QUALITY OF LIFE IN BREAST CANCER PATIENTS AND SURVIVORS

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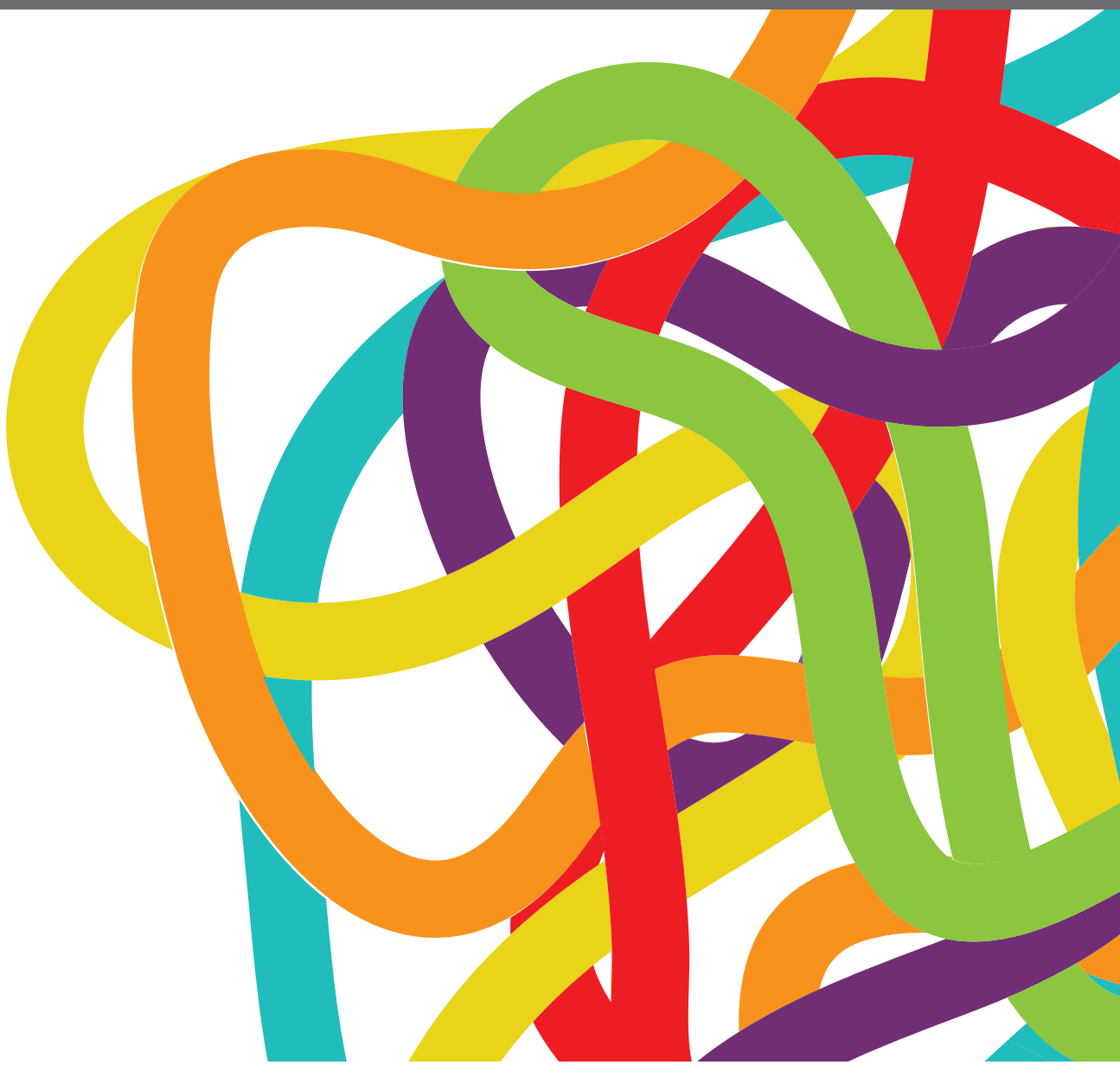

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QUALITY OF LIFE IN BREAST CANCER PATIENTS AND SURVIVORS

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EDITORIAL
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Editorial: Quality of Life in Breast Cancer Patients and Survivors

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Keywords: breast cancer, quality of life, biomarkers, multidisciplinary management, survivorship

An Editorial on the Research Topic

Quality of Life in Breast Cancer Patients and Survivors

Every year more than 2 million women receive a new diagnosis of breast cancer worldwide (1). However, thanks to the increasing effectiveness of the screening programs and treatment protocols, the number of people who die of this disease has declined (Nardin et al.). Nowadays, caregivers are expected not only to prolong their patients' life but also to preserve and improve their patients' wellness before, during, and after the treatment (2). The continuum from initial diagnosis of cancer through the rest of the life (commonly referred to as "survivorship") may evocate different issues and feelings to different subjects at different times (3). The ideal goal of survivorship is to return to, or even improve, the quality of life before diagnosis. This area incorporates a vast spectrum of concerns, such as treatment side effects, sexual life, pregnancy, social activities. In this complex scenario, a precision medicine approach is required to address quality of life issues and influence not only the decision-making but also treatment compliance.

We edited the present Research Topic to offer a new angle on some of the hot subjects about the quality of life in breast cancer survivors. Particular attention has been paid to the selection of articles focusing on patient-specific risk assessment methods and novel treatment strategies.

In a mini-review article, Nardin et al. present an overview of the long-term sequelae of breast cancer therapies. The Authors synthesize some of the most relevant aspects that may impact the quality of life after treatment, including cardiac toxicity related to anthracyclines, trastuzumab, endocrine therapy, and/or chest wall irradiation. In this respect, Axenie and Kurz give a particular emphasis on chemotherapy-induced sensorimotor alterations that may lead to polyneuropathy in a subset of patients. They provide insightful perspectives on the methods to assess these peripheral nervous system deficits and analyze the reliability of what they call "next-generation sensorimotor rehabilitation". We believe that this article will shed fresh light on the use of cutting-edge digital technologies in breast cancer rehabilitation. A well-known unwanted effect of hormone therapy is osteoporosis (4). A nationwide retrospective cohort study on South Korean breast cancer survivors by Lee et al., supports the concept that selective estrogen receptor (ER) modulation with tamoxifen does not increase the risk of osteoporosis before menopause. Another original research article by Li et al. provides insights into the cumulative incidence and risk factors of second primary cancers after diagnosis and treatment of breast cancer. Specifically, they propose a nomogram for individual risk estimation, patient follow-up, and counseling in early-stage ER^{NEG} breast cancer survivors. The results of these studies highlight the significance of performing a tailored risk assessment strategy.

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Multidisciplinarity is essential for the clinical management of breast cancer-related lymphedema (BCRL) (5, 6). This condition that frequently occurs after breast surgery and/or radiation therapy has tremendous implications on women's quality of life (7). A comprehensive review article by Invernizzi, Lopez et al. summarizes the most clinically relevant steps of the lymphatic system ontogenesis to discuss molecular alterations that are likely involved in BCRL pathogenesis and progression. An original elaboration of publicly available genomic data is also provided, allowing for the discussion of the present and future perspectives in biomarker-based patients' risk stratification. In a work by Vatansever et al., a local adaptation of the Upper Limb Lymphedema 27 (ULL-27) questionnaire has been studied. As not all patients can be assessed beyond their mother language, this work may help to implement this useful tool through a cross-cultural approach. Another important concern is represented by breast cancer fatigue (BCF), a condition characterized by persistent physical and/or mental stiffness (8). A prospective clinical study (Invernizzi, de Sire et al.) evaluated the feasibility and the effectiveness of a rehabilitation protocol for BCF, confirming how physical exercise may help improve the quality of life in breast cancer survivors. In line with these remarks, Chung et al. offer insightful viewpoints for the use of a mobile app-based approach to promote physical activity and decrease distress in breast cancer survivors. We would like to highlight that there are still not widely adopted guidelines to measure the muscular strength in these patients (9). For this reason, we have welcomed the study of Dos Santos et al., that evaluated the reliability and agreement for the 10-repetition maximum (10-RM) testing for upper (bench press) and lower (leg press) body strength. They observed that surgery may affect the upper limb but not lower limb strength in breast cancer patients. This paper is one of the few demonstrating that muscle strength increases during re-test, which may suggest significant effects either in technique improvement or muscle strength itself. Universal accessibility to rehabilitation/exercise enhancing centers is a subsequent problem to be considered.

During the past decade, literature embraced the concept that biology, body, and mind are vastly interconnected in both healthy and unhealthy individuals, including breast cancer patients (10). There are several lines of evidence that Tai chi, a Chinese martial art, may have a positive effect on both physical and psychological spheres in breast cancer survivors (11). A thorough update is provided in a systematic review and meta-analysis (Luo et al.) on the positive influence of this low-stress training method in improving the quality of life of breast cancer patients. Regrettably, many patients experience a decrease in cognitive and affective function after the diagnosis of breast cancer (12). This condition often relies on chemotherapy-related changes in brain structure and function (13). Because of its high vulnerability to adverse treatment effects, the hippocampus is becoming the focus of many lines of research (14). Here, Peukert et al. conducted a systematic review analysis of the current literature on hippocampus changes due to breast cancer treatment with subsequent cognitive and affective impairments. Surprisingly, they reveal that hippocampal alterations (e.g. volume loss, deformation, functional connectivity issues) may occur after all major types of treatment. These alterations are associated with cognitive impairments, where working memory, episodic memory, and prospective memory are the most frequently targeted affected domains. Disappointingly, cancer-related affective impairments are less studied and further research would be needed in this field. This is an area where multidisciplinary teamwork between oncologists and neurologist/psychologist is crucial.

Strategies to improve outcomes and subsequent quality of life should be put in place at the very beginning of the clinical workup of breast cancer patients. We believe that a biomarker-based approach in the risk assessment and treatment selection would represent a quantum leap for healthcare providers (15, 16). Based on their broad experience in the quality control of specimens and biomaterials handling in pathology labs, we invited the group of Berrino et al. to write a manuscript for this Research Topic. In their original research article, they provide previously unavailable data that monitored cold formalin fixation (4°C) is better than standard fixation for preserving DNA quality, which is pivotal in biomarkers assessment. An additional and little investigated type of treatment that may influence the outcome of breast cancer survivors is represented by neoadjuvant therapy (17). Chen et al. propose a nomogram based on machine learning that integrates magnetic resonance image data to select breast cancer patients that may benefit more from neoadjuvant chemotherapy. The final aim is the de-escalation of treatments that may impact the quality of life of breast cancer survivors. Beyond this ultimate goal for the physicians, breast cancer survivorship needs attention and efforts by society, communities, social media, and Institutions.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Reliability and Agreement of the 10-Repetition Maximum Test in Breast Cancer Survivors

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The aim of this study was to evaluate the reliability and agreement between the test and retest of the 10-repetition maximum (10-RM) test for leg press and bench press in breast cancer survivors (BCS). Thirty-one BCS participated in this study, age 54.87 ± 5.7 years. All performed 10-RM tests and retests for the leg press 45° and the bench press. For reliability analyses, an intraclass coefficient correlation (ICC) and coefficient of variation (CV) were performed. The limits of agreement were calculated using a Bland-Altman plot with 95% CIs. For absolute and relative error of measurement, we used standard error of measurement and minimally detectable change. The result showed a high reliability for the bench press and leg press; ICC of 0.94 and 0.98, respectively. CV was <10% for both exercises. The systematic error were 1.5 kg (10%) and 6.1 (8%) for the bench press and leg press, respectively. The standard errors of measurements were 0.96 kg (6.08%) and 4.11 kg (5.27%) for the bench press and leg press, respectively. The minimally detectable changes were 2.72 kg (17.20%) and 5.62 kg (7.21%) for the bench press and leg press, respectively. In breast cancer survivors, the muscular strength measurement for the 10-RM test showed a high to very high rate of reliability and agreement, with acceptable error of measurement.

Keywords: muscle strength, muscular measurement, strength training, resistance training, cancer

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INTRODUCTION

The assessment of muscle strength has been used to monitor and prescribe strength training (1). Muscular strength has been associated with high level of functional capacity and to decrease the risk of death from all natural causes (2, 3). The evaluation of muscle strength in breast cancer survivors (BCS) is a significant issue, because breast cancer (BC) treatment could reduce muscle strength after surgery and it may persist over the long-term (4). Therefore, in rehabilitation settings, the assessment of muscular strength is an important strategy to guide exercise prescription in these patients (5, 6).

Muscular strength loss in BCS is one of the side effects of BC treatment (surgery, chemotherapy, and radiotherapy), that could be explained by multiple factors such as: fatigue; lymphedema; decreased in shoulder, elbow, and wrist mobility; pain in the shoulder joint; and psychological changes such as kinesiophobia (7–13). These conditions could interfere with the reliability of maximum force tests and the strength outcomes during resistance training (14). In addition, these side effects of BC treatment pose a challenge for health professionals who work with resistance training for BC patients or survivors.

The one-repetition maximum test (1-RM) is considered the "gold standard" to measure maximum muscle strength in a non-laboratory setting. The 1-RM test is safe and has been applied in studies with BCS and BC patients (15–17). However, there is a lack of data regarding the reliability of this measurement within this population. To our knowledge, a single study presented only the coefficient of variation (CV) data for the bench press and leg press (18). Moreover, the 1-RM receive some criticism during a rehabilitation scenario such as risk of injury (19).

As an alternative to the 1-RM test, some studies with BCS used predictive formulas according to the results of multiple repetition tests (5-10RM) to estimate the maximum strength by 1-RM (20–23). Another method to estimate dynamic muscle strength is the repetition maximum test based on a goal of repetitions, as in the 10-RM test. The 10-RM test has been used to evaluate the load achieved in resistance training (RT) in different populations (24–30). Therefore, taking into consideration the characteristics of BCS, it seems that there is a natural concern with muscular strength tests for upper limbs, and maybe that could interfere on reliability of measurement. For this reason, it is possible that muscular strength for lower limbs could be more reliable than upper limbs. In addition, there is little information on the data of reliability and agreement of muscular strength tests in BCS, thus the performance of reliability studies is necessary.

The objective of this study was therefore to evaluate the reliability and agreement between the test and retest of the 10-RM test in upper and lower limbs in BCS. Our hypothesis was that the 10-RM test is reliable, and that the reliability is higher for the lower limbs.

MATERIALS AND METHODS

Design and Participants

In this reliability and agreement study, 31 BCS were included between February and October 2017. The BCS were contacted via phone calls and face-to-face interactions at the Mastology and Oncology Ambulatory of the University Hospital of the Federal University of Goias, Brazil. The eligibility criteria were: (1) confirmed BC stages I to III; (2) between 40 and 65 years old; (3) being in menopause (31); (4) not involved in any regular exercise program for the last 6 months; (5) completed cancer-related therapies including surgery, chemotherapy and/or radiotherapy at least 6 months prior to enrolling; (6) currently undergoing hormone therapy (tamoxifen or aromatase inhibitor); (7) received medical clearance for exercise training. Patients were excluded from the study if they had musculoskeletal limitations that could compromise exercise performance and/or any uncontrolled chronic disease that could represent a risk to their health.

The study was approved by Research Ethics Committee of the Federal University of Goias (CAAE: 50717115.4.0000.5083), and by the Research Ethics Committee of the Clinical Hospital of the Federal University of Goias (CAAE: 50717115.4.3001.5078). All participants provided written consent.

Procedures

After a measure of body composition, the participants answered medical history and sociodemographic questionnaire and the International Physical Activity Questionnaire (IPAQ—short version) (32). They then performed the 10-RM test at 2 different days within 2– 4 days in between. At day 1, the participants were familiarized with Leg press 45° and Bench press exercises and then performed the 10-RM test (**Figure 1**).

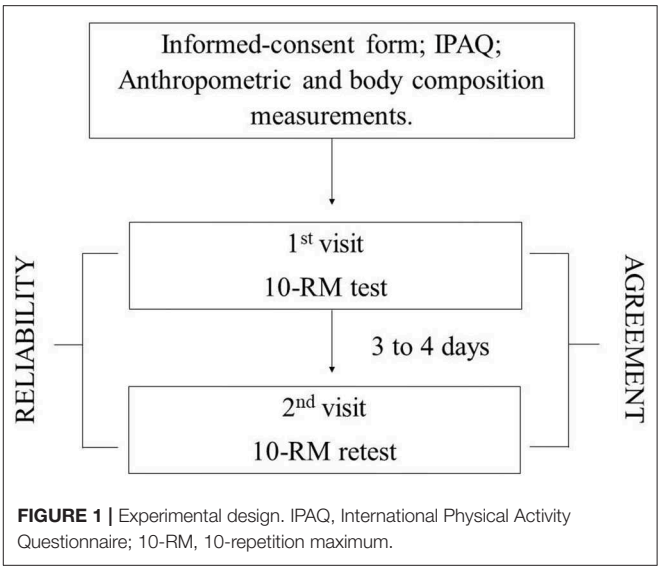

FIGURE 1 | Experimental design. IPAQ, International Physical Activity Questionnaire; 10-RM, 10-repetition maximum.

Anthropometry and Body Composition Assessments

Body mass index (BMI) was calculated based on body mass and height [BMI = weight (kg)/height squared (m^2)]. Fat and lean mass were assessed using dual energy X-ray absorptiometry (DXA) (General Electric Healthcare® model, Madison, WI, USA). Data were analyzed using GE Medical Systems Lunar™ software. A professional technician performed the assessments of DXADuring the DXA, participants remained in a supine position with their lower limbs relaxed, and the upper limbs were positioned along the body with forearms pronated. DXA's were calibrated and tested as recommended by the manufacturer. After analysis of the entire body area, the total body mass, lean body mass and fat mass were registered.

Ten Repetition Maximum Test

The 10-RM test and retests were performed by the leg press 45° (Rocha, Leg Press 45°, Goias, Brazil) and bench press exercises with free-weight, plate-loaded (**Supplementary Material**). Both exercises techniques followed the recommendation from the National Strength and Conditioning Association (NSCA) (33). During the 10-RM test and retest, the participants were informed and supervised by two experienced exercise science professionals. The same exercise science professionals supervised the measurements. The participants had three to five 10-RM attempts for each exercise.

The warm-up consisted of one set of 10 repetitions with 50% of the estimated 10-RM load, by rating of perceived exertion 5–6 (0–10) in the first day. For leg press, the warm-up represented ∼30–40% of their body mass. For bench press, we chose to use only the weight of the barbell (the barbell weighted 6 kg) to perfume the warm-up on the first day. The load used to

perform the warm-up during the 10-RM retest was based on the maximum load achieved on the first day (10-RM test).

The 10-RM load was determined if they were able to complete the 10th repetition but not be able to perform the 11th repetition. If the volunteer were able to performed more than 10 repetitions, the load was increased by 5–10%. The resting interval between each attempt was 3 min, and the resting interval between exercises was 5 min. The cadence was not controlled, but participants were oriented to perform the concentric phase as fast as possible but control the eccentric phase. Leg press 45° was performed first, followed by the bench press. All participants performed the bench press until touching the barbell on the sternum/breast. The 10-RM retest was performed 3–4 days later, using the maximum load achieved on the 10-RM test as reference to perform the first attempt (34).

