Eur J Cancer, Vol. 29A, No. 1, pp. 76–81, 1993.
Printed in Great Britain

0964–1947/93 $5.00 + 0.00

Relations between Epidermal Growth Factor Receptor and Oestrogen and Progesterone Receptors in Breast Cancers of Premenopausal and Postmenopausal Patients in Kuwait

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The levels of cell membrane epidermal growth factor receptor EGFR and cytosol (c) and nuclear (n), oestrogen (E) and progesterone (P) receptors (R) were determined in 132 specimens of primary breast cancers. In the tumours of postmenopausal women an inverse significant correlation was demonstrated between the concentrations of EGFR vs. ERc, ERn, and PRc while no such correlation was noted in the tumours of premenopausal women. Premenopausal and postmenopausal EGFR positive tumours (≥ 10 fmol/mg membrane protein) could be regarded as homogenous with respect to the concentration of ER and PR whose mean values were low and without being significantly different. EGFR negative tumours were heterogeneous with respect to the ER and PR concentrations. Postmenopausal EGFR negative (< 10 fmol/mg membrane protein) tumours had evidently higher mean values of ER and PR than premenopausal EGFR negative tumours, but these differences were statistically significant for oestrogen receptors only. The levels of ER and PR of premenopausal EGFR negative tumours were approximated to the corresponding levels of EGFR positive tumours.
Eur J Cancer, Vol. 29A, No. 1, pp. 76–81, 1993.

INTRODUCTION

THE PRESENCE of oestrogen and progesterone receptors in breast cancer is an important but not fully reliable predictor of its possible response to hormonal treatment, since only 53% of oestrogen receptor (ER)-positive cancers respond with regression to endocrine therapy [1]. Therefore, it is necessary to search for other indices which could improve the discrimination between hormone-responsive and hormone-unresponsive cancers.

The studies on the mechanisms of cellular proliferation have demonstrated that epidermal growth factor receptor (EGFR), which is also present in breast cancer tissue, plays an important role in the recognition of the mitogenic stimulus by a cell [2]. The binding of the mitogenic factor to the extracellular part of the receptor leads to autophosphorylation of its intracellular part, which then becomes an active tyrosine kinase for other proteins which are important in the mitogenic response of the cell [2–4].

The intensity of proliferation processes in breast cancer seems to be the result of a possible action of mitogenic hormones (growth factors) and differentiating hormones (oestrogen and progesterone). In recent reports an inverse relationship between the concentration of EGFR and that of ER and progesterone receptor (PR) in breast cancer tissue has been suggested [5–11], while earlier Fitzpatrick *et al.* [12] failed to observe this correlation. Moreover, Sainsbury *et al.* [13] found a significant correlation between the presence of EGFR and poor prognosis as assessed by Bloom and Richardson score, and with lower patient survival (Sainsbury *et al.* [14]). Determination of receptor availability for the two types of hormones as well as the knowledge of the relationship between their receptors in breast cancer may extend the prognostic possibilities and provide new information for the management of patients with breast cancer.

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Revised 26 Mar. 1992; accepted 28 Apr. 1992.

MATERIALS AND METHODS

The levels of the receptors for epidermal growth factor, oestrogen and progesterone were determined in 132 primary breast cancers of female Arab patients aged 23–80 years. Most patients (62%) were premenopausal and 38% were postmenopausal.

Reagents

Radioactive 2,4,6,7,16,17-^{3}H-oestradiol (^{3}H-E_2) 5.48–5.78 TBq/mmol and mouse ^{125}I-EGF (22.2 TBq/mmol) were from Amersham, and promegestone-17alpha-methyl-^{3}H (^{3}H-R5020) 3.03 TBq/mmol from New England Nuclear. Cold competitors used were h-EGF from Amersham, diethylstilbestrol (DES) from Aldrich and cold R5020 from New England Nuclear. Other chemicals were of analytical grade.

