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Objective and Subjective Breast Cancer Risk: Relationships with Natural Killer Cell Activity and Psychological Distress in Healthy Women

Na-Jin Park, PhD, RN,

University of Alabama at Birmingham School of Nursing, Birmingham, AL

Duck-Hee Kang, PhD, RN, FAAN, and

University of Texas Health Science Center at Houston School of Nursing, Houston, TX

Michael T. Weaver, PhD, RN, FAAN

Indiana University School of Nursing, Indianapolis, IN

Abstract

Background—Breast cancer (BC) risk, based on either known risk factors (objective) or self-assessment (subjective), may influence natural killer cell activity (NKCA) directly or through psychological distress.

Objective—The main objective of this study was to examine the relationships of objective and subjective BC risk with NKCA and a mediating role of psychological distress in a community sample of healthy women.

Methods—In a cross-sectional descriptive study, a convenience sample of 117 healthy women (mean age 36.5 years) completed questionnaires for BC risk and psychological distress and provided blood for NKCA measurement.

Results—Objective and subjective BC risk were positively correlated ($p < .001$). Regression analyses revealed that objective BC risk had a significant negative relationship with NKCA at the 12.5:1 effector-to-target (E:T) cell ratio ($p = .011$), whereas subjective risk was not associated with NKCA at any E:T cell ratio tested. Only subjective risk had a positive association with both BC-specific ($p = .002$) and general ($p < .001$) psychological distress. Psychological distress failed to mediate the relationship between subjective risk and NKCA.

Conclusions—Objective BC risk had a limited but significant relationship with NKCA. Subjective risk was highly associated with psychological distress but was not associated with NKCA.

Implications for Practice—Despite the limited relationships between BC risk and NKCA, the impact of BC risk on other tumor defense mechanisms needs to be further explored. Collective findings in this area will suggest early preventive strategies for monitoring BC risk and maintaining better tumor defense in healthy women.

Correspondence: Na-Jin Park, PhD, RN, School of Nursing, University of Alabama at Birmingham, 1530 3rd Avenue S. NB 314, Birmingham, AL 35294-1210, (najinp@uab.edu).

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Keywords

Breast cancer risk; Risk assessment; Psychological distress; NKCA

Despite significant advances in early detection and treatment, breast cancer (BC) remains one of the most common cancers affecting more than 190,000 American women in 2009.¹ The majority of BC occurs in a sporadic form without hereditary predisposition. Proactive assessment of BC risk and determining its effect on a natural tumor defense function can provide the valuable information for strengthening the human defense against BC in otherwise healthy women.^{2,3}

The two major types of BC risk are objective and subjective BC risk. Objective BC risk is defined as an estimated likelihood for getting BC based on scientifically established risk factors for the disease. Subjective BC risk represents an individual's perception of her chance for developing BC based on her own cognitive appraisal. Objective risk is predictive of resultant health outcomes, but subjective risk is likely to influence psychological and behavioral factors that influence health/health outcomes.⁴ Indeed, perceived subjective risk of BC has been consistently and positively associated with psychological distress in healthy women,^{5,6} whereas objective BC risk has not.⁷ Objective and subjective risk are related but are not equivalent. In previous studies with healthy women, the relationships between objective and subjective BC risk have been mixed by showing either no relationship⁸ or a positive relationship.⁹ We, therefore, posit these two types of BC risk could have different relationships with natural killer cell activity (NKCA).

Natural killer cells are involved in normal defense of tumor cell recognition and lysis.¹⁰ Women with a family history of BC in their first degree relatives (mother, sisters, and daughters) showed lower NKCA than did women without any family history of BC,^{11,12} implying the relationship between BC risk and NKCA. NKCA has also been highly responsive to psychological distress in various populations.¹³ Similarly, women with a family history of BC showed significantly greater psychological distress and stress hormones (e.g., cortisol and catecholamine) than did women without a family history of BC,^{12,14} and, not surprisingly, increased psychological distress and stress hormones were inversely associated with decreased NKCA.^{12,14} Because of the sensitivity to psychological distress and relevance to BC, NKCA is a useful biomarker for assessing the immune impact of BC risk and associated psychological distress. In addition, previous investigations have been limited to the relationship of a single risk factor, family history of BC, with NKCA. Having a family history of BC may simultaneously increase objective¹⁵ and subjective^{7,16} risk of BC development, but previous studies have not clarified which type of BC risk influenced NKCA. Such clarification may be important in determining future strategies for maintaining better NKCA.