Statistical Analyses

Descriptive statistics were presented as mean and standard deviation (SD). The intraclass coefficient correlation (ICC) and coefficient of variation (CV = SD divided by mean of test and retest × 100) was used for evaluation of reliability (35). The ICC form used was a two-way mixed effect, mean of k measurements and consistency agreement (36). The ICC and CV are present as mean and 95% of confidential interval (CI). The analyses of measurement error, absolute and relative, of the 10-RM test and retest was also investigated using the standard error of measurement [(SEM); SEM absolute = SD of the mean test-retest score divided by the square root of 1—ICC; SEM relative = SEM absolute score divided by mean test-retest scores and multiplying by 100] and minimally detectable change [(MDC); MDC absolute = 1.96 × the square root of 2 × SEM; MDC relative = MDC absolute score divided by mean of test-retest scores and multiplying by 100] (37). In addition, the limits of agreement were calculated using a Bland–Altman plot with 95% CIs (38). The Munro's classification of reliability was used to interpret the ICC coefficients: 0.50–0.69 reflects moderate correlation; 0.70–0.89 reflects high correlation; and 0.90–1.00 indicates very high correlation. Statistical analyses were performed using MedCalc Software (version 18.11.6) and Statistical Package for the Social Sciences Software (version 22).

RESULTS

Participants

The sociodemographic, cancer treatment status, and anthropometric characteristics of the participants are presented in **Table 1**.

Reliability and Agreement Between Test and Retest of 10-RM

The comparison between 10-RM test and retest showed high to very high reliability for the leg press 45° and bench press. For the leg press 45° and bench press exercises the ICC were 0.98 and 0.94, respectively. CV was below 10% for both exercises. The results of reliability are presented in **Table 2**.

The agreement between the 10-RM test and retest demonstrated that the results from the retest showed higher

TABLE 1 | Characteristics.

Characteristics	*N* = 31
Age (year)—mean (SD)	54.87 (5.7)
Education—no. (%)	
<8 years of the study	15 (48.4)
>8 years of the study	16 (51.6)
Self-reported race—no. (%)	
Caucasian	20 (64.5)
Non Caucasian	11 (35.5)
Occupation—no. (%)	
Homemaker or cleaner	6 (19.4)
Housewife	16 (51.6)
Nurse	1 (3.2)
Retired	5 (16.1)
Saleswoman	2 (6.5)
Teacher	1 (3.2)
Marital status—no. (%)	
Single	7 (22.6)
Married	16 (51.6)
Divorced	4 (12.9)
Widow	4 (12.9)
Arterial hypertension—no. (%)	9 (29)
Diabetes—no. (%)	3 (9.7)
Months since cancer diagnosis—mean (SD)	40.68 (14.8)
Cancer stage—no. (%)	
I	10 (32.3)
II	17 (54.8)
III	4 (12.9)
Breast surgery—no. (%)	
Lumpectomy	1 (3.2)
Lymphadenectomy	1 (3.2)
Mastectomy and breast reconstruction	1 (3.2)
Mastectomy	13 (41.9)
Mastectomy and quadrantectomy	1 (3.2)
Quadrantectomy	13 (41.9)
Quadrantectomy and breast reconstruction	1 (3.2)
Months since breast surgery—mean (SD)	29.03 (15.4)
Axillary lymph nodes removed—mean (SD)	4.86 (4.3)
Chemotherapy—no. (%)	26 (83.9)
Adjuvant	14 (45.2)
Neoadjuvant	12 (38.7)
Missing data	5 (16.1)
Radiotherapy—no. (%)	28 (90.3)
Hormone therapy—no. (%)	
Tamoxifen	27(87.1)
Aromatase inhibitors	4 (12.0)
Self-reported lymphedema—no. (%)	13 (41.9)
Anthropometry and body composition	
Weight (kg)—mean (SD)	68.67(11.4)
Height (cm)—mean (SD)	157.08 (6.2)
BMI—mean (SD)	27.85(4.5)
Body fat (%)—mean (SD)	46.36(5.90)
Body fat mass (kg)—mean (SD)	31.18(8.33)
Body lean mass (kg)—mean (SD)	35.26(4.64)
Level physical activity (MET-h/wk)—mean (SD)	23.38 (26.40)

SD, standard deviation; BMI, body mass index; MET, metabolic equivalent of task.

TABLE 2 | Analysis of reliability and agreement between 10-RM test and retest.

Exercises	10-RM test (mean ± SD)	10-RM retest (mean ± SD)	CV (95% CI)	ICC (95% CI)	SEM (SEM%)	MDC (MDC%)
Leg press (kg)	74.84 (28.50)	80.97 (29.70)	5.87 (3.19–8.55)	0.98 (0.96–0.99)	4.11 (5.27)	5.62 (7.21)
Bench press (kg)	15.03 (3.79)	16.55 (3.70)	7.27 (4.10–10.45)	0.94 (0.87–0.97)	0.96 (6.08)	2.72 (17.20)

10-RM, 10-repetition maximum; SD, standard deviation; kg, kilogram; CV, coefficient of variation; ICC, intraclass coefficient correlation; CI, confidential interval; SEM, standard error of measurement; MDC, minimally detectable change; LOALB, limits of agreement lower boundary; LOAUB, limits of agreement upper boundary.

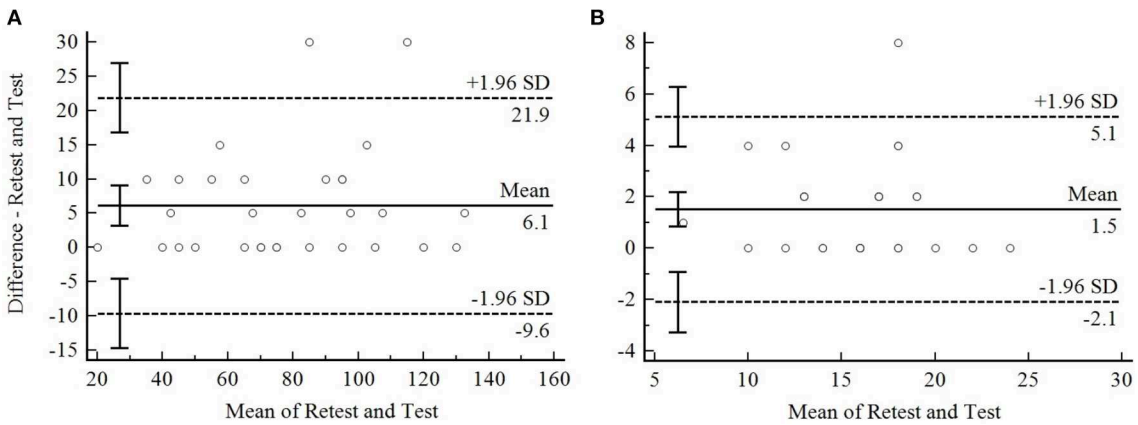

FIGURE 2 | Bland-Altman plot of 10-RM for the leg press 45° **(A)** and the bench press **(B)**. The dotted line represent the limits of agreement upper and lower boundary. The continue line on the center of plot represent the systematic bias. The continue line on the Y axis represent the mean difference between 10-RM retest and test, and on the X axis represent the mean of 10-RM retest and test.

load than the test situation performed at day 1 (systematic bias values in **Figures 2A,B** are positives because the analysis were performed with 10-RM retest as first method and 10-RM test as second method for to build the Bland-Altman plots). The Bland-Altman plots (**Figure 2**) showed the mean difference with 95% IC limits of agreement.

The relative difference between the test and the retest was predicted in 8.3% (limits of agreement for upper and lower boundary 28 and −11%) and 10.3% (limits of agreement for upper and lower boundary 34 and −13%) for the leg press 45° and the bench press, respectively.

The relative and absolute SEM and MDC are presented in **Table 2**.

DISCUSSION

This study aimed to evaluate the reliability and agreement between the 10-RM test and retest for the leg press 45° and bench press exercises in BCS. We found a high to very high rate of reliability and agreement with lower and acceptable CV (CV < 10%), SEM (absolute and relative) and MDC (absolute and relative) between the 10-RM test and retest for both the leg press 45°. and bench press However, a higher value was found in the 10-RM retest situation, for both exercises. To our knowledge, this study is the first to evaluate the 10-RM test reliability in BCS, and the results suggest that 10-RM test could be used to measure muscular strength.

In general, a few studies have previously reported the reliability of test and retest 10-RM. In older people, Farinatti et al. (27) described high reliability of the 10-RM test for the dumbbell bench press (ICC 0.90; typical error 1.61 kg) and knee extension (ICC 0.96; typical error 2.01 kg) in elderly healthy women (68 ± 4 years old). Farinatti et al. (39) reported a high ICC for the barbell bench press in young (22 ± 2 years old) and elderly women (69 ± 7 years old) (0.91 and 0.90, respectively). For the leg press 45°, a high ICC (0.99) was reported in young healthy people (24 ± 3 years old) (40). Monteiro et al. (41) also reported a high ICC for the leg press 45° (0.92) and the bench press (0.90) in adult women (37.6 ± 1.7 years old). Our study found a similar reliability to those studies. Therefore, it seems that the 10-RM test reliability for BCS is similar to that of healthy individuals of different ages.

The CV of 10-RM test showed be <10% for lower and upper limbs. That was similar compare to 1-RM in BCS (18). Winter-Stone et al. (18) reported CV of the 6.6 and 7.5% for the leg press and chest press, respectively. We found a similar CV for the leg press 45° and bench press for 10-RM, 5.87 and 7.27%, respectively. Moreover, our results suggest that lower limbs have a better reliability than upper limb exercise, as we hypothesized. It could be explain by lower capacity of lifting for upper limbs compare to lower limbs, this may be result of sides effects of breast cancer treatments.

The 10-RM retest achieved higher load than 10-RM test situation, which may suggest some training effects either in technique or muscle strength of the first exercise test. A repeated strength measurement could provide a process of the learning of task, improving the ability/skill to perform the

movement. Bernardi et al. (42) showed that skill acquisition to perform maximal voluntary contraction allows better control of neuromuscular system which could provide higher force generation through the trials. Grosicki et al. (43) also found higher value of 1-RM in the second trial than the first trial of assessment in young adults and older people, women and men, for leg press, leg extension and biceps curl. The same behavior was observed by Amarante do Nascimento et al. (44). They found that the second day of testing was higher than first day, but similar with the third day in 1-RM load for bench press and leg extension in elderly women (65 ± 4 years old) (44). Thus, the muscle strength values could be reached in the second or third trial of measurement.

The 10-RM test could be useful in the real word for prescribing or monitoring the load of the training. The use of percentage of 1-RM test may present a large variability in the number of repetition performance. Grosicki et al. (43) showed that using 60% and 80% of 1-RM test the participants were able to perform 28.8 (±9.2)/23.3 (± 16.3) and 17 (±6.5)/12.8 (±7.8) repetitions in younger and older women, respectively. Hence, the session of training would be high or low effort, if use the percentage of 1-RM test. Therefore, it seems that using the load reached from 10-RM test could be more precisely to prescribe and monitor the number of repetitions during the training session, and that may be one advantages of 10-RM test compared to 1-RM test. Another advantage of 10RM test could be a better perception of safety and acceptance in BCS, since there have been reported knesiohpobia, fear of movement (11, 45). In addition, repetition to failure as 10-RM might be used to predict 1-RM loads for the bench press/chest press (46–48) and leg press 45°/horizontal (47–49), with a low error of measurement. However, we did not investigate the accuracy of the 10-RM load to predict a 1-RM load in BCS. Future studies could investigate the accuracy of the 10-RM test to predict a 1-RM load in BC patients and BCS.

STRENGTHS AND LIMITATIONS

The study has important strengths. The tests were supervised by two experienced exercise physiologists/professionals that provided better control of the 10-RM test and guaranteed the safety and confidence for the participants to perform higher load, and the homogeneity of the tests. One limitation of the present study included the lack of assessment of shoulder range of motion during the bench press test. However, we think this limitation was eliminated by the experienced physiologists.

CONCLUSION

In conclusion, muscular strength measurement using 10-RM test has a good to excellent rate of reliability and agreement, with acceptable error of measurement. Due to lack of information about the reliability of 1-RM test in BCS, 10-RM test could be an interesting alternative for diagnosis and prescription in this population. Therefore, the 10-RM test may be used to evaluate the muscular strength in BCS. The new studies with BC patients and BCS could report the reliability of the maximum force production on isoinertial exercises.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the Federal University of Goias (CAAE: 50717115.4.0000.5083), and by the Research Ethics Committee of the Clinical Hospital of the Federal University of Goias (CAAE: 50717115.4.3001.5078). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WS and CV performed the study concept and design. WS and GS supervised the muscle assessments. WS, RS, and WM conducted the analyses. WS wrote the original draft of the manuscript. AV, WM, PG, and CV wrote, reviewed, and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00918/full#supplementary-material

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Exercise Promotion and Distress Reduction Using a Mobile App-Based Community in Breast Cancer Survivors

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Physical activity (PA) enhancement and mental distress reduction are important issues in cancer survivorship care. Mobile technology, as an emerging method for changing health behaviors, is gaining attention from many researchers. This study aimed to investigate the effect of a mobile app-based community on enhancing PA and decreasing distress in breast cancer survivors. We conducted a non-randomized, prospective, interventional study that had a mobile community-later arm and mobile community-first arm. With an Android smartphone app (WalkON®), daily walk steps and weekly distress scores using app-based Distress Thermometer (DT) questionnaires were collected from participants for about 12 weeks. To examine the difference in weekly step counts before and during the community activity, we used a paired *t*-test method. For a comparative analysis, we referred to a previous prospective observational study without a mobile community intervention that had the same setting as the present study. After propensity score matching (PSM), multivariable regression modeling with difference-in-difference (DID) was performed to estimate the effect of the mobile app-based community on PA and mental distress. From January to August 2018, a total of 64 participants were enrolled in this study. In the univariate analysis, after participation in the mobile community, the participants showed a significant increase in total weekly steps ($t = -3.5341$; $P = 0.00208$). The mean of the differences was 10,408.72 steps. In the multivariate analysis after PSM, the mobile community significantly increased steps by 8,683.4 per week (p value <0.0001) and decreased DT scores by 0.77 per week (p value $= 0.009$) in the mixed effect model. In the two-way fixed effect model, the mobile community showed a significant increase in weekly steps by 8,723.4 (p value <0.0001) and decrease in weekly DT by 0.73 (p value $= 0.013$). The mobile app-based community is an effective and less resource-intensive tool to increase PA and decrease distress in breast cancer survivors.

Trial Registration: NCT03190720, NCT03072966

Keywords: telemedicine, breast neoplasms, mobile applications, quality of life, psychological stress, exercise, smartphone, survivorship

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INTRODUCTION

Many cancer survivors experience psychological distress after cancer diagnosis. Depression and anxiety have a prevalence of 11.6 and 17.9%, respectively, among cancer survivors (1). A systematic review reported 22 and 10%, respectively, as the prevalence of depression and anxiety specifically among breast cancer survivors (2). The National Comprehensive Cancer Network (NCCN) guidelines recommend that cancer patients should be managed for their distress regularly according to clinical practice guidelines (3).

Pharmacologic and/or non-pharmacologic interventions can be used to manage depressive symptoms in cancer survivors (4). Exercise is one of the important non-pharmacologic interventions for mental distress in breast cancer survivors. Many breast cancer survivors are not physically active, which is generally associated with poorer health-related quality of life (HRQOL) (5). Interventions to enhance physical activity (PA) may improve mental HRQOL in breast cancer survivors who have completed cancer therapy. Moreover, PA can increase overall survival in breast cancer survivors and decrease treatment-related adverse effects, such as fatigue, thereby leading to better QOL. The Breast Cancer Survivorship Care Guidelines from the American Cancer Society and American Society of Clinical Oncology recommend that breast cancer survivors avoid inactivity and do at least 150 min of moderate or 75 min of vigorous aerobic exercise every week (6).

To enhance PA in cancer survivors, a number of psychological theories related to behavioral change and methodologies have been applied (6). Studies to increase PA in breast cancer survivors have shown that interventions such as direct interviews or regular telephone calls to participants could significantly increase PA levels. However, these interventions are so resource-intensive that clinicians cannot easily perform them in real-world daily practice (7, 8).

Recently, mobile technology, as an emerging method for changing health behaviors, is gaining academic attention (9). Mobile app-based health promotion programs can monitor users' health status and provide health information and feedback, which may lead to behavior change. However, most previous studies have been conducted on general populations, not in cancer survivors. Moreover, although these mobile app-based programs increase PA in cancer survivors, their distress-reducing effects, by increasing PA, have not been investigated.

The present study aimed to investigate the effects of a mobile app-based community program on enhancing PA, and assess whether the mobile community program decreases mental distress through increasing PA in breast cancer survivors.

METHODS

Participants

We conducted a non-randomized, prospective, interventional study (NCT03190720) enrolling female patients who were hospitalized for breast cancer surgery at Asan Medical Center. Patients were included if their ages were between 20 and 60 years and their own Android smartphone was compatible with the free activity-tracking app modified for this study. Patients were excluded if they had metastatic or recurrent breast cancer, or were not capable of using a smartphone. Patients who were pregnant, resided in a foreign country, or were scheduled for adjuvant chemotherapy were also excluded. After enrollment, if adjuvant chemotherapy was decided according to final pathologic reports, these patients were also excluded. Participants were assigned to two arms: (1) mobile community-later arm, in which participants were followed without being registered to a mobile community at first and then registered to the mobile community after about 6 weeks; (2) mobile community-first arm, in which participants were registered to the abovementioned mobile community at first and then dropped from the mobile community and followed after 6 weeks.

We also included a previous prospective study for a comparative analysis (NCT03072966). The previous study was conducted to develop distress-screening algorithms using mobile devices in breast cancer survivors who had never been registered to a mobile community (10), and otherwise, the platform of study design was similar to that of the present study. We used this group of patients as a control group who did not participate in the mobile community.

Written informed consent was obtained from all participants at study enrollment. The study protocol was approved by the institutional review board at Asan Medical Center (2017-0328). These studies were registered on the ClinicalTrial.gov website (NCT03190720, NCT03072966).

Study Setting

During the hospital stay after breast cancer surgery, patients were contacted by a clinical research assistant. After consenting to participate, the participants completed paper-based questionnaires (Distress Thermometer, DT) at baseline. The Android-based app for this study was downloaded to the participants' smartphones.

At the start of the study, patients were allocated to the mobile community-later group. After the 20th participant was followed for 4 weeks, we opened a mobile app-based community and registered 20 participants at the same time. Originally, this study was designed to register participants in the mobile community-later group to the mobile community 6 weeks after enrollment. However, if the first participant goes into the mobile community 6 weeks after enrollment, then this participant would be alone, which means this participant is not in an actual community. Thus, we gathered 20 participants and then opened the mobile community.

Mobile App for Data Collection and Mobile Community

We used a free mobile health care app (WalkOn®, Swallaby Co., Ltd., Seoul, Republic of Korea, http://www.swallaby.com/) as a study platform. This app (10) was modified to obtain users' daily step count and app-based questionnaires on weekly DT (3). The participants were instructed to open and pull-to-refresh the app at least once a week to send the daily walking data to a central database system that archives the anonymized data for

each participant. The participants were also asked to report DT once a week.

