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Significant Impairment in Immune Recovery Following Cancer Treatment

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Abstract

Background—Although immunosuppression from cancer adjuvant therapy has been documented, how these suppressed immune responses recover to baseline values after completion of cancer adjuvant therapy has not been studied systematically.

Objectives—To examine the probability of immune recovery following cancer adjuvant therapy and the potential impact of cancer adjuvant therapy type and cancer stage on immune recovery in newly diagnosed breast cancer patients.

Method—In a repeated-measures design, immune responses were measured 4 times in 80 early stage breast cancer patients: prior to, and at 2, 6, and 12 months from the beginning of cancer adjuvant therapy. Natural killer cell activity (NKCA), lymphokine-activated killer cell activity, lymphocyte proliferation, CD subsets (CD4, CD8, and CD56), and cytokines (IFN-γ, IL-2, IL-4, IL-6, and IL-1α) were selected for their relevance to breast cancer. Immune recovery was defined by the level of immune response reaching to and above baseline levels. Data were analyzed using a multivariate generalized linear mixed model approach.

Results—Delayed immune recovery to pretreatment baseline levels continued to the 12-month time point in all parameters. The percentages of immune recovery ranged from 6% to 76% of the patients, varying among immune parameters. Overall, immune recovery was poorer for IFN-γ, IL-2, IL-4, lymphocyte proliferation and NKCA than for CD subsets and IL-6. The type of cancer adjuvant therapy, not cancer stage, showed selective influence on immune recovery. Chemotherapy or chemo- and radiotherapy combination significantly delayed IL-2 recovery, whereas radiotherapy significantly delayed IL-4 recovery.

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Discussion—Immune recovery following breast cancer adjuvant therapy is delayed significantly for an extended time period in numerous immune parameters. The type of cancer adjuvant therapy has selective influence on immune recovery. Future investigations are warranted to elucidate the time course of immune recovery, clinical significance of poor immune recovery, and factors influencing immune recovery in order to develop potential interventions.

Keywords

immune recovery; breast cancer; cancer treatment

With the exception of skin cancer, breast cancer (BC) is the most common type of cancer and the second leading cause of death from cancer in women in the United States (American Cancer Society, 2007). Treatments for BC include surgery, chemotherapy, radiotherapy, and hormonal and targeted therapy, often combining two or more modalities. With advances in early diagnosis and treatment, the overall survival rates are 88% at 5 years and 80% at 10 years for all stages of BC, and BC is now considered to be a chronic disease (American Cancer Society, 2007). Accordingly, increasing attention has been paid to the long-term effects of BC diagnosis and treatment. However, most attention has been paid primarily to psychosocial outcomes but not to physiological responses.

The immune system is a major regulatory mechanism in the defense of the body. Although cancer diagnosis and adjuvant chemotherapy are known to alter immune responses significantly (Gardner, 1999; van der Most, Currie, Robinson, & Lake, 2006), surprisingly little has been investigated about how altered immune responses recover and how the type of cancer therapy and cancer stage interact with immune recovery over time. These topics may bear even greater significance, given the fact that BC patients are found to have lower baseline immune responses than healthy counterparts (Campbell, Scott, Maecker, Park, & Esserman, 2005; Caras et al., 2004; Konjevic & Spuzic, 1993). Furthermore, poor immune recovery may increase a person's susceptibility to adverse health outcomes. There is evidence that poor recovery of lymphocyte proliferation and lymphocyte count predicted greater disease recurrence in patients with early stage BC (Wiltschke et al., 1995) and poorer disease-free and overall survivals in metastatic BC patients (Nieto et al., 2004; Porrata, Ingle, Litzow, Geyer, & Markovic, 2001).

Immune parameters of this study were selected for the relevance to BC: Natural killer (NK) cell activity, lymphokine-activated killer (LAK) cell activity, CD subsets, lymphocyte proliferation, and cytokines. The NK cells participate in the resistance to and the control of malignancies (Brittenden, Heys, Ross, & Eremin, 1996; Pross & Lotzova, 1993; Whiteside & Herberman, 1995). This NK cell activity was reduced incrementally with advances in BC stage (Konjevic & Spuzic, 1993), and lower levels of NK cell activity were associated with a lack of cortisol diurnal variation, which then predicted a shorter survival in women with breast cancer (Sephton, Sapolsky, Kraemer, & Spiegel, 2000). The LAK cell activity was a sensitive predictor for emotional outcomes in BC patients (Sachs et al., 1995); cytokines, interleukin (IL)-2 and interferons (IFNs) are known to enhance NK cell activity (Sinkovics & Horvath, 2005), but IL-2 was found to be significantly lower in BC patients than in healthy controls prior to cancer treatment (Elsasser-Beile, von Kleist, & Gallati, 1991; Elsasser-Beile, von Kleist, Sauther, Gallati, & Monting, 1993). Lower levels of IL-2 were a predictor for a shorter survival time (Lissoni, Barni, Rovelli, & Tancini, 1991) and increased risk for relapse in BC (Arduino et al., 1996). Similarly, decreased IFN-γ production was correlated significantly with tumor burden (Elsasser-Beile et al., 1993). In contrast, elevated serum IL-6 and tumor necrosis factor (TNF)-alpha levels were significant predictors of shorter disease-free survival and overall survival in metastatic BC patients (Bachelot et al., 2003; Bozcuk et al., 2004).

