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Original Paper

Is Steroid Receptor Profile in Contralateral Breast Cancer a Marker of Independence of the Corresponding Primary Tumour?

D. Coradini,[1] S. Oriana,[2] L. Mariani,[3] R. Miceli,[3] G. Bresciani,[4] E. Marubini[3,5] and G. Di Fronzo[1,6]

[1]Divisione di Oncologia Sperimentale C; [2]Divisione di Oncologia Chirurgica C; [3]Divisione di Statistica Medica e Biometria; [4]Divisione di Oncologia Ginecologica, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan; [5]Istituto di Statistica Medica e Biometria, Università degli Studi, Milan; and [6]Studio della Patologia Cellulare, CNR, Milan, Italy

We compared oestrogen receptor (ER) and progesterone receptor (PgR) profiles between primary and corresponding contralateral breast cancer (CBC) to investigate whether CBC should be considered relapse of a primary or as a feature of the multicentric origin of breast cancer. We adjusted for patient age, menopausal status, histology and adjuvant therapy. In spite of the general application of a cut-off value to dichotomise ER and PgR, we considered them as continuous variables. Moreover, we considered as synchronous cancers only simultaneously occurring lesions. For 399 patients, ER and PgR receptor levels in primary and CBC did not differ significantly, but were significantly correlated within the same patient. The correlation was higher for synchronous than for metachronous lesions when considering ER, but not PgR. The correlation between ER and PgR levels in the same tumour (primary or CBC) appeared stronger than the correlation of either receptor type (ER or PgR) between primary and CBC. Age, histology and adjuvant treatment affected ER concentration, whereas age, menopausal status and histology affected PgR concentration. The analysis indicated that primary and CBC tend to be characterised by a similar steroid receptor profile. The finding may support the hypothesis of CBC as a second primary arising in a common predisposing milieu, rather than a primary-dependent contralateral lesion. In this light, the clinical management of patients with a bilateral breast cancer should be similar to that of a unilateral breast cancer. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

IN SPITE of its relative high cumulative incidence, which varies from 7 to 20% according to the different patient selection and sampling criteria [1, 2], contralateral breast cancer (CBC) is not a sufficiently elucidated feature of the natural evolution of primary breast cancer. In fact, most of its biological and therapeutic aspects remain undefined, unclear or controversial, and there are discordant opinions about the impact of a second primary breast tumour on the overall prognosis. Some clinicians believe that bilateral invasive breast cancer implies a 2-fold risk of relapse for patients and that, regardless of the interval between the first and second cancer, overall survival after a second tumour (which is often small and without axillary node metastases) is worse than that expected for patients with unilateral tumours with similar characteristics [3, 4]. However, others believe that bilateral breast cancer does not imply a significantly worse prognosis than unilateral breast cancer, and many reports [5, 6] indicate that survival of patients with CBC is comparable to that of patients with a unilateral tumour.

Correspondence to D. Coradini.
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A relevant point of disagreement is related to the criteria used to distinguish synchronous (i.e. simultaneous) from metachronous (i.e. occurring at a different time) tumours, since the former include contralateral cancers that appear 1 [7, 8], 3 [9], 6 [5, 10], or 12 [11] months after diagnosis of the first lesion.

The lack of consensus on the management of bilateral breast cancer arises from this controversial approach to the disease and reflects, at least in part, the biological puzzle presented by bilateral cancers. In fact, in spite of increasing knowledge about factors, such as family history [12], which predispose to bilateral breast cancer, the biological nature of the relationship between the two lesions remains uncertain. In fact, it is not clear whether CBC should be considered a sequential event of a primary tumour or rather an independent second primary sharing with the primary localisation a biologically predisposing common substratum. Since the interactions among hormones, steroid receptors and growth factors are relevant for the development of a breast cancer, we compared the receptor profile of the two lesions in a substantial series of patients with synchronous or metachronous breast cancers.

PATIENTS AND METHODS

The study was performed on 399 patients with operable breast cancer who were surgically treated at the Istituto Nazionale Tumori of Milan during the period 1974–1994, who developed a CBC, for whom oestrogen receptor (ER) and progesterone receptor (PgR) concentrations had been determined on both lesions. In particular, 94 patients had simultaneous tumours and 305 developed a metachronous cancer. The series was consecutive with respect to steroid receptor determination on the primary tumour.

