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Immune Defects in Breast Cancer Patients after Radiotherapy

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Abstract

The purpose of this study was to evaluate the immune status of women with stage I–III breast cancer after receiving external beam radiotherapy (RT). Fourteen stage I–III, estrogen or progesterone receptor–positive or–negative (FER/PR +\−), postsurgical breast cancer patients undergoing a

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standard course of chemotherapy and radiation were studied. Complete blood counts (CBC) with differential, phagocytic activity, natural killer (NK) cell functional activity, and tumor necrosis factor-α (TNF-α) and interferon-γ cytokine activity were measured immediately before and for the six weeks following the completion of radiation therapy. Fatigue levels after completion of RT were measured using the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue scale. Nonparametric statistical methods (Wilcoxon rank and Spearman correlations) were used to analyze the data. Compared with postchemotherapy, following the completion of RT, these breast cancer patients showed lymphopenia, low functional activity of natural killer lymphocytes, decreased monocyte phagocytic activity, and decreased TNF-α production but no neutropenia, no anemia, and no change in interferon-γ production. Lymphocyte count did not return to normal by the end of the 6-week post-RT observation period. The severity of lymphopenia and low natural killer cell activity was related to RT area but not radiation dose. Patients did not report significant fatigue levels for the 6 weeks after completing RT.

Significant decreases in the numbers and functions of cells from both the innate and adaptive immune system were detected following a standard course of radiation therapy for the treatment of breast cancer. Immune deficits in lymphocyte populations and TNF-α production, should they persist, may have consequences for immune response to residual or recurrent malignancy following completion of conventional treatment. The use of adjunctive immune therapies which target these specific defects may be warranted in the post-treatment period.

Keywords

breast cancer; fatigue; immune defects; natural killer cell activity; phagocytic activity; radiotherapy; tumor necrosis factor

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This study evaluated the immune status of women after receiving primary treatment for stage I, II, or III breast cancer. Although limited information exists, current data suggest that breast cancer patients who have completed surgery, chemotherapy, and radiotherapy (RT) have immunologic deficits,[1–4] and in some studies, this has been associated with a poor prognosis.[5,6] For more than 20 years, it has been known that local RT for breast cancer causes long-term effects on both the adaptive and the innate immune system. External beam radiation results in reduced secretion of immunoglobulins IgM, IgA, and IgG[7]; lymphopenia for as long as a decade in some patients[8,9]; and a decrease in absolute numbers of T cells.[10,11] Low natural killer (NK) cell activity and low apoptotic cytokine levels have been associated with poor cancer prognosis.[1,4,5,7,10,12–15] However, little is known about the functional activity of NK cells and monocytes or cytokine levels in breast cancer patients following completion of RT.

The immune cells of breast cancer patients have been shown to be impaired in their ability to produce tumor necrosis factor α (TNF-α).[12] The ability to produce TNF-α and interferon-γ (IFN-γ) is associated with tumor regression and increased survival for cancer patients.[13–15] Longer disease-free survival among breast cancer patients is associated with relatively higher circulating levels of TNF-α.[16] Altered secretion of TNF-α and other inflammatory mediators has been associated with persistent posttreatment fatigue in breast cancer survivors.[17–20] Although inflammatory alterations appear to underlie persistent fatigue in breast cancer survivors, the specific relationship between TNF-α levels and fatigue has yet to be elucidated.

The data reported here fill a gap in the knowledge regarding the immune status of breast cancer patients in the first weeks after completion of surgery, chemotherapy, and RT. Knowing more about the status of breast cancer patients' innate and adaptive immune status after completing primary breast cancer treatment will assist in the development and evaluation of novel adjunctive immune therapies. Our long-term goal is the development of immunomodulatory

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therapies to be used in both the neoadjuvant and the adjuvant setting for the immune defects reported here in women who have completed RT for early-stage breast cancer.

Methods

Subjects

Complete blood counts with differential, phagocytosis activity, NK cell activity, and cytokine activity measurements were made in breast cancer patients before and after a standard course of external beam radiotherapy. Women aged 21 to 75 years with stage I, II, or III breast cancer, who had undergone surgery and chemotherapy and were scheduled to begin a standard course of RT, were invited to participate in this observational study at the University of Minnesota and Bastyr University under Institutional Review Board–approved protocols. Patients recruited into the study agreed to avoid taking any products containing known immunomodulating compounds during the 2-week period prior to RT, during RT, and during the 6-week study after RT. Fourteen subjects with newly diagnosed breast cancer were recruited between September 2005 and March 2007. Because the results were consistent among subjects, recruitment was discontinued after the enrolment of 14 participants. A sample size of 12 to 15 is considered adequate to achieve significance in describing intersubject variability.[21] See Table 1 for patient characteristics and treatments.

Protocol

Figure 1 shows the timeline of the observational study.

Complete Blood Count with Differential

The clinical laboratory tests (complete blood count, chemistry, serum pregnancy tests, and urinalysis) were performed at the Department of Laboratory Medicine at University of Washington for Seattle participants and at the University of Minnesota Fairview laboratory for participants recruited in Minneapolis. All samples were hand delivered within 4 hours of blood draw.

NK Cell Functional Activity

To ensure uniform assessment of immune response, immunologic assays were carried out at Bastyr University for subjects recruited from both sites. Blood from patients at the University of Minnesota was collected at the University of Minnesota General Clinical Research Center and shipped overnight to Bastyr University.

Owing to the possibility of blood sample transport conditions from the University of Minnesota to Bastyr University affecting the accuracy of measuring NK cell activity, a blood sample quality control assessment was included. If specimens were more than 48 hours old when they arrived at the Bastyr Tierney laboratory, the lymphocytes were isolated from the blood specimens and cell viability was determined. If the viability was greater than 80%, the assay was performed. If the viability was less than 80%, the specimens were rejected.

Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll–hypaque gradient separation, washed twice in phosphate-buffered saline, and maintained in RPMI 1640, 10% fetal bovine serum (FBS) with 2 mM L-glutamine (L-Gln) and penicillin-streptomycin (1,000 U/mL/1 mg/mL). Monocytes were depleted from PBMC samples by adherence to cell culture flasks for 60 minutes, and nonadherent peripheral blood lymphocytes (PBLs) were collected. NK cell activity of these PBL samples, as measured by the ability to kill K562 tumor target cells (an NK cell–sensitive human tumor cell line), was assessed in triplicate at the effector to target (E:T) ratios of 50:1, 25:1, and 12.5:1 following published methods.[22] Target cells were labeled with $DiOC_{18}3$ and cocultured with PBL effector cell samples for 4 hours.