Furthermore, lymphocyte proliferation was associated inversely with positive nodal status but was impaired in 58% of BC patients (Head, Elliott, & McCoy, 1993).

Although these findings clearly indicate the relevance of cellular immune responses to clinical outcomes in breast cancer, recent studies have been focused largely on molecular or genetic markers of cancer, paying little attention to the significance of cellular immune responses. As a result, it is mostly unknown how and when immune recovery occurs following cancer treatment. In one recent study, it was indicated that impairments in enumerative and functional immune responses persisted for at least 6-12 months following high-dose chemotherapy, although the percentage of patients demonstrating intact immune responses seemed to increase gradually with increasing time (Avigan et al., 2000).

As a first step toward a better understanding of the role of cellular immune responses in long-term clinical outcomes of cancer, the purposes of this study were to: (a) examine how immune responses recover following cancer adjuvant therapy over the first year of cancer diagnosis and treatment and (b) determine what effects the type of cancer adjuvant therapy and cancer stage have on immune recovery in early stage (I-III) BC patients.

The hypothesis was: (a) the probability of immune recovery to or above baseline levels would increase over time after cancer adjuvant therapy; (b) immune recovery over time would differ among the three types of cancer adjuvant therapy (chemotherapy, radiotherapy, or chemotherapy + radiotherapy); and (c) immune recovery over time would differ between two cancer stages (stage 1-2 vs. stage >2). Data were collected as part of a parent study, which was designed to examine the effects of an 8-week integrated intervention of cognitive behavioral modification and exercise training on psychosocial, immune, and symptom outcomes in newly diagnosed BC patients. The effects of intervention were controlled for in the analyses of this study.

Methods

Design

The design of the parent study was a randomized clinical trial with a pre- and post-intervention (8 weeks) design with four repeated measures in women with newly diagnosed breast cancer. Data were collected once before the intervention (baseline), and at 2, 6, and 12 moths from the start of the intervention. Because intervention was coincided with the start of cancer adjuvant therapy, the data in this study represent the baseline data before the start of cancer adjuvant therapy, and post-adjuvant therapy data at 2, 6, and 12 months from the start of cancer adjuvant therapy.

Participants

A total of 1,127 women with breast cancer were screened at the interdisciplinary and other breast clinics within a university health system in the Southeastern region of the United States. Two hundred and thirteen patients were eligible for the study, and 100 patients enrolled in the study. Most women were not eligible for the study because they were not newly diagnosed, were already in the middle of cancer adjuvant therapy, or lived in different cities preventing from participation in the intervention if they were randomized into the intervention group. Among the eligible women, the two most common reasons for declining the invitation to participate were “not having enough time to participate in the study” and “not interested in participating in the study.” The parent study sample size of 90 women (45 women in each group) was based on having a minimum of 80% power to detect differences in NK cell activity (a major immune parameter) between treatment groups, and is not applicable to the aims of these secondary analyses. Since sample size was determined by the parent study for this

secondary analysis, a post-hoc power analysis was performed in order to identify the minimum effect size that could be identified given the available sample. PASS 2008 (Hintze, 2008) was used to perform the calculations, based on the F distribution, which is the test statistic employed for the generalized linear mixed model analyses utilized. Keying on the Time main effect, a . 287 effect size would provide 80% power, given a 5% Type I error rate.

Inclusion criteria were: (a) women 30 years or older with stage I-III BC and receiving chemotherapy and/or radiotherapy; (b) absence of a defined psychosis; (c) no uncontrolled cardiopulmonary or other serious medical conditions that would prohibit moderate intensity exercise; (d) not participating in other structured support or exercise program; (e) ability to comprehend and follow instructions, speak and respond in English; and (f) obtaining medical clearance from a primary medical oncologist to participate in the study. Exclusion criteria were: (a) pregnancy; (b) distance preventing from weekly participation in intervention; (c) diffuse bony metastasis with high risk of pathologic fractures; and (d) lack of access to a telephone. After obtaining written informed consent, patients were stratified first by cancer stage (stage I-IIB vs. III) and randomized into either intervention or wait-list control group using a computer-generated randomization table. The 8-week integrated intervention was implemented at the start of adjuvant therapy, and patients were encouraged to continue their learned activities at home at the completion of the intervention. The control group received usual standard cancer care. Eighty women completed the study: 10 women from each group withdrew from the study for reasons, such as chemotherapy side effects (4), time commitment (2), and other family and personal issues (14). The protocol was approved by the Institutional Review Board.

