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Original article

Immuno-modulatory effects of relaxation training and guided imagery in women with locally advanced breast cancer undergoing multimodality therapy: A randomised controlled trial

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ABSTRACT

Eighty women undergoing multimodality treatment for large (>4 cm) or locally advanced (T3, T4, Tx, N2), breast cancers participated in a randomised controlled trial (RCT) to evaluate the immuno-modulatory effects of relaxation training and guided imagery. Patients underwent chemotherapy followed by surgery, radiotherapy, and hormone therapy. Those in the intervention group were taught relaxation and guided imagery. Patients kept diaries of the frequency of relaxation practice and imagery vividness. On 10 occasions during the 37 weeks following the diagnosis, blood was taken for immunological assays CD phenotyping: T cell subsets (helper, cytotoxic), natural killer (NK) and lymphokine activated killer (LAK) cells, B lymphocytes and monocytes; cytotoxicity: NK and LAK cell activities; cytokines interleukin 1 beta (1β), 2, 4 and 6 and tumour necrosis factor alpha.

Significant between-group differences were found in the number of CD25+ (activated T cells) and CD56+ (LAK cell) subsets. The number of CD3+ (mature) T cells was significantly higher following chemotherapy and radiotherapy, in patients randomised to relaxation and guided imagery. Using a median split, women who rated their imagery ratings highly had elevated levels of NK cell activity at the end of chemotherapy and at follow-up. Significant correlations were obtained between imagery ratings and baseline corrected values for NK and LAK cell activity, and IL1β. Relaxation frequency correlated with the number of CD4+ (T helper) cells, the CD4+:8+ (helper:cytotoxic) ratio, and IL1β levels.

Relaxation training and guided imagery beneficially altered putative anti-cancer host defences during and after multimodality therapy. Such changes, to the best of our knowledge, have not been previously documented in a RCT.

Introduction

The diagnosis of breast cancer is very stressful psychologically.[1–3] Different treatments (surgery, radiotherapy, and chemotherapy) contribute further to what can become clinically significant anxiety and depression.[4–6] These psychological disturbances adversely affect quality of life (QoL) and may be important determinants of survival from cancer.[7–11] Studies have documented an association between QoL scores and prolonged survival of patients with advanced disease,[12–15] and early-stage breast cancer.[16,17]

Chronic stressful events (e.g., bereavement, marital discord) can modulate host defences.[18,19] Immune suppression, both of cell-mediated immunity (e.g., response to mitogens, natural killer (NK) cell activity, T cell subsets, etc.),[20–22] and humoral responses,[23,24] have been documented. In humans, studies have investigated adverse life events (e.g., divorce, bereavement, academic stress) on the incidence of upper respiratory tract infections and reactivation of latent viruses (herpes, Epstein-Barr) as a result of inhibition of putative host defences.[20,25,26] A review by Biondi and Zannino[27] suggested that psychological stress was an important co-factor in the pathogenesis of infectious diseases.

There is a close interaction between the central and autonomic nervous systems and the lympho-reticular compartments in the body.[28–33] Cytokines produced by activated lymphocytes and

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macrophages interleukin (IL) 1, 2, 4 and 6, and tumour necrosis factors α (TNFα), affect brain activity through interaction with receptors in the pre-optic nuclei of the hypothalamus (absent blood brain barrier, specialized transport mechanisms) and other areas of the brain (hippocampus, fronto-parietal cortex, anterior pituitary).[28,29,34,35] Not only are receptors for cytokines (e.g., IL1) found on both glial cells and neurons, but variable in situ production of cytokines in the brain has been documented in rodents, and IL1 implicated in neurological diseases in man[35–38]. This close humoral integration of the immune system and the brain provides the central nervous system with a widespread, sensitive, sensory system for early detection and close monitoring of disparate pathological disturbances in the body.

Activation of the hypothalamic pituitary adrenocorticol axis (HPA) results in enhanced production of corticotrophin releasing factor (CRF). Stress (physical, psychological), acting through the limbic system and fore-brain, induces CRF production and release from the paraventricular nuclei.[28,34] CRF plays a key role in the inhibition of immune reactivity through its release of anterior pituitary immuno-inhibitory hormones (adrenocorticotrophin, β-endorphin, α-melanocyte stimulating hormone) and brain-stem autonomic neuronal and peripheral sympathetic nerve release of noradrenaline, encephalins and neuropeptides.[29,31,39–41] Various lymphoid cells are innervated by the sympathetic nervous system and express receptors for these different hormones.[28,29,31–33] CRF also inhibits growth hormone and prolactin secretion; both hormones are known to enhance cell-mediated immune responses.[28,34] The possible relevance of psychoneuroimmunological mechanisms in cancer has been reviewed recently by Walker et al.[42]

Psychosocial interventions, in animals and man, may alter host defences in ways that are potentially beneficial.[43–46] However, the clinical significance of these intervention-induced changes in patients with malignant disease is unclear, and the optimum interventions have not been defined.[42]

We previously reported the psychosocial effects of relaxation and guided imagery in 96 women with locally advanced breast cancer (LABC) participating in a randomised controlled trial (RCT).[11] Compared to patients receiving treatment as usual, patients randomised to relaxation and guided imagery were more relaxed and easy going (Mood Rating Scale) and their quality of life was better (Global Self-assessment and Rotterdam Symptom Checklist). The intervention also reduced emotional suppression (Courtauld Emotional Control Scale). The incidence of clinically significant mood disturbance was very low, and the incidence in the two groups was similar. Finally, although the groups did not differ for clinical or pathological response to chemotherapy, imagery ratings were correlated with clinical response.