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A control sample with K562 cells only was included to determine spontaneous target cell killing. Following incubation, propidium iodide was added to detect dead cells. The percentage of killed target cells was determined using flow cytometry, and percent specific lysis (PSL) was calculated at each E:T ratio. To standardize NK cell activity so that accurate comparisons were possible between PBL samples from different study participants, lytic units (LUs) were calculated by a previously published and validated software program (Whiteside TL, personal communication, 2007). LU20 values, defined as the E:T ratio at which 20% of target cell death occurs, were extrapolated from dose-response curves of PSL versus log E:T ratio for each blood sample assayed. LUs of NK cell activity, defined as the number of cells required to cause 20% target cell lysis relative to 10^7 effector cells, were determined by the equation 10^7/LU20 and thus increase with increasing lyric activity.

Phagocytic Activity

Phagocytic activity was determined as a functional assessment of polymorphonuclear (PMN) leukocytes and monocytes in whole blood samples collected from study volunteers. Whole blood samples (100 μL) were incubated in triplicate with fluorescently labeled *Escherichia coli* (K-12 strain conjugated to fluorescein isothiocyanate [FITC] and opsonized with antibodies and complement; Invitrogen, Carlsbad, CA) at 37°C for 30 minutes (maximum uptake samples) or 8 minutes (test samples) or left on ice (control). Internalization of FITC-conjugated *E. coli* by the two subpopulations was measured using flow cytometry, gating on monocyte and PMN leukocyte populations. Phagocytic activity (percentage of maximal FITC–*E. coli* uptake) was determined by the following formula:

$$\frac{\text{Mean fluorescence intensity (MFI)}_{\text{test}} - \text{MFI}_{\text{negative control}}}{\text{MFI}_{\text{maximum uptake}} - \text{MFI}_{\text{negative control}}} \times 100$$

Ex Vivo Cytokine Measurement

Blood was collected from participants at each of five time points: within 72 hours prior to the start of RT, within 72 hours following completion of RT, and at 2, 4, and 6 weeks following completion of RT. Isolated PBMCs (1×10^6 cells/mL) were incubated for 24 hours in the presence or absence of 1 μg/mL phytohemagglutinin (PHA). Each of six wells in a 24-well tissue culture plate were inoculated with 1×10^6 cells in 1 mL complete media (RPMI 1640, 2% FBS, L-glutamine, 1× Pen/Strep); three wells included 1 μg/mL PHA and three did not. Following 24-hour incubation, the supernatant was collected and centrifuged to remove cell solids. Following 24-hour incubation, the supernatant was collected and nonadherent cells were removed by centrifuge in an Eppendorf 5415c benchtop microcentrifuge (room temperature, 2 minutes, 3,500*g*). Samples were stored at −80°C until assay. IFN-γ and TNF-α concentrations were determined using enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Plates were read on a SPECTRAmax 384 PLUS plate reader (Molecular Devices, Sunnyvale, CA). Data results were analyzed using *SoftMax Pro* (2006 Molecular Devices Corp.) and *SPSS* (version 15.0; SPSS, Chicago, IL) software.

Statistical Methods

Owing to the small sample size and the nongaussian distribution of the data, nonparametric statistics were used to analyze paired data (Wilcoxon signed rank sum test or Mann-Whitney test). These tests are used to analyze paired data points that are equally likely to go in either direction (positive or negative) and do not assume gaussian distribution or address average absolute difference. Spearman correlations were calculated to detect associations between total RT dose and immune response.

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Results

Subjects

Of 27 women screened for this study, 17 met our diagnostic and temporal inclusion criteria. Of these 17 study subjects enrolled, 14 completed the preRT baseline and the 6-week postRT observational study and are included in this analysis. Four participants were seen at the University of Minnesota, and 10 were recruited from the Seattle area and seen at Bastyr University or the University Health Clinic Specialty Care and Research Center. They ranged in age from 32 to 61 years. Table 1 shows that all study subjects received both chemotherapy and RT; 21% were stage I, 36% stage II, and 43% stage III. Most (71%) had estrogen or progesterone receptor–positive breast tumors, 100% had invasive ductal carcinoma, and 15% also had ductal carcinoma in situ. *HER2/neu* overexpression was present in 23% of the participants' biopsied tissue samples. Hematopoietic growth factor therapy (erythropoietin, granulocyte colony stimulating factor [GCSF], peg-GCSF, and granulocyte-macrophage colony-stimulating factor) was used in 92% of patients. Doxorubicin, cyclophosphamide, and taxanes were the most commonly used chemotherapeutic agents. Total RT doses ranged from 60 to 65 Gy delivered over a range of 30 to 36 days of treatments. Most patients (79%) received a radiation "boost" to surgical sites and the tumor margin area. Of the 10 estrogen receptor–positive patients, 6 initiated antiestrogen hormone therapy after completion of RT, two during and two before RT.

Changes in Red Blood Cell Measures

As shown in Figure 2, the red blood cell (RBC) parameters were not affected by RT. Both total RBCs and hemoglobin were normal before or after RT. Although hematocrit at the preradiation baseline was slightly below normal (35.5%), it returned to normal by 2 weeks after completion of RT. However, none of these differences were statistically significant using a two-tailed, nonparametric Wilcoxon signed rank test.

Changes in White Blood Cell and Differential Counts Associated with RT

Figure 3A shows that WBC had recovered from chemotherapy and was normal in most of the participants (mean WBCs 4.81 thousand/μL + 2.63 SEM) just prior to starting radiation therapy. However, there was a significant decrease in mean total WBCs to 3.4 thou/μL ± 1.13 ($p = .0095$) immediately following completion of RT. WBC count had not returned to normal at 6 weeks post-RT. Basophils and monocytes, but not eosinophils, were significantly reduced after radiation but did not decline into the subnormal range.

Lymphopenia and low NK cell activity were detected immediately following RT and persisted throughout the 6-week post-RT period (Figure 3B). Post-RT lymphopenia was observed in 93% of the subjects. There was a statistically significant decline in absolute lymphocyte count following RT (Wilcoxon signed rank test $p = .0005$). Mean lymphocytes prior to RT were near normal but fell by 39% immediately following RT, declining into the subnormal range. The absolute number of lymphocytes increased within the 6-week post-RT observation period but did not return to pre-RT levels. Although neutrophil counts dropped slightly after RT, they did not drop below normal limits.