Data Collection Procedure

Participants were recruited by posting flyers in the clinic waiting areas, word of mouth, and invitations from clinic nurses, physicians, and research team members. The purpose and procedure of the study, what was expected from them, a need for random assignment, when and how data were to be collected, and potential risks and benefits of the study were explained fully, and questions were answered and clarified prior to obtaining the consent. Consent was obtained at the clinic if the participant preferred to do so. Otherwise, participants took the consent form home and mailed the consent later to the study coordinator. The study was designed in a way that most blood samples could be collected at the time of participant's routine blood work: Additional amount of blood was drawn for the study at the clinical laboratory. If this was not possible, the study coordinator who was a registered nurse traveled to the place preferred by the patient (e.g., home or office) and collected the blood samples by using routine venipuncture technique.

Immune Measurements

Blood samples for immune measurements were collected prior to the start of adjuvant therapy (chemotherapy, radiotherapy, or both) as baseline, and at 2, 6, and 12 months after the start of adjuvant therapy. Baseline samples were collected at least 2-3 weeks after initial surgery to control for any surgery-related impact on immune responses. All blood samples (20 ml) were collected between 0800 and 1200 into heparin-containing vacutainers. All assays were performed following well-established immune assay protocols.

Cell separation—Mononuclear cells (MNCs) were separated using the Ficoll-Hypaque (1.077 sg; Sigma) density-gradient method. MNCs were washed twice with sterile phosphate-buffered saline (PBS without Ca^{2+} and Mg^{2+}, pH 7.4; Gibco), centrifuged at 450 g for 10 min, and were resuspended in complete RPMI 1640 (supplemented with HEPES 25 mM, L-glutamine 2 mM, 50 U Penicillin and 50 μg Streptomycin/ml) at the concentration of 2×10^6

cells/ml. This method has typically yielded more than 98% cell viability (Kang, Coe, & McCarthy, 1996; Kang, Coe, McCarthy, & Ershler, 1997).

Natural killer cell and lymphokine-activated killer cell activity—The standard chromium-51 (Cr-51) release cytotoxicity assay was used using K562 target cells (Kang et al., 1997). Target cells were labeled with 125 μCi Cr-51 for 1 hr at 37° C, were washed and centrifuged twice, and were resuspended at 4×10^4 cells/ml. The MNCs from patients were incubated in triplicate with labeled K562 target cells and 60% heat-inactivated pooled human serum in four effector-to-target cell (E:T) ratios--100:1, 50:1, 25:1, and 12.5:1. Spontaneous and maximal lysis was determined by incubating target cells with medium alone and 10% sodium dodecyl sulfate solution. Following a 4-hr incubation in 5% CO_2 at 37° C, cytotoxicity was determined from supernatant using a gamma counter. Cytotoxicity was calculated as follows: NK cell activity (%) = [(sample release - spontaneous release)/(maximum - spontaneous release)] × 100. The assay sensitivity was ≥ 0.1%, with 2-6% intra-assay coefficient variation in previous work.

For LAK cell activity, a protocol modified from studies by Brenner et al. (Brenner, Gryllis, Gornitsky, Cupples, & Wainberg, 1991) and Nagao et al. (Nagao, Yabe, Xu, & Okumura, 1995) was used: MNCs (1×10^6 cells/ml) were incubated in complete RPMI medium containing 10% fetal calf serum and 100 U/ml recombinant IL-2 for 3 days in a humidified air of 5% CO_2 at 37° C. Next day, LAK cells were washed twice in complete RPMI medium, cells were counted, and the same Cr-51 NK cell assay protocol was used (Brenner et al., 1991). The assay sensitivity and variation remains the same.

Lymphocyte proliferation—The MNCs (1×10^5) were cultured in triplicate with 60% heat-inactivated pooled human serum and phytohemagglutinin at 5 and 10 μg/ml. Following 54 hr-incubation in humidified air with 5% CO_2 at 37°C, cells were pulsed with 1 μCi of tritiated thymidine, incubated for additional 18 hr, harvested onto glass-fiber filters (MASH harvester, Otto Hiller, Madison, WI) and were counted by a liquid scintillation counter (Packard Tricarb 300 CD, Downers Grove, IL). The results indicate the net counts per minute controlling for unstimulated cell counts.

Cytokine production—Whole blood cell culture assay was used, which is thought to be a better method to reflect the *in vivo* condition than using the isolated MNC method. Blood was diluted 1:10 with the complete RPMI medium and was incubated for 4 days at 37°C with 5% CO_2 with phytohemagglutinin 10 μg/ml and lipopolysaccharide 2.5 μg/ml. Culture supernatant was collected and stored in aliquots at -80° C until assayed. Cytokine levels were determined by a standard two-step sandwich enzyme-linked immunosorbant assay (ELISA) using commercial kits. The assay sensitivity for cytokines has been reported to be 0.04 - 5 pg/ml with intra- and interassay % coefficients of variation being 4 - 9.6% for all cytokines (Biosource, San Francisco, CA; R & D Systems, Minneapolis, MN).