In this app, we opened the mobile app-based community for an intervention where users can view other members' daily step count to motivate them and promote health-related activities. Health information on diet and PA was posted on the board in the mobile app-based community once a week.

Statistical Analysis

The balance among the participant demographics, clinicopathological, and intervention variables was compared using a chi-squared test or univariate analysis of variance (ANOVA) according to assigned group.

To examine the effect of the mobile app-based community on weekly step count before and during community activity in the mobile community-later group, we performed a paired *t*-test. To test the persistence of the mobile community effect, we also tested the difference in step counts during and after community activity using a subsample of the community-first group.

Multivariable regression modeling with difference-in-difference (DID) was performed to estimate the effect of the mobile app-based community on PA and mental distress between the control and community-later groups. To reduce the effect of selection bias in the quasi-experimental studies, we employed both mixed and two-way fixed effects models. Dependent variables were the weekly total steps and weekly average scores of DT.

We employed standard DID method to analyze the effect of the mobile community on PA. Regressions included an indicator variable for the time period of PA measurement (i.e., pre- vs. post-mobile app-based community initiation), an indicator variable for community group (control vs. mobile community-later), and the interaction of these two indicators. The indicator for time period corresponded to the difference in steps for participants before vs. after community initiation. The indicator for control and community-later groups corresponded to the difference between the two groups at baseline. The interaction term reflected the DID parameter (i.e., change in steps with the intervention of app-based community relative to change for the control group).

Individual participants (fixed effect) and months (random effect) were included as control variables in a linear mixed-effects model. In the two-way fixed-effects model, the week (fixed effect) was included as control variable instead of the month.

1. Mixed effects model (monthly random effects + individual fixed effects)

$$y_{it} = \beta_1 During\_Community_{it} + \beta_2 Community\_later_i \times During\_Community_{it} + Month_t + Patient\_Fixed_i + \epsilon_{it}$$

2. Two-way fixed effects model (weekly fixed effects + individual fixed effects)

$$y_{it} = \beta_3 Community\_later_i \times During\_Community_{it} + Weekly\_Fixed_t + Patient\_Fixed_i + \epsilon_{it}$$

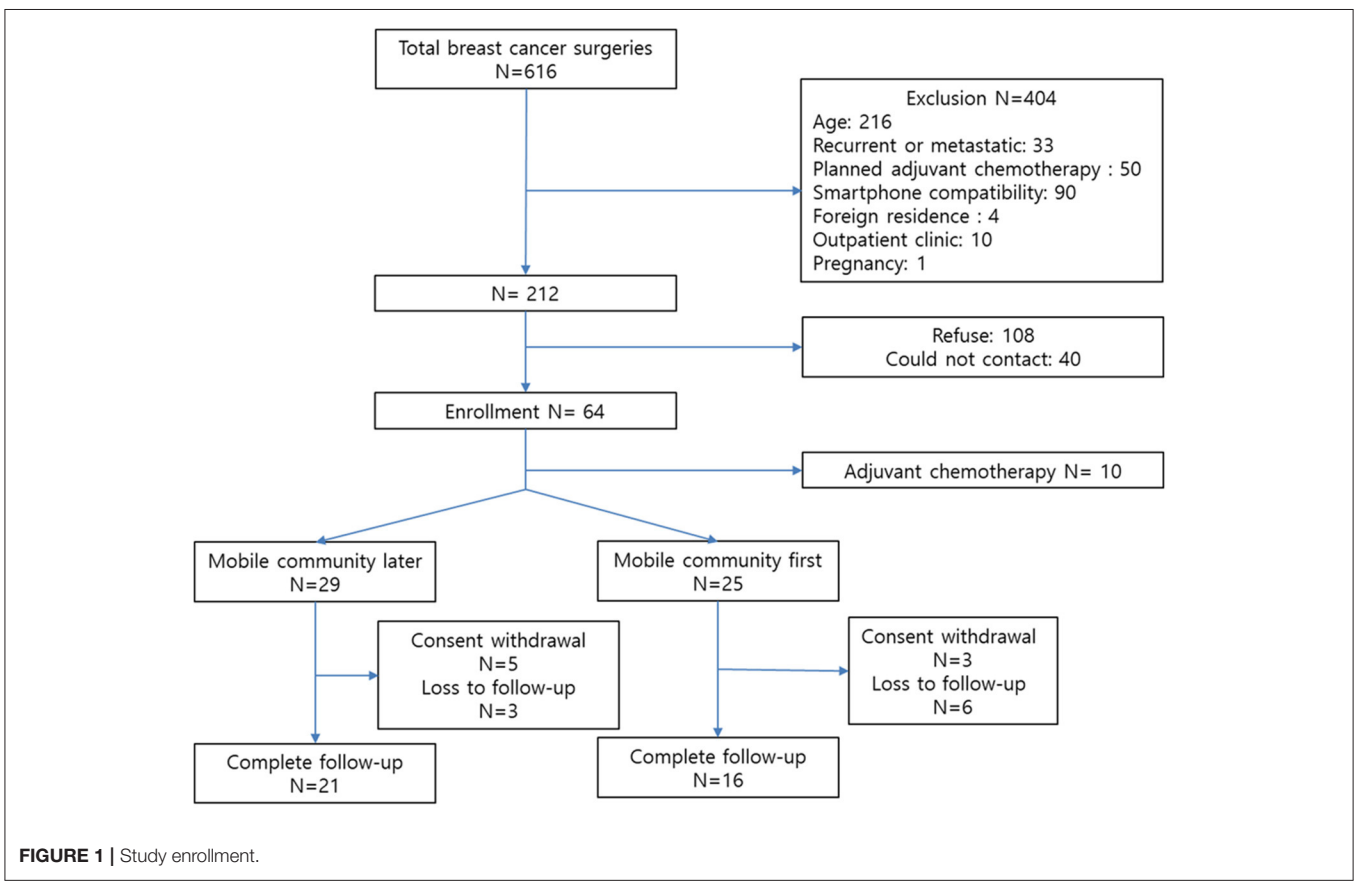

FIGURE 1 | Study enrollment.

As the purpose of this analysis was to estimate the effect of the community compared with the control group, without the confounding effect of the time trend, we used a panel of two groups (except the community-first group). The panel data were constructed using the data before and after 6 weeks from the initiation of the community intervention.

In addition, to decrease the risk of biased estimates of the intervention effect, we defined the logit of predicted probability of intervention as a propensity score using the following patient characteristics: age, marital status, education level, employment status, comorbidity, episode of depression, anti-hormonotherapy, and chemotherapy. The balance check was performed again after matching the control and community-later groups using propensity score matching (PSM). Matching was carried out using a ratio of 1:2, and a caliper distance of 0.025, without replacement based on nearest-neighbor matching. All analyses were conducted using R Software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria), and a p value of <0.05 was considered to indicate statistical significance.

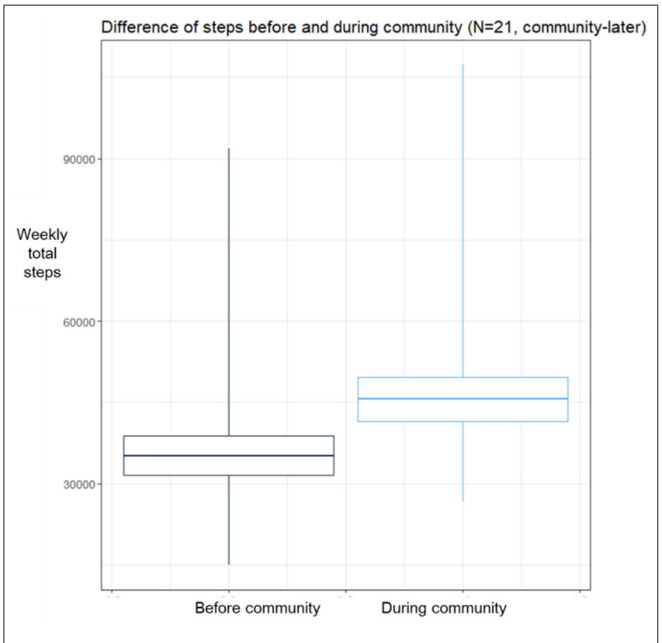

FIGURE 2 | Comparison of weekly step counts. Boxplot means ±1*standard deviation of sample. After-intervention significantly higher than before-intervention period.

RESULTS

Descriptive Statistics

From January to August 2018, a total of 64 participants were enrolled (**Figure 1**). Following enrollment, 10 patients whose adjuvant treatment plans were changed according to postoperative pathologic results were excluded. After 3 months of follow-up, the number of participants in the mobile community-later and -first groups were 21 and 16, respectively. We included 160 patients from the previous prospective study for the comparative analysis (10).

Table 1 summarizes the descriptive statistics of the demographics and clinical characteristics of the participants as absolute and relative frequencies. The mean age of patients was 44.45 (SD 6.42) years. Most patients were married (82.50%) and had completed post-secondary education (71.88%). Of all, 58 patients (36.25%) were employed full-time. A total of 106 patients (66.25) were diagnosed with comorbidities. Only one patient (0.63%) was stratified into the group that experienced an episode of depression; 59 patients (36.88%) were stratified into the group that received chemotherapy; and 134 patients (83.75%) received anti-hormonal therapy.

No difference was seen among patients who were in the control, mobile community-later, and community-first groups in terms of education level, employment, episode of depression, and anti-hormonal therapy. However, age at diagnosis, marital status, comorbidity, and previous chemotherapy were different depending on group.

Improvement of Weekly Step Outcome

Figure 2 shows a comparison of weekly total step counts before and during community activity in the mobile community-later group. After participation in the mobile community, the participants showed a significant increase in total weekly steps

TABLE 1 | Baseline characteristics.

	Full sample	Mobile community-later	Mobile community-first	Control	*P* value
n	160	21	16	123	
Age at diagnosis, years (std)	44.45 (6.418)	43.762 (5.770)	40.313 (5.896)	45.106 (6.418)	0.016
Married, *n* (%)	132 (82.500)	12 (57.143)	15 (93.75)	105 (85.366)	0.015
Bachelor's degree, *n* (%)	115 (71.875)	17 (80.952)	12 (75)	86 (69.919)	0.564
Employed, *n* (%)	58 (36.25)	7 (33.333)	9 (56.25)	42 (34.146)	0.214
Comorbidity, *n* (%)	106 (66.25)	10 (47.619)	8 (50)	88 (71.545)	0.035
Episode of depression, *n* (%)	1 (0.625)	1 (4.762)	0 (0)	0 (0)	0.400
Previous chemotherapy, *n* (%)	59 (36.875)	4 (19.048)	3 (18.75)	52 (42.276)	0.036
Anti-hormonal therapy, *n* (%)	134 (83.75)	16 (76.190)	13 (81.25)	105 (85.366)	0.490

std, standard deviation.

TABLE 2 | Effect of mobile app-based community on weekly steps and mental distress (before PSM).

Panel A: Weekly total steps

	Mixed effects model		Two-way fixed effects model	
	B (SE)	***P* value**	**B (SE)**	***P* value**
Time 1 (vs. time 0)†	4763.3 (1273.8)	0.0002	NA	NA
Community-later group change in steps between times 0 and 1 (vs. control group change)§	6116.3 (1705.1)	0.0003	6177.2 (1697.7)	0.0003
Number of patients	144		144	
Observation (number of weeks * number of patients)	1,770		1,770	
R-squared	0.6852		0.6895	

Panel B: Weekly average DT

	Mixed effects model		Two-way fixed effects model	
	B (SE)	***P* value**	**B (SE)**	***P* value**
Time 1 (vs. time 0)†	0.2918 (0.1926)	0.1303	NA	NA
Community-later group change in DT score between times 0 and 1 (vs. control group change)§	−0.4340 (0.2035)	0.0334	−0.4355 (0.2026)	0.0321
Number of patients	99		99	
Observation (number of weeks * number of patients)	651		651	
R-squared	0.8047		0.8095	

PSM, propensity score matching; SE, standard error; DT, Distress Thermometer; NA, not available. †Time variable, §Difference-in-difference term, examining difference over time between community-later and control groups.

TABLE 3 | Characteristics of all propensity-matched patients.

	Full sample	**Community-later**	**Control**	***P* value**
n	63	21	42	
Age at diagnosis, years (std)	43.3016 (5.3420)	43.7619 (5.7698)	43.0714 (5.1721)	0.6460
Married, *n* (%)	45 (71.4286)	12 (57.1429)	33 (78.5714)	0.0952
Bachelor's degree, *n* (%)	49 (77.7778)	17 (80.9524)	32 (76.1905)	0.7573
Employed, *n* (%)	25 (39.6825)	7 (33.3333)	18 (42.8571)	0.6489
Comorbidity, *n* (%)	37 (58.7302)	10 (47.6191)	27 (64.2857)	0.3196
Episode of depression, *n* (%)	1 (1.5873)	1 (4.7619)	0 (0)	0.3333
Chemotherapy, *n* (%)	8 (12.6984)	4 (19.0476)	4 (9.5238)	0.4234
Anti-hormonal therapy, *n* (%)	49 (77.7778)	15 (76.1905)	33 (78.5714)	1

std, standard deviation.

($t = -3.5341$; $P = 0.00208$). The mean of the differences was 10,408.72 steps.

In the mobile community-first group, the weekly total steps seemed to decrease, but the difference was not statistically significant ($t = 0.98896$; $P = 0.3384$) according to a paired *t*-test (**Supplementary Figure 1**). The mean of the differences was 3,869.103 steps.

DID With Control and Mobile Community-Later Groups Before PSM

Table 2, panel A lists the results from the multivariate regression analysis for weekly total step measure. Controlling for time and individual-idiosyncratic effects, we found that patients treated at the community in the later (post-community initiation) time period were significantly more likely to walk compared with patients treated at the control group in both mixed and fixed effects models.

Compared with the simple before-and-after comparison for the community-later group using paired *t*-test in **Figure 2**, this DID analysis could define the natural time trend of weather change. As shown in the coefficient of Time 1, in the post-community initiation period, the weekly steps for patients were significantly greater by as much as 4,763 steps. Therefore, compared with the increase of 10,000 steps in paired *t*-test, the effect of community in DID analysis was about 6,100–6,200 steps after removing the time trend effect of about 4,500 steps.

We then tested the difference in weekly average DT scores, the second measure of community effect. **Table 2**, panel B lists the results from the multivariate regression analysis for weekly average DT scores. Controlling for time and individual-idiosyncratic effects, we found that patients treated at the community in the later (post-community initiation) time period had significantly lower DT scores compared with patients treated at the control group in both mixed and fixed effects models by as much as 0.4 points.

DID With a Matched Control Group: Weekly Steps and Distress Level

A total of 123 control and 21 community-later patients were eligible for matching. The matching process resulted in a final cohort of 63 patients (42 control and 21 community-later patients) eligible for further analysis. The patient characteristics are summarized in **Table 3**. The mean ages of the control and community-later cohorts were 43.7 and 43.0 years, respectively. Comorbidities were reported by 47.6 and 64.2% in the control and community-later cohorts, respectively. All differences in covariates were statistically insignificant ($P > 0.1$).

For both measures, the differences were statistically significant; our previous results were robust toward the PSM. **Table 4**, panel A lists the results from the multivariate regression analysis for weekly total steps, and **Table 4**, panel B, the results of the effect of community on weekly average DT scores. Controlling for time and individual-idiosyncratic effects,

TABLE 4 | Effect of mobile app-based community on weekly steps and mental distress after PSM.

Panel A: Weekly total steps

	Mixed effects model		Two-way fixed effects model	
	B (SE)	***P* value**	**B (SE)**	***P* value**
Time 1 (vs. time 0)[†]	2128.47 (2053.72)	0.3	NA	NA
Community-later group change in steps between times 0 and 1 (vs. control group change)[§]	8683.40 (1992.87)	0.00001	8723.35 (1977.94)	0.00001
Number of patients	63		63	
Observation (number of weeks * number of patients)	772		772	
R-squared	0.5957		0.6065	

Panel B: Weekly average DT

	Mixed effect model		Two-way Fixed effect model	
	B (SE)	**P value**	**B (SE)**	**P value**
Time 1 (vs. time 0)[†]	0.4537 (0.3384)	0.1812	NA	NA
Community-later group change in DT score between times 0 and 1 (vs. control group change)[§]	−0.7724 (0.2933)	0.009	−0.7328 (0.2916)	0.0126
Number of patients	46		46	
Observation (number of weeks * number of patients)	308		308	
R-squared	0.7606		0.7729	

PSM, propensity score matching; SE, standard error; DT, Distress Thermometer; NA, not available. [†]Time variable, [§]Difference-in-difference term, examining difference in steps over time between community-later and control groups.

the analyses showed that patients treated at the community in the later (post-community initiation) time period were significantly more likely to walk compared with patients treated in the control group in both mixed and fixed effects models by as much as about 8,700 steps.

In terms of DT, controlling for time and individual-idiosyncratic effects, patients treated at the community in the later (post-community initiation) time period had significantly lower DT scores compared with patients treated in the control group in both mixed and fixed effects models by as much as about 0.8 points.

DISCUSSION AND CONCLUSION

Our results indicated that the selected mobile app-based community was effective to increase PA and decrease mental distress in breast cancer survivors. Breast cancer survivors after breast cancer surgery who participated in the mobile community walked significantly more steps per week, by around 7,500 steps, compared with counterparts who did not register in the mobile community. Further, the level of mental distress estimated by DT was significantly lowered in breast cancer survivors in the mobile community compared with the non-community group.

To our knowledge, this prospective study is the first to investigate the effect of mobile app-based community on PA in cancer survivors. Many studies have investigated behavioral changes using mobile phones in general populations (9), but rarely in cancer survivors. Moreover, this study is the first to be performed for the purpose of decreasing mental distress using mobile devices in cancer survivors. Studies using mobile tools have mainly focused on behavior changes or diet in general populations, among whom mental distress is relatively not a bigger issue than in cancer survivors, most of whom experience psychological problems that have huge impacts on them for the rest of their lives (3).

Studies have used mobile tools simply for providing self-monitoring, feedback, or information (11–13). The concept of a mobile community that supports and encourages participants has been used in a few studies. A study that used web- and app-based communities to promote healthy lifestyles showed that these tools significantly decrease body weight, body fat percentage, and waist circumference after 38 weeks of intervention (14). Another study illustrated that "social" apps with group-based collaboration and competition could significantly reduce overall amounts of sedentary lifestyle and increase PA (15). However, although mobile devices have been shown to improve lifestyle and body composition, the actual influence of these small changes on physical health remains questionable. In the present study, a mobile app-based community was used to enhance weekly steps and reduce mental distress in breast cancer survivors. The importance of this finding is that health promotion using mobile devices can have a significant impact on the mental health among breast cancer survivors, with respect to their daily lives.

To develop interventions that can enhance PA in cancer survivors, theory-based research should be conducted (16). Social cognitive theory, for example, includes the important constructs of outcome expectation following a specific behavior (17). Social outcomes are one of the expected outcomes, including social reactions such as approval. Self-evaluative outcomes, meanwhile, include one's own reaction. Another is theory of planned behavior, which suggests that behavior is determined by intention, which in turn is determined by attitude, subjective norm, and perceived behavioral control (18). Subjective norm refers to the perceived social pressure that individuals feel to perform or not perform. In the present study, the mobile community platform seemed to work based on the abovementioned theories to change participants' behavior.