Changes in NK Cell Activity Associated with RT

NK cell lyric activity against K562 tumor targets was also decreased after RT (see Figure 3B). Mean NK cell activity was 15.7 ± 4.6 SEM LU 20 before RT and dropped to 9.01 ± 4.2 SEM LU20 immediately after completion of RT (two-tailed *t*-test; $p = .032$). NK cell activity returned to pre-RT baseline levels by the end of the 6-week post-RT observation period.

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Severity of Lymphopenia and Low NK Cell Activity Was Related to RT Area but Not Dose

Total radiation dose in the 14 subjects was similar among patients, ranging between 60 and 65 Gy, which was administered in fractions over a narrow range of 30 to 36 days. No clear relationship between loss of NK cell activity and total RT dose was detected (Table 2 and Table 3). However, loss of NK cell activity and lymphocyte counts differed when comparing patients who received RT only to the breast ($n = 6$) versus those patients who received both local and regional treatment ($n = 8$). We compared patients who received RT only to the breast ($n = 6$) versus those who received wider area locoregional RT ($n = 8$). Patients who received locoregional RT showed greater lymphopenia and NK cell activity loss following RT compared with those who received only breast RT. Figure 4 shows a mean change in NK cell activity (LU20) and absolute lymphocyte count before and after standard external beam RT. The loss of NK cell activity ($p = .025$) was statistically significant only for those patients who received wider field locoregional RT (Wilcoxon test), and RT-related depletion of lymphocytes was greater in patients who underwent locoregional RT (46% decrease) compared with patients who only had breast RT (22% decrease). These data suggest that locoregional RT, involving a larger field and more lymphatic exposure, may have more immunologic consequences compared with RT to the breast alone.

Changes in Monocyte and PMN Phagocytic Activity Associated with RT

Mean phagocytic activity of monocytes, but not PMN leukocytes, declined markedly after completion of RT (Figure 5; PMN data not shown). Despite the clear drop in the mean monocyte phagocytic activity, the difference between pre-RT baseline and the first post-RT measure taken within the first 72 hours after completion of RT, this trend in decreasing phagocytic activity was not statistically significant. Some recovery of monocyte phagocytic activity appears to occur within the 6 weeks post-RT observation period.

Ex Vivo Cytokine Measurements: TNF-α and IFN-γ

RT resulted in a measurable reduction in the capacity to produce TNF-α following PHA stimulation (Figure 6), yet IFN-γ production was unaffected. Between study participants' pre-RT and post-RT blood draw, the average adjusted TNF-α production in PHA-stimulated PBMCs decreased by 46% from 2,847.9 to 1,309.9 pg/mL ($p = .035$, Wilcoxon rank signed test, 95% confidence interval). Differences in IFN-γ production were not significant at any time point (data not shown).

Fatigue Scores Pre- and Post-RT

Using the Functional Assessment of Cancer Therapy (FACT)–Functional Assessment of Chronic Illness Therapy (FACIT) questionnaire, the breast cancer patients in this study did not report significant fatigue at any time point. The maximum score on this instrument is 52, which indicates the absence of fatigue symptoms. Mean post-RT scores over the 6 weeks following RT had a narrow range from 38 to 43.

Conclusions and Discussion

The main findings of this study are that women with stage I, II, or III breast cancer after completing RT showed (1) lymphopenia and low NK cell activity throughout the 6 weeks after the completion of RT, (2) depressed phagocytic activity of monocytes, and (3) a transient but measurable depression of TNF-α (but not IFN-γ) production by PHA-stimulated PBMCs. Patients who received RT to both breast and regional RT showed more immune deficits compared with those who received only breast RT.

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The interpretation of these data may be limited by the fact that all study subjects received myelosuppressive systemic chemotherapy prior to RT. However, mean RBCs, hemoglobin, hematocrit, and WBCs were normal at the start of RT. What is striking about the results of this study is that although the mean WBCs were in the normal range prior to starting RT, total WBC and lymphocyte populations decreased after 6 weeks after RT, as did NK cell functional activity.

Persistent lymphopenia and low NK cell activity may have consequences for risk of relapse. Low NK cell activity and low TNF-α levels are associated with increased risk of recurrent cancer.[1,4,5,7,10,12–15] Our data suggest that women may be at immunologic risk following RT. The immune defect was restricted to lymphocytes and monocytes and their functional activity. The fact that lymphocytes and monocytes were higher after chemotherapy that was completed at least 4 to 6 weeks before RT began suggests that these changes are related to a specific immunologic effect of RT. The patients in this study did not report significant changes in fatigue, despite depression of TNF-α levels in the post-RT period.

These data may shed light on potential clinical implications of the immune defects of women after standard breast cancer therapy and contribute to the development and evaluation of adjunct immune therapies in the oncologic treatment sequence. Medicinal mushrooms, especially *Trametes versicolor* (turkey tail mushroom), show therapeutic promise as immune modulators.[23–26]

This study has several limitations, including (1) a small sample size of 14 breast cancer patients who were observed just before and after RT and (2) the potential confounding effect of previous systemic chemotherapy and growth factor therapy during chemotherapy. However, a consistent pattern of immune deficits in NK cell and cytokine activity, as well as persistent lymphopenia, emerged in more than 70% of the subjects and appeared to be temporally related to RT. Ideally, a study identifying radiologic effects on immune function would include participants who only received RT as an adjuvant therapy. However, a large proportion of early-stage breast cancer patients currently receive adjuvant chemotherapy prior to RT. These data describe the immune consequence of the standard of care sequence in oncology care in Seattle and Minneapolis. The immune defects detected in these breast cancer patients were not present after completion of chemotherapy but rather occurred after the initiation of RT.

This study is the first to describe changes in multiple hematologic and immune functional and immune cell population subset parameters in breast cancer patients before and after RT for early-stage breast cancer. Although the mechanisms underlying the immunologic effects of RT are poorly understood, these results help characterize the nature of the immune defects observed during the course of conventional therapy. Both low NK cell activity and TNF-α levels have been associated with poorer prognosis and worse symptomatology in cancer patients.[27]

Lymphopenia, low NK cell activity and low phagocytic activity of monocytes, and depressed TNF-α were observed following standard external beam chest RT for breast cancer and may have consequences for anticancer immune competence in the weeks and months following completion of standard treatment. The decreases observed in NK cell activity after RT could be due to a decrease in NK cell numbers in peripheral blood and/or in NK cell functional activity. This will be an important question to address in future studies. These novel data emphasize the importance of research on immunomodulators to maintain white cell function in breast cancer patients. Immune therapies that increase lymphocyte counts, NK cell activity, TNF-α production, and phagocytic activity may be warranted in the post-RT period.