Cell subsets—Direct immunofluorescence method was used. Cells suspended in PBS supplemented with 0.1% sodium azide were stained using fluorescein (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies against CD4, CD8, CD56, and CD3+ (pan T cells) surface markers. Cells were incubated on ice for 30 min, washed with PBS, and analyzed using a flow cytometer (FACScan, Becton-Dickinson, Franklin Lakes, NJ) at the FACS Shared Core Facility on campus.

Statistical Analysis

The purpose of this study was to evaluate immune recovery, relative to pretreatment levels, after breast cancer adjuvant therapy. Of particular interest was (a) immune recovery over time

and (b) how different cancer adjuvant therapy types and cancer stage might affect recovery, after controlling for selected covariates which might influence recovery. The response variable, Immune Recovery to baseline or above, was binary in nature and was evaluated at 2, 6, and 12 months after baseline. Immune Recovery was coded as 1 if immune response was equal or greater than the baseline value, and as 0 if immune response was less than the baseline value. The analysis method, therefore, included (a) within-subjects (repeated measures), (b) between-subjects effects, (c) covariates, and (d) dichotomous response variables. A Generalized Linear Mixed Models (GLMM) approach, as implemented in SAS PROC GLIMMIX (SAS Institute Inc., 2005), was used to analyze the data. This approach accommodates the binomial distribution of the response variables through use of a logit link function, models dependencies produced by the repeated measures, and incorporates time-varying covariates and factors (Little, Schnabel, & Baumert, 2000; McCullagh & Nelder, 1997; McCulloch & Searle, 2001). Multivariate models were examined for NK, LAK, and lymphocyte proliferation immune parameters to accommodate the multiple target-to-effector ratios or stimulations used for those parameters.

Each model was composed of first-order type of cancer adjuvant therapy (chemotherapy, radiotherapy, or both), cancer stage (Stage 2 or less, greater than 2), hormone replacement (no, yes), menopause (pre-, peri, postmenopause), and time (2, 6, and 12 months) effects, as well as all significant second-order effects involving the time factor. Models were of the form $\eta = \mathbf{X\beta} + \mathbf{Z\gamma}$, where $\mathbf{X\beta}$ is the fixed model component, composed of a matrix of predictor values ($\mathbf{X}$) and vector of estimated regression weights ($\boldsymbol{\beta}$). The $\mathbf{Z\gamma}$ term is a random term, representing the overdispersion effect. For example, the predictor ($\mathbf{X}$) effects contained in the model for CD4% included Time, Age, Cancer Adjuvant Cancer Therapy, Cancer Stage, Hormone Replacement, Menopause, and the Time by Cancer Adjuvant Cancer Therapy interaction. A random multiplicative over-dispersion parameter was incorporated into each model. The GLIMMIX default estimation method (residual pseudo-likelihood with a subject-specific expansion) was employed. Residuals were examined for conformity to assumptions, and model fit statistics were examined to determine best fit covariance structure. Predictor collinearity was examined, and all Variance Inflation values were below 1.3, indicating no problems in terms of collinearity.

Probability of immune recovery was estimated using $\widehat{\pi}_{\text{Recovery}_k} = \left(1+e^{-\widehat{\eta}_k}\right)^{-1}$ where $\sim \widehat{\eta}_k \sim$ is the model-estimated (that is, least-squares) estimate for the logit response for level k of the effect of interest (that is, cancer treatment or time), after controlling for all other variables in the model (SAS Institute Inc., 2005). Statistically significant interactions were followed up with simple main effects analyses. Missing values were relatively limited to 5 – 13 across all time points and all immune parameters and were not included within analyses. Significance level was set at 0.05, with adjustment for multiple comparisons using the SIMULATE adjustment option implemented within PROC GLIMMIX (SAS Institute Inc., 2005).

Results

Participant Characteristics

Demographic characteristics and medical information of the participants are presented in Tables 1 and 2. Participants had a mean age of 49.5 years and tended to be Caucasian (75%), postmenopausal (57.5%) and working full-time or part-time (81.3%). Participants were overweight on average (mean body mass index = 28.8), had stage I-III BC, and most frequently received both chemo- and radiotherapy. Typical regimens of chemotherapy included about 40% of the patients receiving Adriamycin 60mg/m^2 + Cytoxan 600 mg/m^2 together every 2-3 weeks for 4 doses and about 53% of the patients receiving either Adriamycin 60mg/m^2, Taxol 145mg/m^2, and Cytoxan 600 mg/m^2 sequentially every 2-3 weeks for 3 doses each, or

Adriamycin 50mg/m^2 + Cytoxan 500 mg/m^2 together every 2-3 weeks for 4 doses followed by Taxotere 75mg/m^2 every 2-3 weeks for 4 doses. Type of chemotherapy regimen influence on immune recovery was examined, but there was not a sufficient sample to statistically analyze three different chemotherapy regimens. On the other hand, typical radiotherapy included a total dose of 45-65 Gy over 6 weeks with 15-20 Gy boost dose toward the end.