This study had much strength, including its longitudinal tracking involving multiple time points for both walking activity and mental health. We collected enough data points before and after the initiation of community activity. The study was not randomized; as such, while this research did have the advantage of mimicking the real-life choice of selecting whether to enroll in the community or not, causality could not be assumed.

Notably, we used statistical approaches, namely, DID and PSM, that allowed us to model the effects of intervention without a natural time trend and a group difference at baseline. DID is a statistical technique used in econometrics and quantitative research that attempts to mimic an experimental research design using observational study data, by studying the differential effects of an intervention on a treatment group vs. a control group in a natural experiment (19). In our PSM, our pseudo-control and community-later groups were extremely well-matched statistically at baseline. However, future research using a randomized control trial is required to confirm whether involvement in the community is causally linked with better mental health and weekly walking activity.

As the number of cancer survivors has increased around the world (20), the importance of cancer survivorship care has been emphasized. Various technologies developed recently have the potential to launch a new chapter in cancer survivorship care. As a part of such efforts, we conducted this prospective study as well as launched a randomized controlled study to develop distress screening algorithms using mobile device-based PA data in breast cancer survivors in the Distress Reduction by Activity Tracking and Activity Enhancement by Mobile Support Group in Oncology study (DRAAGON study, NCT03783481). We expect these studies to provide new tools to enhance the level of the management of cancer survivorship.

The limitations of the present study should be noted. First, all participants were female breast cancer patients. Thus, our findings can only be generalized to this group of patients. Second, this study was performed in a single tertiary hospital in the Republic of Korea. Third, we did not conduct a randomized controlled trial. Therefore, inherent bias may have influenced the results of the study. Finally, the long-term effects of a mobile community on PA and mental distress have not been demonstrated; the follow-up period of this study was relatively short.

In conclusion, the mobile app-based community is an effective tool to increase PA in breast cancer survivors, which may lead to a decrease in mental distress. The potential role of mobile devices in the management of distress among breast cancer survivors should be further investigated in future.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board at Asan Medical Center (2017-0328). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IC, JL, YM, and SC designed the study and provided input throughout the study. YP, HC, HP, and ML collected the data. SC, BS, and S-HA provided clinical expertise. MJ, DC, SL, and IC analyzed portions of the data. IC and MJ wrote the manuscript along with contributions from all of the authors. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.01505/full#supplementary-material

Supplementary Figure 1 | Comparison of weekly step counts in the mobile community-first group. Boxplot means ±1*standard deviation of sample.

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ORIGINAL RESEARCH
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Risk of Second Primary Cancers Among Long-Term Survivors of Breast Cancer

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Purpose: The current study explored the risk of developing second primary cancers (SPCs) among long-term early-stage breast cancer survivors and identified risk factors to build an externally validated clinical prediction model.

Methods: The cumulative incidence of SPCs was calculated by Gray method among survivors of early-stage initial primary breast cancer (IPBC). Comparisons of treatment-related risk by selected organ sites were performed. A nomogram was established to estimate the individual risk of developing SPCs based on the multivariate Fine and Gray risk model. Decision curve analysis (DCA) was used to evaluate clinical usefulness of the model.

Results: The cumulative incidence of developing SPCs after early-stage IPBC was 7.43% at 10 years, 14.41% at 15 years, and 20.08% at 20 years. Radiotherapy was associated with elevated risks of any SPCs and with elevated risks of lung cancer (SHR: 1.109; $P = 0.045$), breast cancer (SHR: 1.389; $P < 0.001$), and AML (SHR: 1.298; $P = 0.045$). Chemotherapy was significantly associated with a declined risk of any SPCs, with decreased risks of lung (SHR: 0.895; $P = 0.015$) and breast cancers (SHR: 0.891; $P < 0.001$), as well as elevated risks of other leukemias (SHR: 1.408; $P = 0.002$). HR-positive status was associated with decreased risks of any SPCs; with decreased risks of breast (SHR: 0.842; $P < 0.001$) and ovarian cancers (SHR: 0.483; $P < 0.001$); and with elevated risks of urinary tract cancers (SHR: 1.214; $P = 0.029$).

Conclusion: We found that the cumulative incidence of developing SPCs increased over time and did not plateau. Risk factors for developing SPCs identified by our study were not consistent with those of previous studies. The prediction model can help identify individuals at higher risk of SPCs.

Keywords: cancer risk factor, risk model, survival, breast cancer, second primary cancers

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INTRODUCTION

Breast cancer is worldwide the leading cancer among women (1). Advances in early systematic screening, effective treatments, and supportive care have caused an elevated proportion of breast cancer survivors (2). For some survivors, these survival benefit have been diluted by the late long-term effects of initial cancer and its therapy, with second primary cancers (SPCs) comprising

TABLE 1 | Patient characteristics and clinicopathological variables with stratified events.

Risk factors		Stratified events, no. (%)			
	Total	Censored	Death from IPBC	Death from other causes	SPCs
	(250,764)	(173,737)	(20,170)	(26,572)	(3,0285)
Age					
20–40	23,537	18,316 (10.54%)	2,646 (7.47%)	314 (1.18%)	2,261 (7.47%)
41–60	131,791	102,951 (59.26%)	9,996 (48.09%)	4,280 (16.11%)	14,564 (48.09%)
61–70	56,494	35,866 (20.64%)	4,288 (27.89%)	7,894 (29.71%)	8,446 (27.89%)
71–80	38,942	16,604 (9.56%)	3,240 (16.56%)	14,084 (53%)	5,014 (16.56%)
Race					
White	207,149	142,231 (81.87%)	16,455 (83.73%)	23,106 (86.96%)	25,357 (83.73%)
Black	22,030	15,262 (8.78%)	2,114 (8.52%)	2,074 (7.81%)	2,580 (8.52%)
Other	21,585	16,244 (9.35%)	1,601 (7.75%)	1,392 (5.24%)	2,348 (7.75%)
Marital status					
Married	160,206	115,200 (66.31%)	12,298 (63.9%)	13,355 (50.26%)	19,353 (63.9%)
Single	32,416	23,617 (13.59%)	2,808 (11.51%)	2,506 (9.43%)	3,485 (11.51%)
Divorced	58,142	34,920 (20.1%)	5,064 (24.59%)	10,711 (40.31%)	7,447 (24.59%)
Laterality					
Right	126,809	87,974 (50.64%)	10,261 (50.06%)	13,413 (50.48%)	15,161 (50.06%)
Left	123,955	85,763 (49.36%)	9,909 (49.94%)	13,159 (49.52%)	15,124 (49.94%)
Location					
Central portion	14,614	9,642 (5.55%)	1,376 (5.8%)	1,839 (6.92%)	1,757 (5.8%)
Upper-inner quadrant	26,492	18,529 (10.66%)	2,043 (10.86%)	2,632 (9.91%)	3,288 (10.86%)
Lower-inner quadrant	13,472	9,006 (5.18%)	1,142 (5.73%)	1,590 (5.98%)	1,734 (5.73%)
Upper-outer quadrant	93,876	65,550 (37.73%)	6,828 (38.26%)	9,912 (37.3%)	11,586 (38.26%)
Lower-outer quadrant	17,581	12,300 (7.08%)	1,409 (6.91%)	1,780 (6.7%)	2,092 (6.91%)
Other	84,729	58,710 (33.79%)	7,372 (32.45%)	8,819 (33.19%)	9,828 (32.45%)
Histological type					
IDC	196,061	136,173 (78.38%)	15,604 (78.1%)	20,631 (77.64%)	23,653 (78.1%)
ILC	16,219	11,295 (6.5%)	1,575 (5.52%)	1,676 (6.31%)	1,673 (5.52%)
Mixed	19,768	13,817 (7.95%)	1,769 (7.88%)	1,796 (6.76%)	2,386 (7.88%)
Other	18,716	12,452 (7.17%)	1,222 (8.5%)	2,469 (9.29%)	2,573 (8.5%)
Grade					
Well	44,593	30,982 (17.83%)	1,683 (19.78%)	5,937 (22.34%)	5,991 (19.78%)
Moderate	107,443	73,030 (42.03%)	9,082 (43.33%)	12,209 (45.95%)	13,122 (43.33%)
Poor	93,890	66,675 (38.38%)	8,913 (34.41%)	7,880 (29.66%)	10,422 (34.41%)
Undifferentiated	4,838	3,050 (1.76%)	492 (2.48%)	546 (2.05%)	750 (2.48%)
Stage *					
I	87,312	54,392 (31.31%)	4,165 (48.54%)	14,054 (52.89%)	14,701 (48.54%)
II	126,028	94,712 (54.51%)	9,152 (40.71%)	9,834 (37.01%)	12,330 (40.71%)
III	37,424	24,633 (14.18%)	6,853 (10.74%)	2,684 (10.1%)	3,254 (10.74%)
Surgery					
BCS	135,904	95,767 (55.12%)	8,114 (40.23%)	13,354 (50.26%)	18,669 (61.64%)
Mastectomy	114,860	77,970 (44.88%)	12,056 (59.77%)	13,218 (49.74%)	11,616 (38.36%)
HR					
Negative	49,400	36,315 (20.9%)	3,128 (19.76%)	3,972 (14.95%)	5,985 (19.76%)
Positive	201,364	137,422 (79.1%)	17,042 (80.24%)	22,600 (85.05%)	24,300 (80.24%)
Chemotherapy					
With	120,333	74,077 (42.64%)	7,901 (57.73%)	20,871 (78.55%)	17,484 (57.73%)
Without	130,431	99,660 (57.36%)	12,269 (42.27%)	5,701 (21.45%)	12,801 (42.27%)
Radiotherapy					
With	113,100	76,559 (44.07%)	9,703 (41.6%)	14,238 (53.58%)	12,600 (41.6%)
Without	137,664	97,178 (55.93%)	10,467 (58.4%)	12,334 (46.42%)	17,685 (58.4%)

**Stage classification according to the 8th edition of AJCC staging.*

SPCs, Second primary cancers; IPBC, initial primary breast cancer; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; Mixed, mix of IDC and ILC; HR, Hormone receptor; BCS, Breast conserving surgery.

one of the most potentially life-threatening sequelae (3). Previous population-based researches have examined the risk of developing SPCs among initial primary breast cancer (IPBC) survivors compared to the general population. However, the results from these studies were inconsistent in risk estimation, with an elevated risk range from 15 to 45% for any types of SPCs (2). It is difficult to find an exact estimation of how frequently SPCs occur or the likelihood that IPBC survivors will develop one (4).

Risk stratification by age and race have been extensively explored, demonstrating that survivors of premenopausal age at initial diagnosis and black women had an elevated risk of developing SPCs (2). Each of these previously used methods has inherent limitations when attempting to ascribe causation, especially when several risk factors are involved (5). Therefore, the patterns of SPC development are still poorly understood. Clinicopathological factors have also been proposed to explain the elevated risks. Only a few researches estimated the effect of initial treatment on the development of SPCs (2, 6). The results from these researches were inconsistent, and prediction models of developing SPCs were not provided for survivors.

The purpose of the current research is to estimate cumulative incidence of SPCs and examine risk factors of developing SPCs in long-term early-stage breast cancer survivors in the presence of competing risks. Furthermore, we built an externally validated competing nomogram to help select patients at increased risk of developing SPCs.

METHODS

Inclusion and Exclusion Criteria

Only Female breast cancer patients in the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) registry with histologically confirmed early-stage (stage I–III) who survived for 5 years and more were retrospectively reviewed from 1990 to 2010. In total, 250,764 eligible female patients at 20–80 years old with complete clinicopathological information were included. The inclusion and exclusion criteria was showed in flow chart (**Supplemental Figure 1**). The follow-up time for SPCs for each patients began 5 years after the IPBC diagnosis and ended at diagnosis of SPCs, death from IPBC or the end of follow up (December 2017), or death from other causes.

Variable Declaration

Age was regrouped into four subpopulation (20–40, 41–60, 61–70, and 71–80). Race was regrouped into white race, black race and other race. Marital status was regrouped into married status, single status or divorced status. The hormone receptor (HR) status was stratified to HR positive (estrogen receptor (ER) or progesterone receptor (PR) was positive) and HR negative (both ER and PR were negative). Histology was divided as invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), mixed (mix of IDC and ILC), and other. Surgery was regrouped as breast conserving surgery (BCS) (including partial mastectomy, lumpectomy excisional biopsy, and segmental mastectomy) and mastectomy (including total mastectomy, modified radical mastectomy, radical mastectomy, extended radical mastectomy). Topography and morphology were used to explore the organ site specific risk using International Classification of Diseases for Oncology (ICD-O).

Study Design and Methods

The cumulative incidence of SPCs was calculated based on the Gray method with a competing risk framework: deaths from IPBC or other causes, whichever occurred first, was regarded as competing event (7). The Kaplan-Meier method was constructed

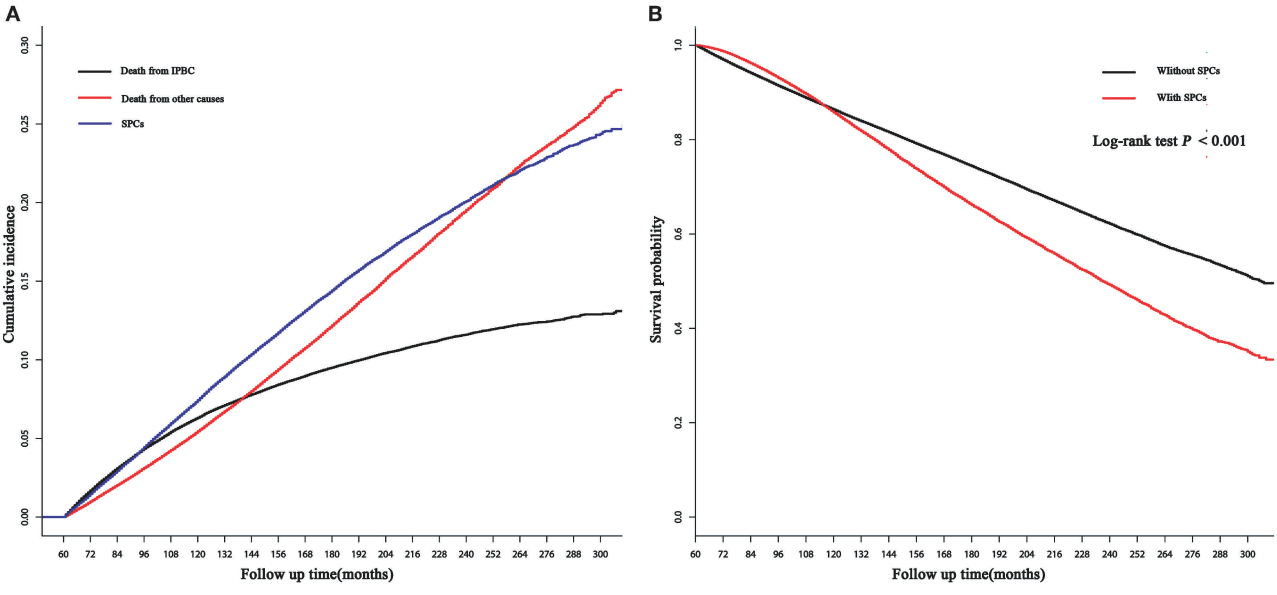

FIGURE 1 | (A) Cumulative incidence of second primary cancers (SPCs), death from initial primary breast cancer (IPBC), and death from other causes in the entire cohort based on the Gray method; **(B)** overall survival (OS) between survivors with and without SPCs in the entire cohort based on the Kaplan-Meier method.

to estimate difference in overall survival (OS) between survivors with and without SPCs.

We randomly divided the entire cohort into a development cohort (75%) and another validation cohort (25%) for development and validation of the competing risks nomogram. Standardized mean differences (SMDs) was used to assess distributional differences in the baseline variables between the development and validation cohorts. Values of $P > 0.1$ imply a potential difference between development and validation cohort (8).

Variable Selection

The forward and backward stepwise methods was used to select the predictive variables from the development cohort for the prediction model based on the Akaike information criterion (AIC) (9). To further reduce the final model, multivariate Fine and Gray competing risks regression model was used to exclude variables based on a backward selection algorithm with a $P > 0.05$. Furthermore, based on all of the selected features, independent effects of initial cancer treatment (chemotherapy and radiotherapy) and HR status on the risk of developing SPCs in selected organ sites were also examined based on the multivariable competing risk model (7).

Validation of the Prediction Model

We assessed the calibration for risks of developing SPCs by comparing the observed risks based on the Gray method with the mean predicted risks predicted risks from the prediction model. Likewise, an external validation was performed in the validation cohort. The C-index was also used to quantify the discrimination ability of the prediction model.

Risk Stratification Ability

The decision curve analysis (DCA) in the validation cohort was used to examine the clinical utility and net benefits of competing risks model for developing SPCs. DCA is a suitable method for evaluating alternative diagnostic and prognostic strategies that has advantages over other commonly used measures and techniques (10). We divided the survivors into three subgroups by the 25th and 75th percentile risk score of the nomogram-based estimated SPC risks. We then calculated the cumulative incidence using the Gray method for each subgroup and compared them across the different risk subgroups (7).

All statistical analysis were conducted using R software (https://www.r-project.org/). Significance level set as $P < 0.05$.

RESULTS

Patient Characteristics

250,764 early-stage IPBC patients who survived >5 years between 1990 and 2010 were included in the entire cohort. Of those patients, 30,285 (12.08%) patients developed SPCs. Twenty thousand one hundred and seventy (8.04%) patients died from IPBC, and 26,572 (10.60%) died from other causes. Second breast cancers represented 13,105 (43.27%) of all SPCs followed by gastrointestinal (GI) at 4,325 (14.28%), lung at 3,203 (10.58%), female genital tract at 2,923 (9.65%), skin at 1,467 (4.84%), central nervous system at 1,333 (4.40%), leukemias at 1,253 (4.14%), urinary tract at 1,192 (3.94%), and lymphoma 546 at (1.80%). The median latency from diagnosis of IPBC to subsequent diagnosis of SPCs was 116 months (25–75% interquartile range, 86–153 months). The detailed information of population is summarized in **Table 1**. In the entire cohort, cumulative incidence of SPCs, in the presence of competing risks of death, was 7.43, 14.41, 20.08% at 10, 15, and 20 years (**Figure 1A**). There is a significant difference in OS between survivors with and without SPCs (85.77 vs. 86.37% at 10 years, 66.16 vs. 74.39% at 15 years, 49.21 vs. 62.16% at 20 years, $P < 0.001$; **Figure 1B**).

We randomly divided entire cohort into two parts: a development cohort (188,073 patients) and a validation cohort (62,691 patients). Baseline characteristics, such as initial diagnosis age, race and treatment-related factor, were similarly distributed in the development and validation cohorts (**Supplemental Table 1**).

Identifying Factors Associated With SPC Risk

The pre-specified variable selection process selected eight variables for inclusion in the multivariable Fine and Gray model:

TABLE 2 | Factors associated with development of second primary cancer risks.