Methods

Tumour specimens were frozen in dry ice shortly after surgical removal, and were stored for 1 week in dry ice until processed. The specimens were powdered in liquid nitrogen, dispersed in 5 vol. of TEDG buffer (10 nmol/l Tris, 1.5 nmol/l EDTA, 0.5 nmol/l dithiothreitol, 10% glycerol, pH 7.4) in ultraturrax homogeniser (Janke and Kunkel) and centrifuged at 800 *g*. The pellet fraction was saved for nuclear oestrogen (ERn) and

progesterone (PRn) receptor determination, the supernatant was centrifuged at 105 000 *g* for 1 h, and the pellet was used as crude membrane fraction for EGFR determination. The remaining high-speed supernatants (cytosols) were stored at −70°C up to 1 week for oestrogen and progesterone receptor assays. Cytosolic oestrogen (ERc) and progesterone (PRc) receptors were assayed using the dextran-coated charcoal procedure in triplicate sample binding assay at a single saturating concentration of ^{3}H-E_2 (7.5 nmol/l) or ^{3}H-R5020 (10 nmol/l) in the presence or absence of a 100-fold molar excess of nonlabelled competitor DES or R5020. The nuclear fraction was washed three times with 5 vol. of TEDG buffer to remove cytosol protein, and was then extracted with 3 vol. of 0.6 molar KCl in TEDG buffer for 1 h, the suspension was centrifuged at 105 000 *g* for 30 min to obtain a soluble nuclear extract. ERn and PRn were determined by exchange dextran-coated charcoal methods. The temperature and times used for exchange of ERn and PRn were 30°C (1 h) and 4°C (24 h), respectively. Details of ERn, and PRn determinations were described elsewhere [15].

The crude membrane fraction was washed with TEDG buffer from the remaining cytosol protein and resuspended in 0.025 mol/l phosphate buffer (pH 7.0 with addition 0.002 mol/l $MgCl_2$ and 0.15 mol/l NaCl); 100 µl of the suspension containing an equivalent of 100 µg of membrane protein were incubated with ^{125}I-EGF (2 nmol/l) in the presence or absence of 200 nmol/l EGF (final volume 120 µl) for 1 h at room temperature. The reaction was stopped by the addition of 2 ml of ice-cold phosphate buffer containing bovine serum albumin (5 mg/ml) and centrifuged at 15 000 *g* for 15 min. The supernatant was discarded and the amount of ^{125}I-EGF bound to pellets was counted in a Beckman gamma counter. Specific binding of ^{125}I-EGF was calculated by subtraction of nonspecific binding from the total binding, and the results were expressed as fmol/mg of membrane protein. In several tumours the EGFR, were determined by saturation analyses using 0.2–18 nmol/l concentrations of ^{125}I-EGF. The data were subjected to Scatchard analysis to estimate the affinity, the linearity of binding and the number of binding sites.

Tumour tissues were assumed to be positive for EGF-R, ERc, ERn, PRc and PRn when the concentrations of the respective receptors were equal to or greater than 10 fmol/mg of tumour cytosol or cell membrane protein. Protein content was assayed by Lowry *et al.* [16] method using bovine albumin as a standard. The details of ERc and PRc determination were described before (Paszko *et al.* [15]).

RESULTS

The analysis of specific binding of increasing concentrations of ^{125}I-EGF to crude cell membranes of breast cancer (100 µg of membrane protein) showed clearly a two-stage type of this process. In general, at the 2–4 nmol/l concentrations of ^{125}I-EGF the first stage of membrane saturation with the ligand occurred, then the amount of bound ^{125}I-EGF rose steeply reaching again a plateau at the concentrations above 8 nmol/l (Fig. 1). Scatchard's curve of ^{125}I-EGF specifically bound to the membranes calculated from the saturation curve in the range 0.2–4 nmol/l was a straight line which suggested a homogenous character of the binding sites (mean linearity correlation coefficient - r = 0.923 (0.02 S.E.) range 0.71–0.996; n = 15). On the other hand, Scatchard's curve plotted on the basis of the saturation analysis of concentrations from 0.2 to 18 nmol/l had an evident two-component character. The dissociation constant (K_d) for the first type of binding sites was 2.7 (0.25 S.E.) nmol/l on average (15 cases) and that for the second type was K_d > 10 nmol/l. The studies presented below of the content of EGF receptor concerned bonds of high affinity in which the dissociation constant was in the range from 1.1 to 4.4 nmol/l.