Immune Recovery over Time

The first hypothesis was that the probability of immune recovery to or above baseline levels would increase over time after cancer adjuvant therapy. First, the percentages of the participants with immune recovery to baseline or above ranged from 17% to 62% at 2 months, 11% to 76% at 6 months, and 6% to 76% at 12 months, varying among immune parameters (Tables 3 and 4). Accordingly, for CD4%, IFN-γ, IL-2, IL-6, LAK cell activity, and lymphocyte proliferation responses, the probability of immune recovery was significantly different across three different time points, $p \leq .05$ (Table 5). The IL-2 and lymphocyte proliferation responses, for example, showed further decrement in the probability of immune recovery at 12 months than earlier time points, whereas IL-6 showed a higher probability of recovery at 12 months. Immune recovery of CD4%, IFN-γ, and LAK cell activity differed across three time points in subgroups of women based on the type of cancer adjuvant therapy received. For example, CD4% immune recovery was significantly lower at 12 months in women who received chemotherapy, whereas immune recovery of LAK cell activity was significantly higher at 12 months in women who received both chemotherapy and radiotherapy (Table 5). Therefore, this hypothesis was supported only partially.

Immune Recovery by the Type of Cancer Adjuvant Therapy

The second hypothesis was that immune recovery over time would differ among the three types of cancer adjuvant therapy (chemotherapy, radiotherapy, or chemotherapy and radiotherapy). The type of cancer adjuvant therapy had significant effects on the probability of IL-2 and IL-4 immune recovery across all three time points (Table 6). The recovery of IL-2 responses was significantly poorer in women who received chemotherapy or both chemotherapy and radiotherapy than women who received radiotherapy only. In contrast, the recovery of IL-4 responses was significantly poorer in women who received radiotherapy than women who received chemotherapy or both chemotherapy and radiotherapy. For the recovery of CD4% and LAK cell activity, the type of cancer adjuvant therapy had significant impact on immune recovery at only the 12-month time point: The recovery of CD4% and LAK cell activity was significantly poorer in women who received chemotherapy than women who received radiotherapy or both chemotherapy and radiotherapy. For other immune parameters tested in this study, the type of cancer adjuvant therapy did not show significant impact on immune recovery at any time point. Therefore, this hypothesis was supported partially.

Immune Recovery by Cancer Stage

The third hypothesis was that immune recovery over time would differ between two cancer stages (stage 1-2 vs. stage > 2). The probability of immune recovery to baseline or above was not significantly different by cancer stage for any immune parameters (Table 7). In all immune parameters tested in this study, the probability of immune recovery was similar between women with breast cancer stage ≤ 2 and women with breast cancer stage >2 at any time point. Therefore, this hypothesis was not supported.

Discussion

The findings of this study indicate that immune recovery to pretreatment levels within the first year of cancer adjuvant therapy is impaired significantly in large proportions of BC patients. Furthermore, there is considerable variability in the extent of immune recovery among different

immune parameters and patients. Not surprisingly, the type of adjuvant therapy showed significant effects on immune recovery, but those effects also varied based on immune parameters. Cancer stage, on the other hand, did not show any significant effects on immune recovery in this study, perhaps because we did not have the patients with advanced metastatic disease.

The percentages of the patients with immune recovery to baseline or above ranged from 6% to 76% across all three time points in this study. Significant delays in immune recovery and large variability persisted at 12 months, which was at least 6-10 months after the completion of any adjuvant therapy. It is interesting to note that enumerative parameters of CD subsets (with the exception of CD4% in chemotherapy patients) and a proinflammatory cytokine, IL-6, seem to recover faster than other parameters that are better known to be a tumor-defense mechanism (NK cell activity) and cytokines that enhance tumor-defense mechanism (IFN-γ and IL-2). The differential speed of recovery among different CD subsets we found is consistent with previous findings: CD8+ and CD16+/CD56+ cells recovered faster than CD4+ cells (Belka et al., 1999; Santin et al., 2000); and CD4+ cell recovery remained below baseline at 4 months after a low dose radiotherapy (Belka et al., 1999). In advanced BC patients, high-dose chemotherapy led to an inversion of CD4/CD8 ratio and significant reductions in lymphocyte proliferation that persisted for 6-12 months (Avigan et al., 2000). Prolonged reductions in lymphocyte proliferation were also reported in early stage BC patients (Wiltschke et al., 1995). Furthermore, immune recovery did not always improve with time. For example, the probability of recovering IL-2 responses to baseline or above was only about 19% at 12 months (Table 5), lower than that of earlier time points. A similar decreasing trend was noted in lymphocyte proliferation responses. These findings suggest that cancer adjuvant therapy may have a delayed impact on recovery of certain immune responses far beyond the immediate period after completion of therapy.