	SHR	95% CI	*p*
Age			
20–40	ref		
41–60	1.206	1.100–1.323	<0.001
61–70	1.648	1.494–1.818	<0.001
71–80	1.332	1.197–1.482	<0.001
Race			
White	ref		
Black	1.101	1.015–1.194	0.021
Other	0.993	0.914–1.079	0.860
Histological type			
IDC	ref		
ILC	0.974	0.881–1.077	0.600
Mix	1.092	1.004–1.188	0.039
Other	0.942	0.867–1.024	0.160
Stage*			
I	ref		
II	0.945	0.896–0.996	0.034
III	0.814	0.750–0.884	<0.001
HR			
Negative	ref		
Positive	0.880	0.829–0.933	<0.001
Chemotherapy			
With	ref		
Without	0.88	0.832–0.931	<0.001
Radiotherapy			
With	ref		
Without	1.161	1.109–1.217	<0.001

p values obtained from the multivariable Fine and Gray competing model.
**Stage classification according to the 8th edition of AJCC staging.*
SHR, Subdistribution hazard ratio; IDC, Infiltrating duct carcinoma; ILC, Invasive lobular carcinoma; Mixed, mix of IDC and ILC; HR, Hormone receptor.

age at initial diagnosis, race, laterality, histology, stage, HR, chemotherapy, and radiotherapy. Except laterality, all variables were included in the final model. Compared to a reference age group of 20–40 years, survivors of an elderly age had substantially elevated risks of SPCs [subdistribution hazard ratio (SHR) of 1.206 (95% CI: 1.100–1.323; $P < 0.001$) in the 41–60 group, 1.648 (95% CI: 1.494–1.818; $P < 0.001$) in the 61–70 group, and 1.332 (95% CI: 1.197–1.482; $P < 0.001$) in the 71–80 group]. Black women survivors had slightly elevated risks of developing SPCs compared with white survivors (SHR = 1.101; 95% CI: 1.015–1.194; $P = 0.021$). Survivors with mixed histology had an increased risk (SHR = 1.092; 95% CI: 1.004–1.188; $P = 0.039$) compared with survivors with IDC. Increasing IPBC stage was also associated with decreased risks of SPCs, with an SHR of 0.945 (95% CI: 0.896–0.996; $P = 0.034$) for stage II and 0.814 (95% CI: 0.750–0.884; $P < 0.001$) for stage III. HR positive status had significantly decreased risks by 12.0% compared to HR negative status (SHR = 0.880; 95% CI: 0.829–0.933; $P < 0.001$). In the present study, survivors treated with radiotherapy had elevated risks of developing SPCs compared to unirradiated survivors

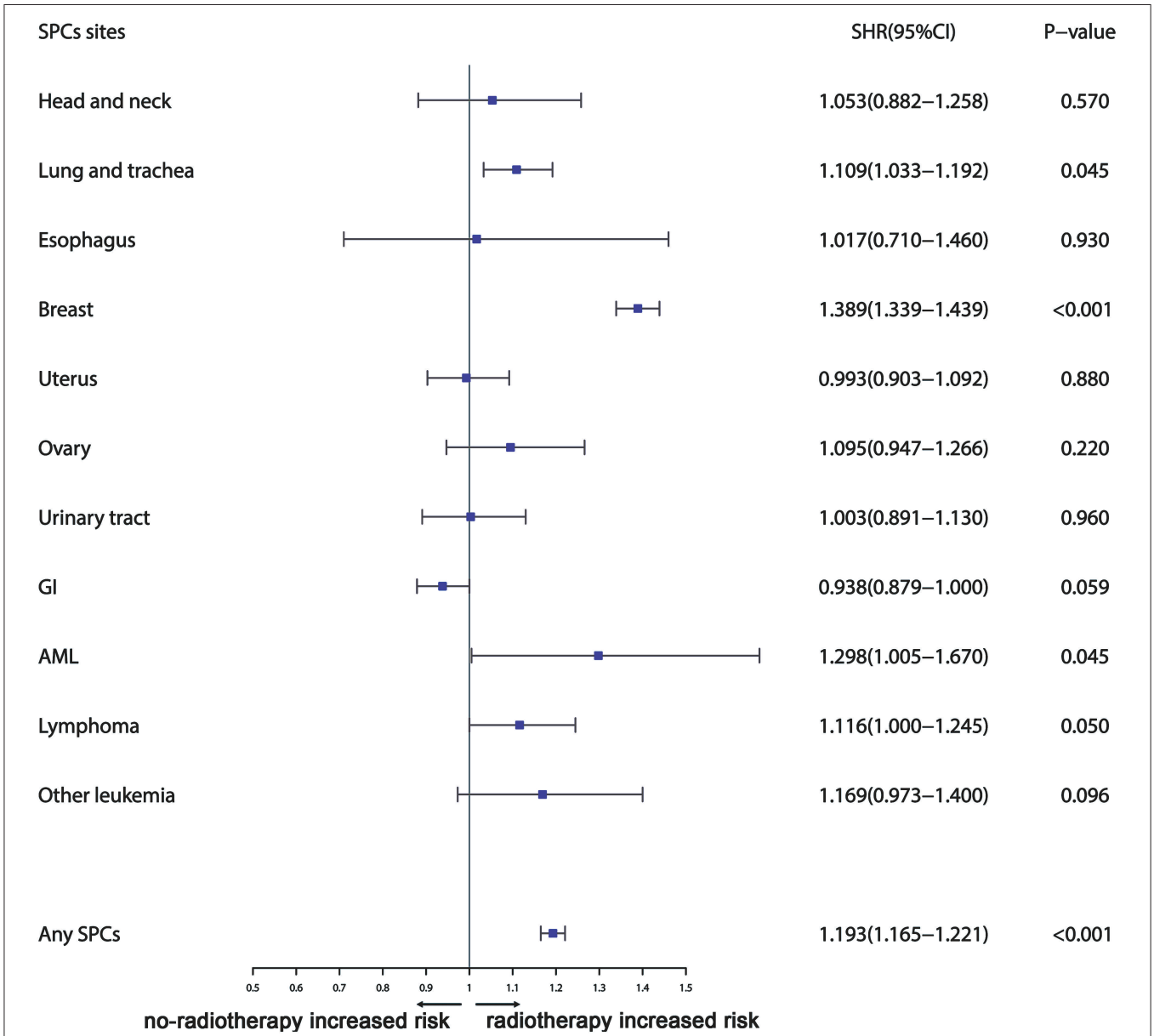

FIGURE 2 | The forest plot comparing radiotherapy-related risk by selected organ sites. Head and neck: ICD-O codes C00-C14. Esophagus: ICD-O codes C15. Lung and trachea: ICD-O codes C33-C34. Breast: ICD-O codes C50. Uterus: ICD-O codes C54-C55. Ovary: ICD-O codes C56-C57. Urinary tract: ICD-O codes C63-C68. GI: Gastrointestinal, ICD-O codes C16-C26. AML: acute myeloid leukemia, ICD-O morphology codes 9860-9911. Other leukemia: ICD-O morphology codes 9912-9989. Lymphoma: ICD-O morphology codes: 9590-9837. SHR: Subdistribution hazard ratio. 95% CI: confidence interval.

(SHR = 1.161; 95% CI: 1.109–1.217; $P < 0.001$). Patients with chemotherapy had a modest decreasing risk of developing SPCs (SHR = 0.880; 95% CI: 0.832–0.931; $P < 0.001$; **Table 2**).

Comparisons of Treatment and HR Status Related Risk by Organ Sites

Furthermore, the effects of initial cancer-treatment (chemotherapy and radiotherapy) and HR status on the SPCs risk in selected organ sites were estimated based on the multivariable Fine and Gray risk model. We found that, after adjusting for age, race, histology, IPBC stage, HR, and chemotherapy, patients with radiotherapy had an elevated risk of any SPCs and with increased risks of lung cancer (SHR = 1.109; 95% CI: 1.033–1.192; $P = 0.045$), breast cancer (SHR = 1.389; 95% CI: 1.339–1.439; $P < 0.001$), and acute myeloid leukemia (AML) (SHR = 1.298; 95% CI: 1.005–1.670; $P = 0.045$). The results were shown in a forest plot (**Figure 2**). Patients with chemotherapy had a decreased risk of any SPCs and with decreased risks of lung (SHR = 0.895; 95% CI: 0.818–0.979; $P = 0.015$) and breast (SHR = 0.891; 95% CI: 0.854–0.930; $P <$

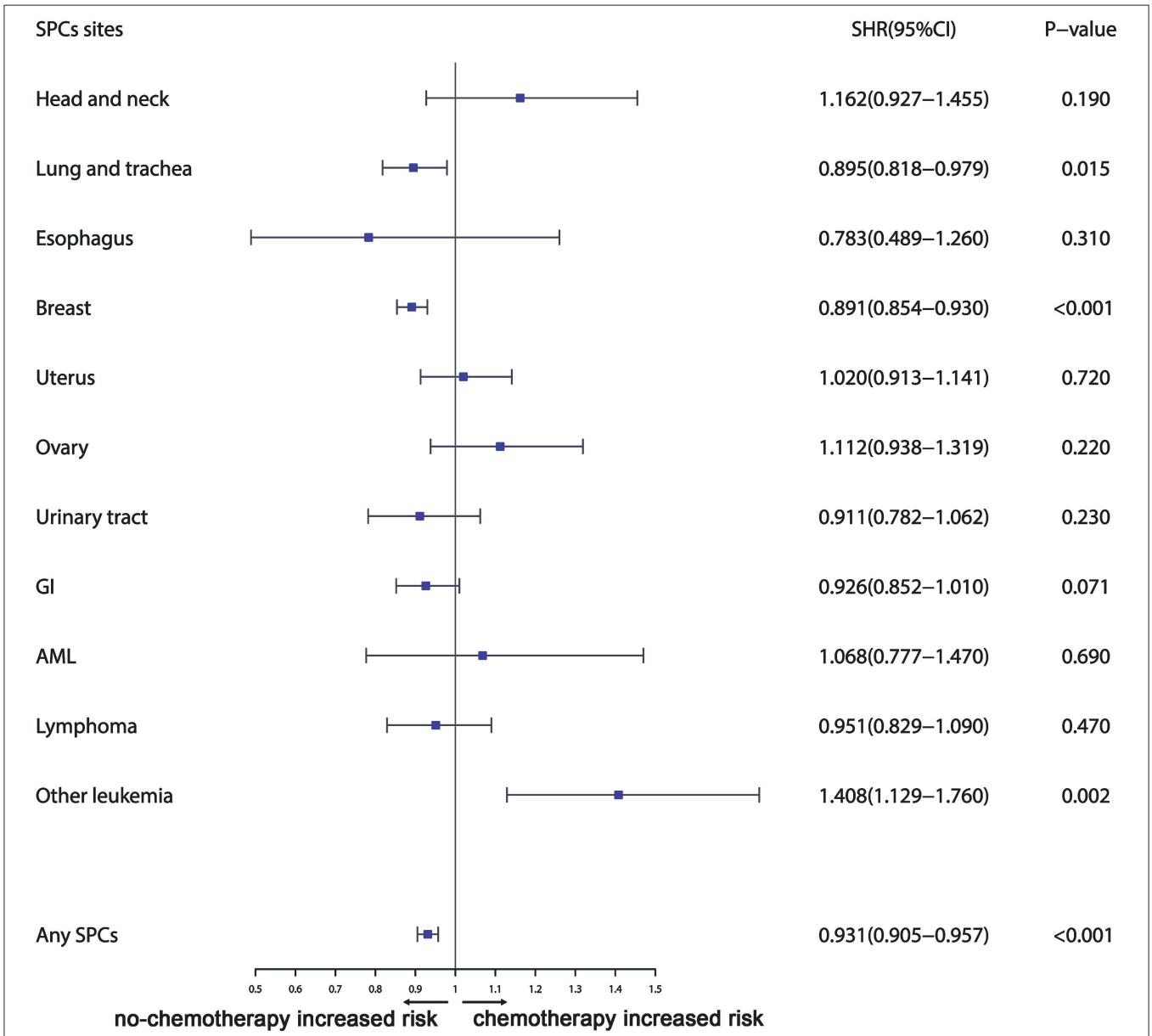

FIGURE 3 | The forest plot comparing chemotherapy-related risk by selected organ sites. Head and neck: ICD-O codes C00-C14. Esophagus: ICD-O codes C15. Lung and trachea: ICD-O codes C33-C34. Breast: ICD-O codes C50. Uterus: ICD-O codes C54-C55. Ovary: ICD-O codes C56-C57. Urinary tract: ICD-O codes C63-C68. GI: Gastrointestinal, ICD-O codes C16-C26. AML: acute myeloid leukemia, ICD-O morphology codes 9860-9911. Other leukemia: ICD-O morphology codes 9912-9989. Lymphoma: ICD-O morphology codes: 9590-9837. SHR: subdistribution hazard ratio; 95% CI: confidence interval.

0.001) cancers, and with elevated risks of other leukemias (SHR = 1.408; 95% CI: 1.129–1.760; $P = 0.002$), after adjusting for age, race, histology, IPBC stage, HR, and radiotherapy. The results were shown in a forest plot (**Figure 3**). After adjusting for initial age of IPBC diagnosis, race, histology, IPBC stage, radiotherapy, and chemotherapy, HR-positive status patients had a declined risk of any SPCs and with decreased risks of second breast (SHR = 0.842; 95% CI: 0.807–0.879; $P < 0.001$) and ovarian cancers (SHR = 0.483; 95% CI: 0.415–0.563; $P < 0.001$), with elevated risks of urinary tract cancer (SHR = 1.214; 95% CI: 1.020–1.444; $P = 0.029$). The results were shown in a forest plot (**Figure 4**). The risk of developing SPCs by selected organ sites was summary in **Table 3**.

Establishment and Validation of the Competing Risks Nomogram

The established nomogram based on the multivariable Fine and Gray model shows the relative importance of each independent variable: age was the vital predictors of developing SPCs, followed by the IPBC stage, radiotherapy, race, HR status, histology,

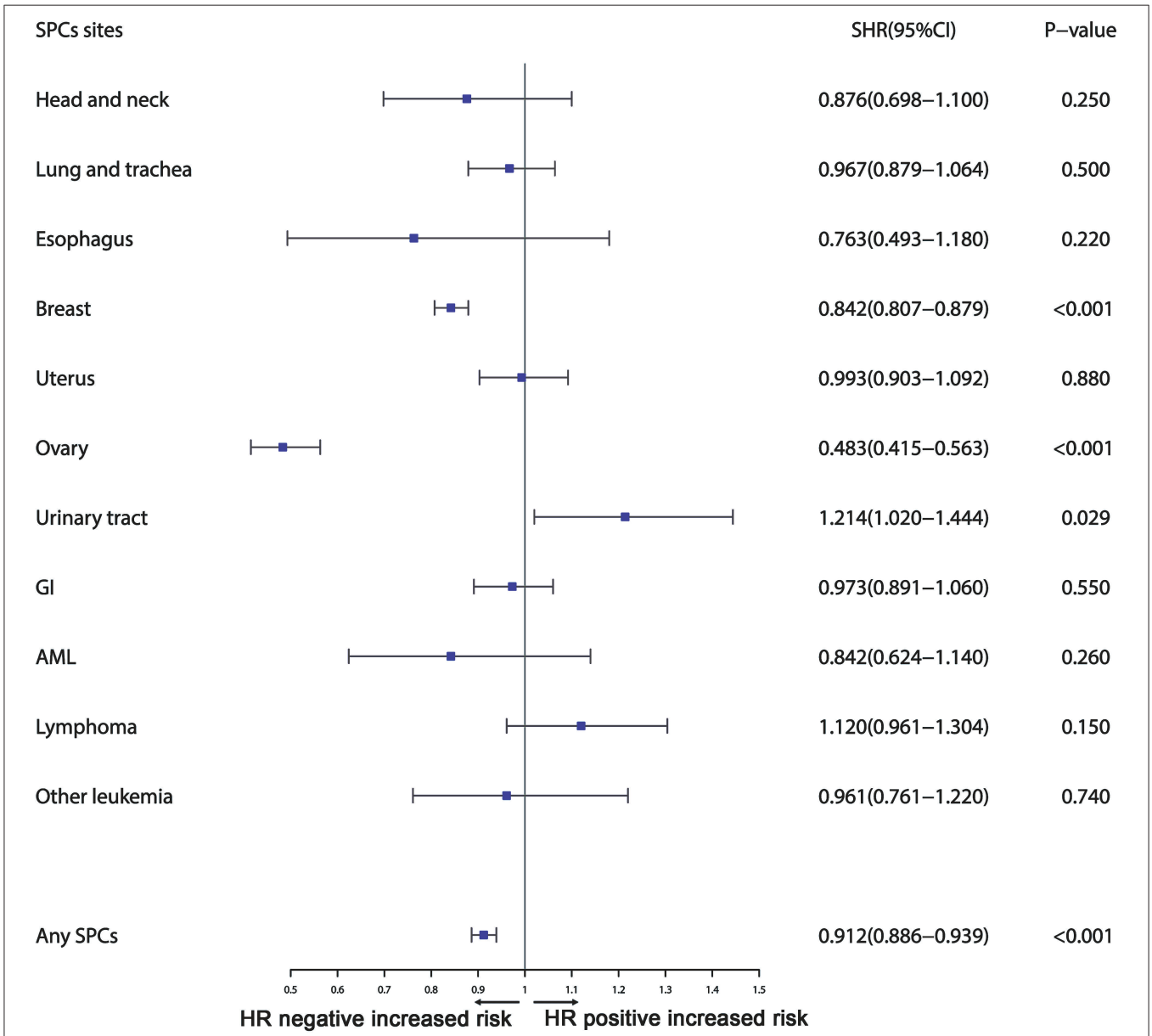

FIGURE 4 | The forest plot comparing effect of HR status on second primary cancer risks by selected organ sites. Head and neck: ICD-O codes C00-C14. Esophagus: ICD-O codes C15. Lung and trachea: ICD-O codes C33-C34. Breast: ICD-O codes C50. Uterus: ICD-O codes C54-C55. Ovary: ICD-O codes C56-C57. Urinary tract: ICD-O codes C63-C68. GI: Gastrointestinal, ICD-O codes C16-C26. AML: acute myeloid leukemia, ICD-O morphology codes 9860-9911. Other leukemia: ICD-O morphology codes 9912-9989. Lymphoma: ICD-O morphology codes: 9590-9837. SHR: Subdistribution hazard ratio; 95% CI: confidence interval.

TABLE 3 | Factors associated with development of second primary cancer risks by organ sites within the entire cohort.