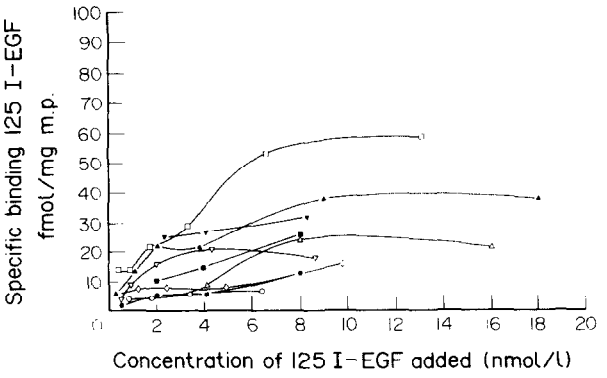

Fig. 1. Specific binding of ^{125}I-EGF to the cell membranes of breast cancers. At the concentrations of 0.2–4.0 nmol/l of ^{125}I-EGF the bindings exhibited one class of binding sites with K_d = 2.62 (0.25) nmol/l (range 1.1–4.4). At the wider range of concentrations 0.2–18.0 nmol/l several tumours exhibited two classes of binding sites with K_d ranged between 2–4 and 10–160 nmol/l, respectively.

The EGFR concentrations in crude cell membranes of breast cancer were in the range from 0 to 415 fmol/mg of membrane protein (mg membrane protein). The distribution of EGFR values in the cell membranes of the studied breast cancers was as follows: The greatest groups of tumours were those ranging between 0 and 4 fmol/mg membrane protein (36.5%), then those ranging from 5 to 9 fmol/mg membrane protein (26.8%), and from 10 to 20 fmol/mg membrane protein (24.7%). The values between 21 and 100 fmol/mg membrane protein and over 100 fmol/mg membrane protein accounted only for 8 and 4%, respectively. The arithmetical mean of EGFR (S.E.) calculated for all tumour values was 15.5 (3.7) fmol/mg membrane protein (132 cases), and 22.3 (5.4) fmol/mg membrane protein when zero values were excluded (88 cases).

The mean concentration of EGFR was in tumours of postmenopausal women (Table 1) about 2-fold as high as in those of premenopausal women (21.6 and 11.8, respectively), but this difference was statistically non-significant. The mean concentrations of ERc and ERn in tumours in postmenopausal women (73.5 and 67.2 fmol/mg protein, respectively) were about threefold as high as in the premenopausal women (21.5 and 22.8 fmol/mg protein), and these differences were statistically significant. On the other hand, the mean PRc and PRn concentrations were not significantly different (45.5 vs. 33.4 fmol/mg cytosol protein for PRc and 13.4 vs. 7.3 fmol/mg nuclear protein for PRn).

The EGFR concentrations in the tumours, and the corresponding concentrations of the receptors of steroid hormones (ERc, ERn, PRc and PRn) were subjected to Spearman's rank correlation analysis. In the tumours in postmenopausal women and in all cases a statistically significant, inverse correlation was found between the concentrations of EGFR and the receptors of oestrogens (ERc and ERn), while in the tumours of premenopausal women no such correlation was revealed (Table 2). Inverse correlation between EGFR and PRc was significant in the tumours in women after menopause, but not significant in the tumours in premenopausal women. In the tumours of all women this correlation was not significant. The correlation

Table 1. The values of EGFR, ER and PR in breast cancers of premenopausal and postmenopausal women

Group of patients	Type of receptor (fmol/mg protein)				
	EGFR	ERc	ERn	PRc	PRn
Premenopausal					
Mean (S.E.)	11.8 (2.6)	21.5 (3.2)	22.8 (3.6)	33.4 (7.3)	7.3 (1.5)
Median	6.5	18	11	5	0
n	82	82	81	82	72
Postmenopausal					
Mean (S.E.)	21.6 (8.9)	73.5 (19.2)	67.2 (17.7)	45.5 (12.7)	13.4 (4.3)
Median	6	28.5	27	7.5	0
n	50	50	47	50	45
Wilcoxon test (*P*)	0.98	0.004	0.04	0.38	0.77