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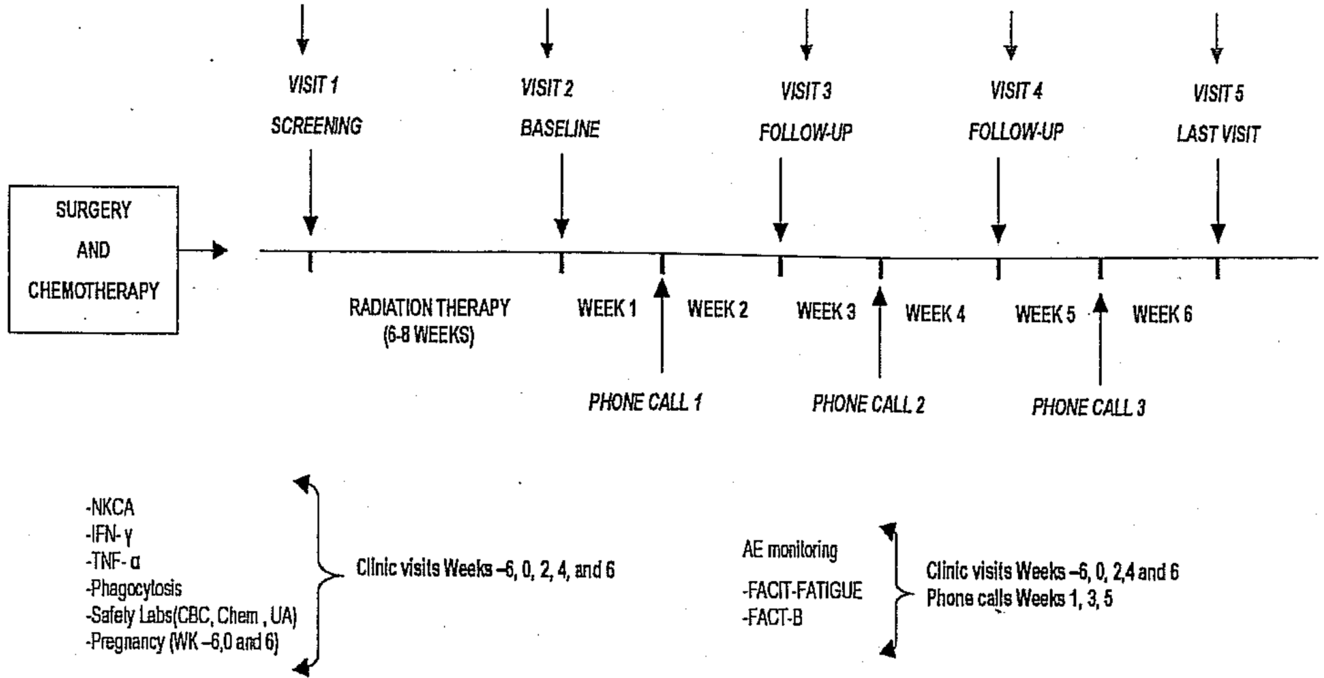

Figure 1.
Breast cancer participant study timeline. AE = adverse event; CBC = complete blood count; Chem = chemistry; FACIT = Functional Assessment of Chronic Illness Therapy; FACT-B = Functional Assessment of Cancer Therapy-Breast; IFN = interferon; NKCA = natural killer cell activity; TNF-α = tumor necrosis factor α; UA = urinalysis.

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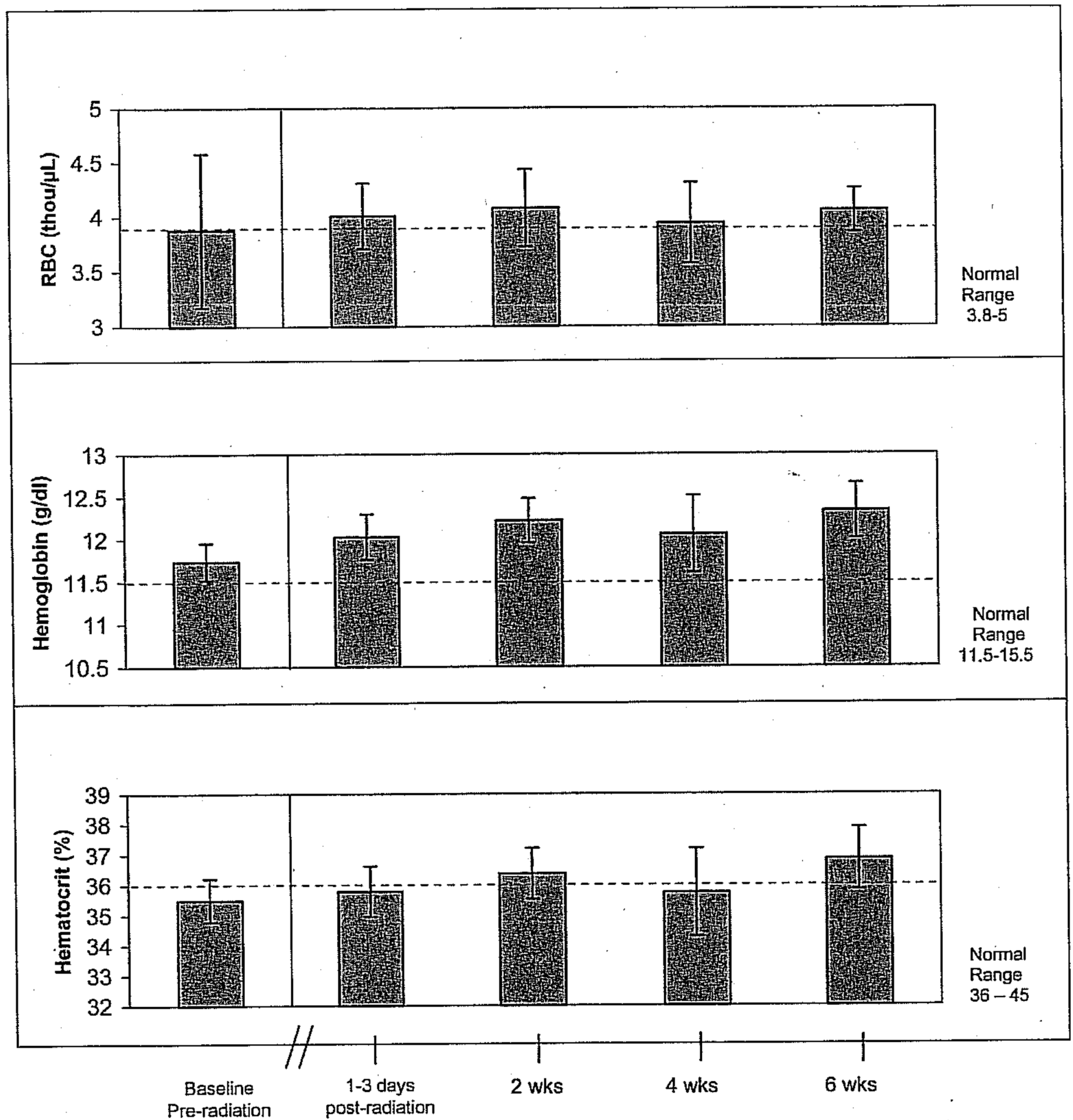