Although a family history of BC is an important risk factor, eight out of nine women who develop BC do not have an affected first-degree relative.¹⁵ This fact indicates that a large proportion of variance in BC development comes from other factors, supporting a need for a more comprehensive BC risk assessment that incorporates multiple risk factors and their potential interactions.¹⁷ Several models of objective BC risk assessment are available, and most widely used are the Gail model¹⁸ and the Claus model.¹⁹ Each model has been developed on a unique combination of risk factors from a different epidemiological data set: The Gail model incorporates a family history of BC in first-degree relatives along with non-genetic factors such as age, race, and menstrual history in the risk assessment, whereas the Claus model is primarily focused on the extensive family history of BC in first- and second-degree relatives.²⁰ In general, the Gail model is more appropriate for women at risk for sporadic BC representing most BC cases, whereas the Claus model fits better for women at risk for hereditary BC with

genetic predisposition.²⁰ Taken together, the Gail model remains to be a useful basic model for BC risk assessment in general populations.²¹ The Gail model has been further modified on the basis of more recent large validation studies^{22,23} to improve the accuracy of objective BC risk assessment. Based on the modified Gail model, the National Cancer Institute (NCI) developed a computerized BC risk assessment tool, which was used to assess the objective BC risk in this study.

Therefore, the specific aims of this study were to: (1) Examine the relationship between objective and subjective BC risk; (2) Determine the associations of objective and subjective BC risk with NKCA; and (3) Examine the mediating role of psychological distress in the relationship between subjective BC risk and NKCA in a community sample of healthy women at varying levels of BC risk. Because smoking, alcohol consumption, inadequate sleep, and hormone supplements have been associated with lower levels of NKCA,^{24–28} these factors were entered as potential covariates in the analyses. Although the findings of menopausal status on NKCA were inconsistent²⁹, it was also included as a potential covariate, but age was excluded because this was already included in the assessment of objective BC risk.

Conceptual Framework

This study was guided by the model of stress and physiological responses³⁰ and the transactional model of stress and coping.³¹ The model of stress and physiological responses explains how various forms of stress alter neuroendocrine and immune responses triggering local and general adaptations but leading to negative health consequences when adaptation is exhausted. Objective BC risk in this study may represent a form of physical stress, which can decrease NKCA and lower natural tumor defense function. On the other hand, the transaction model of stress and coping emphasizes the significance of individual's cognitive appraisal process in determining the meaning of an event and the use of coping resources. For this study, subjective BC risk indicates primary cognitive appraisal of a woman for her own BC risk and is defined as perceived belief of personal threat or harm for developing BC.³² This personal appraisal of BC risk is likely to trigger different levels of BC-specific distress along with general psychological distress, which then can influence NKCA. The indicators constituting objective BC risk (age, age at menarche, age at first live birth, number of first-degree relatives with BC, the number of previous breast biopsies, and race/ethnicity) are the factors that accumulate over time preceding NKCA assessment, whereas subjective BC risk and psychological distress are most likely affected by those factors for objective BC risk. Therefore, we conceptualized that both types of BC risk were the predictors of NKCA and that psychological distress was a potential mediator in the relationship between subjective BC risk and NKCA (Figure). The findings of this study will enhance our understanding of biobehavioral linkages of BC risk and NKCA in otherwise healthy women and may inform future studies of BC risk.

Methods

Design

A cross-sectional, descriptive design was used to achieve the aims of the study. Data were collected at one time. BC risk and psychological distress were conceptualized as predictors or a mediator of NKCA.

Sample

A prior power analysis was used to determine the sample size, guided by Cohen.³³ With 0.05 significance level, 0.85 power, 0.13 effect size, and 5 independent variables, the sample size

was calculated ranging from 100 to 126. We targeted the maximum sample size for recruitment to ensure the proposed study power when data were lost or unusable for analyses.

A convenience sample of 126 healthy women was recruited by public advertisements from a state university health system in the southeastern region of the United States. Inclusion criteria were: (a) woman, (b) ages of 20 years or older, (c) no personal history of BC, and (d) ability to read and write English to complete questionnaires. Exclusion criteria were: (a) diagnosis of any prior or concurrent cancer, (b) presence of chronic or acute infectious illnesses, (c) having known mental illnesses and/or substance abuse, (d) presence of immune disorders (e.g., autoimmune disease or HIV-positive status), or (e) current use of immunosuppressive drugs (e.g., corticosteroids). The initial eligibility to participate in the study was determined by potential participants themselves using the criteria stated above. After obtaining consent, more detailed baseline information was collected by the investigator. Nine women were found to take an immunosuppressant and thus were excluded from the study, reducing the final sample size to 117.