Table 2. Correlation between concentrations of EGFR and ERc, ERn, PRc, PRn in breast cancers in women assessed by Spearman rank test

	Premenopausal			Postmenopausal			All cases		
Receptors	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
EGFR vs. ERc	−0.142	0.199	82	−0.39	0.005	50	−0.26	0.003	132
EGFR vs. ERn	−0.040	0.71	81	−0.32	0.029	47	−0.17	0.05	128
EGFR vs. PRc	−0.031	0.77	82	−0.31	0.029	50	−0.143	0.100	132
EGFR vs. PRn	−0.137	0.24	72	−0.23	0.121	45	−0.16	0.07	117

r: correlation coefficient; *P*: probability; *n*: number of cases.

between the concentrations of EGFR and PRn in all studied groups of tumours was not significant.

Accepting 10 fmol/mg membrane protein as the range of EGFR detectability the breast cancers were divided arbitrarily into the so-called EGFR low or negative (below 10 fmol/mg membrane protein) and EGFR high or positive (equal to or over 10 fmol/mg membrane protein). In all breast cancers the mean concentration of EGFR positive values was 37.3 (9.8) fmol/mg membrane protein. In EGFR positive cancers in postmenopausal patients the mean concentrations of EGFR was over 2-fold as high as that in EGFR positive cancers in premenopausal women (60.1 (25.8) and 25.5 (6.0) fmol/mg membrane protein; Table 3) and this difference was significant in Wilcoxon test ($P = 0.047$).

In EGFR negative breast cancers of all patients (Table 3; group F) the concentrations of receptors of steroid hormones (ERc, ERn, PRc and PRn) were 2.6–4.4 times higher than in EGFR positive cancers (E), and these differences were statistically significant with exception of PRn values (Table 3; $P = 0.075$). In the breast cancers of postmenopausal women analogous differences between EGFR negative and EGFR positive groups (D, C) were 3–14.7 times greater, and were statistically significant, with the exception of PRn. In the breast cancers of premenopausal women these differences in the concentrations of steroid hormone receptors were less evident (only 1.4–2.4 times higher) and statistically not significant (A, B).

In the EGFR positive breast cancers in premenopausal (A), postmenopausal (C), and all patients (E) the mean concentrations of ERc, ERn, PRc and PRn not differed significantly, respectively although the mean PRc concentration in postmenopausal EGFR positive cancers (C) was lower (3.5–4.8 times) than the mean PRc value in the remaining groups (A, E). In EGFR negative breast cancers of postmenopausal women (D) the concentrations of ERc, ERn, PRc and PRn were higher (1.6–4.0 times), respectively, than in EGFR negative cancers of premenopausal women (B), but these differences were statistically significant for oestrogen receptors only (Table 3; B, D).

Of interest is the observation that the mean concentrations of the investigated steroid hormone receptors in premenopausal and postmenopausal EGFR positive and premenopausal EGFR negative tumours were not significantly different (Table 3; groups A, B; A, C; B, C). On the other hand the mean concentrations of oestrogen receptors in postmenopausal EGFR negative tumours were evidently significantly higher than in postmenopausal and premenopausal EGFR positive tumours and premenopausal EGFR negative tumours (Table 3; groups D, C; D, A; D, B). In respect to the progesterone receptors the above-mentioned relationships were not so unequivocal as compared to the oestrogen receptors. Only the mean concentrations of PRc in postmenopausal EGFR negative tumours were significantly higher as compared to the premenopausal and postmenopausal EGFR positive tumours (Table 3; A, D; C, D).

The evaluation of the receptor status of the cancer depending on the location of the receptors in the cytosol (ERc) or in the nuclear fraction (ERn) does not always reflect the actual stage, e.g. a part of the tumours negative for ERc PRc may be ERn PRn positive, and conversely. In view of this, it would be more correct to express the receptor status (known as cell receptor, cl) of a tumour on the basis of receptor presence ($\geq$ 10 fmol/mg protein) in the cytosol or in the nuclear fraction or in both these fractions.