Figure 2.
Red blood cell (RBC) parameters are unaffected by radiotherapy in stage I–III breast cancer patients (n = 14).

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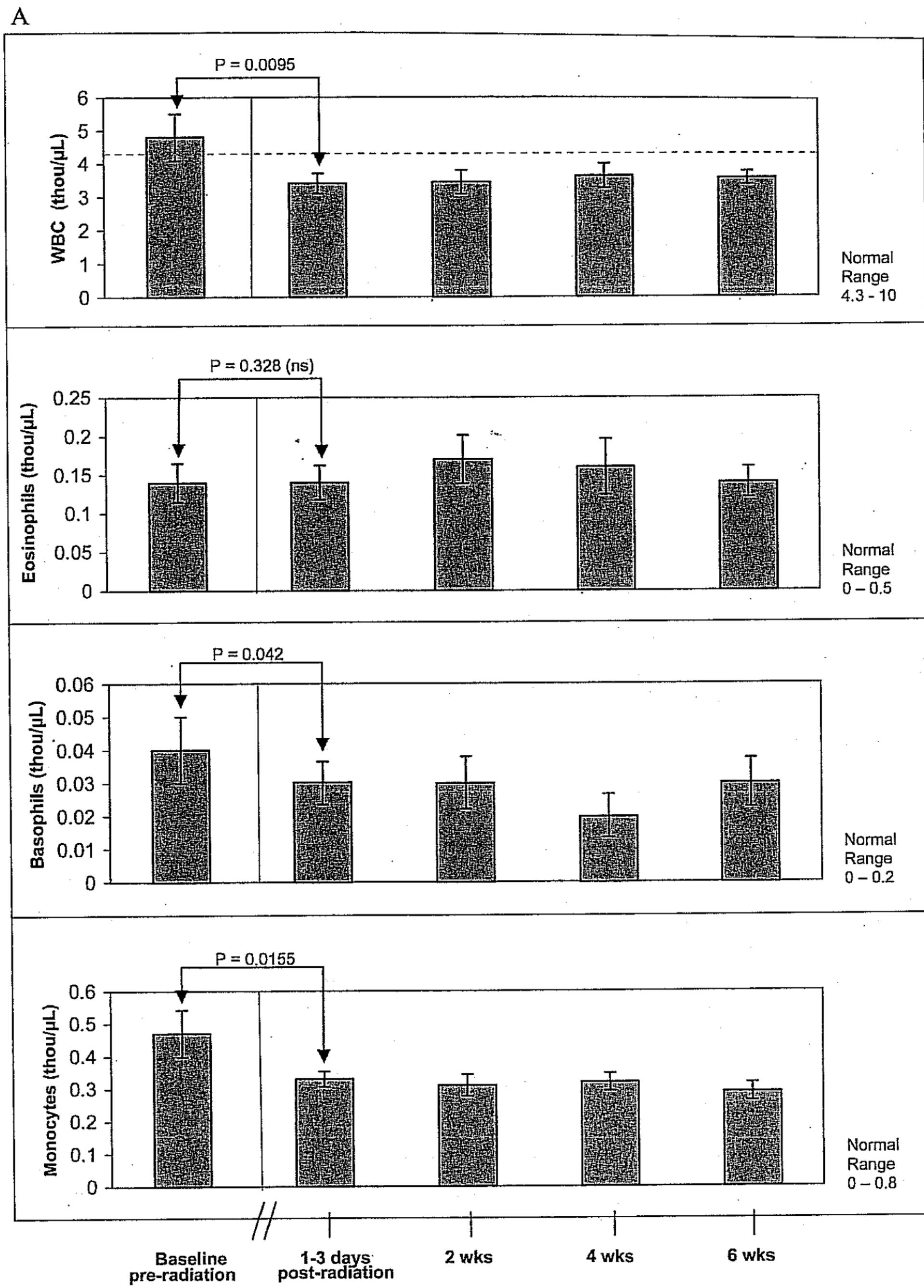

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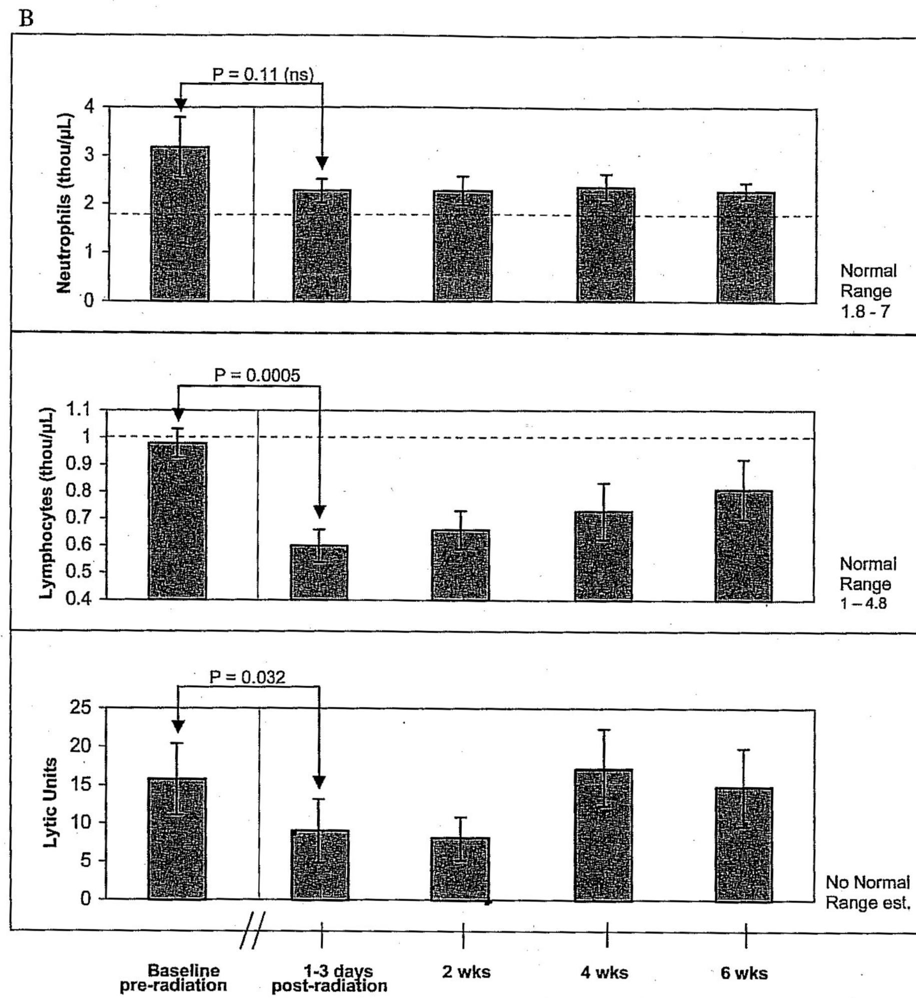