Procedures

Participants were recruited by multiple strategies: posting flyers and brochures in the public areas; advertising the study in a campus newsletter; and by word of mouth. To ensure recruiting a sufficient number of women at increased BC risk from the community, two sets of recruitment announcements were publicized at the same time: one set for women with at least one first-degree relative with BC, and the other set for women without any first-degree relative with BC. When interested individuals called in, the investigator informed them of the eligibility criteria so that they could self-determine own eligibility. This procedure protected the callers from providing the data prior to signing the informed consent. Once the caller self-determined, a meeting was arranged between 09:00 am and 11:30 am in the School of Nursing. At the meeting, the purpose and procedure of the study and the potential risks and benefits of participating in the study were thoroughly explained before obtaining the consent. After informed consent was obtained, the participant's eligibility for the study was reexamined by the investigator. Those who did not meet the eligibility criteria were excluded from the study, but they received a study incentive for their visit. The eligible participant completed a set of questionnaires, and a registered nurse collected a blood sample (15 ml) using routine venipuncture techniques. Participants received \$20 for their time. The study protocol was approved by the University Institutional Review Board and the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command for the Department of Defense.

Instruments

Objective BC risk was measured using the NCI Breast Cancer Risk Assessment Tool v.2 for Health Care Providers. This tool was developed based on the modified Gail model,^{22,23} and the risk was calculated as a percentage of lifetime risk (to age 90) for BC. The tool is accessible free-of-charge online at <http://bcra.nci.nih.gov/brc/> or as a CD. We used the CD version of the NCI tool specifically designed to estimate the objective BC risk for women 20 years and older.³⁴ The modified Gail's risk assessment tool includes questions on the following key risk factors: current age (<50, or ≥50), age at menarche (<12, 12–13, or ≥14 years), age at first live birth (<20, 20–24, 25–29, or nulliparous or ≥30 years), number of first-degree relatives with BC (0, 1, or ≥2), the number of previous breast biopsies (0, 1, or ≥2) with at least one biopsy with atypical hyperplasia (yes, no, or not sure), and race/ethnicity (Caucasian and others, or African American). With older age, earlier menarche, later first live birth or no birth, greater number of first-degree relatives with BC, more previous breast biopsies with atypical hyperplasia, and being Caucasian, the tool generates higher percentages of lifetime risk depending on the combination of these risk factors. Higher percentages of lifetime risk indicate higher levels of

objective BC risk with the range of 0 – 100%. The utility of this Gail's risk assessment tool has been validated in healthy women.^{22,23}

Subjective BC risk was assessed using the revised Perceived Susceptibility Scale.³² This scale contains three items with a 5-point Likert response format ranging from 1 (*strongly disagree*) to 5 (*strongly agree*). The possible total score range is 3 – 15, and higher scores indicate higher levels of subjective BC risk. Although construct validity and reliability were originally confirmed in 618 healthy women age 50 and over,³² this measure, in original and modified version, has been widely used among women younger than 50 from culturally diverse groups of women with acceptable reliability.^(e.g., 35) In this study, the internal consistency reliability was 0.91. Although items on the revised Perceived Susceptibility Scale for subjective BC risk were not specifically referring to a period preceding NKCA measurement, the items (e.g., it is likely that I will get breast cancer) were construed to draw a response that would have been thought through and stable over a period. The test-retest correlation for this scale over 6 weeks was $r = .62$,³² supporting the stability of the responses over time. Given the limited availability of other valid instruments for assessing subjective BC risk, this scale has been recommended as a valuable means for measuring perceived subjective BC risk with high validity and reliability.⁴

Psychological distress was measured for both BC-specific distress and general distress. BC-specific distress was measured with the Impact of Event Scale (IES).³⁶ The IES is a 15-item scale that assesses the frequency of intrusive thoughts and avoidance regarding a specific stressor during *the past seven days*. The specific stressor used in this study was “the fear of developing BC.” Participants were asked to indicate how frequently each item had been true with respect to their fear of developing BC in the past seven days using a 4-point weighted rating scale (0 = *not at all*, 1 = *rarely*, 3 = *sometimes*, and 5 = *often*). A total score (range 0–75) obtained by adding the two subscales was used to determine BC-specific distress. Higher total scores correspond to higher levels of BC-specific psychological distress. Content validity and reliability of the scale have been confirmed in various populations,^{36,37} including women at increased risk of BC.³⁸ In this study, Cronbach's alpha for internal consistency reliability was 0.93.