Blood samples were taken from the first 80 patients recruited to this study, and the effects of the intervention on host defences of these patients are reported here.

Aim

The aim of this study was to evaluate the immunological effects of relaxation and guided imagery in women undergoing multimodality treatment for LABC. We wished to document the effects on those parameters of cell-mediated immunity and natural cytotoxicity, and their associated regulatory cytokines, which are believed to play a crucial role in tumour-host interactions.[47–50]

Materials and methods

Patients studied

A consecutive series of 81 women under 75 years of age with large (>4 cm) or LABC (T3, T4, Tx N2) took part. 80 agreed to participate (99% acceptance). Detailed inclusion/exclusion criteria can be found in Walker et al.[11] Ethical approval for the study was obtained from the Joint Committee of the University of Aberdeen and Aberdeen Royal Hospitals NHS Trust. Patients gave signed informed consent. Patients received the following chemotherapy [CVAP]: doxorubicin (50 mg/m^2), cyclophosphamide (1 g/m^2), vincristine (1.5 mg/m^2) and prednisolone (40 mg/day for 5 days)]. This was given three weekly for 6 courses.

Study design

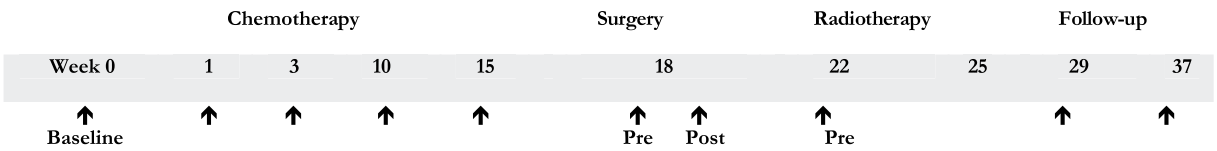
This was a randomised, controlled, trial. Following diagnosis, and before the first cycle of chemotherapy, patients were randomised to relaxation and guided imagery, with standard information and support (experimental group), or to standard information and support only (control group). Randomisation was stratified for menopausal status and was carried remotely using permuted blocks.

All patients received chemotherapy, surgery, and radiotherapy (Fig. 1). Following surgery, they took tamoxifen (20 mg daily) for 5 years. Blood samples were taken for immunological analysis at the 10 time points indicated in Fig. 1. All blood samples were taken at 12 noon ± 2 h. The first sample (baseline) was taken 3 days before commencement of chemotherapy. Samples 2–5 were taken during chemotherapy, 6 the day before surgery and 7 was collected 2 or 3 days after surgery. Sample 8 was taken before the women received radiotherapy. Samples 9 and 10 were taken 4 weeks and 12 weeks after completion of radiotherapy.

The primary study endpoint was week 37 (12 weeks post radiotherapy). Secondary endpoints were week 18 (post chemotherapy/pre surgery) and week 29 (4 weeks post radiotherapy).

Study setting

The study was carried out in the Behavioural Oncology Unit, Aberdeen Royal Infirmary. Patients could telephone or attend the Unit at any time to discuss matters of concern; considerable efforts were made to coordinate treatments and investigations to suit the women. We cultivated a professional, but informal, atmosphere to make the women feel welcome and comfortable. Patients met their peers in the Unit to discuss issues of common interest and concern. Family members and friends were welcome.



Legend: Time course for the different therapeutic modalities used. Blood samples for immunological evaluations were collected at the time points marked ↑.

Fig. 1. Time course for the different therapeutic modalities used. Blood samples for immunological evaluations were collected at the time points marked.

Experimental intervention

Patients in the experimental group were taught progressive muscular relaxation and cue-controlled relaxation training[51] by means of five individual live training sessions and audio-recordings for regular home practice. The patients were also given advice about guided imagery. This entailed visualizing host defences destroying tumour cells, or in some other way improving health, whilst relaxed. Women received a portfolio of cartoons depicting this process and they were encouraged to generate their own visual images. Patients were asked to record each day the number of times they had listened to the recording and to rate imagery vividness (scale of 1–10). Further details of the intervention and its effects on QoL during neoadjuvant chemotherapy have been previously reported.[11] Essentially, relaxation and guided imagery enhanced global QoL, improved mood, reduced social conformity, and enhanced emotional expression.

Immunological assays

Blood mononuclear cell preparations

Peripheral blood mononuclear cells (viability greater than 95%; predominately (85%) lymphocytes) were isolated from heparinised venous blood on Ficoll-Hypaque gradients (Pharmacia Biotech, Milton Keynes, UK) (400 g for 40 min), washed twice in Hanks balanced salt solution and re-suspended at 1×10^7 per ml in TCM [TCM: RPMI 1640 in 20% heat inactivated (56 C, 1 h) foetal calf serum (Life Technologies, Glasgow, UK)] and 10% dimethyl sulphoxide (Sigma chemicals, Poole, UK), immediately cryopreserved using a KRYO 10 series II controlled rate freezer (Planar products, Sunbury on Thames, UK). When required for assays which were done with collected blocks of samples, the cells were thawed in a 37 C water bath, washed twice with TCM, re-suspended at 2×10^6 per ml in TCM (10% foetal calf serum). For NK and LAK cell assays, the cells were incubated at 37° C for at least 3 h before being used in the assays.

CD immunophenotyping

Cell subsets were assayed using FACS (fluorescent-activated cell sorter) analysis (EPICS prophile II, Coulter Corporation, Luton, UK), and the appropriate mouse monoclonal antibodies (Mabs) reactive with lymphocyte surface CDs (cluster differentiation antigens). The following mononuclear cell subsets were assayed by FACS: total lymphocyte pool (CD2+), mature T lymphocytes (CD3+), T helper cells (CD4+), T cytotoxic cells (CD8+), B lymphocytes (CD19+), activated T cells (CD25+), NK cells (CD16+), LAK cells (CD56+) and monocyte-macrophages (CD14+).