The type of cancer adjuvant therapy had a differential influence on immune recovery in selective immune measures. Chemotherapy had significant selective immunosuppressive impact on the recovery of IL-2 responses at all times and CD4% at 12 months. In contrast, radiotherapy had significant selective immunosuppressive impact on the recovery of IL-4 responses. Immunosuppression from both chemo- or radiotherapy has been reported by others, including a depletion of CD4+, CD8+ and CD56+ subsets, impaired NK cell activity and lymphocyte proliferation in various cancer patients (Santin et al., 2000; Steele, 2002; Verastegui, Morales, Barrera-Franco, Poitevin, & Hadden, 2003). The NK cell activity has been found to decrease significantly during and following chemotherapy in BC patients, although NK cell numbers were less affected and tended to return to baseline before subsequent cycles of chemotherapy (Beitsch, Lotzova, Hortobagyi, & Pollock, 1994; Sewell et al., 1993; Solomayer et al., 2003).

Direct comparison of three different types of cancer adjuvant therapy on immune responses has rarely been conducted. Santin et al. (Santin et al., 2000) reported no significant differences in immune suppression between radiotherapy alone and radiotherapy combined with cisplatinum in cervical cancer patients, but the sample size was extremely small ($n = 8$). While time between completion of adjuvant therapy and blood draw for immune function measurement differed among adjuvant therapy types in this study, those differences are an unlikely explanation for differential responses because the patterns of IL-2 and IL-4 responses to adjuvant therapy types remained similar across time points. It appears that chemotherapy or combination of chemo- and radiotherapy suppresses Th1-type cytokine response, whereas radiotherapy suppresses Th2-type cytokine response.

Cancer stage had no significant effects on immune recovery in this study. Findings from prior studies have been mixed in that some found incrementally lower NK cell activity with advances

in BC stage particularly among those with metastatic BC (Konjevic & Spuzic, 1993), whereas others did not find stage-dependent reductions in immune responses (Campbell et al., 2005). One likely reason for not observing the effect of cancer stage in this study is that advanced stage metastatic BC patients were not included in the study.

Clinical significance of differential and impaired immune recovery is unclear at present and warrants further long-term in-depth investigations. The impact of impaired immune recovery in early stage BC patients may be more subtle than clinical outcomes documented in advanced metastatic BC patients. In advanced BC patients, early immune recovery of lymphocyte count or lymphocyte proliferation responses predicted better survival and lower recurrence of disease (Nieto et al., 2004; Porrata et al., 2001); and lower levels of IL-2 but higher levels of IL-6 were associated with significantly negative survival outcomes (Bachelot et al., 2003; Bozcuk et al., 2004). Even in early BC patients, lower lymphocyte proliferation was correlated with greater tumor burden (tumor size and axillary lymph node involvement) and those who had not recovered their lymphocyte proliferation response to baseline values in 12 months from surgery developed a far greater rate of metastatic disease (61%) within subsequent 2 years than those who had recovered lymphocyte proliferation above the baseline (2%) (Verastegui et al., 2003; Wiltschke et al., 1995). Similarly, decreased intracellular IFN-γ and IL-2 expression in T cells was correlated with increased number of tumor cells in bone marrow (Campbell et al., 2005), and lower baseline IL-2 levels were associated with a greater tumor relapse rate, 33.3% vs. 4.7% in early stage BC patients (Arduino et al., 1996).

Although clinical outcomes from delayed immune recovery in early stage BC patients might be mostly nonlife-threatening outcomes, such as infections, they can compromise patients' quality of life. Such infections were documented in 11-17% of early BC patients (median follow-up of 69 months; (Henderson et al., 2003) and 47% of metastatic BC patients (median follow-up of 40 weeks) (Slamon et al., 2001). Thus, research needs to be extended to determine clinical significance of delayed and poor immune recovery in early stage BC cancer patients employing large sample sizes and longer follow-up periods to gain sufficient statistical power. In addition, investigators need to simultaneously assess potential psychosocial, behavioral, disease-related and biological factors that might contribute to variability in immune recovery. These findings may provide a basis for future interventions to facilitate a timely recovery of immune responses and perhaps improve clinical outcomes in early stage BC patients.

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Table 1

Demographic characteristics of participants (N = 80)

Variable	M (SD)	*n* (%)
Age (years)	49.5 (8.7)	
Range	32-70	
Education (years)		
Less than high school		1 (1.3)
Graduated from High School		13 (16.3)
Trade School/some college		28 (35.0)
Bachelor's degree		17 (21.3)
Some Graduate school/Graduate degree		15 (18.8)
Other		6 (7.5)
Employment Status		
Not employed		15 (18.8)
Part-time		12 (15.0)
Full-time (40 hr/week)		53 (66.3)
Body Mass Index	28.8 (6.3)	
19-24.9		28 (35.0)
25 – 29.9		17 (21.3)
30 – 46.6		31 (38.8)
Missing		4 (5.0)
Ethnicity		
African American		18 (22.5)
Native American		2 (2.5)
Caucasian		60 (75.0)
Religion		
Protestant		62 (77.5)
Catholic		12 (15.0)
Jewish or Muslim		2 (2.4)
None or Missing		4 (5.0)
Marital Status		
Single		5 (6.3)
Married or living as married		58 (72.5)
Separated, Divorced, or Widowed		16 (20.0)
Missing		1 (1.3)
Menopausal Status		
Premenopause		22 (27.5)
Postmenopause		46 (57.5)
Perimenopause		12 (15.0)