General distress was measured using the 37-item short version of the Profile of Mood States (POMS), which was tested for high reliability and validity.³⁹ This Likert-style adjective checklist retains the original six subscales of the following mood states: depression, tension/anxiety, anger, fatigue, vigor, and confusion. Participants were asked to rate the degree to which an adjective had applied to their moods during *the past week* on a 5-point rating scale (from 0 = *not at all* to 4 = *extremely*). A total score, computed by summing the six subscale scores with vigor scored inversely, was used for this study. Higher total scores (range 0–148) indicate higher levels of general distress. This shortened version of POMS have been widely used to measure psychological distress among cancer patients⁴⁰ and healthy women at increased risk of BC⁴¹ with good content validity and reliability. In this study, Cronbach's alpha was 0.96 for the overall scale and ranged from 0.83 (confusion) to 0.91 (depression) for six subscales.

BC-specific and general distress was treated independently in this study, because each measure demonstrates different aspects of psychological distress: BC-specific distress represents the cognitive appraisal of distress, whereas general distress represents the emotional appraisal of distress. This perspective was supported by a weak correlation between the two ($r = .33$) in this study.

NKCA

NKCA indicates how effectively natural killer cells lyse tumor cells. All blood samples were collected between 09:00 am and 11:30 am to minimize the potential effects of circadian

variation on immune response. The assay was conducted with freshly isolated peripheral blood mononuclear cells (PBMCs) from 15 ml whole blood collected into sterile, preservative-free, and heparinized vacutainers via routine venipuncture. The same investigator performed all NKCA assays immediately following sample collection in the laboratory at the School of Nursing.

Cell Separation—PBMCs were separated by layering heparinized blood onto Ficoll-Hypaque (specific gravity of 1.077–1.080 gm/ml; Mediatech, VA) and centrifuging at 1500 rpm for 30 minutes. The PBMCs at the interface between plasma and Ficoll were carefully harvested for NKCA assay, were washed twice with sterile cell culture medium (Roswell Park Memorial Institute [RPMI] 1640, Mediatech, VA), and were centrifuged at 1200 rpm for 12 minutes. Cells were resuspended at the final concentration of 2×10^6 cells/ml with complete medium (RPMI 1640 supplemented with HEPES 25 mM, L-glutamine 2 mM, Penicillin 50 unit and Streptomycin 50 µg/ml). The cell viability was determined using trypan blue staining, which is known to yield >98% viability of PBMCs.^{42,43}

NKCA Assay—Following the gold standard chromium-51 (Cr-51) release cytotoxicity assay protocol,⁴⁴ we determined NKCA *in vitro* using K562 target cells (2 to 3 days old) from a human erythroleukemia cell line. K562 target cells (The American Type Culture Collection, Manassas, VA) were labeled with 125 µCi Cr-51 radioisotope for 1 hour at 37°C, were washed and centrifuged twice, and were resuspended to a density of 4×10^4 cells/ml with complete medium. The 100 µl PBMCs (2×10^6 cells/ml) were then incubated in triplicate in 96-well microtiter plates with 50 µl 60% heat-inactivated fetal bovine serum and 50 µl radioisotope labeled K562 target cells in four effector-to-target (E:T) cell ratios: 100:1, 50:1, 25:1, and 12.5:1. Spontaneous and maximum release control was determined by incubating target cells with medium alone and 10% sodium dodecyl sulfate solution. After a 4-hour incubation in 5% CO₂ at 37°C, cytotoxicity was determined by measuring the release of radiolabel in the culture supernatant using a gamma counter. NKCA was calculated as follows: $\text{NKCA (\%)} = \frac{(\text{sample release} - \text{spontaneous release control})}{(\text{maximum release control} - \text{spontaneous release control})} \times 100$. Higher percentages of NKCA indicate better cytotoxicity. At higher E:T cell ratios, higher cytotoxicity is expected than at lower E:T cell ratios because more effector cells (i.e., immune cells) are available for every tumor target cell. The average coefficient of variation (CV) of NKCA assays was 7.29%, indicating a high quality of the data. The CV is a measure of variability of an assay, and a percentage lower than 15–20% is considered to indicate a better control of quality of the assay.⁴⁵

NKCA is typically measured at multiple E:T cell ratios, but largely two different formats have been used in reporting NKCA data across studies. Some investigators prefer to report a proxy value called a lytic unit by compiling the data across all E:T cell ratios.^(e.g., 12) However reducing the information to a single lytic unit score may compromise the ability to detect relationships under study.⁴⁶ Therefore, other investigators have used multiple E:T cell ratios to keep important information which might otherwise be missed. The use of multiple E:T cell ratios is believed to be more useful to verify dose-specific responses to a phenomenon under study and to enhance sensitivity to detect fine differences that may emerge only at a certain E:T cell ratio.^{46,47} We support the use of multiple E:T cell ratios and analyzed the NKCA data accordingly.