Goat antimouse immunoglobulin fluoresceine-isothiocyanate (FITC) – conjugated serum was used. Reagents were obtained from the Scottish Antibody Production Unit, Lanark, UK, Becton Dickinson, Oxford, UK and DAKO, High Wycombe, UK. Fifty microlitre of lymphocytes were added to LP3 tubes (Deneley Instruments, Burgesshill, UK) containing optimum titre (1:10–1:20) of Mab 5 μl in 45 μl phosphate buffered saline (PBS) with 5% goat serum (heat inactivated) and 0.1% sodium azide (Sigma Chemicals, UK). After incubation on ice for 40 min, cells were washed in 2 ml of PBS containing 1% goat serum and 0.1% sodium azide, centrifuged at 250 g for 6 min, washed twice before addition of the secondary FITC – conjugated goat antimouse immunoglobulin. After further 40 min incubation, cells were washed free of unbound antibody, re-suspended in 400 μl ice-cold PBS and underwent FACS analysis. The results obtained from flow cytometry were determined from the percentage of positive cells within the lymphocyte bitmap and the gated channel as a measure of intensity and absolute numbers calculated from the total lymphocyte counts obtained concurrently.

Natural cytotoxicities

Nartural killer (NK) and lymphokyne activated killer (LAK) cell activities were documented in trial participants using isolated blood mononuclear cells and 51chromium (51CR) (Amersham International, UK) – labelled target cells [K562 (NK sensitive) and Daudi (LAK sensitive)] in 4 h in vitro cytotoxicity assays, as previously described.[48,52] K562 is a widely used target cell for human NK cells – derived from chronic myeloid leukaemic cells (lacks class I and II MHC proteins and does not express Epstein-Barr nuclear antigen). The Daudi cell line is derived from a patient with Burkitt's lymphoma and is relatively "NK resistant".[48] Both cell lines were established, mycoplasma free and available in-house.

Labelled (51CR 7.4–9.2 MBq) K562 and Daudi cells (200 μl) and effector lymphocytes (1:5–1:40) were set up in duplicates (100 μl) in LP2 tubes (Sterilin, Staffordshire, UK). Target cells alone were set up as controls. Tubes were centrifuged at 150 g for 2 min, incubated in a 5% CO_2 incubator for 4 h, then re-suspended and further centrifuged at 200 g for 5 min. 150 μl of supernatant was transferred to new LP2 tubes; the latter and the pellet with the residual supernatant were then placed in a gamma counter (LKB Wallace 1282 compugamma counter). Spontaneous release of 51CR from target cells was less than 10%. Maximal lysis of target cells was determined with lysol and was constant at 70% 51CR release. The percentage target cell lysis was plotted against effector to target ratios and lytic units calculated. These were defined as the number of effector cells required to produce 50% of lysis of target cells (35% of 1000 target cells obtained by extrapolation graphically and computed by linear regression).

Serum cytokines

IL1β, IL2, IL4, IL6, and TNFα were documented in the serum of patients in the trial using the enzyme-linked immunosorbent assay (ELISA) as previously documented[53]. Commercial kits for quantitative measurement of IL4 were obtained from Gemzyme Corporation, Cambridge, UK and for the other cytokines from Medgenix, High Wycombe, UK. Briefly, the assays were solid phase enzyme amplified sensitivity immunoassays performed in microtitre plates, employing the quantitative sandwich technique. A substrate solution added to the wells developed a colour in proportion to the amount of cytokine in the serum sample used in the initial step. Intensity (absorbance) of the colour was measured at 450 nm and 490 nm (reference filters 650 nm) with Dynatek MR500 microplate reader. A standard curve was constructed using standard points for which absorbances were less than 1.5, plotting the absorbance against the standard concentration of cytokine. If the control or sample had an absorbance greater than the standard readings at 4540 nm, a second standard curve at 490 nm was constructed. Concentration (pg/ml) was expressed as means plus or minus one standard error of the mean (±1SEM) (intraplate coefficient of variation was <5%). Concentrations were plotted graphically for each variable so that changes in each of the variables over time in relation to chemotherapy, surgery and radiotherapy could be evaluated visually.

Statistical analyses

Means and standard errors were plotted graphically for each variable so that changes in each of the variables over time in relation to chemotherapy, surgery and radiotherapy can be evaluated visually for the experimental and control groups. Statistically significant between-group differences (* <0.05; ** <0.01) are indicated in Fig. 4.

The study was planned to detect a medium effect size (0.45). Formal statistical testing of differences between the two groups over time was carried out using repeated measures analysis of covariance (RMANCOVA) for all available time points simultaneously, and

