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Specialist Interest Articles

Proliferative Activity, Histological Grade and Benefit from Adjuvant Chemotherapy in Node Positive Breast Cancer

Susan M. O'Reilly, Richard S. Camplejohn, Rosemary R. Millis, Robert D. Rubens and Michael A. Richards

The influence of S-phase fraction (SPF), measured by DNA flow cytometry, and histological grade on outcome following adjuvant chemotherapy was analysed for 214 patients with node positive breast cancer treated at Guy's Hospital who were entered into the Guy's/Manchester trial of combination chemotherapy with cyclophosphamide/methotrexate/5-fluorouracil (CMF) vs. no adjuvant treatment. Adjuvant CMF significantly improved relapse-free survival (RFS) for premenopausal patients whose tumours had an SPF of 10% or less (control vs. CMF, $P = 0.05$) and premenopausal patients whose tumours had an SPF over 10% (control vs. CMF, $P = 0.003$). No significant improvement in RFS attributable to CMF was seen for either subgroup of postmenopausal patients. When patients were divided into subgroups based on histological grade of tumour, an improvement in RFS attributable to CMF was seen for premenopausal patients with well differentiated (grade 1 or 2) tumours (control vs. CMF, $P = 0.03$) and premenopausal patients with poorly differentiated (grade 3) tumours (control vs. CMF, $P = 0.006$). Again, no improvement in RFS was noted for any subgroup of postmenopausal patients defined by tumour grade.
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INTRODUCTION

ADJUVANT CYTOTOXIC chemotherapy is given to patients with breast cancer in an attempt to eradicate micrometastases and thus prolong survival. There is good evidence that such treatment improves prognosis for premenopausal patients with node positive disease [1, 2]. In addition, adjuvant chemotherapy may have a similar effect in node negative disease [3], although longer follow-up is needed to confirm this [4, 5].

DNA flow cytometry can be used to measure the proportion of cells in S-phase (SPF) and thus estimate the proliferative activity of the tumour. While patients with faster proliferating tumours have a poorer prognosis [6], these tumours may be more likely to respond to chemotherapy [7, 8]. Sulkes *et al.* [7], using a ^{3}H-thymidine labelling index to measure SPF, showed an association between more rapid tumour proliferation and response to chemotherapy for metastatic disease. More recently, Remvikos *et al.* [8] demonstrated a relation between high SPF, measured by flow cytometry, and response of the unresected primary tumour to chemotherapy. In the adjuvant setting, Bonadonna *et al.* [9] reported that chemotherapy for node negative breast cancer appears to improve prognosis preferentially in the group of patients with rapidly proliferating tumours, with little effect in those patients whose tumours were slow growing.

Histological grade also correlates with prognosis in node positive breast cancer, patients with poorly differentiated tumours having a shorter relapse-free survival (RFS) and survival than those with well differentiated tumours [10]. Fisher *et al.* [11] have suggested that patients with well differentiated tumours do not benefit from adjuvant chemotherapy, while patients from all age groups with poorly differentiated tumours benefit significantly from such treatment.

We have analysed the influence of SPF (measured by DNA flow cytometry) and histological grade on outcome following adjuvant chemotherapy for patients with node positive breast cancer who were entered into a randomised trial of combination chemotherapy vs. no adjuvant treatment.

PATIENTS AND METHODS

214 patients with breast cancer who presented to the Guy's Hospital Breast Unit between October 1979 and December 1985 were entered into the Guy's/Manchester adjuvant chemotherapy trial. All patients had a total mastectomy and axillary clearance and had histological evidence of axillary node involvement. Patients were randomised to receive either no adjuvant therapy (107 patients) or a combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) given every 28 days for twelve

Correspondence to S.M. O'Reilly.
S.M. O'Reilly is at the Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Rd, London W6 8RF; R.R. Millis, R.D. Rubens and M.A. Richards are at the ICRF Clinical Oncology Unit, Guy's Hospital; and R.S. Camplejohn is at the Richard Dimbleby Department of Cancer Research, United Medical and Dental Schools, St Thomas' Hospital, London, U.K.