Data Management and Analysis

Data were analyzed using the Statistic Package for Social Science (SPSS) version 13.0 for Windows (Chicago, IL). Because BC-specific distress scores were not normally distributed, data were log transformed, and no violations were identified with the remedy. Pearson correlation coefficients were used to examine the relationship between objective and subjective

BC risk. Hierarchical multiple regression analyses were used to examine the contributions of objective and subjective BC risk to NKCA controlling for the selected covariates. Major covariates included were current status of smoking (yes or no), alcohol use (yes or no), and average hours of sleep per day during the previous week, and hormonal factors including current use of birth control pills, patches, or injections (yes or no), hormone replacement therapy (yes or no), and menopause status (pre- or post-menopause). At each E:T cell ratio, bivariate correlations between potential covariates and NKCA were run, and those showing significant correlations at $p < .05$ were entered into the regression model. Those showing significant contributions to the regression model of NKCA at each E:T cell ratio were selected as covariates. The selected covariates were sleep hours, current birth control use, and menopause status for NKCA at 100:1 E:T cell ratio and only current birth control use for NKCA at the rest of E:T cell ratios. Accordingly, these covariates were entered in the first step of regression model to control their contributions to each E:T cell ratio of NKCA. Bivariate relationships between the selected covariates were examined and none exceeded $r = .35$, eliminating concern for multicollinearity among the selected covariates. In the next step, we entered a set of objective and subjective BC risk.

To determine the mediating role of BC-specific and general psychological distress in the relationship between subjective BC risk and NKCA, a series of three regression analyses were performed as specified by Baron and Kenny.⁴⁸ First, psychological distress was regressed on subjective risk; second, each E:T cell ratio of NKCA was regressed on subjective risk; and finally NKCA was regressed on subjective risk and psychological distress simultaneously. To support a mediating role, the first and second regression models must be significant in the hypothesized direction. Then, the relationship between psychological distress and NKCA should be significant after controlling for the relationship of subjective risk with NKCA; and, at the same time, the relationship between subjective risk and NKCA must be significantly reduced after controlling for the relationship of psychological distress with NKCA. All analyses were performed with the alpha level at 0.05.

Results

Sample Characteristics and Descriptive Statistics

The mean age of the participants was 36.5 years (range = 20–71; $SD = 12.1$ years). The majority of the participants identified themselves as Caucasian (55.6%) or African American (41.0%). Over half of the participants had at least a college degree, worked full-time, and were married or living together with a partner. Sixty four women (54.7%) did not have any first-degree relatives with BC, and 53 women (45.3%) had at least one first-degree relative with BC including 5 women having more than one affected first-degree relative.

Thirty two participants (27.4%) were currently using birth control pills, patches, or injections. There were 93 premenopausal (79.5%) and 24 postmenopausal women. Thirty three women (28.2%) reported to have smoked at least 100 cigarettes in their entire life, but only ten (8.5%) reported to be current smokers. On average, participants slept 7 hours per day (range 4–10, $SD = 1.2$) during the past week. As indicated in Table 1, the mean level of objective BC risk was low with 11.94%, and the mean subjective BC risk was low to moderate with 7.55 out of the maximal score of 15. The levels of both BC-specific and general psychological distress were generally low. NKCA showed a dose response based on the E:T ratios, but their levels were very low.

Objective and Subjective BC Risk: Relationship with NKCA

First, Pearson correlation coefficient indicated a significant positive correlation between objective and subjective BC risk ($r = .323$, $p < .001$). Other initial bivariate correlational

analyses indicated that objective BC risk showed a significant negative correlation with NKCA at the 12.5:1 E:T cell ratio ($p = .01$), whereas subjective BC risk did not show any significant correlation with NKCA. For psychological distress, BC-specific distress showed no correlation with NKCA, but general distress showed a significant negative correlation with NKCA at the 100:1 E:T cell ratio ($p = .027$) (Table 2).

The hierarchical multiple regression analyses are summarized in Table 3. When potential covariates of selected health behaviors and hormonal factors were tested, current birth control use, menopause status, and sleep hours showed significant correlations with NKCA. A greater number of hours of sleep, not using birth control pills, patches, or injections, and postmenopausal status were significantly associated with higher levels of NKCA at the 100:1 E:T cell ratio, accounting for 19.7% of the variance in NKCA at the 100:1 E:T cell ratio. After controlling for a set of covariates, however, variance explained by objective and subjective BC risk for NKCA became insignificant at this E:T cell ratio ($\Delta R^2 = 0.005$, $p = .730$). The current use of birth control pills, patches, or injections was associated with lower NKCA at all four E:T cell ratios ($p = .004 - .048$). After controlling for current birth control use, objective and subjective BC risk together showed a statistically significant contribution to only at the 12.5:1 E:T cell ratio of NKCA ($\Delta R^2 = 0.057$, $p = .034$). Further analyses indicated that only objective BC risk was a significant predictor for NKCA at this ratio ($p = .011$), whereas subjective BC risk did not show any association with NKCA, controlling for current birth control use.