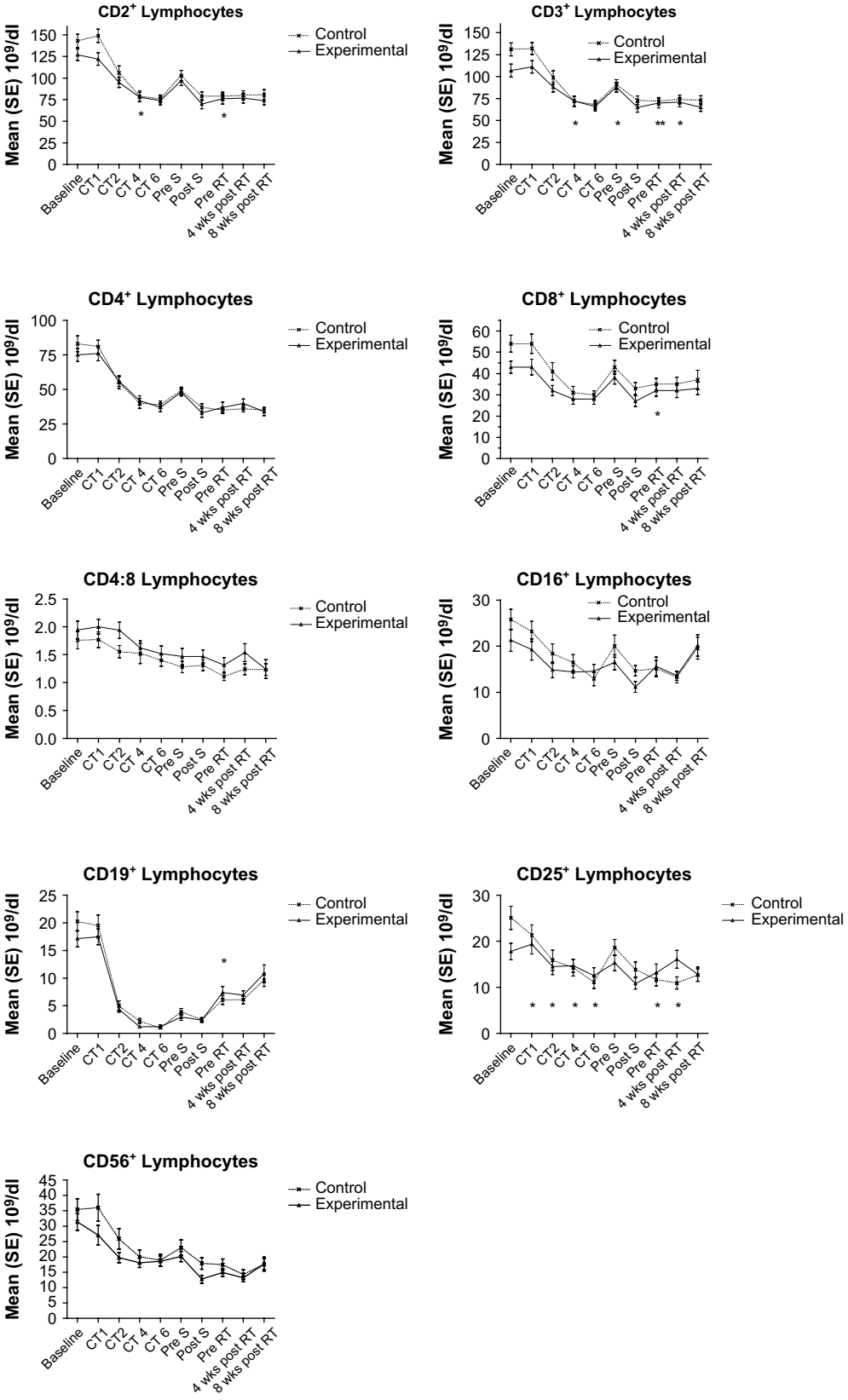


Fig. 2. Immunophenotyping of lymphocyte subsets in the experimental and control groups. CT 1, Before the first cycle of chemotherapy; CT 2, Before the second cycle of chemotherapy; CT 4, Before the fourth cycle of chemotherapy; CT 6, Before the sixth cycle of chemotherapy; Pre S, Post chemotherapy/pre surgery: secondary endpoint; Post S, After surgery; Pre RT, Before radiotherapy; 4 weeks post radiotherapy, 4 weeks after completion of radiotherapy: secondary endpoint; 8 weeks post radiotherapy, 8 weeks after completion of radiotherapy: primary endpoint; * = <0.05 (adjusted for baseline differences); ** = <0.001 (adjusted for baseline differences).

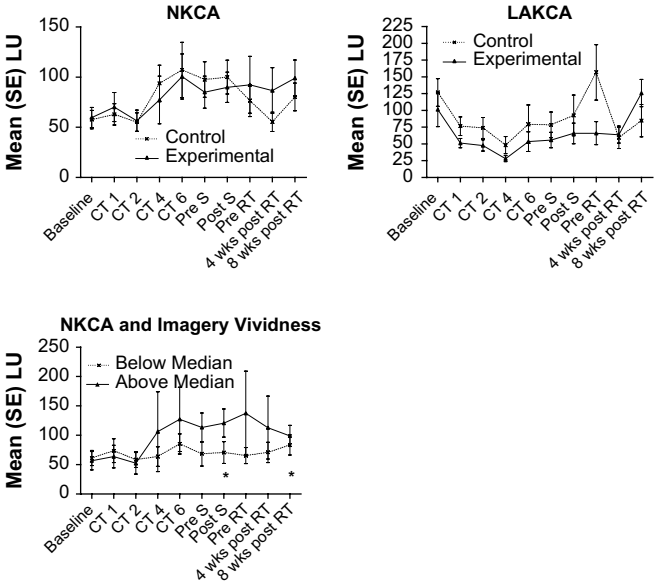


Fig. 3. NK and LAK cell activity in the experimental and control groups, and NK cell activity and imagery vividness in the experimental group. CT 1, Before the first cycle of chemotherapy; CT 2, Before the second cycle of chemotherapy; CT 4, Before the fourth cycle of chemotherapy; CT 6, Before the sixth cycle of chemotherapy; Pre S, Post chemotherapy/pre surgery: secondary endpoint; Post S, After surgery; Pre RT, Before radiotherapy; 4 weeks post radiotherapy, 4 weeks after completion of radiotherapy: secondary endpoint; 8 weeks post radiotherapy, 8 weeks after completion of radiotherapy: primary endpoint; * = <0.05 (adjusted for baseline differences); ** = <0.001 (adjusted for baseline differences).