Figure 3.

A, Pre- and postradiotherapy total white blood cell and differential count for neutrophils, eosinophils, basophils, and monocytes in stage I–III breast cancer patients ($n = 14$). There were significant decreases in mean total white blood cells (WBC), monocytes, and basophils after radiotherapy (RT). WBC levels failed to return to baseline by the end of the 6-week study. *B*, Lymphocytes and natural killer cell activity pre- versus postRT ($n = 14$). Neutrophil counts decreased after RT but did not drop below normal levels. Postradiation lymphopenia and low natural killer cell activity were observed and persisted throughout the 6-week post-RT period. The *red dotted line* indicates normal ranges. Normal ranges for natural killer cell activity have not been established. ns = no significance.

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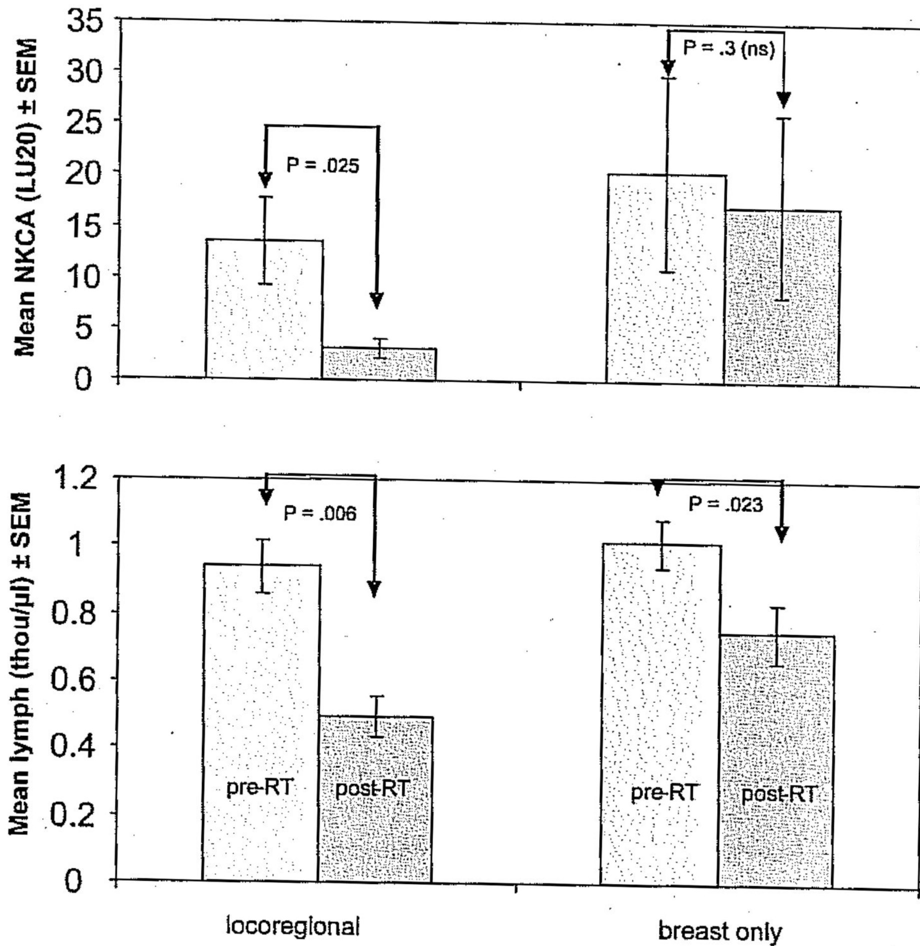

Figure 4.

Mean change in natural killer cell activity (NKCA) (LU20) and absolute lymphocyte count before and after standard external beam radiotherapy (RT). Here we compare patients who received RT only to the breast ($n = 6$) versus those who received wider area locoregional RT ($n = 8$). Patients who received locoregional RT showed greater lymphopenia and NKCA loss compared with those who received only breast RT.

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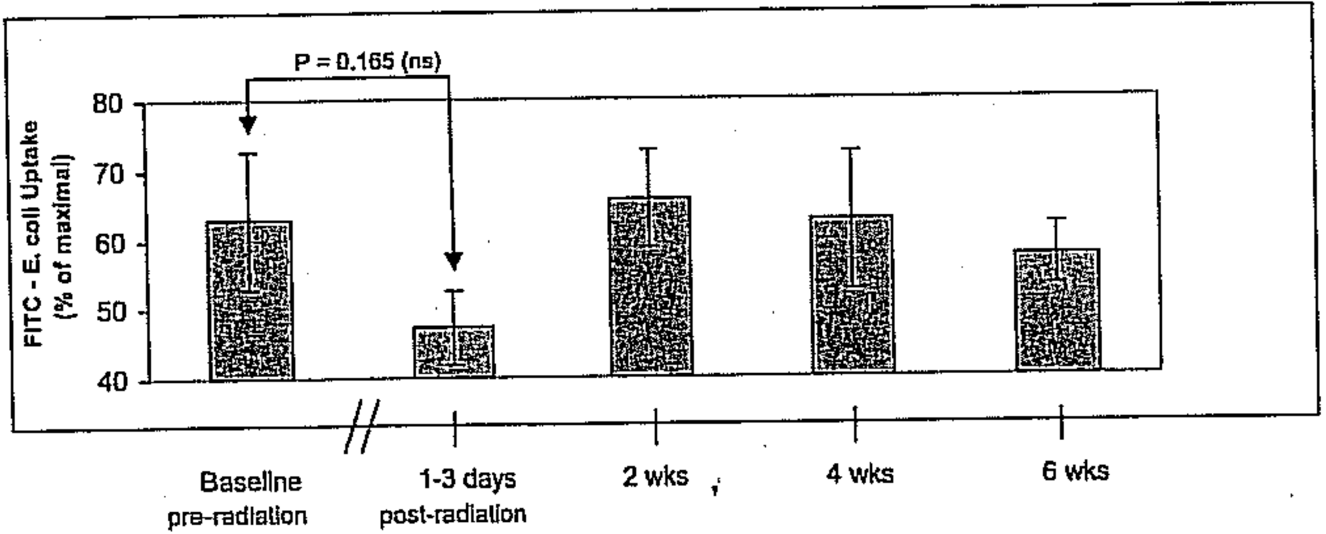