At the time of treatment of the primary breast cancer, patient age ranged from 26 to 85 years (median 51 years), 247 (62%) of the cases had pathological node-negative tumours, and 255 (64%) had a tumour diameter of ≤ 2 cm. Node-negative patients received surgery with or without radiotherapy. In addition to surgery, node-positive patients (38%) received adjuvant systemic treatment: tamoxifen (Tam) in approximately 12% of cases and chemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen) in approximately 80% of cases. Only a few patients received hormonal and chemical treatments. The interval between the primary and metachronous CBC ranged from 1 to 211 months (median 50 months).

Receptor determination

Tumour samples, obtained from primary and contralateral lesions, were immediately frozen and stored in liquid nitrogen. As described elsewhere [13], ER and PgR concentrations were assayed by the dextran-coated charcoal technique, quantified by Scatchard analysis, and expressed as fmol/mg of cytosolic protein. To avoid the loss of biological information for the use of a cut-off value to dichotomise ER and PgR [14], we considered ER and PgR concentrations as continuous variables.

Statistical methods

After a preliminary check for the existence of linear association patterns, the association between ER and PgR within the same lesion (primary or contralateral tumour) or between primary tumour and CBC was investigated by computing Pearson's correlation coefficients. ER and PgR levels in primary tumour and CBC were compared by applying multiple regression models for Gaussian correlated data. This type of analysis was adjusted for possible confounding factors such as age, menopausal status (pre- or postmenopause), histology (ductal, lobular, medullary cancers) and adjuvant systemic treatment after the primary tumour (Tam or CMF regimen). Further details concerning the modelling strategy are given in the Appendix. All the analyses relied on log-transformed ER and PgR measurements.

The degree of association between primary and CBC for histological characteristics, although not of primary interest, was estimated by Cramer's V. A zero value of the statistic indicates the total lack of association, whereas a value equal to 1 indicates a perfect association. The standard 5% significance level was adopted in all analyses.

RESULTS

Patient and tumour characteristics, relevant for the analysis, are reported in Table 1. A total of 160 patients were premenopausal at detection of the primary and the contralateral tumour, whereas 50 women changed their menopausal status during the interval between the two lesions (we considered patients postmenopausal if their last menses had occurred more than 2 years prior to lesion detection or if they had undergone hystero-adnexectomy). Tumour histology was similar in the primary and CBC. Moreover, Cramer's V was significantly greater than zero for synchronous ($V = 0.58$, $P < 0.0001$) and metachronous tumours ($V = 0.38$, $P < 0.0001$), supporting the presence of a moderate degree of association between the primary tumour and CBC for histology.

Table 2 shows that marginal distribution of ER and PgR concentrations were similar in primary and CBC. Correlation estimates between receptor measurements in primary and metachronous CBC showed no clear trend over the interval

Table 1. *Main patient and tumour characteristics*

	Number of patients (%)	
	Synchronous (94 cases)	Metachronous (305 cases)
Menopause		
Pre	30 (31.9)	130 (42.6)
Post	64 (68.1)	125 (41.0)
Pre→Post*		50 (16.4)
Histology of primary cancer		
Infiltrating ductal carcinoma	70 (74.5)	210 (68.9)
Infiltrating lobular carcinoma	17 (18.1)	54 (17.7)
Medullary	2 (2.1)	11 (3.6)
Other	5 (5.3)	30 (9.8)
Histology of contralateral breast cancer		
Infiltrating ductal carcinoma	69 (73.4)	210 (68.9)
Infiltrating lobular carcinoma	14 (14.9)	69 (22.6)
Medullary	1 (1.1)	4 (1.3)
Other	10 (10.6)	22 (7.2)
Adjuvant systemic treatment†		
None		189 (62.0)
Chemotherapy		95 (31.1)
Hormonal		17 (5.6)
Both		4 (1.3)

*Patients whose status changed between primary and contralateral breast cancer. †Only treatment preceding the occurrence of contralateral breast cancer are reported.

