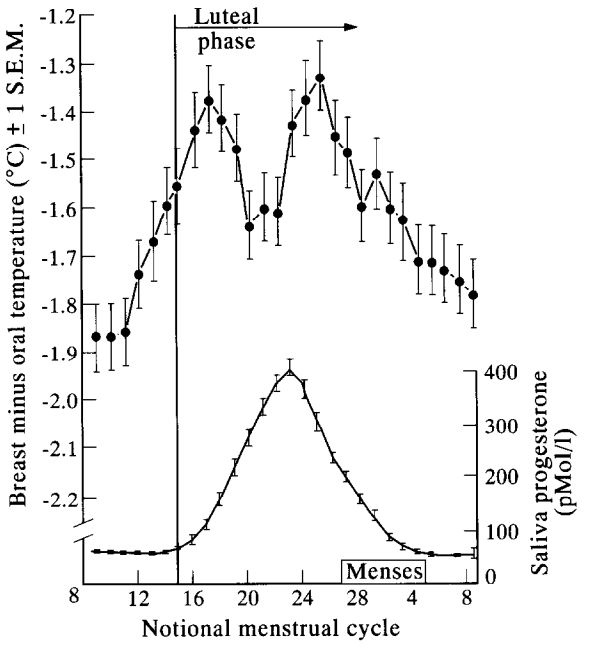

Figure 2. Luteal heat cycle of the breast in cancer-associated cases (3-day means ± 1 S.E.).

Subject database

Breast luteal heat cycle profiles from 50 control breasts were compared with 41 cancer-associated breasts from women of similar age and parity. Subject details of these two groups are documented in Tables 1 (controls) and 2 (cancer-associated breasts). Ten of the cancer-associated breasts were ipsilateral (after cancer lumpectomy) and 31 contralateral (to a previously excised carcinoma), as defined by Jensen and associates [6]. Herein, the cancer-associated breasts, whether ipsi or contra, are described as one entity because the data were similar. Exclusions included mastectomy sites and irradiated breasts, and patients currently on cytotoxic drugs or with anovulatory cycles (luteal progesterone averaging <200 picomoles/l over a 3-day span at the luteal maximum).

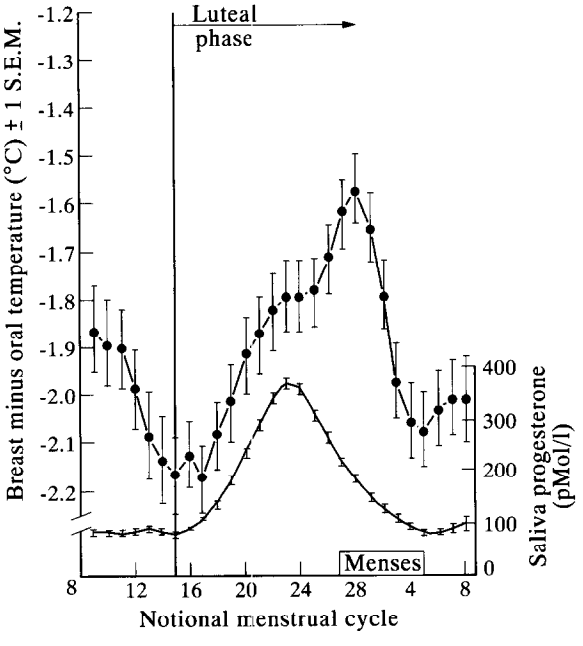

Figure 1. Luteal heat cycle of the breast in controls (3-day means ± 1 S.E.).

Rhythm analysis

A cosine function with a 28-day period was fitted [11] to the average luteal heat cycle profiles, representing normal and cancer-associated breasts. This enabled the two series to be summarised into a few parameters (i.e. phase, amplitude and level) with associated error estimates.

Linear discrimination analysis

The candidate signals (see Results) were entered into a linear discrimination programme using multiple regression (MINITAB Software) [12]. This uses a 'C-p' and R^2 (adjusted) statistic to optimise the signal/noise ratio of the regression equation.

Neural net analysis

In order to try to improve on the sensitivity and specificity of the linear discrimination model, the data sets from the control and cancer-associated breasts were subjected to neural net analysis [13]. This is a well accepted, powerful tool for pattern recognition.

RESULTS

Introduction

The two data series giving the grouped results for control breasts (50) and cancer-associated breasts (41) are plotted in Figures 1 and 2. In each case the y axis expresses the breast surface temperature as a difference from the higher oral (body) temperature. It has, therefore, a negative value. In this way, individual body temperature "set point" variation is compensated for. Initial inspection of the data indicates that while both groups exhibit a luteal heat cycle, the cancer-associated breasts are persistently hotter and have an earlier-phased cycle with a smaller amplitude. Rhythm analysis was conducted to confirm any statistical validity of these initial impressions.