Table 1. Relation between SPF, tumour grade and other clinical and pathological features

	SPF < 10%	SPF > 10%	Grade 1/2	Grade 3
Menopausal status				
Pre	42	27	54	44
Post	28	29	39	31
Treatment				
Control	38	25	47	34
CMF	32	31	46	41
No. of positive nodes				
1–3	36	28	53	37
> 3	34	28	40	38
ER status*				
Negative	6	19	12	29
Positive	58	37	75	43
Tumour size (cm)				
≤ 2	18	15	28	19
> 2	49	39	59	54
Grade†				
1/2	37	14	—	—
3	15	36	—	—

χ^2 test: *$P = 0.002$ for ER status vs. SPF and $P < 0.001$ for ER status vs. grade; †$P < 0.001$.

cycles (107 patients). Details of the study design and outcome at 8 years have been reported [2].

DNA flow cytometry was performed on nuclear suspensions prepared from 50 μm sections cut from formalin-fixed paraffin-embedded tissue from the primary tumour [6, 12]. At least 10 000 cells were scanned to construct each histogram, which was considered interpretable if the coefficient of variation (CV) was less than 8%. The mean CV was 5.0%. The DNA index was calculated by measuring the position of any aneuploid G1 peak relative to the normal G1/G0 peak, with a DNA index of 1.0 indicating the presence of only diploid cells. For DNA diploid tumours, SPF was calculated using the method of Baisch *et al.* [13]. For aneuploid tumours with a DNA index greater than 1.2 a modification of this method was used to calculate SPF for the aneuploid cells alone [14].

Tumour size, measured clinically, was recorded for all cases.

Table 2. Influence of adjuvant chemotherapy on RFS at 5 years for subgroups of patients defined by tumour grade, SPF and menopausal status

Patients	Control	CMF	*P* (logrank)
Premenopausal			
SPF ≤ 10%	42%	72%	0.05
SPF > 10%	10%	55%	0.003
Grade 1/2	35%	74%	0.03
Grade 3	18%	60%	0.006
Postmenopausal			
SPF ≤ 10%	33%	60%	0.2
SPF > 10%	42%	53%	0.75
Grade 1/2	38%	55%	0.4
Grade 3	37%	46%	0.5

Oestrogen receptor (ER) status was determined with the dextran-coated charcoal ligand binding assay [15], with a value of 10 fmol/mg cystosol protein taken as positive. The histological type of all tumours at diagnosis was documented and infiltrating ductal carcinomas were graded by one pathologist (R.R.M.) using the criteria of Bloom and Richardson [10].

The relations between variables were analysed with the χ^2 test. RFS was measured from the date of primary treatment to date of first relapse (locoregional or metastatic) [16] and RFS curves were calculated with the Kaplan–Meier method. The logrank test was used to assess the influence of potential prognostic factors on RFS.

RESULTS

Blocks of paraffin-embedded tissue from the primary tumour of 200 of the 214 patients entered into the study were available for DNA flow cytometry. Interpretable histograms were obtained for 161/200 (81%) of tumours. 48 tumours (30%) were diploid and 113 (70%) aneuploid, with 19 tumours having more than one aneuploid cell population. SPF could be calculated for 126 tumours. The median SPF was 8.5%, range 1.3–36%.

The prognostic significance of SPF in node positive breast cancer was examined within the group of patients who received no adjuvant therapy. To select the level of SPF that best separated groups of patients, the effect of all possible SPF cutoff was analysed [17]. It was possible to separate patients into groups at high and low risk of relapse with cut-offs of around 10% giving the best separation (SPF 10% or less, 5 year RFS 45%; SPF over 10%, 5 year RFS 23%; logrank test, $P < 0.01$). Tumour grade was also a significant predictor of RFS within the controls (grade 1/2, 5 year RFS 45%; grade 3, 5 year RFS 25%; logrank test, $P = 0.03$).