Mediating Role of Psychological Distress

Specific aim 3 of the study was to examine the mediating role of psychological distress in the relationship between subjective BC risk and NKCA. To test mediation, it was necessary to find a significant relationship between subjective BC risk and NKCA in the first step. However, as can be seen in Table 4, subjective BC risk failed to show a significant relationship with NKCA at any ratio in this study sample and precluded further testing of this mediating role. Therefore, psychological distress, either BC-specific or general, did not mediate the relationship between subjective BC risk and NKCA. Instead, higher subjective BC risk was significantly associated with higher levels of both BC-specific ($p = .002$) and general ($p < .001$) psychological distress but not with NKCA at any E:T cell ratio tested.

Discussion

The main findings of this study were the significant negative association of objective BC risk with NKCA at the 12.5:1 E:T cell ratio and the positive associations of subjective BC risk with both BC-specific and general psychological distress. However, subjective BC risk had no association with NKCA in this community sample of healthy women at varying levels of BC risk. These findings partly extend previous findings on a family history of BC and low immune responses of NKCA and T helper cell 1-type cytokines (e.g., interferon-gamma, interleukin (IL)-2, and IL-12).^{11,12,49} The previous studies were based on one single factor of BC risk, a family history of BC, whereas current study was based on an entire set of multiple risk factors determined by the modified Gail model for objective BC risk assessment and included subjective BC risk assessment. Despite the different approaches for objective BC risk assessment, the findings were similar to indicate that increased objective BC risk is associated with decreased NKCA to a certain degree in currently healthy women at varying levels of objective BC risk.

The negative association of objective BC risk with NKCA was observed in only one E:T cell ratio of 12.5:1. This suggests that the overall association of objective BC risk with NKCA may be modest at best in this cohort of women. Part of this finding may be attributed to relatively small variability in the levels of objective BC risk in this sample. Although the mean level of objective BC risk (11.9%) was similar to the mean level (12.03%) of lifetime BC risk in the

general population of American women,¹ the variability was limited. Future studies need a larger sample size with various individual and environmental backgrounds to determine the true relationship between objective BC risk and NKCA and other BC-relevant biological parameters.

This study was one of the first to explore simultaneously the independent contribution of objective and subjective BC risk to immune cell function. Objective BC risk showed a significant negative association with NKCA at one low E:T cell ratio. However, we found no significant association between subjective BC risk and NKCA at any E:T cell ratio. The levels of subjective risk in this study sample were relatively low to moderate with total mean of 7.55 (range 3–15) and item mean of 2.52 (range 1–5). This mean total score was slightly higher than the mean score previously reported (6.51 to 7.47) in 618 healthy women age 50 and over.³² Although the range of age was 20–71 in our sample, the mean age was 36.5 years ($SD = 12.1$). Within our younger sample, variability in the levels of subjective BC risk was limited, which may be a subsequent influence of the limited variability of objective risk, thereby potentially contributing to the lack of the association of subjective BC risk with NKCA.

In the present study, subjective BC risk was positively associated with both BC-specific and general psychological distress, which was consistent with previous findings employing both types of psychological distress.⁶ Other studies have shown that subjective BC risk was positively associated with BC-specific distress, but not with general psychological distress.^{7,9} The multi-item-based assessment of subjective BC risk in the present study differed from the typical one-item-based assessment in other studies, which may partly explain the discrepancy in findings.⁴ In contrast, psychological distress was not associated with objective BC risk (data not shown) but was associated with NKCA at a high E:T cell ratio in this study.

Our findings of the positive correlation between objective and subjective BC risk in women with and without a family history of BC may add new information to current knowledge base because of the different approaches for assessing both types of BC risk from previous studies. In previous studies with women with a family history of BC, data on this relationship mostly measured by the typical one-item-based assessment for subjective risk and the Claus model-based objective risk assessment have been inconsistent.^{7,9} When both the Gail and Claus models were used for objective BC risk assessment in women with a family history of BC, subjective BC risk was positively related with objective risk assessed only on the Claus model, but not on the Gail model.⁷ In this study, we used the Gail model-based objective risk and the multi-item-based subjective risk assessment and found the positive relationship between the two in healthy women including both with and without a family history of BC.