Table 1. *Control breasts*

Subject no.	Age at study (years)	Parity	No. of breasts studied	Menstrual cycle length (days)	Salivary progesterone luteal peak (pMol/l)*	Clinical assessment + Breast symptoms
1	37	5 + 0	2	27	382	None
2	45	3 + 0	2	28	279	Fibroadenoma
3	43	2 + 0	2	26	395	None
4	41	2 + 0	2	28	207	Inverted nipple
5	35	2 + 0	2	28	342	None
6	36	3 + 0	2	32	201	None
7	41	1 + 0	2	28	387	None
8	39	2 + 0	2	25	322	None
9	32	2 + 0	2	27	264	Lipoma, mastalgia
10	38	4 + 2	2	29	496	Simple nipple lesion
11	32	3 + 0	2	29	438	Fibroadenoma
12	40	4 + 2	2	28	401	None
13	33	1 + 0	2	34	230	Slight mastalgia
14	37	2 + 0	2	31	357	Fibroadenoma
15	42	2 + 0	2	29	349	Some lumpiness
16	45	2 + 0	2	24	437	None
17	28	1 + 2	2	27	541	Fibroadenoma
18	39	2 + 0	2	33	394	Some lumpiness
19	35	1 + 0	2	29	305	Some lumpiness
20	38	2 + 0	2	29	565	None
21	42	2 + 0	2	36	284	Mastalgia
22	40	3 + 1	2	23	213	Fibroadenoma
23	48	3 + 1	2	28	224	Some lumpiness
24	38	1 + 0	2	26	381	None
25	45	2 + 0	2	27	279	None
Mean (total)	38.8	2.3 + 0.3	50	28.4	346	

*Three-day peak average.

Rhythm analysis

The results of this procedure (see Patients and Methods for details) are given in Table 3. The control breasts showed a stronger rhythm (50% versus 36%) than the cancer-associated breasts. In both groups, the probability that the data could be represented by a straight line (i.e. no rhythm) was rejected at least at the $P<0.007$ level. The amplitude was less in the cancer-associated breasts, but the difference was only 1.5 S.E. of the mean. However, the rhythm-adjusted average temperatures of the cancer-associated group were significantly higher and exhibited a significantly earlier luteal phase than the controls (Table 3).

These menstrual rhythm parameter differences could have been used singly to provide a basis for identifying the cancer-associated breast but their power was weak. For example, the rhythm-adjusted average breast temperature used alone as a predictor had a sensitivity of only 54% and a specificity of 68%. Accordingly, we turned to multipredictor models, such as linear discrimination [12] and neural net [13]. The input predictors and methods are outlined below.

Linear discrimination analysis

Candidate signals were identified as follows:

(i) Rhythm parameters of each subject's data, as discussed above and summarised in Table 3.
(ii) Selected statistics, identified by the inspection of the control and test time plots in Figures 1 and 2 for example, periovulation temperature days (the mean temperature recorded on days 14–18 of the menstrual cycle).
(iii) Evaluation of the 'progesterone responsiveness' statistics seen by correlating daily breast temperature values with the paired saliva concentrations occurring 3 days earlier.

In the programme, both individual candidate signals and all combinations were tried iteratively, searching for the most powerful discrimination between the normal and cancer-associated breasts. Seven predictors were found to be most effective. In order of power, when used together, these were:

(a) Progesterone–breast temperature response gradient obtained by cross correlating the two variables
(b) Amplitude of the breast temperature rhythm
(c) Average cycle temperature
(d) Periovulation breast temperature
(e) Amplitude of basal body temperature rhythm
(f) Crest day of oral temperature
(g) Progesterone/oral temperature response gradient.

Noteworthy is the fact that the latter three parameters (e, f and g) measure a different progesterone response phenomenon. Using these seven predictors, the resulting regression equation has a C–p value of 2.8, an adjusted R^2 of 42%, and a P value <0.00001, allowing the normal breasts to be distinguished from the cancer-associated breasts with an 87.5% sensitivity and 80% specificity. The best single predictor for distinguishing control