There was no significant association between either SPF or

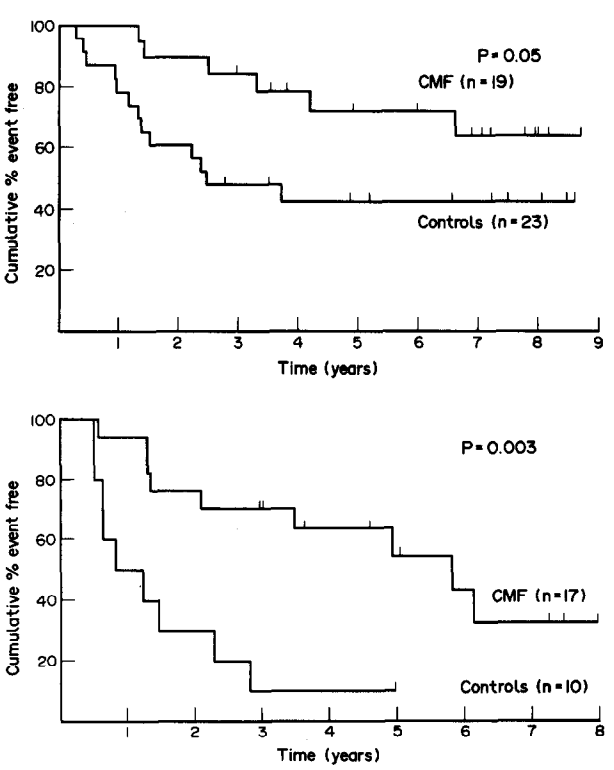

Fig. 1. Influence of adjuvant CMF on RFS for premenopausal patients whose tumours had SPF 10% or less (upper) or over 10% (lower).

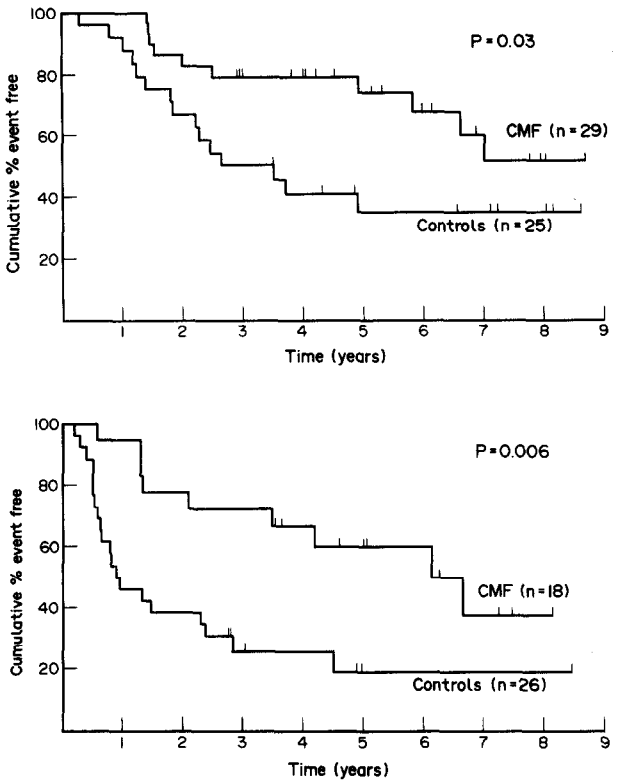

Fig. 2. Influence of adjuvant CMF on RFS for premenopausal patients with grade 1/2 (upper) or grade 3 (lower) tumours.

grade and menopausal status, tumour size or number of nodes involved by tumour (Table 1). The distributions of SPF values and tumour grade were similar in the control and treatment arms of the study. Tumours with a high SPF were significantly more likely to be (ER) negative, as were grade 3 tumours.

The relation between proliferative activity, histological grade and RFS was analysed for both premenopausal and postmenopausal patients (Table 2). The influence of adjuvant chemotherapy on RFS was examined separately for patients with slowly proliferating tumours (SPF 10% or less) and those with rapidly proliferating tumours (SPF over 10%). Adjuvant CMF significantly improved RFS for premenopausal patients whose tumours had an SPF of 10% or less (Fig. 1, upper) and for premenopausal patients whose tumours had an SPF over 10% (Fig. 1, lower). No significant improvement in RFS attributable to CMF was seen for either subgroup of postmenopausal patients (SPF 10% or less, control vs. CMF, $P = 0.2$; SPF over 10%, control vs. CMF, $P = 0.75$).

When patients were divided into subgroups based on histological grade of tumour, an improvement in RFS attributable to CMF was also seen for premenopausal patients with well differentiated (grade 1 or 2) tumours (Fig. 2, upper) and premenopausal patients with poorly differentiated (grade 3) tumours (Fig. 2, lower). Once again, no improvement in RFS was noted for either subgroup of postmenopausal patients defined by tumour grade (grade 1 or 2, control vs. CMF, $P = 0.04$; grade 3, control vs. CMF, $P = 0.5$).