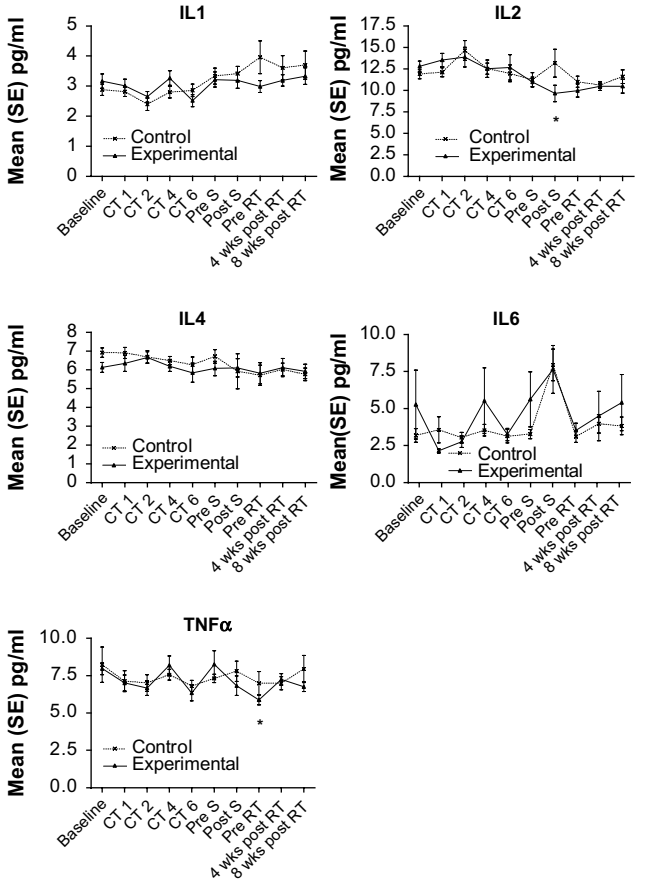


Fig. 4. Serum cytokine levels in the experimental and control groups. CT 1, Before the first cycle of chemotherapy; CT 2, Before the second cycle of chemotherapy; CT 4, Before the fourth cycle of chemotherapy; CT 6, Before the sixth cycle of chemotherapy; Pre S, Post chemotherapy/pre surgery: secondary endpoint; Post S, After surgery; Pre RT, Before radiotherapy; 4 weeks post radiotherapy, 4 weeks after completion of radiotherapy: secondary endpoint; 8 weeks post radiotherapy, 8 weeks after completion of radiotherapy: primary endpoint; * = <0.05 (adjusted for baseline differences); ** = <0.001 (adjusted for baseline differences).

repeated measures analysis of variance for the primary and secondary endpoints, with baseline value as covariate as recommended by Vickers and Altman.[54] Patients in the control group tended to have higher numbers of various T cell subsets (Fig. 2).

The relationship between self-assessed imagery ratings, relaxation frequency and changes in host defences at the end of chemotherapy, and at the end of the study, were evaluated using Kendall's τ.

The data were analyzed using SPSS MS for Windows (version 12). Alpha was set at 0.05 (2-tailed). In line with the recommendations of Bacchetti[55] and Goodman,[56] Bonferroni corrections were not used.

Results

Patient characteristics

Eighty of 81 consecutive eligible patients took part. One patient withdrew as she felt that relaxation and imagery conflicted with her religious convictions.

The two groups did not differ significantly in terms of age, T stage, N status, tumour size (product of the two largest perpendicular diameters on caliper measurement), menopausal status, and oestrogen receptor status (Table 1a and Table 1b).

Compliance with relaxation and guided imagery

Analysis of the daily diary recordings indicated that 10 (25%) of the women practiced on average at least 1.5 times per day during the 18 weeks of chemotherapy; 10 (25%) practiced between once and 1.5 times per day; 12 (30%) practiced between 0.5 and 0.99 times per day; 2 (2.5%) practiced less than 0.5 times per day, and 6 (7.5%) practiced minimally or not at all.

Circulating T lymphocyte subsets (Fig. 2)

The number of samples available at each time point varied from 70 to 79 (88%–99%). Complete data for all 10 time points were available for 64 patients.

When the experimental and control groups were compared simultaneously at all time points, there was a significant time by

Table 1a
Menopausal status and tumour characteristics of the patients (categorical variables)

		Control group	Experimental group	χ^2	*P* value
Tumour stage	2	10	12	1.537	0.674
	3	22	23		
	4	7	5		
	x	1	0		
Nodal stage	0	26	23	2.110	0.348
	1	11	16		
	2	3	1		
Menopausal status	Pre-menopausal	22	20	0.201	0.654
	Post-menopausal	18	20		
Oestrogen receptor status	Negative	20	15	1.759	0.415
	Low positive	7	10		
	High positive	7	10		
	Unknown	6	5		

Table 1b
Age and clinical tumour size(continuous variables)

		Control	Experimental	*t*	*p*
Age	Mean (years)	50.45	49.38	0.417	0.678
	Std. deviation	11.806	11.240		
Tumour size	Mean (cms^2)	36.175	40.812	0.811	0.420
	Std. deviation	23.883	27.181		

intervention interaction in the number of CD25+ and CD56+ lymphocyte subsets (Table 2). Post chemotherapy/pre surgery, the experimental group had higher numbers of CD3+ lymphocytes, and post radiotherapy the experimental group had higher numbers of CD3+ and CD25+ lymphocytes (Table 3). None of the differences were statistically significant at the final endpoint.