Table 2. Oestrogen (ER) and progesterone receptor (PgR) concentration distributions in primary and contralateral breast cancer (CBC)

	Primary	CBC
ER		
25th percentile	10	12
Median	38	49
75th percentile	123	121
PgR		
25th percentile	1	4
Median	22	39
75th percentile	163	167

Table 3. Unadjusted and adjusted correlation estimates

	Unadjusted	Adjusted
ER: primary tumour versus CBC		
Synchronous lesions	0.52	0.46
Metachronous lesions	0.31	0.19
PgR: primary tumour versus CBC		
Synchronous lesions	0.34	0.29
Metachronous lesions	0.27	0.24
ER versus PgR: synchronous lesions		
Primary tumour	0.43	0.42
CBC	0.40	0.38
ER versus PgR: metachronous lesions		
Primary tumour	0.53	0.55
CBC	0.62	0.64

ER, oestrogen receptor; PgR, progesterone receptor; CBC, contralateral breast cancer.

between the two lesions. Accordingly, we subsequently only distinguished between synchronous and metachronous lesions. Corresponding correlation estimates unadjusted and adjusted for covariates are reported in Table 3. All estimates were significantly greater than zero. In particular, considering ER content, both unadjusted and adjusted correlation estimates were higher for synchronous than for metachronous lesions, and the difference between the two adjusted estimates was statistically significant (0.46 versus 0.19, $P = 0.0124$).

Similar trends were observed for PgR content, although the difference between the adjusted estimates obtained for synchronous (0.29) and metachronous lesions (0.24) was not statistically significant. Considering the correlation between ER and PgR concentration, adjusted estimates substantially overlapped unadjusted estimates. When a single correlation coefficient between ER and PgR levels was estimated for all lesion types (synchronous or metachronous; primary tumour or CBC), the figure obtained was 0.55.

Table 4 shows the results obtained with the multiple regression models including the factors significantly affecting ER or PgR levels. As further explained in the Appendix, ER and PgR mean levels, as well as the effect of the investigated covariates on receptor levels, did not significantly differ for primary and contralateral synchronous or metachronous tumours. Consequently, in the final models, one intercept term and one coefficient for each variable category was sufficient to describe the covariate effects. The factors significantly affecting ER concentration were age, histology and adjuvant treatment. ER levels increased with age in a quadratic manner, showing that the older the patient, the higher the receptor levels. Owing to the curve of the relationship, the same increment in age implied different effects on ER level, depending on the age at diagnosis. For instance, the estimated increase for ER level was 2.4, 1.7 and 1.3 times for a 10-year increment in women aged 30, 50 or 70 years, respectively.

As regards histology, ER levels were significantly reduced in medullary tumours. Single measurements varied from 1 to 17 (median 3.5) fmol/mg of cytosolic protein, compared with a range of 1–1490 (median 46) fmol/mg of cytosolic protein for all other histotypes. A 4.3-fold average reduction was estimated by the regression model.

Table 4. Results obtained with the multiple regression models for oestrogen receptor (ER) and progesterone receptor (PgR)

	Parameter estimate	Standard error	Likelihood ratio test χ^2	Degrees of freedom	*P*
ER model					
Intercept	−1.8368	1.1197			
Age					
Linear effect	0.1420	0.0413	11.80	1	0.0007
Quadratic effect	−0.0008	0.0004	4.51	1	0.0344
Histology			13.66	1	0.0002
Medullary versus others	−1.4673	0.3969			
Adjuvant treatment			2.65	3	0.0483
Chemotherapy versus none	0.2163	0.1758			
Hormone therapy versus none	−0.7585	0.3974			
Chemo-/hormone therapy versus none	−1.3560	0.8247			
PgR model					
Intercept	1.5707	0.7996			
Age					
Linear effect	0.0302	0.0126	5.74	1	0.0172
Menopause			7.73	1	0.0058
Pre versus post	0.8124	0.2923			
Histology			10.54	1	0.0013
Medullary versus others	−2.1286	0.6557			

Finally, a significant effect was observed for adjuvant treatment. In particular, after chemotherapy, ER levels showed a slight and non-significant increase. Conversely, ER concentration was reduced by a factor of 2.1 in the group of patients treated with hormone therapy. The reduction was also greater after combined chemo- and hormone therapy (3.9 times), but not statistically significant, due to the small number of women who received such treatment. We also investigated whether the effect of hormone therapy varied according to the time elapsing between the primary and contralateral tumour. We found that the earlier the occurrence of CBC, the greater the degree of reduction in ER levels, and the reduction tended to disappear with time.

As regards PgR concentration, the factors that significantly affected the variable were age, menopause and histology. In particular, PgR levels increased with age, whereas a 2.3-fold reduction in PgR content was estimated for postmenopausal women. Since there was no significant interaction between age and menopause, the aforementioned findings seem contradictory at first sight. To understand this point better, we plotted smoothed PgR residuals (from a regression model not including age and menopause) versus age, as shown in Figure 1. The plot showed a complex association pattern between age and PgR concentration. In fact, the relationship was characterised by a roughly linear increase in PgR levels up to 45 years, followed by a sharp reduction in the subsequent 5–6 years and again an increase starting from 51 years, which is the age when most women enter menopause. Therefore, the significance of age and menopause could be explained by considering that 'age' was necessary to describe the pattern of variation in PgR levels observed for young or elderly women, whereas the fall in PgR concentration observed in middle-aged patients could be explained by the inclusion of menopause status.