Figure 5.

Pre- and postradiotherapy mean percent phagocytic activity of monocytes showing a trend toward decreasing phagocytic activity after radiotherapy (RT). Some recovery of monocyte phagocytic activity appears to occur within the 6-week post-RT observation period. FITC = fluorescein isothiocyanate.

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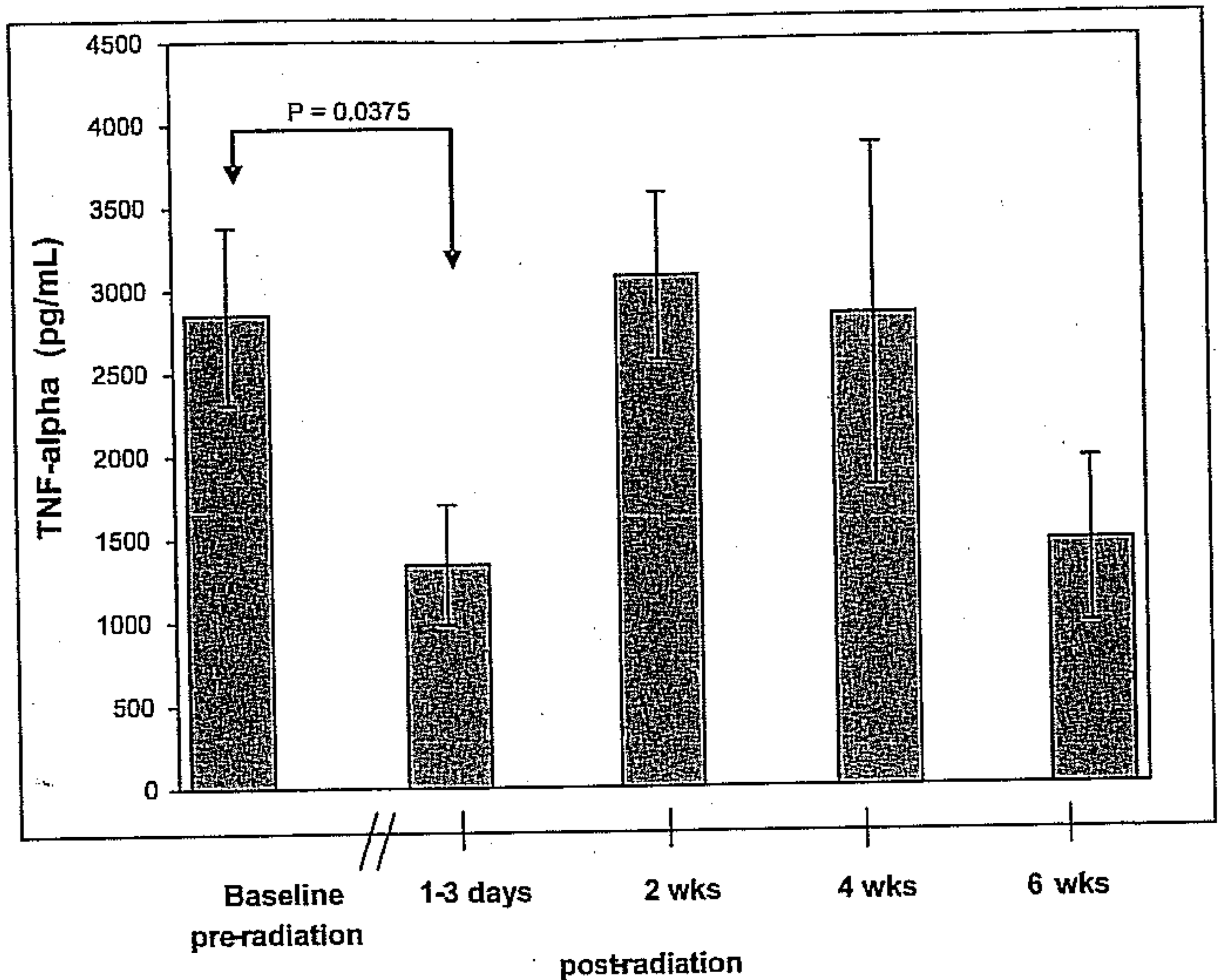

Figure 6.
PHA-stimulated tumor necrosis factor α (TNF-α) production in peripheral blood mononuclear cells at each study time point (mean ± SEM). Production levels between visit 1 (preradiation baseline) and visit 2 (postradiation) were significantly different. (When the four subjects recruited from the University of Minnesota were removed from analysis, the *p* value was greated than .05.)