	Any SPC	Head and neck	Lung and trachea	Esophagus	Breast	Uterus
Variable	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**
Age						
20–40	Ref	Ref	Ref	Ref	Ref	Ref
41–60	1.121 (1.073–1.172)	1.525 (1.053–2.21)	4.808 (3.583–6.451)	1.593 (0.675–3.76)	0.754 (0.714–0.797)	3.48 (2.6–4.659)
61–70	1.475 (1.406–1.547)	1.747 (1.175–2.598)	10.05 (7.475–13.51)	2.296 (0.934–5.65)	0.662 (0.621–0.706)	4.391 (3.256–5.92)
71–80	1.200 (1.139–1.264)	1.681 (1.103–2.562)	7.183 (5.310–9.715)	2.651 (1.037–6.78)	0.434 (0.402–0.468)	3.574 (2.618–4.88)
Race						
White	Ref	Ref	Ref	Ref	Ref	Ref
Black	1.117 (1.072–1.164)	0.550 (0.36–0.841)	0.990 (0.866–1.133)	0.904 (0.453–1.8)	1.366 (1.291–1.446)	1.103 (0.931–1.306)
Other	0.950 (0.910–0.990)	1.106 (0.821–1.49)	0.649 (0.555–0.759)	0.782 (0.379–1.62)	1.079 (1.015–1.146)	1.111 (0.944–1.307)
Histological type						
IDC	Ref	Ref	Ref	Ref	Ref	Ref
ILC	0.983 (0.935–1.034)	1.072 (0.749–1.536)	0.937 (0.807–1.088)	1.276 (0.664–2.45)	1.009 (0.931–1.093)	0.802 (0.646–0.996)
Mix	1.065 (1.021–1.111)	0.939 (0.672–1.311)	0.902 (0.786–1.034)	0.717 (0.333–1.54)	1.217 (1.143–1.295)	0.944 (0.791–1.127)
Other	1.024 (0.983–1.067)	0.655 (0.446–0.962)	1.100 (0.974–1.242)	0.807 (0.408–1.6)	1.045 (0.983–1.112)	0.996 (0.84–1.181)
Stage						
I	Ref	Ref	Ref	Ref	Ref	Ref
II	0.939 (0.915–0.964)	0.970 (0.786–1.196)	0.991 (0.913–1.076)	1.169 (0.761–1.8)	0.881 (0.846–0.917)	1.007 (0.904–1.122)
III	0.802 (0.769–0.836)	0.943 (0.692–1.284)	0.930 (0.819–1.057)	1.428 (0.772–2.64)	0.651 (0.61–0.695)	0.898 (0.761–1.06)
HR						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.912 (0.886–0.939)	0.876 (0.698–1.100)	0.967 (0.879–1.064)	0.763 (0.493–1.18)	0.842 (0.807–0.879)	1.009 (0.893–1.141)
Radiotherapy						
Without	Ref	Ref	Ref	Ref	Ref	Ref
With	1.193 (1.165–1.221)	1.053 (0.882–1.258)	1.109 (1.033–1.192)	1.017 (0.71–1.46)	1.389 (1.339–1.439)	0.993 (0.903–1.092)
Chemotherapy						
Without	Ref	Ref	Ref	Ref	Ref	Ref
With	0.931 (0.905–0.957)	1.162 (0.927–1.455)	0.895 (0.818–0.979)	0.783 (0.489–1.260)	0.891 (0.854–0.93)	1.02 (0.913–1.141)
	Ovary	**Urinary tract**	**GI**	**AML**	**Lymphoma**	**Other leukemia**
Variable	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**	**SHR (95% CI)**
Age						
20–40	Ref	Ref	Ref	Ref	Ref	Ref
41–60	0.912 (0.718–1.158)	3.436 (2.234–5.283)	2.325 (1.900–2.840)	1.356 (0.791–2.330)	2.657 (1.865–3.784)	2.795 (1.517–5.15)
61–70	1.041 (0.798–1.358)	6.714 (4.338–10.39)	4.840 (3.943–5.940)	2.522 (1.445–4.400)	4.928 (3.436–7.066)	7.167 (3.877–13.25)
71–80	0.931 (0.694–1.249)	5.507 (3.517–8.622)	5.579 (4.528–6.880)	2.763 (1.501–5.090)	5.146 (3.562–7.436)	9.389 (5.022–17.56)
Race						
White	Ref	Ref	Ref	Ref	Ref	Ref
Black	0.499 (0.357–0.696)	0.965 (0.766–1.216)	1.206 (1.071–1.360)	1.032 (0.661–1.610)	0.895 (0.719–1.114)	1.004 (0.713–1.41)
Other	0.791 (0.601–1.041)	0.552 (0.416–0.732)	1.319 (1.182–1.470)	1.306 (0.878–1.940)	0.715 (0.567–0.901)	0.8 (0.551–1.16)
Histological type						
IDC	Ref	Ref	Ref	Ref	Ref	Ref
ILC	1.004 (0.726–1.388)	1.002 (0.788–1.274)	0.937 (0.816–1.080)	0.577 (0.306–1.090)	0.932 (0.74–1.173)	0.858 (0.583–1.26)
Mix	0.796 (0.582–1.088)	0.934 (0.748–1.166)	0.942 (0.831–1.070)	0.809 (0.490–1.330)	1.015 (0.833–1.236)	1.018 (0.73–1.42)
Other	0.864 (0.662–1.127)	0.822 (0.652–1.037)	0.980 (0.871–1.100)	1.138 (0.753–1.720)	0.938 (0.768–1.146)	1.101 (0.803–1.51)
Stage						
I	Ref	Ref	Ref	Ref	Ref	Ref
II	0.968 (0.823–1.14)	1.011 (0.881–1.16)	1.016 (0.942–1.090)	0.93 (0.684–1.260)	0.903 (0.798–1.023)	0.953 (0.775–1.17)
III	0.746 (0.575–0.967)	0.875 (0.702–1.09)	1.015 (0.904–1.140)	1.629 (1.101–2.410)	0.744 (0.606–0.913)	1.317 (0.998–1.74)

(Continued)

TABLE 3 | Continued

Variable	Ovary SHR (95% CI)	Urinary tract SHR (95% CI)	GI SHR (95% CI)	AML SHR (95% CI)	Lymphoma SHR (95% CI)	Other leukemia SHR (95% CI)
HR						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.483 (0.415–0.563)	1.214 (1.02–1.444)	0.973 (0.891–1.060)	0.842 (0.624–1.140)	1.12 (0.961–1.304)	0.961 (0.761–1.22)
Radiotherapy						
Without	Ref	Ref	Ref	Ref	Ref	Ref
With	1.095 (0.947–1.266)	1.003 (0.891–1.13)	0.938 (0.879–1.000)	1.298 (1.005–1.670)	1.116 (1–1.245)	1.169 (0.973–1.4)
Chemotherapy						
Without	Ref	Ref	Ref	Ref	Ref	Ref
With	1.112 (0.938–1.319)	0.911 (0.782–1.062)	0.926 (0.852–1.010)	1.068 (0.777–1.470)	0.951 (0.829–1.09)	1.408 (1.129–1.76)

p values obtained from the multivariable Fine and Gray competing model.

Head and neck: ICD-O codes C00-C14. Esophagus: ICD-O codes C15. Lung and trachea: ICD-O codes C33-C34. Breast: ICD-O codes C50. Uterus: ICD-O codes C54-C55. Ovary: ICD-O codes C56-C57. Urinary tract: ICD-O codes C63-C68. GI: ICD-O codes C16-C26. AML: acute myeloid leukemia, ICD-O morphology codes 9860-9911. Other leukemia: ICD-O morphology codes 9912-9989. Lymphoma: ICD-O morphology codes: 9590-9837.

Stage classification according to the 8th edition of AJCC staging.

SPCs, Second primary cancers; SHR, Subdistribution hazard ratio; IDC, Infiltrating duct carcinoma; ILC, Invasive lobular carcinoma; Mixed, mix of IDC and ILC; HR, Hormone receptor.

and chemotherapy (**Figure 5**). The validated C-index of this prediction model in the development cohort was 0.59 (95% CI: 0.56–0.61). The C-index in the validation cohort was 0.58 (95% CI: 0.55–0.62). Calibration plots for internal (development cohort) and external (validation cohort) validation of the prediction nomogram were shown in **Supplemental Figure 2**. Point assignment and risk score in the nomogram was summarized in **Supplemental Table 2**.

Risk Stratification: Variation of SPC Risks Based on the Prediction Model

Cumulative incidence of developing SPCs across different risk subgroups defined nomogram-predicted risk score, which shows a wide stratification of the SPC risks at 15 years, from 12.01% for the 25th interquartile group to 17.42% for the 75th interquartile group with a statistical significance according to the Gray test ($P < 0.001$), demonstrated a well-discrimination among low and high risk subgroups (**Supplemental Figure 3**). The decision curve analysis using the 15-year risk of SPCs from the competing risks nomogram in the validation cohort to inform clinical decisions was better than the strategies of treat all or treat none across a wide range of thresholds between 0.01 and 0.24 (**Figure 6**).

DISCUSSION

In the present study, we calculate the cumulative incidence of SPCs among survivors of early-staged IPBC in the presence of competing events, evaluate risk factors for developing SPCs based on the multivariate Fine and Gray model, and build and externally validate a clinical prediction model. Our study supports and expands on previous studies demonstrating an elevated standardized incidence ratio (SIR) for SPCs following an IPBC, especially among elderly, early-stage, HR-negative, and irradiated survivors compared with the general population. To our knowledge, this is the first available nomogram for developing SPCs in IPBC survivors in the presence of competing events, which was helpful in individual risk estimation, patient follow-up and counseling. The DCA inform clinical decisions was better than the strategies of treat all or treat none across a wide range of thresholds between 0.01 and 0.24, which shows the higher clinical utility of our risk prediction model.

The previous studies demonstrated that young patients had a higher SIR than elderly patients (11). Inconsistent with those studies, our study found that elderly survivors have higher risks of developing SPCs. Previous studies is not directly comparing SPCs rates between older and younger population. To calculate age-adjusted standardized rates, one must first have the age-specific rates of disease for each of the populations to be compared. Studies based on SIR analysis, which is obtained by dividing the observed number of cases of breast cancer by the "expected" number of cases (12). Additionally, a high SIR does not necessarily imply a high cancer burden, given that the expected incidence of second cancers may be low (13). Overall breast cancer incidence increases with age, so the difference between the observed and expected risks of developing SPCs in the elderly group will be lower (12). And more young patients have a higher risk of mortality from IPBC, preventing the development of SPCs (13). More young patients have a higher risk of mortality from IPBC, preventing the development of SPCs (14). In addition, SIR study was not enough to ascribe causation when several risk factors are implicated (5).

Few studies have explored the effect of the extent of the initial disease on the development of SPCs. We found that increased patients with higher IPBC stage had a declined risk of SPCs, necessarily attributed to higher possibility of mortality from IPBC before SPCs occur (15). Consistent with previous studies, our study found that patients with HR-positive breast cancer had a declined SPCs risk. Of note, 60–90% of germline mutation BRCA1-associated breast cancers are HR negative, which may be a possible explanation for the increased second

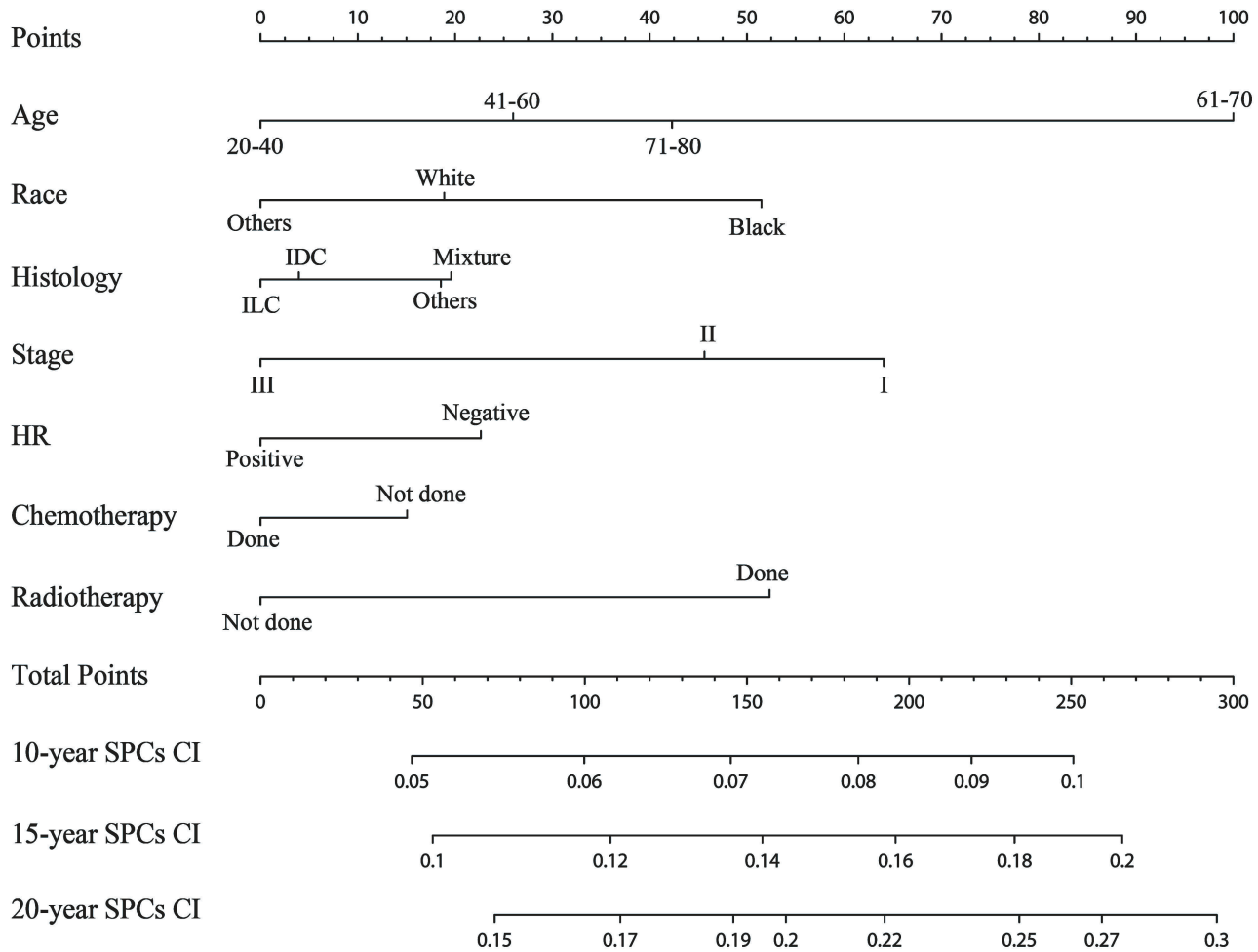

FIGURE 5 | Competing risks nomogram for predicting the 10-, 15-, and 20-year risk of developing second primary cancers. The competing risks nomogram provides a method to calculate 10-, 15-, and 10-year probability of cumulative incidence (CI) of developing second primary cancers (SPCs) on the basis of a patient's combination of covariates. To use, locate the patient's age at initial diagnosis, draw a line straight up to the points axis to establish the score associated with that age. Repeat for the other five covariates (race, histology, stage, HR, chemotherapy, and radiotherapy). Add the score of each covariate together and locate the total score on the total points axis. Draw a line straight down to the 10-, 15-, and 20-year SPCs cumulative incidence axis to obtain the individual probability.

SPCs in HR-negative IPBC patients (16). BRCA1 and BRCA2 mutation patients had a respective 4.5- and 3.4-fold elevated risk of developing contralateral breast cancer (17). Previous studies found that Endocrine therapy approximately reduces 33% second breast cancer (18). We found that HR-positive patients were associated with an increased risk of second urinary tract cancers, which may be explained by hormone use. A Dutch study also found that hormonal therapy and shared etiological risk factors were associated with elevated risks of developing second urinary tract cancers (13).

In the present study, we compared treatment-related SPC risks by selected organ sites. A study estimated that 9% of any SPCs and 25% of the irradiation-associated site SPCs were ascribed to radiation therapy (19). A meta-analysis demonstrated that breast cancer patients with radiotherapy had an elevated overall risks of second non-breast cancer (20). In the present study, we found that patients with radiotherapy had an elevated risk of any SPCs and with elevated risks of lung, breast, and AML, which was consistent with the previous study. A study based on a SEER dataset demonstrated the risk of secondary malignancies and concluded that SPCs were significantly higher for cases that received chemotherapy after adjusting for known confounders (21). A population-based study including 58,068 Dutch patients demonstrated that patients with chemotherapy had a decreased risks of developing second non-breast cancers and colon and lung cancer (13). Our result was consistent with the Dutch study, finding that chemotherapy was associated with a modest protective effect of developing SPCs. Organ specific analysis showed that patients with chemotherapy had an elevated risks of leukemia (excluding AML). Given a SEER chemotherapy sensitivity of only 68% (22), our results should be treated with

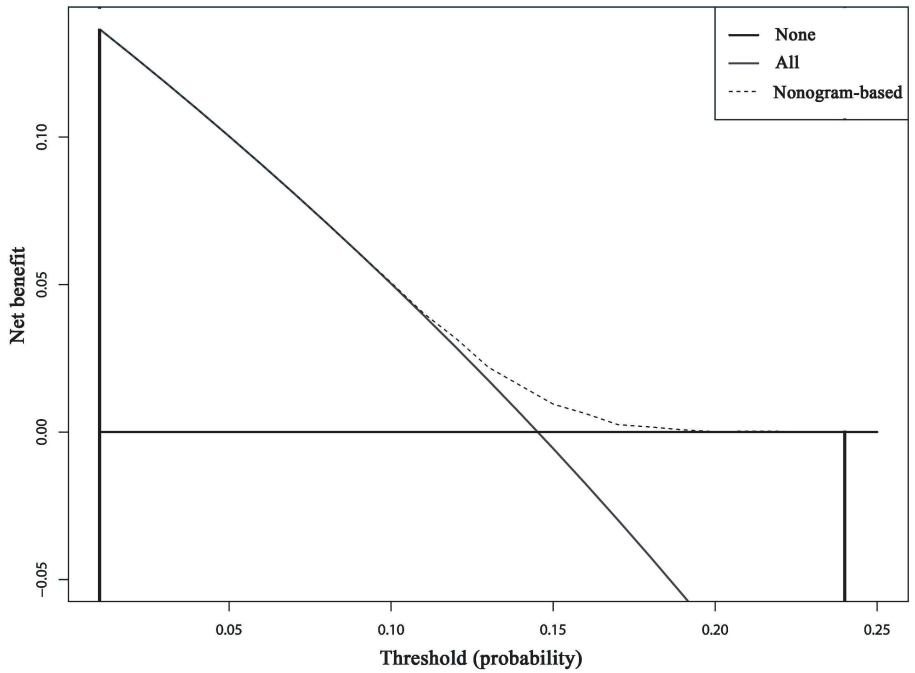

FIGURE 6 | Decision curve analysis for the competing risks nomogram for 15-year second primary cancer risks in the validation cohort. The X-axis is the risk threshold probability that changes from 0 to 1 (right truncated at 0.25) and the Y-axis is the calculated net benefit for a given threshold probability. The dashed lines depict the net clinical benefit of the competing risks–based selection strategy for intervention, whereas the gray and black curves display the net benefits in the alternative strategies of treating all patients (gray) vs. treating no patients (black) in the cohort.

caution and need to be further confirmed in other population-based datasets.

A previous study also identified that black breast cancer survivors had a higher risk of developing SPCs (23). SPCs reflect not only the late effects of cancer and its treatment but also the influence of shared lifestyle, genetic susceptibility, environmental exposures, and gene-environment interactions (3). A Spanish cohort study demonstrated that smoking history, obesity, and high blood pressure were risk factors for SPCs (24). SEER does not provide all the above-listed information, which may lead to the lower C-index observed in our prediction model. Despite the lower C-index, our competing nomogram has a stratification ability to classify the cohort into subgroups with distinct risks of SPC development. SEER does not provide information of regimens. We recognize that the treatment regimens data is an inevitable limitation of our study.