Finally, as observed for ER, PgR levels were also lower (about 8.4 times) in medullary carcinomas than in other histotypes. Single measurements varied from 1 to 29 (median 1) fmol/mg, compared with 1–4425 (median 55) fmol/mg for all other histotypes. No effect on PgR content was observed for adjuvant treatment.

DISCUSSION

One of the main open questions about bilateral cancer is whether CBC should be considered as a contralateral relapse of a primary tumour or as an independent second primary, feature of the multicentric origin of breast cancer. The question is not trivial because, according to the interpretation, the clinical viewpoint and the management of the patient might change [3, 7].

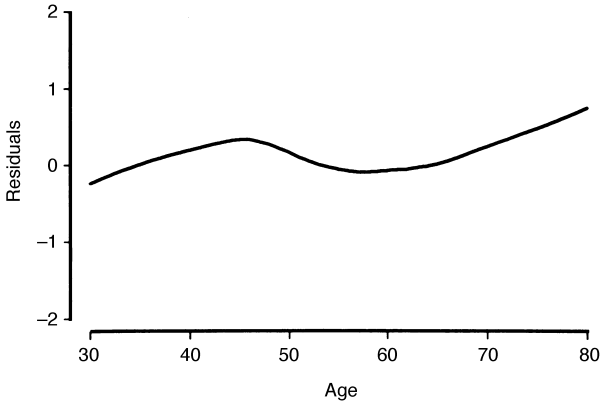

Figure 1. Smoothed progesterone receptor (PgR) residuals (obtained from a regression model not including age and menopause) versus age.

The hypothesis about the multicentric origin of breast cancer was formulated by Foote and Stewart [15]. Afterwards, many other clinicians faced the issue, and in particular Gallager and Martin [16] provided histopathological evidence of the multicentric origin of breast cancer.

To shed further light on the matter, in the present study we compared the hormone steroid receptor profile, of prognostic importance in breast cancer, of a primary and a corresponding CBC. To avoid spurious results due to the effect of those factors (such as age, menopausal status, histology and adjuvant therapy) likely to influence steroid receptor levels [17], the analysis took them into account. Moreover, to avoid the loss of useful information due to the choice of an arbitrary cut-off value, we considered ER and PgR concentration as continuous variables.

The analysis showed that mean receptor levels did not differ markedly between the primary and CBC. Furthermore, it indicated the existence of an overall correlation between steroid receptor concentration in the primary tumour and CBC. In particular, the correlation between ER concentration in the primary and CBC was higher for synchronous than for metachronous lesions. For synchronous tumours, our estimates overlapped with those reported by Kiang and colleagues [18]. In contrast, the correlation between PgR content in the primary and CBC was not statistically influenced by the interval between diagnosis of the two lesions. Finally, the correlation between ER and PgR levels in the same tumour (primary or CBC) appeared stronger than the correlation of either receptor type (ER or PgR) between the primary and the contralateral lesion. The latter finding was probably related to the mechanism of action of oestrogens: by binding to its receptor, oestradiol induces PgR synthesis. Therefore, the production of PgR is functionally related to the presence of ER, whereas no functional relationship exists between a primary and the corresponding CBC, thereby justifying the weak correlation observed.

The above findings differ from the results of a previous study [19] which showed that, regardless of adjuvant treatment, receptor positivity was less frequent in distant metastasis (59% for ER and 39% for PgR) than in the corresponding primary (75% for ER and 62% for PgR). The overlap in hormone receptor profiles between primary and CBC, together with the different findings previously mentioned for distant metastases and CBC, seem to rule out the hypothesis of CBC as a primary dependent lesion, and rather support the concept of a multicentric origin of bilateral cancer.