Despite an extensive body of the literature documenting low immune responses under high psychological distress,¹³ our data showed a limited relationship between psychological distress and NKCA. Bivariate correlational analyses showed that only general distress was negatively correlated with NKCA at the 100:1 E:T cell ratio, but BC-specific distress was not. The limited variability and low levels of psychological distress, particularly of BC-specific distress, are the likely reasons for the weak relationship. Previous studies in healthy women with a family history of BC reported a significant inverse relationship between general distress and NKCA,^{11,12,14} but BC-specific distress was not examined. In addition to subjective BC risk, the significant relationship of psychological distress with NKCA needs to be further explored.

Among selected covariates, we found sleep hours, birth control hormone use, and menopause status had a significant relationship with NKCA. Fewer hours of sleep had significant negative association with NKCA at the 100:1 E:T cell ratio, which was similar to previous findings.²⁶ Postmenopausal status was associated with higher levels of NKCA at the 100:1 E:T cell ratio, compared with premenopausal status. Although age was not treated as a covariate because age

was already included in the assessment of objective BC risk, when we ran additional analyses, age was positively correlated with NKCA at 100:1 E:T cell ratio ($r = .271, p = .003$). Moreover, a dichotomous classification of age (younger or older than 50) as graded in objective BC risk assessment also showed the significant relationship of the age group ($r = .205, p = .029$) with NKCA at 100:1 E:T cell ratio. It seems that the age factor is already accounted into the contribution of objective BC risk to NKCA, minimizing the reason for treating age as an independent covariate in this type of the study. NKCA is generally believed to be decreased with the advanced age, but the empirical findings are inconclusive, and a decline in NKCA tends to occur in very advanced age, typically above 75 years.⁵⁰ Smoking and alcohol consumption are thought to have negative impact on NKCA,^{24,25} but we did not find significant relationships with NKCA, possibly because of a very small number of the current smokers (8.5%) in the sample and a crude screening method for smoking and alcohol consumption. The most robust covariate was the current use of hormonal supplements. Hormone use has a significant negative association with all ratios of NKCA, similar to the previous findings on oral contraceptives.²⁸ Therefore, selective covariates need to be controlled for in future studies, but the hormone use needs to be investigated most carefully for its contribution to NKCA and perhaps other immune responses as well.

Limitations

We note several limitations of this study. Because of a cross-sectional design with only one time measurement, it is not possible to confirm a causal relationship between BC risk and NKCA. The study also has a limited generalizability. The convenience sample was recruited from one community setting in one region of the United States. As a result, the sample was relatively homogeneous with a limited variability for objective BC risk, limiting the likelihood for discovering true relationships between objective and subjective BC risk and NKCA and the mediating role of psychological distress. In addition, comprehensive assessment tools for objective BC risk are limited. Although the NCI tool was the best available tool for the study, this tool was not refined for risk assessments in African American women, women with one or more second-degree relatives with BC, or women with a family history of ovarian cancer, early-onset BC, or inherited genetic mutations.³

Summary and Future Recommendations

The findings of this study contribute to the understanding of two different types of BC risk and their relationship and their individual contribution to NKCA and psychological distress in healthy women at various levels of BC risk. Objective BC risk has a limited but significant association with NKCA, whereas subjective BC risk is associated with psychological distress but does not have a significant relationship with NKCA. NKCA is an important biomarker in tumor defense. Continued validations and replications of similar findings will strengthen the importance of monitoring BC risk and examining its impact on NKCA and other BC relevant immune cell functions. A longitudinal and prospective design with a long-term follow-up will better clarify the causal relationships between BC risk, immune functions, and ultimately clinical outcomes in a large number of women at varying levels of BC risk. Although subjective BC risk was not associated with NKCA in this study, the relationships between specific psychological distress, sleep, and hormones on immune functions should be further explored.

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Figure.
Conceptual Framework of the Study

Table 1

Descriptive Statistics for Study Variables

Study Variable	N	Possible range	Actual range	Mean (SD)	Median
Objective BC Risk (%)	117	0–100	3.7–31.5	11.94 (5.26)	11.4
Subjective BC Risk	117	3–15	3–15	7.55 (3.16)	8.0
1. It is likely that I will get breast cancer	117	1–5	1–5	2.64 (1.15)	
2. My chances of getting breast cancer in the next few years are great.	117	1–5	1–5	2.24 (1.03)	
3. I feel I will get breast cancer sometime during my life.	117	1–5	1–5	2.67 (1.24)	
BC-Specific Distress	117	0–75	0–62	10.93 (15.02)	3.0
General Distress	117	0–148	0–114	34.18 (21.93)	29.0
NKCA (%)		0–100			
NKCA 100:1	114		2.38–28.81	12.45 (5.85)	11.69
NKCA 50:1	115		1.02–19.88	8.02 (3.89)	7.15
NKCA 25:1	115		1.28–15.28	5.95 (2.98)	5.45
NKCA 12.5:1	115		0.63–11.43	5.09 (2.19)	5.14

Note. Due to missing data or deleting extreme outliers, some data had less than 117 samples.