Table 2. Cancer-associated breasts

Subject no.	Age at study (years)	Parity	No. of breasts studied	Menstrual cycle length (days)	Salivary progesterone luteal peak* (pMol/l)	Cancer diagnosis (histopathology)	Treatment				Time (months) between surgery and study
							Surgery	X-ray	Cytotoxic	Other	
1	43	1 + 0	2	22	345	Infiltrating and lobular	L^x	No	No	No	3
2	43	3 + 0	1	28	439	Intraduct and infiltrating	M^x	No	No	Tam†	108
3	44	5 + 0	1	28	199	Infiltrating ductal	L^x	Yes	Yes‡	No	11
4	34	2 + 0	1	29	538	Poorly differentiated	L^x	Yes	Yes§	No	13
5	39	1 + 0	1	33	427	Infiltrating ductal	L^x	Yes	No	Tam‖	8
6	29	0 + 0	2	28	¶	Infiltrating ductal	L^x	No	No	No	17
7	40	4 + 0	2	25	355	Undifferentiated	L^x	No	No	No	54
8	37	1 + 0	1	25	453	Bilateral scirrhous	$L^x \times 2$	One side	No	No	50
9	41	2 + 0	2	27	811	Intraduct	L^x	No	No	No	55
10	41	2 + 0	1	21	297	Tubular	L^x	Yes	Yes‡	No	12
11	32	0 + 0	1	19	585	Infiltrating ductal	M^x	No	No	No	19
12	42	2 + 1	2	26	321	Infiltrating ductal	L^x	No	No	No	6
13	39	2 + 0	2	27	433	Infiltrating ductal	L^x	No	No	No	61
14	41	2 + 0	1	27	367	Intraduct and infiltrating	M^x	No	No	No	72
15	40	2 + 0	1	24	387	Infiltrating ductal	L^x	Yes	No	No	11
16	37	2 + 0	1	35	939	Infiltrating ductal	M^x	No	No	No	4
17	45	1 + 0	1	24	240	Infiltrating ductal	M^x	No	No	No	84
18	37	1 + 0	2	26	404	Adenocarcinoma	L^x	No	No	No	1
19	44	2 + 0	1	25	330	Infiltrating ductal	M^x	No	No	No	156
20	41	3 + 3	2	23	383	Tubular	L^x	No	No	No	1
21	34	2 + 0	1	26	367	Intraduct only	M^x	No	No	No	17
22	42	0 + 0	1	25	679	Infiltrating lobular	M^x	No	No	No	17
23	40	2 + 0	1	29	276	Intraduct and infiltrating	L^x	Yes	No	No	12
24	44	5 + 0	2	33	328	Infiltrating ductal	L^x	No	No	No	13
25	41	3 + 1	1	27	252	Intraductal	L^x	Yes	No	No	15
26	39	1 + 2	2	29	229	Adenocarcinoma	L^x	No	No	No	30
27	45	2 + 1	1	24	362	Intraduct and infiltrating	M^x	No	No	No	36
28	44	1 + 1	1	26	248	Intraduct and infiltrating	M^x	No	No	No	7
29	29	1 + 0	1	31	306	Ductal	M^x	Yes	No	No	15
30	46	2 + 0	1	27	453	Lobular	M^x	No	No	No	37
31	36	2 + 0	1	24	472	Intraduct	M^x	No	No	No	22
Mean (total)	39.6	1.9 + 0.3	41	26.5	394						

L^x, lumpectomy; M^x, mastectomy.

*Three-day peak average; †Stopped 6 years before study; ‡Stopped 6 months before study; §Stopped 10 months before study; ‖Stopped for study; ¶No progesterone values available; ovulatory dates inferred from oral temperature curve. Tam, tamoxifen.

Table 3. Rhythm analysis of averaged luteal heat cycles of the breast in preneoplastic disease

Type of breast	Number examined	Percentage of rhythm*	*P* value†	Amplitude of fit (°C ± 1 S.E.M.)	Rhythm adjusted average temperature (°C ± 1 S.E.M.)	Estimated day of crest (Menses = 1) ± 1 S.E.M. (days)
Normal	50	50%	<0.001	0.21 + 0.04	−1.94‡ ± 0.03	26.5 ± 0.9
Cancer-associated	41	36%	0.007	0.15 + 0.04	−1.60 ± 0.03	22.2 ± 1.2

*Per cent variance accounted for by the 28-day cosine fit to the averaged data by least squares; †Probability that the averaged series could be represented by a straight line; ‡Breast temperatures are a negative quantity in °C since oral temperature (blood temperature) has been subtracted from the observed value.

and preneoplastic breasts was the periovulation breast surface temperature (mean of days 14–18) $P<0.0001$.