As a side issue, the study supplied additional information concerning the influence of age, menopausal status, histology and adjuvant therapy on steroid receptor profile. The analysis indicated that age, histology and adjuvant treatment significantly affected ER concentration, whereas age, menopausal status and histology significantly affected PgR concentration. The findings concerning the effect of age on ER level can be explained by a partial or total saturation of free ER due to the presence of endogenous or oestradiol in young women and are in agreement with our previous report [20]. As regards PgR, we observed a progressive increase in PgR concentration up to 45 years of age (related to the

synthesis induced by oestradiol after binding to ER), followed by a sharp reduction in the interval between 45 and 51 years of age (probably related to the progressive disappearance of the endogenous oestrogenic stimulus), and a further increase in PgR concentration in elderly women (probably due to an alternative production of oestrogens in adipose tissue by aromatase activity).

In agreement with previous reports [21, 22], the present study showed an association between steroid receptor concentration and histotype. Notably, in medullary carcinoma we observed very low ER and PgR levels, indicating medullary carcinoma as a typical hormone-independent tumour, despite its well-known low metastatic potential.

Noteworthy is the observation that adjuvant hormone therapy, but not chemotherapy, affected ER levels. In fact, CMF acts on all proliferating cells, regardless of their steroid receptor profile, according to its non-specific mechanism of action. Conversely, in the Tam-treated group, we observed that the earlier a CBC arises, i.e. the shorter the interval between the two lesions, the lower the ER level. This finding suggests that Tam therapy selectively inhibits the growth of tumour subclones characterised by a high ER concentration and that such a selection decreases with time. However, the lack of individual information on treatment duration does not enable us to assess whether the time-varying effect of Tam depends on the variable treatment length in different patients or on the appearance of resistant subclones.

In conclusion, overall the results of the study seem to support the hypothesis that CBC is an independent second primary that shares a biologically predisposing common substratum with the primary lesion, rather than being a sequential event of a primary tumour. In this light, i.e. considering CBC as an independent second primary, clinical management of patients with a bilateral breast cancer should be similar to that of a unilateral breast cancer.

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APPENDIX

Our analysis relied on a mixed linear model [23] approach, a generalisation of the standard linear model. The generalisation was that the response data are allowed to exhibit correlations and non-constant variability.

Log-transformed ER and PgR measurements were regarded as the response variable. Distinct hormone receptor measurements within the same patient (receptor profile) were regarded as possibly correlated data. Patient age and menopausal status at the time of lesion detection (either primary tumour or CBC), lesion histology and adjuvant systemic treatment after the primary tumour were handled as explanatory variables (covariates). In particular, menopause, histology and systemic therapy were categorised as described in Patients and Methods and entered into the model by means of (0–1) indicator variables. Two kinds of parameters were therefore estimated: covariance parameters, regarding the variance–covariance structure of hormone receptor data, and fixed-effects parameters, describing covariate effects on mean receptor levels. The models were fitted by using the SAS® MIXED Procedure [24]. Parameter estimates were obtained with the restricted maximum likelihood (REML) method, as usually recommended in mixed linear models. Model assumptions were verified by examining model residuals.

The covariance structure of the data was studied first. ER and PgR measurements were analysed separately when assessing the correlation between measurements in primary and contralateral tumours and jointly when estimating the correlation between ER and PgR. As a first step, a completely general (unstructured) covariance matrix parameterised in terms of variances and correlations was adopted for the profile of individual measurements. Furthermore, covariance parameter estimates were allowed to differ in synchronous and metachronous lesions, in the latter case according to the time (in years) elapsing between the occurrence of the primary tumour and

the contralateral tumour. The intercept term and regression coefficients were allowed to vary for primary and contralateral lesions, in such a way as to account for a possible variation in mean receptor levels or differential covariate effects in the two types of lesions. Statistical testing (based on likelihood ratio tests), as well as inspection of the estimates obtained as formerly specified, led us to simplify the above covariance structure. Notably, no clear trend in correlation estimates over time was evident for ER or PgR. Consequently, as shown in Table 3, we only distinguished between synchronous and metachronous lesions. Furthermore, for ER or PgR measurements, variance terms were found to be homogeneous for primary and contralateral lesions, either synchronous or metachronous.

Having thus defined the covariance structure of the data, covariate effects on mean receptor levels were investigated. For this purpose, ER and PgR measurements were analysed separately. As a first step, homogeneity of covariance effects and of mean (adjusted) receptor levels, respectively, in primary and contralateral lesions, was tested. A backward selection procedure was then adopted to identify covariates significantly affecting receptor levels. Finally, after examination of regression coefficients, categories for the variable 'histology' were collapsed into 'lobular' and 'other' histotypes. Fixed-effect parameters estimated by the models thus obtained are reported in Table 4. Covariance parameter estimates were substantially stable in the different models.