Table 2

Bivariate Correlations between Psychological Variables and NKCA

	Objective BC risk	Subjective BC risk	BC-specific distress ^a	General distress
NKCA 100:1	.023	-.106	-.146	-.207*
NKCA 50:1	-.042	-.085	-.091	-.022
NKCA 25:1	-.157	-.070	-.065	-.049
NKCA 12.5:1	-.238*	-.061	-.111	-.112

Note.

*
 $p < .05$.^a Natural log transformed data were used.

Table 3
Relationships of Objective and Subjective BC Risks with NKCA controlling for Covariates

	Model	R ²	AdjR ²	B	β	p
NKCA 100:1						
Step 1: Covariates	$F(3,106) = 9.909$, $p < .001^{***}$	0.219	0.197			
Sleep Hours				1.486	0.305	.001**
Current Birth Control Use				2.955	0.228	.013*
Menopause Status				3.965	0.280	.003**
Step 2: BC Risk	$F(5,104) = 5.995$, $p < .001^{***}$	0.224	0.186			
Objective Risk				−0.016	−0.015	.881
Subjective Risk				−0.115	−0.063	.515
$\Delta R^2 = 0.005, \Delta F = 0.316$ (ns)						
NKCA 50:1						
Step 1: Covariates	$F(1,113) = 8.728$, $p = .004^{**}$	0.072	0.063			
Current Birth Control Use				2.313	0.268	.004**
Step 2: BC Risk	$F(3,111) = 3.079$, $p = .030^*$	0.077	0.052			
Objective Risk				−0.012	−0.016	.865
Subjective Risk				−0.079	−0.064	.506
$\Delta R^2 = 0.005, \Delta F = 0.308$ (ns)						
NKCA 25:1						
Step 1: Covariates	$F(1,113) = 6.962$, $p = .010^*$	0.058	0.050			
Current Birth Control Use				1.595	0.241	.010*
Step 2: BC Risk	$F(3,111) = 3.261$, $p = .024^*$	0.081	0.056			
Objective Risk				−0.085	−0.150	.120
Subjective Risk				−0.005	−0.006	.953
$\Delta R^2 = 0.023, \Delta F = 1.386$ (ns)						
NKCA 12.5:1						
Step 1: Covariates	$F(1,113) = 3.988$, $p = .048^*$	0.034	0.026			
Current Birth Control Use				0.899	0.185	.048*

	Model	R ²	AdjR ²	B	β	p
Step 2: BC Risk	$F(3,111) = 3.702,$ $p = .014^*$	0.091	0.066			
Objective Risk				-0.103	-0.247	.011 [*]
Subjective Risk				0.021	0.031	.748
$\Delta R^2 = 0.057, \Delta F = 3.472 (p = .034^*)$						

Note.

^{*}
 $p < .05,$

^{**}
 $p < .01,$

^{***}
 $p < .001,$ ns = not significant.

Current birth control use: 0 for yes & 1 for no.

Menopause status: 0 for premenopause & 1 for postmenopause.

Mediating Role of Psychological Distress in the Relationship between Subjective BC Risk and NKCA using Series of Regressions

Table 4

Overall model	AdjR ²	B	β	p
Step 1: SR → Distress				
SR → BCSD ^a	<i>F</i> (1,115)=10.359**	0.075	0.128	0.287 .002**
SR → GD	<i>F</i> (1,115)=13.038***	0.094	2.212	0.319 < .001***
Step 2: SR → NKCA				
SR → NKCA 100:1	<i>F</i> (1,112)=1.269 (ns)	0.002	-0.195	-0.106 .262
SR → NKCA 50:1	<i>F</i> (1,113)=0.832 (ns)	-0.001	-0.104	-0.085 .364
SR → NKCA 25:1	<i>F</i> (1,113)=0.555 (ns)	-0.004	-0.067	-0.070 .458
SR → NKCA12.5:1	<i>F</i> (1,113)=0.416 (ns)	-0.005	-0.042	-0.061 .520
Step 3:				
Not Performed				
SR & BCSD ^a → NKCA				
SR & GD → NKCA				

Note.

**
p < .01,

p < .001, ns = not significant.

^aNatural log transformed data were used.

BCSD: Breast cancer-specific distress, GD: General distress, SR: Subjective breast cancer risk.