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Table 1

Breast Cancer Patient Study Participants

Patient	Age (yrs)	Stage	ER	PR	HER 2/neu	Chemotherapy Protocol	Hematopoietic Drugs	Hormonal Therapy and Initiation Timeline	Dose (cGy) and Location	Total Dose (cGy)
1	42	2	Y	Y	N	Doxorubicin	EPO	Anastrozole, letrozole	Breast	6,000
						Cyclophosphamide	Filgrastim	initiated pre-RT	5,000 to L chest wall	
						Paclitaxel			1,000 boost to surgical scar	
2	37	1	N	N	N	Doxorubicin	Pegfilgrastim	None	Breast	6,400
						Cyclosphosphamide			5,000 to R tangent breast	
						Paclitaxel			1,400 boost to R tangent breast	
3	42	3	N	N	P	Doxorubicin	Pegfilgrastim	None	Locoregional	6,040
						Cyclophosphamide			5,040 to whole R breast	
						Paclitaxel			1,000 intramammary boost	
						Trastuzumab			5,040 to IMC	
									5,040 to R SCF	
4	38	3	Y	Y	N	Doxorubicin	None	Letrozole initiated	Locoregional	6,040
						Cyclophosphamide		post-RT	1,000 to mastectomy scar	
						Paclitaxel			5,040 to R SCF	
									4,500 to PAB	
									5,040 to R CW tangents	
									5,040 R IM electron field	
5	32	1	Y	N	I	Doxorubicin	Filgrastim	Tamoxifen initiated	Breast	5,400
						Cyclophosphamide	Epoetin alfa	post-RT	5,400 to LCW	
						Paclitaxel			4,680 to L SCF	
						Trastuzumab				
6	52	1	Y	Y	N	Doxorubicin	GM-CSF	Tamoxifen initiated	Breast	6,640
						Cyclophosphamide		during RT	1,600 scar boost	
									5,040 to UOQ of L breast	
7	35	2	Y	Y	N	Doxorubicin	EPO	Tamoxifen initiated	Breast	6,000
						Cyclophosphamide	Pegfilgrastim	post-RT	5,000 to R breast	

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Patient	Age (yrs)	Stage	ER	PR	HER 2/neu	Chemotherapy Protocol	Hematopoietic Drugs	Hormonal Therapy and Initiation Timeline	Dose (cGy) and Location	Total Dose (cGy)
						Paclitaxel			1,000 to tumor bed and excisional bx scar	
8	39	2	N	N	N	Doxorubicin	Pegfilgrastim	None	Locoregional	6,000
						Cyclophosphamide			4,600 to L breast IM	
						Paclitaxel			4,600 to L SCF/axilla	
									690 to PAB	
									1,400 boost to tumor bed	
9	49	3	Y	Y	N	Doxorubicin	Pegfilgrastim	Letrozole initiated post-RT	Locoregional	6,640
						Cyclophosphamide			6,640 to L chest wall, axilla, and paraclavicular region and scar margins	
						Docetaxel				
10	47	3	Y	Y	N	Doxorubicin	Filgrastim	Tamoxifen initiated post-RT	Locoregional	6,040
						Cyclophosphamide	Epoetin alfa		6,040 to R chest wall	
						Paclitaxel			5,040 to paraclavicular region	
11	60	2	N	N	P	Doxorubicin	Pegfilgrastim	None	Breast	6,300
						Cyclophosphamide			1,260 boost to lumpectomy site	
						Paclitaxel			5,040 to R breast	
12	59	3	Y	Y	N	Doxorubicin	None	Anastrozole initiated pre-RT	Breast	6,440
						Cyclophosphamide			1,440 boost to L chest wall scar	
						Paclitaxel			5,080 IM	
						Gemcitabine			5,040 to L breast	
									5,040 to L supraclavicular and axillary area	
13	42	2	Y	Y	P	Doxorubicin	Pegfilgrastim	Tamoxifen initiated post-RT	Breast	6,440
						Cyclophosphamide			1,400 boost to lumpectomy scar	
						Docetaxel			5,040 to R breast	
14	61	3	Y	Y	I	Doxorubicin	Pegfilgrastim	Anastrozole initiated during RT	Locoregional	6,520
						Cyclophosphamide			5,080 IM	
						Docetaxel			5,040 to L chest wall	
									1,400 boost to L chest wall	
									5,040 to L supraclavicular and	

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Patient	Age (yrs)	Stage	ER	PR	HER 2/neu	Chemotherapy Protocol	Hematopoietic Drugs	Hormonal Therapy and Initiation Timeline	Dose (cGy) and Location	Total Dose (cGy)
									axillary area	

bx = biopsy; CW = chest wall; EPO = erythropoietin; ER = estrogen receptor; GM-CSF = granulocyte-macrophage colony-stimulating factor; I = indeteminant; IM = internal mammary; IMC = internal mammary chain; LCW = left chest wall; N = negative; P = positive; PAB = posterior axillary boost; PR = progesterone receptor; RT = radiotherapy; SCF = supraclavicular field; UOQ = upper outer quadrant.

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Table 2

Effect of Radiation Dose on Lymphocyte Counts (thou/μL)

Dose (cGy)	Stage	Surgery	HER2	Pretreatment Lymphocytes	Posttreatment Lymphocytes	Δ Lymphocytes
5,400	1	M	I	0.7	0.34	−0.36
6,000	2	M	N	1.07	0.84	−0.23
6,000	2	L	N	1.22	0.68	−0.54
6,000	2	M	N	0.85	0.62	−0.23
6,040	3	M	P	1	0.57	−0.43
6,040	3	M	N	0.83	0.25	−0.58
6,040	3	M	N	1.24	0.79	−0.45
6,300	2	L	P	0.7	0.5	−0.2
6,400	1	L	N	1.06	0.8	−0.26
6,440	2	L	P	1	1.1	0.1
6,520	3	L	N	1.1	0.5	−0.6
6,520	3	M	I	0.7	0.5	−0.2
6,640	1	L	N	1.04	0.58	−0.46
6,640	3	M	N	1.25	0.39	−0.86

I = indeterminant; L = lumpectomy; M = mastectomy; N = negative; P = positive.

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Table 3

Effect of Radiation Dose on Natural Killer Cell Activity

Dose (cGy)	Stage	Surgery	HER2	Presentment LU20	Posttreatment LU20	Δ LU
5,400	1	M	I	17.45	0.78	−16.67
6,000	2	M	N	48.63	53.52	4.89
6,000	2	L	N	11.42	1.74	−9.68
6,000	2	M	N	20.82	2.51	−18.31
6,040	3	M	P	9.20	1.08	−8.12
6,040	3	M	N	37.09	3.8	−33.29
6,040	3	M	N	3.72	1.94	−1.78
6,300	2	L	P	5.96	9.26	3.3
6,400	1	L	N	50.79	34.61	−16.18
6,440	2	L	P	0.26	1.45	1.19
6,520	3	L	N	3.66	1.45	−2.21
6,520	3	M	I	3.47	2.49	−0.98
6,640	1	L	N	4.93	2.16	−2.77
6,640	3	M	N	2.54	9.35	6.81

I = indeterminant; L = lumpectomy; LU = lytic units; M = mastectomy; N = negative; P = positive.

Lytic units = number of natural killer cells required to cause 20% target cell lysis relative to 10^7 effector cells (K562), determined by the equation 10^7/LU 20.