Neural net analysis

A data file was created randomising the training set of breast temperature data from 50 normal breasts and the test set of 41 cancer-associated breasts. This master data file was then divided, with 70% (n = 64) of the vectors in the training set and 30% (n = 27) in the test set, the network being designated CH70. Each of the vectors selected for the training and test set files were chosen at random from the master file of the total 50 plus 41 = 91 exemplar vectors. In this way, no bias or priority

was given to specific vectors which could potentially cause exaggerated performance levels during testing. The network was trained over the input vectors until all of the exemplars were correctly classified. During the training session, the test set was periodically run to determine the effectiveness of the currently weighted interconnects. The maximum classification accuracy was recorded and the weight vectors were stored. At the end of training, the weight vectors that produced the maximum historical classification were then recalled and assigned to the network. The test set was run again over the network and the results were recorded. This set of weight matrices represented the best overall classification of the algorithm. A confusion matrix was generated showing the percentage of correctly and incorrectly classified vectors. From this confusion matrix, standard metrics such as sensitivity and specificity, were calculated. After training to define the boundaries of the solution space, the test set was then run through the network. The results were as follows:

Patterns correctly classified: 25/27

Classification accuracy of the best training set: 90.6%

Classification accuracy of the test set: 92.6%

Sensitivity: 90.9%

Specificity: 93.8%

The above figures compare favourably with a sensitivity of 73% and a specificity of 81% obtained from a linear discrimination of the same training and test sets.

DISCUSSION

The case has been made in the Introduction that premenopausal cancer occurs in the breast which is histopathologically highly abnormal and has a 6-fold risk of being the seat of a new primary. Surgeons sometimes object to this scenario on the basis that they do not often see second primaries after limited surgery; but the 6-fold risk would mean that half the survivors would develop a second primary in 16 years, and this frequency may well be masked by the high mortality from the primary disease.

If this scenario is accepted, the cancer-associated breast can be regarded as a "herald model" for breast cancer and, if so, its detection could be important as a new screening modality for precancer. From the evidence and data available, it is believed that the cancer-associated breast signal, as reported here, provides a basis for new precancer testing initiatives. Chemoprevention of breast cancer using an anti-oestrogen, such as tamoxifen, is currently the centre of considerable discussion, and, in certain countries, under investigation [14]. It is reasonable to suggest that the "chronobra test" provides an appropriate rational entry criterion for such studies. If an "adverse" result for the patient occurs, the test can always be repeated if considered necessary, since it is a non-invasive procedure.

1. Department of Health and Social Security. Breast cancer screening report to the Health Ministers of England, Wales and Northern Ireland, Chairman A.P.M. Forrest. HMSO, London, 1987.
2. Simpson HW, Griffiths K. The diagnosis of breast pre-cancer by the Chronobra. *Chronobiol Internat* 1989, **4**, 355–393.
3. Simpson HW, Mutch F, Halberg F, Griffiths K, Wilson D. Bimodal distribution of epitheliosis in cancer mastectomies. *Cancer* 1982, **50** (II), 2417–2422.
4. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. *N Engl J Med* 1985, **312**, 146–151.
5. Bostwick DG, Sole Balcells F, Cooner WH, *et al.* Benign prostatic hyperplasia and cancer of the prostate. In Cocket ATK, ASOV, Chatelain C, *et al.*, eds. *Proc Int Consultations on BPH*, Geneva Switzerland 1992, 139–159.
6. Jensen HM, Rice JR, Wellings SR. Preneoplastic lesions in the human breast. *Science* 1976, **191**, 295–297.
7. Chaudary MA, Millis RR, Hoskins EOL, *et al.* Bilateral primary breast cancer; a prospective study of disease incidence. *Br J Surg* 1984, 71, 711–714.
8. Eremin O. Second primary breast cancer. *Br Med J* 1988, **296**, 1755–1756.
9. Simpson HW, Griffiths K, McArdle C, Pauson AW, Hume P, Turkes A. The luteal heat cycle of the breast in health. *Breast Cancer Res Treat* 1993, **27**, 239–245.
10. Read GF, Bradley JA, Wilson DW, George WD, Griffiths K. Evaluation of luteal phase salivary progesterone levels in women with benign breast disease. *Eur J Cancer Clin Oncol* 1985, **21**, 9–17.
11. Halberg F, Johnson EA, Nelson W, Runge W, Sothern R. Autorhythmometry procedures for physiological measurements and their analysis. *The Physiology Teacher* 1972, **1**, 1–11. Published by The American Physiology Society.
12. Minitab software from 3081 Enterprise Drive, State College, PA 16801, U.S.A.
13. Lippmann RP. An introduction to computing with neural nets. IEEE ASSP Magazine, 1987, 4–27.
14. Powles TJ, Tillyer CR, Treleaven JG. Chemoprevention of breast cancer. *Breast Cancer Res Treat* 1989, **14**, 23–31.

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