Table 3. The values of oestrogen and progesterone receptors in breast cancers with low or high concentrations of EGFR

Kind of cases / EGFR levels		Steroid receptors fmol/mg p. ERc		ERn		PRc		PRn	
Premenopausal									
(A) High EGFR** 25.5 (6.0) fmol/mg protein	Mean*	14.5	(2.3)	18.4	(3.9)	21.0	(9.2)	4.0	(1.6)
	Median	13		9.3		0		0	
	n	31		30		31		28	
(B) Low EGFR** < 10 fmol/mg protein	Mean*	25.8	(4.9)	25.5	(5.3)	41.1	(10.2)	9.4	(2.2)
	Median	19		11		6		0	
	n	51		51		51		44	
Postmenopausal									
(C) High EGFR** 60.1 (25.8) fmol/mg protein	Mean*	10.2	(2.7)	20.5	(11.0)	4.4	(1.6)	5.7	(2.6)
	Median	7.5		0		0		0	
	n	16		16		16		15	
(D) Low EGFR** < 10 fmol/mg protein	Mean*	103.2	(26.8)	91.3	(25.2)	64.8	(17.9)	17.2	(6.2)
	Median	44		41		17		0	
	n	34		31		34		30	
All cases									
(E) High EGFR** 37.3 (9.8) fmol/mg protein	Mean*	13.0	(1.8)	19.1	(4.6)	15.4	(6.2)	4.6	(1.4)
	Median	9.0		6		0		0	
	n	47		46		47		43	
(F) Low EGFR** < 10 fmol/mg protein	Mean*	56.7	(11.8)	50.4	(10.6)	50.5	(9.4)	12.6	(2.9)
	Median	25		22		9.0		0	
	n	85		82		85		74	
Statistics between groups***, *P*									
	A, B	0.128		0.996		0.232		0.125	
	C, D	0.00003		0.0028		0.003		0.33	
	E, F	0.00007		0.033		0.0065		0.075	
	A, C	0.309		0.179		0.367		0.834	
	A, D	0.00001		0.0022		0.016		0.178	
	B, C	0.061		0.23		0.071		0.347	
	B, D	0.00009		0.001		0.159		0.900	
	A, E					Statistically	n.s.	>	0.05
	C, E								

*Mean (S.E.); **High ≥ 10 fmol/mg membrane protein, Low < 10 fmol/mg membrane protein. ***Wilcoxon test.

In view of the presence or absence of oestrogen and progesterone receptors the studied 130 cases of breast cancer could be divided into four groups (Fig. 2). The greatest group (49.3%) had tumours cl-ER positive and cl-PR positive, the mean EGFR concentration was 5.7 (0.6 S.E.) fmol/mg membrane proteins. The second largest group (31.5%) had tumours containing only cl-ER but no cl-PR. The mean EFGR concentration was 7.9 (1.5 S.E.) fmol/mg membrane protein, and these mean values were not significantly different ($P = 0.133$, Student's t-test) from the mean EGFR values in the first group. In the group containing only cl-PR (6.1%) but no cl-ER the mean EGFR concentration was 16.6 (5.2 S.E.) fmol/mg membrane protein. The greatest mean concentration of EGFR [70.9 (25.2 S.E.) fmol/mg membrane protein] was present in the group of tumours (13.1%) without cl-ER and cl-PR. The mean values of EGFR in both of the last groups were not significantly different ($P = 0.15$), but they differed from the mean EGFR values in the two first groups, and the differences were statistically significant ($P < 0.0002$).

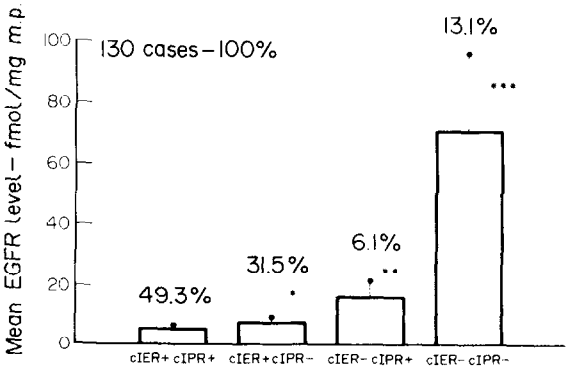

Fig. 2. EGFR level and simultaneous occurrence of cl-ER and cl-PR in breast cancers. Cellular (cl) receptors were assessed on the bases of their presence (+) or absence (−) in cytosol, nuclear or in both fractions; (+) − ≥ 10 and (−) − < 10 fmol/mg protein, probabilities as compared to the cl-ER+ cl-PR+ group; * $P = 0.045$, ** $P = 0.0001$, *** $P < 0.0001$.