DISCUSSION

Adjuvant chemotherapy acts on micrometastases and therefore the response to such treatment can only be measured indirectly by comparing RFS and survival of treated and untreated groups of patients. As cytotoxic chemotherapy acts by killing dividing cells, its effect might be expected to be greatest in rapidly proliferating tumours. We have reported that in the Guy's/Manchester trial adjuvant CMF improved prognosis only for premenopausal patients [2]. The benefit attributable to chemotherapy in that trial was seen in all subgroups of premenopausal patients defined by nodal status, tumour size and steroid receptor status. No subgroup of postmenopausal patients benefited from treatment. We have now established that, while the magnitude of the effect is greater for patients with rapidly proliferating or poorly differentiated tumours, CMF does significantly improve prognosis for premenopausal patients with slow growing or well differentiated tumours. Again, neither subgroup of postmenopausal patients benefited from treatment.

Two other centres have related outcome following adjuvant chemotherapy to the proliferative activity of the primary tumour, one using thymidine labelling index and the other using flow cytometry. In neither case, however, was this analysis done for premenopausal node positive patients randomised either to receive chemotherapy or no adjuvant treatment. Bonadonna *et al.* [9] measured the thymidine labelling index of the primary tumour for 54 women with ER negative, node negative breast cancer treated as part of the Milan trial comparing no adjuvant therapy with twelve courses of CMF. For this small number of patients, they commented that CMF appeared to act maximally in those with a high thymidine labelling index, with little or no effect in the subgroup whose tumours had a low index. No further analysis on the effectiveness of treatment within menopausal groups was reported. In node positive breast cancer, the Milan group reported that the risk of relapse and death was related to thymidine labelling index for patients receiving CMF with or without doxorubicin. However, the effect of chemotherapy vs. no adjuvant treatment was not assessed within subgroups defined by the thymidine labelling index [18].

Hedley *et al.* [19] examined the prognostic significance of SPF measured by flow cytometry within treatment groups for patients entered into the Ludwig Breast Cancer Studies I–IV. Premenopausal patients all received adjuvant CMF, with some groups also receiving prednisone or undergoing oophorectomy. No difference in outcome between the groups was observed and there was no difference in the effect of SPF on RFS within the treatment groups. Postmenopausal patients were randomised to a control arm or to treatment with either tamoxifen plus prednisone or tamoxifen, prednisone and CMF. While adjuvant therapy did improve RFS, the effect of this treatment did not appear to be related to SPF. The Ludwig group also reported a similar effect when the prognostic significance of histological grade on clinical outcome was examined [20].

The relation between response to adjuvant chemotherapy and histological grade has also been analysed by Fisher *et al.* [11] using results from the national Surgical Adjuvant Breast and Bowel Project (NSABP). Patients either received no adjuvant treatment, single-agent l-phenylalanine mustard (L-PAM), L-PAM plus 5-fluorouracil (PF) or a combination of L-PAM, 5-fluorouracil and methotrexate (PMF). A statistically significant improvement in survival for patients aged less than 50 years who received PF or PMF was seen only for those who had poorly differentiated tumours with at least four involved axillary nodes. A similar result was observed for patients aged over 50 years.

In summary, this is the only report which examines the impact of both the proliferative activity and the histological grade of tumours on outcome following adjuvant chemotherapy for a group of patients in whom both premenopausal and postmenopausal women were randomised to a treatment or control arm.

We have extended our previous finding that all subgroups of premenopausal patients benefit significantly from such treatment.

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Effect of Peri-operative Chemotherapy on the Quality of Life of Patients with Early Breast Cancer

Gwendoline M. Kiebert, J. Hanneke C.J.M. de Haes, Jacob Kievit and Cornelis J.H. van de Velde

Since chemotherapy is assumed to have a negative impact on quality of life, the impact of peri-operative chemotherapy on physical, psychological and social well-being and on the activity level of patients with early stage breast cancer was investigated. 24 women received peri-operative chemotherapy and 29 did not. They were interviewed 2 months and at a mean of 12 months post-surgery. Although the treated group reported more fatigue and considered hair loss a severe side-effect, no differences were found in overall physical and psychological well-being, perceived social interaction and activity level at 2 months. 1 year after surgery no differences were found between the two groups. Although no substantial effects of peri-operative chemotherapy on quality of life were demonstrated, patients almost unanimously considered peri-operative chemotherapy the most burdensome aspect of the treatment because of alopecia.
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INTRODUCTION

CONSENSUS ABOUT the best treatment regimen for early stage breast cancer has not been reached [1–5]. In 1986 the EORTC Breast Cancer Cooperative Group started a multi-centre randomised study (EORTC 10854) to test the hypothesis that a single peri-operative dose of adjuvant combination chemotherapy increases overall survival compared with an untreated control arm. However, side-effects of chemotherapy have a negative