Table 2

Medical information of participants (N = 80)

Variable	N	n (%)
Stage (TNM classification)	80	
0 *		1 (1.3)
I		21 (26.3)
II		45 (56.3)
III		11 (13.8)
Unknown		2 (2.5)
Estrogen & Progesterone Receptor	80	
Positive-positive		59 (73.8)
Negative-negative		17 (21.3)
Mixed		1 (1.3)
Unknown		3 (3.8)
Adjuvant Therapy	80	
Chemotherapy		20 (25.0)
Radiotherapy		26 (32.5)
Chemo + radiotherapy		33 (41.3)
No adjuvant therapy		1 (1.3)
Hormonal therapy		60 (75.0)
Chemotherapy Regimen	53	
AC		21 (39.6)
ACT/ATC		28 (52.8)
Other (e.g., CMF)		4 (7.6)
Recurrence	80	
Yes		3 (3.8)
No		74 (92.5)
Unknown		3 (3.8)

Notes. One woman was originally staged as stage 1, but it was later changed to stage 0. AC = Adriamycin 60mg/m^2 + Cytoxan 600 mg/m^2 together every 2-3 weeks for 4 doses; ATC = Adriamycin 60mg/m^2, Taxol 145mg/m^2, and Cytoxan 600 mg/m^2 sequentially every 2-3 weeks for 3 doses each; ACT = Adriamycin 50mg/m^2 + Cytoxan 500 mg/m^2 together every 2-3 weeks for 4 doses followed by Taxotere 75mg/m^2 every 2-3 weeks for 4 doses

Table 3

Descriptive percentages of participants with immune recovery to or above baseline immune responses at 2, 6, and 12 months by cancer treatment group

Immune parameter	Chemotherapy 2 mo (%)	Chemotherapy 6 mo (%)	Chemotherapy 12 mo (%)	Radiotherapy 2 mo (%)	Radiotherapy 6 mo (%)	Radiotherapy 12 mo (%)	Chemo-+Radiotherapy 2 mo (%)	Chemo-+Radiotherapy 6 mo (%)	Chemo-+Radiotherapy 12 mo (%)
CD4%	41	41	18	57	52	70	38	50	53
CD56%	41	76	76	57	71	75	62	57	70
CD8%	41	65	71	57	67	65	59	70	67
IFN-γ	33	28	24	17	48	27	58	50	25
IL-1α	53	59	62	27	27	29	33	30	44
IL-2	18	11	6	39	32	18	39	21	12
IL-4	56	56	35	17	13	23	27	33	35
IL-6	61	67	71	39	61	73	45	70	59
NK 12.5:1	35	22	18	30	48	32	41	42	39
NK 25:1	28	22	17	17	30	41	47	42	41
NK 50:1	28	28	17	22	48	41	41	27	35
NK 100:1	28	28	28	35	43	45	41	24	16
LAK 12.5:1	33	33	22	39	39	45	41	55	56
LAK 25:1	22	28	11	36	43	55	34	48	53
LAK 50:1	33	39	22	39	43	41	30	44	44
LAK 100:1	33	33	28	52	52	36	33	44	56
LP5	33	25	7	43	40	25	48	38	36
LP10	33	33	29	57	30	25	33	25	43

Notes. NK = natural killer cell activity 12.5:1 – 100:1; LAK = lymphokine-activated killer cell activity 12.5:1 – 100:1; LP5 and LP10 = lymphocyte proliferation stimulated with Phytohemagglutinin 5-10 μg/ml. Sample size may vary slightly due to missing data.

Table 4

Descriptive percentages of participants with immune recovery to or above baseline immune level at 2, 6, and 12 months by cancer stage

Immune parameter	Stage 1-2 2 mo (%)	Stage 1-2 6 mo (%)	Stage 1-2 12 mo (%)	Stage > 2 2 mo (%)	Stage > 2 6 mo (%)	Stage > 2 12 mo (%)
CD4%	52	48	49	29	50	50
CD56%	59	70	76	48	59	68
CD8%	59	65	67	43	73	68
IFN-γ	33	40	22	50	52	33
IL-1α	36	31	39	36	46	52
IL-2	31	20	10	42	25	17
IL-4	33	36	33	28	25	27
IL-6	51	66	67	40	67	65
NK 12.5:1	36	40	30	36	38	35
NK 25:1	29	34	35	40	33	35
NK 50:1	29	38	35	36	25	26
NK 100:1	35	38	35	36	17	13
LAK 12.5:1	38	48	45	41	36	43
LAK 25:1	32	44	47	32	36	35
LAK 50:1	35	44	41	32	39	30
LAK 100:1	41	42	41	36	48	48
LP5	41	35	21	47	37	37
LP10	41	30	30	42	26	42

Notes. NK denotes natural killer cell activity 12.5:1 – 100:1; LAK denotes lymphokine-activated killer cell activity 12.5:1 – 100:1; LP5 and LP10 denote lymphocyte proliferation stimulated with Phytohemagglutinin 5-10 μg/ml. Sample size may vary slightly due to missing data.