DISCUSSION

The studies on binding of ^{125}EGF to cell membranes of breast cancer have shown that they contained two types of sites specifically binding EGF. The first type is a high affinity site (K_d = 2.7 (0.25) nmol/l) which can be saturated with a 2–4 nmol/l concentration of ^{125}I-EGF in membranes containing 100 mg of protein. The sites with lower affinity (K_d = > 10 nmol/l) were saturated in the same amount of membranes with ^{125}I-EGF concentration over 15 nmol/l. Two similar types of specific EGF binding to cell membranes of breast cancers were described by Nicholson *et al.* [9]. However, Fitzpatrick *et al.* [12, 17], Pekonen *et al.* [10], Cappelletti *et al.* [7] and Battaglia *et al.* [5, 6] mentioned only one type of binding site, but they used, a more narrow range of ^{125}I-EGF concentrations for saturation of binding sites (usually up to 5 nmol/l) in their experiments. The dissociation constant of the EGFR complex with growth factor in the cell membranes of breast cancers are regarded as ranging from 0.2 to 3 nmol/l. Our data also fall within this range.

The specific EGF binding sites of high affinity are usually called receptors (EGFR). The mean EGFR concentration in cell membranes of all breast cancers was in our material 15.5 (3.7 S.E.) fmol/mg of protein, median 6 fmol/mg membrane protein and the range of concentrations was, as a rule, from 0 to 135, with the exception of one case in which it was 415 fmol/mg membrane protein.

The frequency and distribution of EGFR values in cell membranes of breast cancers in our material, similar to that of other authors, had a unimodal character with a progressive fall of the proportion of tumours with high values of these parameters.

The ranges of EGFR concentrations in our material were similar to those reported by other authors Cappelletti *et al.* [7], Nicholson *et al.* [9], and Fitzpatrick *et al.* [12], but the mean concentrations were different. In the material reported by Nicholson *et al.* [8] the mean EGFR value was twice as high as in our study, and in the material of Fitzpatrick *et al.* [12, 17] and Battaglia *et al.* [5, 6] it was by one order of values lower.

Comparing the values of EGFR concentrations in breast cancers with the ERc and PRc values several authors found an inverse correlation between them, but Fitzpatrick *et al.* [12] failed to observe this correlation. Our study estimated by Spearman's test showed the presence of a statistically significant inverse correlation between EGFR and ERc, ERn or PRc in the tumours of postmenopausal women, while in the tumours of premenopausal women these correlations were not significant. In the tumours of premenopausal and postmenopausal patients there was no significant correlation between EGFR and PRn values.

The statistical estimation of the relationship between EGFR and ER and PR in all tumours jointly showed a significant correlation between EGFR and ER values only, while this correlation was not found for EGFR and PR. This is understandable in the light of the difference found between the groups of premenopausal and postmenopausal women.

A comparison of EGFR concentrations in the tumours with concomitant presence of oestrogen and progesterone receptors or with concomitant absence of both these receptors confirmed the negative correlation between EGFR and ER but not fully confirmed the analogous correlation with PR (Fig. 2). The absence of progesterone receptor in the group of cl-ER positive cl-PR negative tumours was not associated with significantly increased EGFR content in comparison to tumours cl-ER positive cl-PR positive (5.7 fmol/mg membrane protein and 7.9 fmol/mg membrane protein). On the other hand, the absence of oestrogen receptor in tumours was associated with a statistically significant rise of EGFR concentration (16.6 fmol/mg membrane protein). The absence of both receptors in the tumours (group cl-ER negative cl-PR negative) was associated with a striking but not significant increase in EGFR concentration (70.9 fmol/mg membrane protein).