Natural cytotoxicity (Fig. 3)

The number of samples available at each time point varied from 66 to 75 (83%–94%) for NK cells and 45 to 60 (56%–75%) for LAK cells. For LAK cells, complete data were available for baseline and the three endpoints for 29 patients and this was used in the RMANCOVA. For NK cells, complete data for all 10 time points were available for 47 patients.

When NK and LAK cell activity, using all 10 time points simultaneously, were compared, no significant differences were found (Table 4). However, the experimental group had significantly higher LAK cell activity than the control group at the end of the study (Table 3).

Within the experimental group, good imagers (good imagery versus poor imagery determined by a median split) had significantly higher NK cell activity during the study period (Table 4). Those patients who rated their imagery as good maintained a high level of cytotoxicity following the fourth cycle of chemotherapy (Fig. 3).

Systemic cytokines

The number of samples available for analyses at baseline, before the third cycle of chemotherapy and at the three endpoints varied from 70 to 79 (88%–99%). For the other time points, the number of samples ranged from 40 to 48. Complete data for baseline and the three endpoints were available for 68–70 (85%–88%) patients and the RMANCOVAs, therefore, were carried out on the latter dataset.

There were no significant differences in the circulation of IL1β, IL2, IL4, and IL6 or TNFα in the two groups (Tables 3 and 5). There was an enhanced acute phase response, in the peri-surgical period, in both groups, with comparable increases in the level of IL6 (Fig. 4).

Table 2
CD Immunophenotyping of lymphocyte subsets: repeated measures analyses of covariance for all time points simultaneously

	Time			Time × intervention			Between-groups			*n*
	F	df[a]	*p*	*F*	df[a]	*p*	*F*	df	*p*	
CD2+	1.015	5.16	0.410	0.687	5.16	0.638	0.020	1	0.887	64
CD3+	0.871	5.29	0.505	0.470	5.29	0.809	0.086	1	0.770	63
CD4+	0.768	5.82	0.592	0.413	5.82	0.865	0.606	1	0.439	64
CD8+	1.114	5.11	0.353	0.364	5.11	0.876	0.021	1	0.886	64
CD4:8+	2.234	6.35	**0.036**	0.953	6.35	0.400	1.819	1	0.182	64
CD16+	1.091	6.65	0.368	0.922	6.65	0.485	0.061	1	0.806	62
CD19+	2.540	4.04	**0.040**	1.219	4.04	0.303	1.123	1	0.293	64
CD25+	2.353	6.33	**0.028**	2.475	6.33	**0.021**	1.817	1	0.183	63
CD56+	2.602	5.48	**0.021**	2.231	5.49	**0.045**	0.984	1	0.325	62

[a] With Huynh Feldt correction as required.

Table 3
CD immunophenotyping of lymphocyte subsets: analyses of covariance at the primary and secondary endpoints

	df	Post-chemotherapy		4 Weeks post radiotherapy		Final endpoint	
		F	*p*	*F*	*p*	*F*	*p*
CD2+	1	2.717	0.104	3.765	0.057	2.285	0.135
CD3+	1	5.333	**0.024**	5.718	**0.020**	3.236	0.077
CD4+	1	1.682	0.201	3.251	0.076	1.490	0.223
CD8+	1	1.770	0.188	3.195	0.078	2.312	0.133
CD4:8	1	0.000	0.996	0.188	0.666	0.845	0.361
CD16+	1	0.043	0.836	1.520	0.222	1.085	0.301
CD19+	1	0.501	0.481	2.096	0.152	2.384	0.127
CD25+	1	1.444	0.288	15.964	**<0.0001**	3.246	0.076
CD56+	1	0.508	0.479	1.183	0.281	2.169	0.146
IL1β	1	0.950	0.333	1.873	0.176	1.326	0.254
IL2	1	0.884	0.350	1.203	0.277	2.312	0.133
IL4	1	0.123	0.727	1.740	0.192	3.675	0.059
IL6	1	0.018	0.895	2.896	0.093	0.690	0.409
TNFα	1	0.690	0.409	0.022	0.882	0.507	0.479
NKCA	1	0.118	0.733	0.871	0.354	0.355	0.544
LAKCA	1	0.026	8.82	1.222	0.277	4.556	**0.040**

Host defences, relaxation practice and imagery ratings

Changes post chemotherapy/pre surgery: Significant correlations between imagery ratings and change from baseline were obtained for NK cell ($\tau = .320$, $p = 0.022$) and LAK cell activites ($\tau = .437$, $p = 0.030$). Frequency of relaxation practice and the number of blood CD4 + T lymphocytes correlated negatively ($\tau = -.267$, $p = 0.034$).

Changes 4 weeks post radiotherapy: Frequency of relaxation practice correlated negatively with changes in CD4+ ($\tau = -.258$, $p = 0.048$) and CD4+:8+ratios ($\tau = -.266$, $p = 0.042$).

Changes 8 weeks post radiotherapy: Significant correlations between imagery ratings and change from baseline were obtained for NK cell activity ($\tau = .319$, $p = 0.020$). Relaxation frequency ($\tau = .308$, $p = 0.018$) and imagery ratings ($\tau = .308$, $p = 0.019$) correlated significantly with blood IL1β levels.