Cumulative incidence of developing SPCs elevated over time and did not plateau. There is a significant difference in OS between survivors with and without SPCs. Consistent with previous studies, our study found that HR negative with radiotherapy and black race were significantly associated with increased risks of SPCs. In contrast, chemotherapy was associated with a modest protective effect. Inconsistent with previous reports, we found that elderly patients was associated with an elevated risk of developing SPCs. For the first time, we found that lower IPBC stage was also associated with elevated risk of developing SPCs. Furthermore, an externally validated clinical prediction model was established to help select high-risk patients.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

DL conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents, materials and analysis tools, prepared figures and/or tables, and authored or reviewed drafts of the paper. SW and XT conceived and designed the experiments, performed the experiments, analyzed the data. CZ, NZ, YC, and DX performed the experiments and authored or reviewed drafts of the paper. YY conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.01426/full#supplementary-material

Supplemental Figure 1 | The flow chart of the detailed inclusion and exclusion criteria.

Supplemental Figure 2 | Internal (**A**: development cohort) and external (**B**: validation cohort) validation plots of the competing risks nomogram. The X-axis is average predicted probabilities of the competing risks nomogram. The Y-axis is the observed cumulative incidence probabilities for the respective cohort. Vertical lines are 95% CIs of the cumulative incidence. Dashed lines are the reference lines, which indicate where an ideal nomogram would lie.

Supplemental Figure 3 | Cumulative incidence of second primary cancers (SPCs) by different risk subgroups defined by the estimated nomogram-predicted risk score. The marginal cumulative incidence of SPCs was calculated, and the difference of the cumulative incidences across distinct risk subgroups was tested using the Gray method.

Supplemental Table 1 | Comparisons of patient characteristics of the study population in the development and validation cohorts.

Supplemental Table 2 | Point assignment and risk score in the nomogram.

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Cold Formalin Fixation Guarantees DNA Integrity in Formalin Fixed Paraffin Embedded Tissues: Premises for a Better Quality of Diagnostic and Experimental Pathology With a Specific Impact on Breast Cancer

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Formalin fixation and paraffin embedding (FFPE) represent the standard method to preserve tissue specimens for diagnostic pathology, however formalin fixation induces severe fragmentation of nucleic acids. We investigated whether formalin fixation at 4°C could preserve DNA integrity in FFPE specimens. Paired samples from 38 specimens were formalin fixed at room temperature (*stdFFPE*) and at 4°C (*coldFFPE*), respectively. Two independent cohorts were prospectively collected, cohort A (collected 6 years prior to the study, $n = 21$), cohort B (collected at time of the study, $n = 17$). DNA was extracted and its integrity evaluated with a qPCR-based assay that produces a normalized integrity index, the QC score (ratio between the quantity of a long and a short amplicon of the same gene). We observed higher QC scores in *coldFFPE* compared to *stdFFPE* samples (mean values: 0.69 vs. 0.36, $p < 0.0001$) and *stdFFPE* breast cancer specimens showed the most detrimental effect overall. Comparable QC scores were obtained between *coldFFPE* tissues of both cohorts; conversely, DNA integrity of *stdFFPE* was significantly lower in cohort A compared to cohort B ($p < 0.0001$). Of note, QC scores of *stdFFPE* (but not of *coldFFPE*) samples were significantly reduced following 6 months of storage ($p = 0.0001$). Monitored formalin fixation at 4°C outperforms standard fixation in ensuring high-quality DNA, which is key to feasibility of downstream high-throughput molecular analyses. An important effect was observed over storage time, thus suggesting a likely better preservation of archival samples when this cold fixation protocol is used.

Keywords: fixation, cold formalin, DNA fragmentation, molecular diagnostics, breast cancer, diagnostic accuracy, oncology, biomarkers

INTRODUCTION

The harmonization of pre-analytic procedures represents the corner stone of optimal diagnosis in pathology (1, 2). The preanalytical phase in pathology includes different steps, spanning from transportation of tissue specimens from surgical theaters to pathology laboratories, grossing, and fixation of tissues (type of fixative and duration of fixation are key features in this respect). Tissue fixation in formalin with generation of formalin-fixed paraffin embedded (FFPE) tissues blocks represents the standard method for tissue specimen processing and archival in diagnostic pathology at present. Of note, heterogeneous tissue handling methods typically lead to inefficiency and poor reproducibility in pathology laboratories. Tissue fixation in formalin, with the generation of formalin-fixed paraffin embedded (FFPE) tissue blocks, represents the standard method for tissue specimen processing and archival in diagnostic pathology. With the tremendous advances of precision medicine pathologists face the challenge to integrate morphology and immunophenotyping with genetic and epigenetic analyses, which require purification of good quality nucleic acids from FFPE blocks. In addition, FFPE tissue blocks preserved in pathology archives may constitute the substrate for comprehensive "omics" strategies, in the context of both prospective and retrospective experimental studies.

It is widely accepted that formalin fixation exerts a blasting effect on both DNA and RNA, with damages comprising fragmentation, non-canonical cross-linkage and base alterations, with critical proportional consequences related with storage time (3, 4). Of note, Polymerase-Chain Reaction (PCR)-based next generation sequencing (NGS) methods are strongly influenced by formalin artifacts, reducing the library performance. The extensive fragmentation of DNA purified from FFPE samples usually leads to lower coverage of unique reads in whole genome and whole exome sequencing approaches (5), but it may also decrease the success rate of amplicon-based methods due to reduced size of DNA templates (6). Moreover, the low quality of DNA from FFPE samples also stems from formalin induced base artifacts within the sequences, generating false mutation calls, in particular in sub-clonal experiments (7).

We have previously reported on a protocol, i.e., cold formalin fixation, which improves RNA quality without interfering with the formalin ability to preserve both tissue structure and antigen reliability for immunohistochemical analyses (2). This modified formalin fixation may represent an easy alternative to conventional formalin fixation ensuring a better preservation of analytes. In addition, a standard method is needed to identify those DNA samples reaching the minimal parameter to obtain robust results. For the large majority of genomic downstream applications, the assessment of DNA size distribution, the ratio between long and short fragments as well as the amplifiability of samples can contribute as parameters to define a quality score (8).

Based on these premises, the aim of our study was to comprehensively characterize a series of DNA samples purified from tissues derived from the same surgical resection but processed with two different fixation protocols (standard fixation vs. cold fixation at 4°C), and to assess whether results would differ based on the tissue of origin. Fluorometric, spectrophotometric and qPCR-based methods were applied to assess DNA integrity.

MATERIALS AND METHODS

Sample Collection and Sampling Procedures

The 38 paired samples included in the study were *ad hoc* collected from surgical specimens handled by under-vacuum packing and cooling (VPAC), as previously described (2, 9, 10). All of the surgical specimens enrolled in this study were at least 2 cm in size to allow for proper parallel sampling of comparable size and thickness. Informed consent was obtained from all individual participants included in the study (protocol "Profiling" 001-IRCC-00IIS-10, approved by the Ethical Committee of Fondazione Piemontese per l'Oncologia-Istituto di Ricerca e Cura a Carattere Scientifico of Candiolo). All experimental procedures were performed in accordance with relevant guidelines and regulations. It should be noted, however, that the sampling did not affect the diagnostic process as the adopted procedure did not require additional samplings, rather included a variation of tissue processing for parallel samples, already validated in a previous study as non-interfering with morphological evaluation (2). Of note, all of the tissue samples were available to the pathologist in charge of signing out the final diagnosis. Each tumoral lesion was sampled in parallel as follows: (i) *standard fixation procedure (stdFPPE):* samples were fixed for 24 h in 4% neutral-buffered formalin (NBF) at room temperature (RT). Subsequently, samples were processed to paraffin embedding with an automatic tissue processor and embedded in paraffin wax; (ii) *cold fixation procedure (coldFFPE):* the sample was immersed in pre-cooled 4% NBF and fixed at 4°C for 24 h. For this protocol, specimens were dehydrated in ethanol 95% at 4°C for 4 h and then followed the same *stdFFPE* sample processing without the first ethanol 95% step by using the automated tissue processor. All of the methods were performed in accordance with the relevant guidelines and regulations.

Out of the 38 cases, 21 pairs were collected and fixed 6 years prior to the present study (Cohort A, collected between 2012 and 2013) and 17 samples prospectively collected at time of the present study (Cohort B, collected in early 2019) (**Figure 1**). Cohort A included 11 breast carcinomas of no special type, three colorectal adenocarcinomas, three cases of lung adenocarcinoma, two gastric adenocarcinomas, and two thyroid follicular carcinomas (**Supplementary Table 1**). Cohort B was composed of eight cases of colorectal adenocarcinoma, two Gastro-Intestinal Stromal Tumors (GISTs), two lung adenocarcinomas, one intrahepatic cholangiocarcinoma, one splenic metastasis of endometrial carcinoma, one high grade serous-papillary ovarian carcinoma, one case of pleomorphic undifferentiated sarcoma and one adenocarcinoma of the gallbladder (**Supplementary Table 1**).

Nucleic Acid Extraction and Quantification

A pathologist evaluated histological and pathological features of the cases included in both cohorts and the tumor area was identified on the hematoxylin and eosin

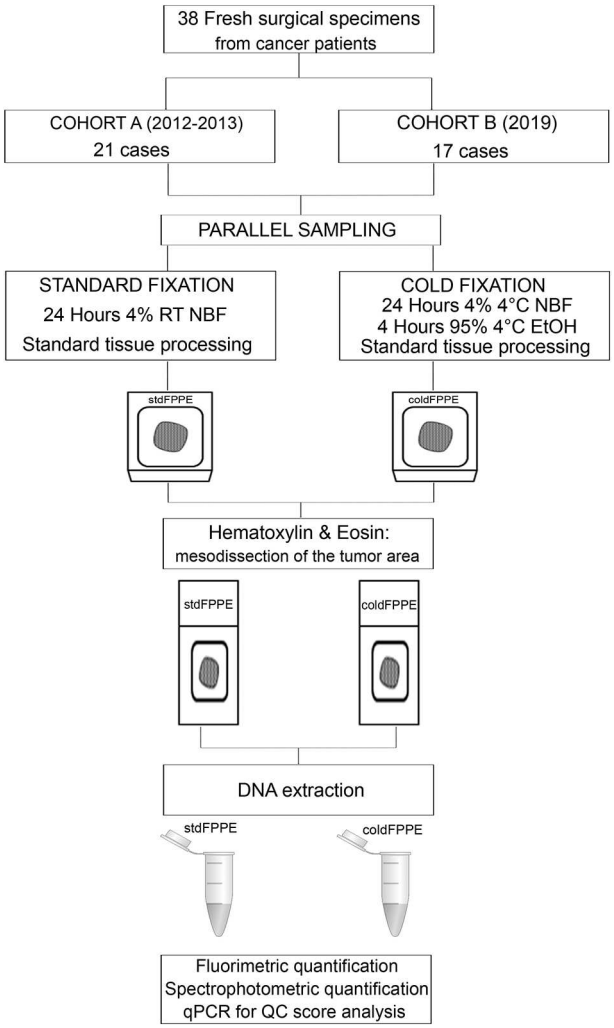

FIGURE 1 | Design of the study. The full cohort comprised 38 cancer specimens that were collected and sampled in parallel to allow standard fixation (i.e., at room temperature) and cold formalin fixation (i.e., at 4°C). Following processing and tissue sectioning the H&E slides were reviewed to identify the tumor area that was mesodissected for DNA extraction. Two independent cohorts were prospectively collected: Cohort A, whose sampled were collected 6 years prior to the study and DNA extraction performed at present time; Cohort B, whose samples were collected at time of the present study with contextual DNA extraction. For 14 samples from Cohort B, i.e., corresponding to those samples for which at least 6 months elapsed from collection, two DNA extractions were performed: at baseline (at time of collection/fixation) and after 6 months of storage/archival. On the total 90 DNA samples we performed fluorometric and spectrophotometric quantifications and we ran a qPCR with the DEPArray™ FFPE QC Kit. RT, room temperature; NBF, neutral buffered formalin; EtOH, ethanol.

(H&E) stained slide before proceeding to the experimental procedure for both *stdFFPE* and *coldFFPE* samples. Data were analyzed anonymously. Five 8-μm thick sections were dissected and DNA samples were purified from all the 38 FFPE pairs for a total of 72 DNA samples. In addition, in 14 cases from cohort B for which at least 6 months elapsed from collection paired *stdFFPE* and *coldFFPE* samples underwent a second DNA extraction 6 months after collection/fixation.

DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, following an overnight 56°C tissues lyses allowing a complete tissue digestion without fragmenting the DNA. DNA was eluted in 40 μL of nuclease-free water and quantified using the Qubit 3.0 Fluorimeter (Life Technologies, Wilmington, DE, USA) following the protocol of High Sensitivity DNA Kit (Life Technologies, Eugene, OR, USA). To check for any possible contaminant on the purified samples, DNA concentration was also evaluated using the DeNovix DS-11 UV-Vis Spectrophotometer (DeNovix, Wilmington, DL, USA) to obtain the 260/230 and the 260/280 nm ratios.

DNA Fragmentation Analyses

DNA fragmentation was evaluated by using the DEPArray™ FFPE QC Kit (Menarini-Silicon Biosystem, Bologna, Italy). This qPCR-based kit is a multiplex reaction composed of two primers pairs flanking two regions of 54 and 132 bp of the same genomic locus. To quantify the amount of the produced amplicons, a standard curve for each primer pair was generated. To infer the DNA integrity level, the ratio between the quantity of the long amplicon (e.g., most conserved DNA) and the short amplicon is calculated. This ratio, named QC score, is a normalized number tending either to 1, in a context of highly conserved DNA, or to 0, in a scenario of diffuse DNA degradation.

Briefly, the standard curve points were produced with serial 1:10 dilutions from an undiluted standard with a known concentration of 20,000 pg/μL. To perform the assay, 8 μL of DNA with a concentration of 2 ng/ μL were added in two mix composed by 10 μL of the Master Mix and 2 μL of the Short or Long Primer mix for a final volume of 20 μL. The thermal cycle was run on a RotorGene Q instrument (Qiagen) and contemplated an initial step of DNA denaturation for 10 min at 95°C followed by 40 cycles of 20 s at 95°C denaturation and 1 min at 57.5°C annealing/extension with the detection of FAM fluorescence signal at 520 nM. To obtain optimal results, slope and R^2 parameters of the standard curve may be between 3.1 and 3.6 and 0.99, respectively. The fluorescence unit threshold was set at 0.04 for all the experiment.

All samples and standards were analyzed with technical triplicates in each experiment. The amount of amplified DNA was checked analyzing the quantity of the short and long amplicons assay products. By comparing the average Ct of the three replicates with the standard curve the relative quantification (RQ) of PCR product was obtained using the formula $10^{(CTsample-INTERCEPTstdcurve/SLOPEstdcurve)}$ for both the short and long amplicon. The QC score was obtained by dividing the long amplicon RQ by the short amplicon RQ.

Statistical Analyses

Statistical analyses were carried out using GraphPad Prism statistical software v8.0 (GraphPad Software, La Jolla California USA). After normality test, we applied both the unpaired and paired distribution test to evaluate significant differences in QC scores between the two-fixation methods.

The unpaired distribution test was used to assess differences by considering the QC scores as independent measurement of uncorrelated samples, whereas the paired distribution test evaluated the statistical distribution considering the different fixation of the same sample as repeated and correlated data. The Pearson's correlation coefficient test (r) was used to determine the correlation between QC scores of corresponding *stdFFPE* and *coldFFPE* samples to determine any intra-specimen influences.

We first analyzed the total cohort by merging the data obtained from the two sets and then by separately considering cohort A and cohort B. *p*-values of <0.05, with 95% confidence intervals (CI), were considered as statistically significant.

RESULTS

Cases and Tissue Morphology

The H&E sections of corresponding *stdFPPE* and *coldFFPE* samples were evaluated by pathologists blinded to the experimental procedures of fixation. Only tumor cell areas were selected for dissection and DNA purification. Tumor cell composition was comparable between corresponding samples for each case; minor differences were observed in terms of relative percentage of immune cells populating the intertumoral stroma. Necrotic areas were carefully avoided through mesodissection. Only two sample pairs were characterized by large necrotic areas interspersed among tumor cell clusters and most likely contained a non-negligible degree of necrosis in the extracted material, nevertheless no evident technical-derived alterations were detected. Of note, the pathologists involved in the assessment of morphological features did not raise observations on fixation artifacts in any of the samples.

DNA Quantity and Absorbance Quality

To determine any influence of the different fixation protocol on DNA quantity and quality, we both applied fluorometric and spectrophotometric methods to evaluate the DNA amount. DNA concentration and ratios are reported in **Supplementary Tables 1**, **2**.

From the standard fixed samples, we purified a mean level of 4.2 (range: 0.2–15.6 μg) and 16.2 μg (1.9–57.7 μg) of DNA analyzed with Qubit or DeNovix instruments, respectively. No significant differences ($p = 0.2$) were detected by comparing these data with the DNA quantity obtained from *coldFFPE* samples, which were slightly higher by the fluorometric (mean: 4.9 μg, range: 0.3–16.4 μg) and spectrophotometric measurement (mean: 20.37 μg, range: 2.4–55.8 μg).

When considering Cohort A only, a significantly higher DNA quantity was detected for *coldFFPE* compared with *stdFFPE* samples (mean values: 4.1 μg vs. 2.1 μg, $p = 0.04$) using the Qubit fluorometer instrument, which was not appreciated in Cohort B. For both fixation methods the nucleic acid yield was significantly higher in Cohort B than in Cohort A ($p = 0.03$ and $p < 0.0001$ for *coldFFPE* and *stdFFPE* samples, respectively), however the difference was less evident when comparing *coldFFPE* than *stdFFPE* specimens (40% of reduction against 70%) (**Figure 2A**).

No correlation between DNA amount and type of tumors was detected. Finally, the fixation protocol did not influence the DNA quality, defined as the absorbance ratio between 230/260 and 260/280 nm, which resulted comparable between the pairs.

DNA Fragmentation Analysis

The qPCR-based DEPArray™ FFPE QC Kit returned a QC score for each sample, a normalized evaluation of DNA amplifiability. From a technical standpoint, the mean R^2 scores of the standard curves were 0.994 and 0.993 for the short and the long amplicon, respectively, and the line slopes were comprised in the optimal range defined by the manufacturer's protocol.

All DNA samples were successfully amplified, and a QC score was generated. More in detail, the *stdFFPE* DNA samples were characterized by mean QC score of 0.36 (range: 0.03–0.86), which was significantly lower than the corresponding value in *coldFFPE* samples (mean: 0.69, range: 0.34–1.05). By comparing the QC score distributions of the two sets of samples, both the paired and unpaired tests reached the statistical significance ($p < 0.0001$) (**Figure 2B**). We wondered whether the intrinsic fragmentation of the samples could have influenced this difference. In this context, the Pearson correlation test described an independent trend for the QC value for the two different fixation methodologies in the same sample ($r = 0.45$) (**Figure 2C**).