The classification of breast cancers into EGFR positive and EGFR negative is usually arbitrary, and thus considerable differences appear in the definition of the borderline between these groups. Fitzpatrick *et al.* [12] regarded as EGFR positive such breast cancers which contained at least 1 fmol/mg membrane protein, and in which specific binding accounted for 15% or more of total EGF binding. Similarly, Battaglia *et al.* [5, 6] estimated the borderline values of EGFR positive at 1.5 fmol/mg membrane protein while Cappelletti *et al.* [7] accepted as EGFR positive cases with concentrations of 30–45 fmol/mg membrane protein.

The borderline value of EGFR accepted by us (10 fmol/mg membrane protein) divided all tumours into EGFR positive and EGFR negative groups. The first group was characterised by low and the second by high concentrations of steroid hormone receptors. EGFR positive group could be regarded as homogenous with respect to the concentrations of steroid hormone receptors. The mean concentrations of these receptors in premenopausal and postmenopausal EGFR positive tumours were very similar and did not differ significantly, though the mean EGFR value of postmenopausal EGFR positive tumours was evidently higher than in premenopausal ones (Table 3; groups A, C).

The EGFR negative group of breast cancers is not homogenous with regard to the content of steroid hormone receptors. In postmenopausal EGFR negative tumours the mean concentrations of oestrogen and progesterone receptors were evidently higher than in premenopausal EGFR negative tumours but these differences were statistically significant for oestrogen receptors only. The premenopausal EGFR negative group in respect to the content of oestrogen and progesterone receptors was rather approximating the premenopausal and postmenopausal EGFR positive group.

Postmenopausal EGFR negative tumours evidently formed a separate group differing from the remaining groups of tumours in its very high oestrogen and, partially, progesterone receptors content.

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Eur J Cancer, Vol. 29A, No. 1, pp. 81–86, 1993.
Printed in Great Britain

0964–1947/93 $5.00 + 0.00

Predicting Septic Complications of Chemotherapy: An Analysis of 382 Patients Treated for Small Cell Lung Cancer without Dose Reduction after Major Sepsis

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The incidence and risk of septic complications in 382 patients treated for small cell lung cancer with combination chemotherapy at a single centre have been analysed. Full protocol doses were employed throughout with no dose reduction after episodes of severe or life-threatening sepsis (SLTS). 50 (13%) patients experienced 66 episodes of SLTS associated with 1978 cycles of chemotherapy (3.2% cycles affected). 20 (5.2%) patients died due to sepsis (SD) of whom only 4 had experienced SLTS with a previous cycle of treatment. The others died as a result of their first septic episode. A model comprising four variables, age (≤ 50 or > 50 years), Karnofsky performance status (KP ≤ 50 or > 50), treatment (two- or three-drug regimen) and previous sepsis (SLTS or no SLTS with previous cycles) was found to satisfactorily describe the incidence of SLTS and SD in the study population and once validated in another patient groups this model should allow identification of high-risk individuals before treatment starts. If so, we propose that high-risk patients (age > 50 years, KP ≤ 50, treatment with three-drug regimen) receive 50% of protocol doses in the first cycle of treatment with escalation to 75% and eventually 100% doses in subsequent cycles if sepsis does not supervene. Those with one or two risk factors present run a relatively low risk of SLTS or SD and we consider that full-dose chemotherapy should be used throughout in these individuals.
Eur J Cancer, Vol. 29A, No. 1, pp. 81–86, 1993.

INTRODUCTION

CANCER CHEMOTHERAPY is commonly attended by myelosuppression. This is reflected in reduced numbers of granulocytes and platelets in the peripheral blood and sometimes leads to infection or haemorrhage, both potentially fatal complications of treatment. Bleeding can usually be prevented if prophylactic platelet transfusions are employed when the platelet count falls to less than $20 \times 10^9/1$ and intravenous broad-spectrum antibiotic therapy often allows full recovery from even severe episodes of sepsis. However, sepsis related deaths do occur and non-fatal episodes of infection are a major contribution to the morbidity of cytotoxic chemotherapy. In efforts to reduce the incidence of these complications of treatment, physicians commonly reduce the dose of chemotherapy after an episode of severe infection and although such empiric alterations in planned dose are commonplace, there is no published data to either support or reject the hypothesis that dose reduction prevents further septic episodes. These issues are important since any reduction in