Discussion

To the best of our knowledge, this is the first RCT evaluating relaxation and guided imagery in patients with locally advanced breast cancer undergoing multimodality therapy. Patients were evaluated serially at 10 time points over a period of 37 weeks during which they received chemotherapy, surgery, and radiotherapy. Our findings suggest that relaxation therapy and guided imagery can alter aspects of host defences during and after prolonged multimodality therapy.

RMANCOVA revealed that women randomised to relaxation and guided imagery had an increase in the number of activated (CD25+) T cells. These lymphocytes, through enhanced expression of the α chain for IL2, are primed for rapid proliferation and differentiation. The functional significance of these changes is unclear; activated T lymphocytes can induce tumour cell death and to inhibit tumour growth.[47–49,57] No studies, were done to establish whether these were CD8+ CD25+ tumour antigen/peptide specific cytotoxic T lymphocytes. Also, no characterisation was carried out to establish the presence of CD25+ CD4+ regulatory T lymphocytes.[58] These changes have not been previously documented in patients with cancer undergoing psychological interventions or in other situations where these interventions have been used.

The two groups differed in the number of CD56+ cells during the study, although not significantly at any of the three endpoints.

Table 4
NK and LAK cell activities: repeated measures analyses of covariance for all variable time points simultaneously

	Time			Time × intervention			Between-groups			*n*
	F	df[a]	*p*	*F*	df[a]	*p*	*F*	df	*p*	
NKCA	1.478	8.00	0.164	0.265	8.00	0.875	0.065	1	0.800	47
NKCA[b] and imagery ratings	1.272	3.30	0.292	1.153	3.30	0.337	10.301	1	**0.005**	22
LAKCA[c]	0.227	2.00	0.798	1.132	8.00	0.324	0.485	1	0.492	29

[a] Huyn Feldt correction as required.
[b] Above versus below median for imagery ratings.
[c] Time points: baseline, pre surgery, post-radiotherapy and final follow-up.

The RMANCOVA using all 10 time points was not significant for CD3+ (mature T cells), although the experimental group had significantly higher numbers of CD3+ lymphocytes at the end of chemotherapy, and 4 weeks after the end of radiotherapy.

A meta-analysis carried out by Herbert and Cohen,[59] documented that stress is associated with reduced numbers of circulating T cells and subsets. Studies have confirmed the susceptibility of T cell subsets (CD3+, CD4+ and CD8+) to stress, both in healthy individuals and women with breast cancer undergoing treatment.[26,46,60] Studies carried out by us on healthy volunteers undergoing relaxation training have demonstrated functional changes in T lymphocytes (responses to polyclonal activators).[44] Also, Kiecolt-Glaser et al.[45] were able to minimize the fall in circulating T helper cells in medical students undergoing relaxation training prior to, and at the time of, major examinations. More recently, Naito et al.[61] found in students who practiced self-hypnosis that the percentage of CD3(−) CD56(+) NK cells and CD3(+) CD4(+) T cells were maintained, and CD3(+) CD8(+) T cell percentages, shown previously to decline with examinations, increased.

The largest RCT published, to date, evaluated the effects of a group intervention (relaxation therapy and educational components) in 227 women with Stage 2 or 3 breast cancer.[62] T cell proliferation to polyclonal mitogens remained stable or increased in patients randomised to the intervention, whereas responses decreased in the control group. However, the intervention did not affect CD3+, CD4+ or CD8+ counts, but CD25+ and CD56+ lymphocyte subsets were not measured. Blood samples were taken at baseline and 4 months later, thereby, precluding detailed sequential analyses. The authors concluded that the intervention had significant psychological, behavioural and biological effects.

A number of key regulatory cytokines in the circulation, which control systemic mononuclear cell activity, namely the pro-inflammatory Th1 (IL1β, IL2, IL6, TNFα) cytokines, the anti-inflammatory Th2 (IL4) and the monocyte-macrophage (IL1, IL6, TNFα) cytokine levels, were measured. Functional receptors for IL1β, IL2, IL6, and TNFα have been documented in the brain.[28,29,34,35] Although in situ interactions and production in the brain are not possible to measure, systemic levels may reflect brain-immune interactions.[36–38] In view of the complex and prolonged therapeutic interventions, with their impact on pathophysiological and psychological processes, it is not surprising that there were no significant effects. However, relaxation frequency and imagery ratings were positively correlated with IL1β levels at the final endpoint. IL1β induces host-defence generated 'sickness behaviour' acute phase response and affects the HPA axis and the release of CRF.[35]

At the end of the study, the women undergoing intervention had higher LAK cell activity, compared with controls. Moreover, before surgery, significant correlations between imagery ratings and baseline corrected values were obtained for LAK cell activity. Thus, in spite of major multimodality therapy, the experimental group appeared to be benefiting, as LAK cells are recognized as having an anti-cancer role.[48,49] Such findings have not been documented previously. These observations, provide tangible evidence as to how relaxation and/or guided imagery may be beneficial in patients with cancer, at least in those with no evidence of overt metastases and suppression of host defences.[63,64]

To assess further the psychological interventions employed, intra-group analysis was carried out on NK cell activity. Natural cytotoxicity has an important role in preventing tumour cell dissemination and immune surveillance in animal tumour models. Its role in man, is less well established.[48,65] Repeated measures ANOVA showed that imagery ratings were strongly related to NK cell activity. Patients with high vividness ratings had significantly higher levels of natural cytotoxicity (Fig. 3 and Table 4). Fawzy et al.[66] have shown in patients with melanoma that a brief psycho-educational intervention also altered NK cell activity. Anderson et al.,[62] however, did not find an effect on CD56+ cells and NK activity. Fawzy and his group went on to demonstrate that their psychological intervention was associated with a prolongation of survival.[43] Other RCTs have found that psychological interventions prolonged survival,[67–69] although negative results have been reported.[7,9,70] Mental imaging is one of the commonest forms of alternative therapy employed in cancer management.[71]