Table 5

Model-estimated treatment group probability of immune recovery over time by the type of cancer adjuvant therapy

Variable	Treatment	Time Point 2 mo Probability	6 mo Probability	12 mo Probability	p
CD4	Chemo	0.45	0.46A	0.18B	.04
	Radiation	0.65	0.60	0.78	.28
	Chemo+Radio	0.46	0.58	0.62	.36
CD56	Combined	0.60A	0.71A	0.76B	.13
CD8	Combined	0.60	0.71	0.71	.18
IFN-γ	Chemo	0.33	0.26	0.23	.83
	Radiation	0.21A	0.53B	0.31	.05
	Chemo+Radio	0.51A	0.43	0.21B	.04
IL-1α	Combined	0.34	0.36	0.42	.43
IL-2	Combined	0.48A	0.34B	0.19C	<.001
IL-4	Combined	0.22	0.22	0.22	.99
IL-6	Combined	0.45A	0.63B	0.67B	.04
NK (MV)	Com bined	0.34	0.35	0.33	.92
LAK (MV)	Chemo	0.33	0.37	0.24	.26
	Radiation	0.49	0.45	0.45	.91
	Chemo+Radio	0.33A	0.50	0.58B	<.02
LP (MV)	Combined	0.44A	0.32B	0.29B	.03

Notes. Pairs of probability with different letters (e.g., A and B, A and C, B and C) are significantly different at $p < .05$.

Combined: Collapsed over types of cancer adjuvant therapy as interaction with time was not statistically significant.

NK: natural killer cell activity. LAK: lymphokine-activated killer cell activity. LP: lymphocyte proliferation. MV: Multivariate model tested.

'Chemo+Radio' indicates both chemotherapy and radiotherapy.

Table 6

Model-estimated time point probability of immune recovery by the type of cancer adjuvant therapy

	Time point (month)	Chemotherapy Probability	Radiotherapy Probability	Chemo+ Radiotherapy Probability	p
CD4	2	0.45	0.65	0.46	.39
	6	0.45	0.60	0.58	.59
	12	0.18A	0.77B	0.62B	.002
CD56	Combined	0.66	0.70	0.73	.77
CD8	Combined	0.63	0.68	0.59	.66
IFN-r	2	0.33	0.21	0.51	.17
	6	0.25	0.53	0.43	.27
	12	0.23	0.31	0.21	.82
IL-1α	Combined	0.56	0.25	0.33	.10
IL-2	Combined	0.11A	0.73B	0.29A	.004
IL-4	Combined	0.41A	0.09B	0.26A	.002
IL-6	Combined	0.67	0.53	0.56	.56
NK (MV)	Combined	0.27	0.37	0.38	.65
LAK (MV)	2	0.33	0.37	0.24	.54
	6	0.49	0.45	0.45	.66
	12	0.33A	0.50	0.58B	.05
LP (MV)	Combined	0.29	0.37	0.38	.82

Notes. Pairs of treatment group with different letters (e.g., A and B, A and C, B and C) are significantly different at $p < .05$.

Combined: Collapsed over types of cancer adjuvant therapy, as interaction with time was not statistically significant.

NK: natural killer cell activity. LAK: lymphokine-activated killer cell activity. LP: lymphocyte proliferation. MV: Multivariate model tested.

Table 7

Model-estimated probability of immune recovery by cancer stage

Variable	Timepoint (month)	Stage 1 - 2 Probability	Stage > 2 Probability	p
CD4	Combined	0.54	0.52	.86
CD56	Combined	0.74	0.64	.31
CD8	Combined	0.70	0.66	.69
IFN-r	Combined	0.28	0.37	.31
IL-1α	Combined	0.36	0.39	.82
IL-2	Combined	0.20	0.50	.07
IL-4	Combined	0.29	0.17	.11
IL-6	Combined	0.65	0.52	.20
NK (MV)	Combined	0.36	0.32	0.63
LAK (MV)	Combined	0.43	0.39	.74
LP(MV)	Combined	0.31	0.39	.52

Notes. Combined: Collapsed over types of cancer adjuvant therapy, as interaction with time was not statistically significant.

NK: natural killer cell activity. LAK: lymphokine-activated killer cell activity. LP: lymphocyte proliferation. MV: Multivariate model tested.