We have previously documented significant modulation of host defences after major surgery for malignant disease in the peri-operative phase.[72] The IL1β response to surgery is short lived, with increased serum levels appearing within 2–4 h of trauma and returning to normal levels by 12 h after surgery. Soluble receptors for IL2 (produced following receptor-ligand interaction, proteolytic cleavage of the alpha subunit, and entry into the circulation) are elevated after 72 h following surgery but gradually returns to normal levels within 3 weeks of surgery. In uncomplicated surgery (for example no sepsis) TNFα is not usually detected in the serum. IL6, however, is elevated after trauma, peaking on day 2 and returning to normal levels by day 7. This was seen in sample 7 of IL6 in our study (Fig. 4).

Table 5
Serum cytokine levels: repeated measures analyses of covariance for all variable time points simultaneously

	Time			Time × intervention			Between-groups			*n*
	F	df[a]	*p*	*F*	df[a]	*p*	*F*	df	*p*	
1L1	2.559	2.26	0.078	0.914	2.26	0.413	0.365	1	0.548	68
1L2	0.874	3.00	0.455	0.276	3.00	0.837	0.673	1	0.415	70
1L4	1.342	3.00	0.262	0.854	3.00	0.466	0.299	1	0.586	70
1L6	2.344	3.00	0.074	1.803	3.00	0.148	0.008	1	0.931	70
TNFα	0.234	2.91	0.872	1.589	2.91	0.195	0.347	1	0.558	70

[a] With Huynh Feldt correction as required.

We also have documented impaired NK and LAK cell activity with significant suppression just prior to, and following, surgery with a gradual return to normal activity by seven days (NK cells) to 21 days (LAK cells). There was no fall in circulating CD16+ NK cells and a transient fall of CD56+ LAK cells.[73] Mature and activated T cells (CD3+, CD25+) have been shown to fall following surgery, but return to normal values by the tenth postoperative day. T helper cells are also known to fall in the postoperative period.[49]

Thus, the significant changes in immune parameters may have an impact on the measurements obtained from the post surgical sample but should not have had an effect on the sample obtained 4 weeks later.

A number of studies have documented the substantial and prolonged lymphopenia which results following radiotherapy.[74] Although both B and T cells are susceptible, reduction in circulating T lymphocytes is usually more pronounced and chronic; CD3+ mature T lymphocytes are more radioresistant compared with CD4+ T helper and CD8+ T cytotoxic cells. NK cells are radiosensitive, but regenerate rapidly. The critical factor inducing lymphopenia is the volume of blood irradiated.[74]

In the current study, there did not appear to be a selective reduction of circulating lymphocyte subsets and natural cytotoxicity four weeks and eight weeks post radiotherapy. However, subtle changes in immune reactivity (for example in dendritic cell function and antigen presentation) were not studied.

It is well established that anti-cancer drugs, including steroids, may have significant effects on the immune response both in inhibiting and augmenting host defences.[75] Both humoral and cellular immune functions have been shown to be inhibited to a variable degree. Suppression of natural cytotoxicity occurs following treatment with cyclophosphamide, adriamycin and vincristine, agents used in the current study (including prednisolone). There was no overt change in NK cell activity, but a tendency for a reduction in LAK cell activity. In a previous study we demonstrated a fall in NK and LAK cell activities with chemotherapy, although this was transient and cytotoxicity had returned to pre-treatment levels by the beginning of the next treatment cycle. There was no progressive decline in natural cytotoxicity documented.[76]

Studies both in man and animals have documented augmentation of certain immune responses with chemotherapy. Adriamycin, cyclophosphamide and vincristine have been shown in animals to enhance CD8+ cytotoxic cells, as well as inhibition (cyclophosphamide) of T regulatory cells, augmenting cell-mediated immune responses. Adriamycin has been documented in inducing the release of IL1β by macrophages.[74]

Chemotherapy-induced tumour cell necrosis can lead to in situ accumulation of previously cryptic tumour-associated antigens and their uptake and presentation by resident or migratory dendritic cells. The latter can generate CD8+ cytotoxic T cells in regional lymph nodes.[77] Chemotherapy-induced cellular damage also generates danger signals and release of heat shock proteins and proinflammatory cytokines such as TNFα and IL1β.[78]

Thus the multimodal therapeutic approach used in this study comprised there different forms of treatment each of which is known to have important and significant effects on host defences, as discussed above. However, as both groups had identical interventions, there is no reason to believe that there was a differential effect on host defences in the two groups.

In this study, we evaluated the effects of the intervention on a wide range of host defences. It is known that neurohormonal factors modify host defences, and we have recently reviewed this evidence.[42] However, evaluation of these parameters fell beyond the scope of this large trial.

The patients, in either group, had a low rate of psychiatric morbidity during chemotherapy (5–7% psychometric or clinical criteria used).[10] The fact that the control group also showed such a low incidence of clinically significant distress is likely to have made it particularly difficult to demonstrate between-group differences in host defences.

In conclusion, the findings demonstrate the potential of inexpensive, simple, easily taught and acquired techniques, to alter putative anti-cancer host defences despite prolonged and major medical and surgical interventions. The relative contributions of relaxation and imagery require further evaluation, and the clinical significance and possible long-term benefit in breast and other cancers need further study.

Conflict of interest statement

None declared.

Ethical approval

Ethical approval was obtained from the Joint Committee of the University of Aberdeen and Aberdeen Royal Hospitals NHS Trust.

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