When samples were clustered according to the time of fixation (Cohort A vs. Cohort B) we observed that the QC scores of *coldFFPE* samples were substantially comparable between the two cohorts, regardless of time of fixation ($p = 0.79$), whereas the QC score of *stdFFPE* samples was significantly lower in Cohort A compared to the more recent prospectively accrued samples of Cohort B ($p < 0.0001$) (**Figure 2D**). Within Cohort A, *stdFPFPE* samples harbored the highest level of DNA fragmentation overall, with a mean QC level of 0.12 (range: 0.03–0.27), in contrast with the corresponding *coldFFPE* samples that showed a 6-fold increase in the QC average (mean QC: 0.63, range: 0.24–1.02; $p < 0.0001$). On the other hand, the DNA purified from the 17 paired samples of Cohort B displayed a relatively better preservation in both *stdFFPE* and *coldFFPE*, nevertheless *coldFFPE* samples still showed a statistically significantly higher QC distribution ($p = 0.0016$ and 0.038, paired and unpaired *t*-tests respectively, **Figure 2D**).

DNA Integrity and Site of Origin of the Tumor

We checked any possible association between the site of origin of the tumor and the DNA preservation efficiency of the two processing methods. The heterogeneity of our population allowed to reach enough numerosity to obtain statistically informative results only for breast, colorectal, and lung carcinomas. We observed that the DNA purified from breast carcinomas showed higher degrees of fragmentation compared to colorectal and lung adenocarcinomas (**Figure 3**). We obtained the lowest QC scores from *stdFFPE* breast

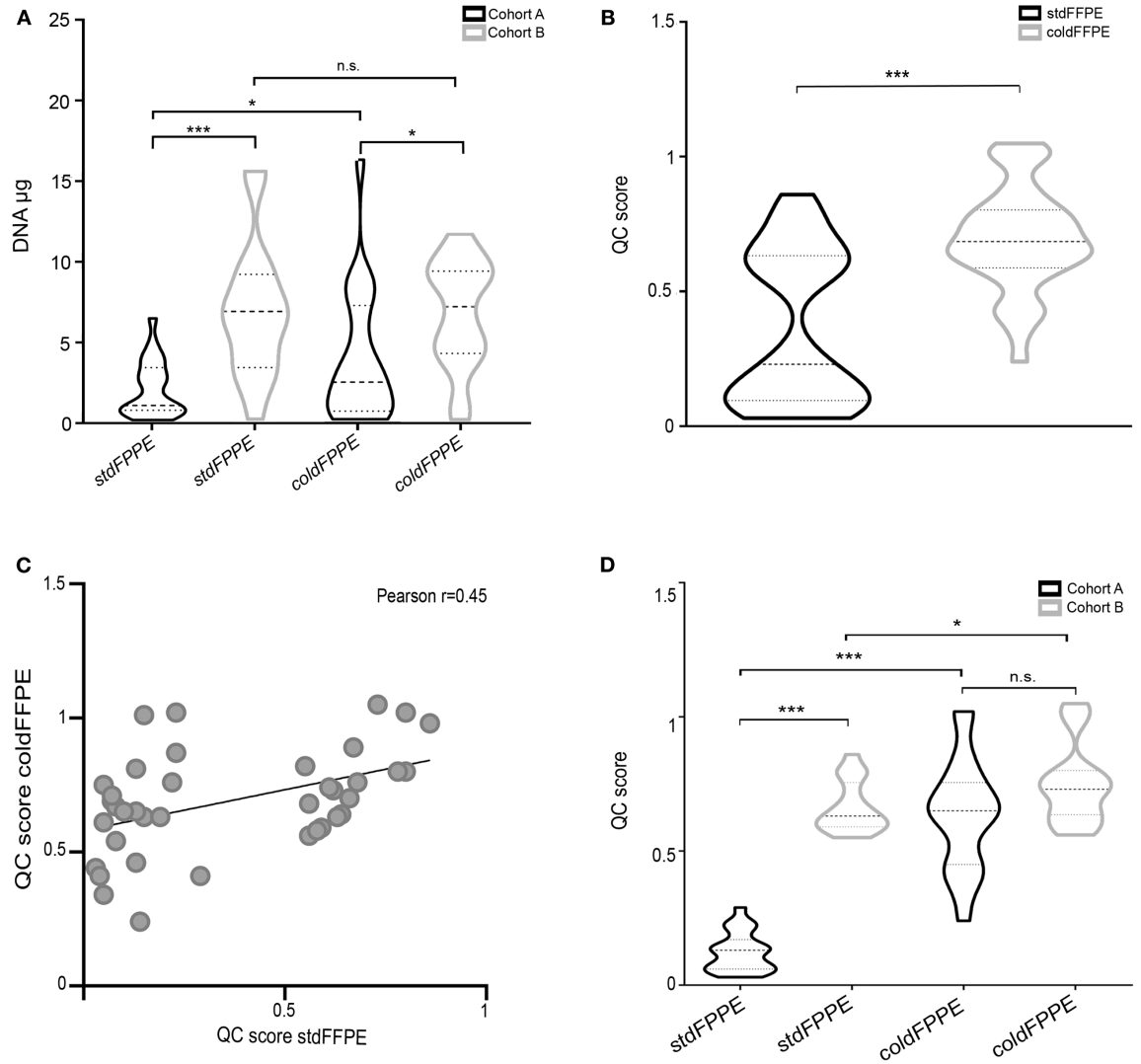

FIGURE 2 | Quantity and quality of DNA extracted from parallel samples. **(A)** DNA quantification of samples. Violin plots representing the DNA µg purified from the specimens, grouped according to the fixation methods (*stdFFPE* and *coldFFPE*) and the time of cohort collection [Cohort A (collected 6 years prior to the study with DNA extraction at present time); Cohort B (collected at time of the present study with contextual DNA extraction)]. Cohort A was characterized by higher DNA yields for *coldFFPE* compared to *stdFFPE* samples (4.1 µg vs. 2.1 µg, $p = 0.04$). As for Cohort B, violin plots showed a median-around density distribution for both *stdFFPE* and *coldFFPE* samples that was significantly higher compared the same fixation protocol of Cohort A ($p = 0.03$ for cold fixation and $p = 0.0001$ for standard fixation). **(B)** Violin plots representing the QC score distribution in the groups clustered according to fixation method. The *stdFFPE* cohort was characterized by heterogeneous QC scores with several samples in the low range (wider diameters of the black violin) and the DNA fragmentation was statistically significantly higher than in *coldFFPE* (gray violin). **(C)** Dot plot illustrating the Pearson correlation between the QC scores of corresponding samples fixed with the two protocols. The absence of a robust correlation suggests the reduced influence of the intra-individual fragmentation on the QC score. **(D)** QC distribution among the sample sets. Violin plots representing the QC score of the samples, which are grouped according to the fixation methods (*stdFFPE* and *coldFFPE*) and the time of collection [Cohort A (collected 6 years prior to the study with DNA extraction at present time); Cohort B (collected at time of the present study with contextual DNA extraction)]. The QC scores of *coldFFPE* samples are comparable between the two cohorts, regardless of time of collection/fixation ($p = 0.79$), whereas the QC score of *stdFPPE* samples are significantly lower in Cohort A compared to Cohort B ($p < 0.0001$). Notably, the DNA purified from Cohort B samples displayed a better preservation in both *stdFFPE* and *coldFFPE*, nevertheless *coldFFPE* samples still showed a higher QC distribution. n.s. not significant, $^{*}p < 0.05$, $^{***}p < 0.001$.

carcinoma samples, and these values were significantly lower compared to all the other samples, either *stdFFPE* or *coldFFPE* ($p = 0.0009$). By focusing on the same samples treated with cold formalin, breast specimens confirmed the tissue-specific DNA degradation, compared with lung and colon cancer tissues ($p = 0.02$), more pronounced within the standard fixed samples ($p = 0.0002$). By investigating the intrasample QC score ratio between *stdFFPE* and *coldFFPE,* the highest advantage from cold formalin fixation was observed for DNAs purified from breast carcinoma specimens, with average 7.7-fold increase in the long amplicon in the *coldFFPE* compared to the *stdFFPE* samples (**Figure 3**).

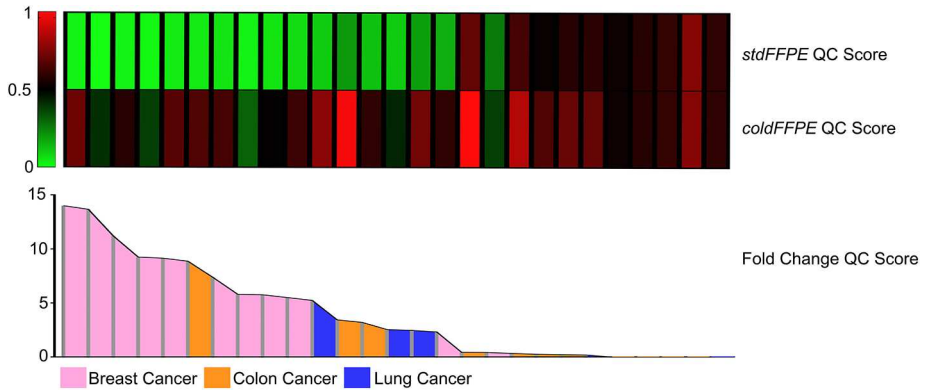

FIGURE 3 | QC score and QC score fold change in breast, colon, and lung carcinoma samples. The heatmap represents the QC score for purified DNA from *stdFFPE* and *coldFFPE* samples. The histogram shows the fold changes of the QC score in *coldFFPE* compared to the corresponding *stdFFPE* samples. The order of the samples in the heatmap is defined by the color in the histogram. All the breast *stdFFPE* samples shows the lowest QC score level, which is less heterogenous among the *coldFFPE* specimens. Nine of the first ten samples with and increased level of DNA integrity have a mammary site of origin, confirming the specific improvement for breast cancer tissue.

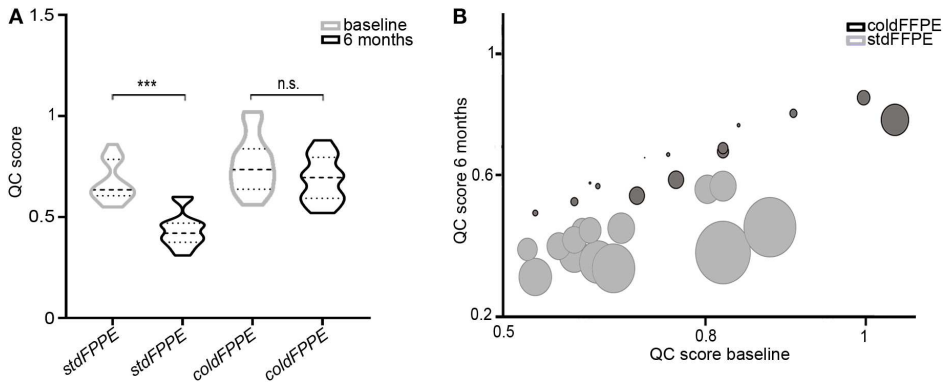

FIGURE 4 | QC score distribution in the subgroup analysis of the 14 tissue pairs with a second DNA extraction after 6 months from collection/fixation. **(A)** Violin plots representing the QC scores in a head to head comparison between DNA extraction at baseline and after 6 months, subdivided by standard fixation (*stdFFPE*) and cold fixation (*coldFFPE*). Significantly lower QC scores are observed after 6 months in *stdFFPE* samples, whereas comparable QC scores are displayed for *coldFFPE* samples. **(B)** Bubble plot of the QC score for each patient. The plot showed the basal QC score on the Y axes and the 6-month QC score on the X axes, paired for each sample. The bubble size illustrates the drop in terms of QC score for each couple of DNA samples purified after 6 months of archival. n.s. not significant, $^{***}p < 0.001$.

DNA Integrity Following 6 Months of Storage

Based on the lowest level of DNA integrity observed in *stdFFPE* samples of Cohort A and on the better values of QC scores detected in *stdFFPE* samples of Cohort B, we hypothesized that the time of storage could have had an impact on DNA integrity. In subgroup analysis of the 14 tissue pairs that underwent a second extraction after 6 months from collection and fixation we were able to perform a head to head comparison of the QC score values of DNA extracted following FFPE storage of 6 months to those of the DNA extracted at time of collection (**Supplementary Table 2**). We observed significantly lower QC scores for the *stdFFPE* samples ($p < 0.0001$; with a mean percentage loss of 35%) and comparable QC scores for the *coldFFPE* samples ($p = 0.131$, with a mean percentage loss of 9%) (**Figure 4**).

DISCUSSION

Our experimental study shows that formalin fixation of tissue specimens at 4°C (cold fixation) leads to a better preservation of DNA as exemplified by the significantly lower levels of fragmentation in DNA samples obtained from *coldFFPE* as compared to those from *stdFFPE* tissue samples. In addition, the results in our hands suggest an important impact of time of storage of FFPE samples on DNA integrity that can be circumvented by cold fixation. This seems to be particularly important for breast cancer specimens, which showed the most detrimental effect with standard fixation overall.

It is universally acknowledged that optimal DNA quality is obtained from fresh frozen tissue specimens, which represents also the backbone of tissue biobanking. Nevertheless, systematic freezing in pathology laboratories is seldom feasible on a routine basis, being restricted mainly to research Institutes where a proper Biobank is in place with dedicated personnel. On the other side we should take into account that diagnostic pathology is constantly reshaping due to the contribution of several molecular assays (including NGS-based approaches) to the diagnostic process, leading to either a better definition of the lesion, or a better prognostic and/or predictive stratification of the disease (precision medicine). For instance, depending on the site of origin and on the histologic type of a tumoral lesion genetic alterations such as mutations, translocations and gene expression signatures are currently being assayed in clinical practice [reviewed in (11)]. As pointed out by Schillaci et al. the primary goal of radiological and pathological evaluation is to increase the quality of life of oncological patients, both through the reduction and the invasiveness of the methods as well as by the accuracy of molecular analyses (12). Feasibility, robustness and reproducibility of molecular assays are therefore key in this respect and these features are strictly dependent on the integrity of DNA/RNA, which derives from a proper management of tissue during the preanalytical phase, including formalin fixation. Although several molecular assays have been designed and optimized on FFPE tissue samples and robustness has been shown, there are important scenarios in which genomic analyses on DNA extracted from FFPE samples are challenging. High throughput sequencing methods, such as Tumor Mutation Burden, somatic detection of *BRCA1* and *BRCA2* single nucleotide variants and copy number variations that can address patient to targeted therapeutic approaches (13), are strongly influenced by DNA integrity (14, 15). The pilot study for the 100,000 Genome Project excluded significant number of samples due to the poor quality of DNA extracted from FFPE samples, and the enrolled tissues revealed coverage unbalance after sequencing (16).

Cold formalin fixation has been used in other studies, mainly focused on RNA integrity. Our group (2) and others (17) have highlighted a better preservation of RNA molecules, whereas very little is known in terms of DNA integrity. Our systematic approach that analyzed the DNA fragment distribution in paired tissues with different formalin fixation methods provides direct evidence of a lesser degree of DNA fragmentation in *coldFFPE* samples, which showed significantly higher QC score values compared to *stdFFPE* DNA samples. Of note, the degree of statistically significant difference between *coldFFPE* and to *stdFFPE* DNA samples was higher in Cohort A, composed of specimens collected 6 years prior to the present study and whose DNA was recovered at time of the present study, compared to the Cohort B, composed of samples collected and extracted at present time. It is important to note that QC scores of the *coldFFPE* were comparable between the two cohorts, thus demonstrating consistency in obtaining high quality DNA from *coldFFPE* samples, whereas we observed relatively higher QC scores in *stdFFPE* DNA samples belonging to the prospective cohort, which rendered the difference with *coldFFPE* samples less evident, even though still statistically significant.

These data prompted us to assess whether an effect due to length of FFPE block storage could have contributed to the difference. This would be particularly relevant, as archival FFPE tissue blocks are a source of retrospective and prospective samples for translational research (18, 19) and, if a better method of preservation is identified, pathology archives may become an invaluable source of good quality DNA for future studies. Guyard et al. reported a systematic quantification of the time-dependent degradation of DNA in FFPE specimens and they detected a loss of both quantity and quality of DNA extracted from the same FFPE samples stored over a period of several years (4). In our study, we ran a subgroup analysis on 14 samples that underwent a second DNA extraction after 6 months from collection and fixation. We demonstrated a significant QC score loss in *stdFFPE* samples compared to the first extraction, whereas comparable results were obtained between first and second DNA extraction for *coldFFPE* samples, hence strongly suggesting a better preservation of DNA in tissue specimens fixed in cold formalin. As a possible mechanism contributing to this phenomenon one could hypothesize that cold formalin fixation may be able to reduce both the first enzymatic degradation of DNA by blocking the DNAse activity (20) and the formalin-dependent crosslinks that lead to DNA fragmentation (21).

Another important parameter derived from our data relates to the tissue type. We detected the highest advantage for DNA integrity in *coldFFPE* breast cancer specimens, as compared with *coldFFPE* specimens derived from colorectal, lung, and thyroid carcinoma samples. A tissue specificity for the DNA quantity and quality has been already reported by other groups. Bonin et al. showed variable yield and quality of nucleic acid extraction for different tissue (22) and Guyard et al. (4) reported a reduced DNA integrity in colorectal cancer FFPE tissues, probably associated with physiological characteristics, compared with lung, and urothelial tumors. The fat tissue the mammary gland is composed of may hamper an efficient formalin fixation, yet our results seem to suggest that breast cancer specimens may benefit form cold formalin fixation.

Our study has some limitations, mainly related to the limited sample size. Even though we were able to validate our results in two independently collected series of cases, we cannot rule out the possible association about tissue specificity for the less represented tumor types here included. Second, we could not systematically monitor the DNA fragmentation trend in all of the specimens over time. Nevertheless, the consistent data obtained in all the 14 sample pairs of the subgroup analysis we were able to perform strongly supports the contention of a better DNA preservation in cold fixed samples.

Finally, our analyses were carried out on parallel sampling of lesions from surgical samples only. We have preliminary data in our hands (unpublished results) demonstrating that cold fixation in core biopsies of breast carcinomas results in optimal morphology and reliable protein detection by immunohistochemistry-based assays. Further studies are

warranted to ascertain whether the advantage in terms of DNA integrity demonstrated for FFPE samples of surgical specimens is also observed in core biopsy and cytology samples, which are smaller in size and may undergo a shorter duration of formalin fixation.

Despite these limitations, the precise quantification of the degree of DNA fragmentation that we performed with a normalized evaluation of the QC score revealed a clear benefit for DNA preservation in samples fixed in cold (4°C) formalin. Hence, we here provide a method to limit DNA degradation that is likely to (i) ensure a better performance of molecular diagnostic tests, (ii) enable a higher complexity of genomic analysis on DNA extracted from FFPE samples, (iii) anticipate a paradigm shift in pathology laboratories with the creation of FFPE archival samples that may be better preserved over time and foster therefore a higher accuracy and throughput of predictive molecular pathology assays, which have the potential to ultimately impact on the quality of life of breast cancer patients in the era of precision medicine.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available in the **Supplementary Materials**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved Ethical Committee of Fondazione Piemontese per l'Oncologia- Istituto di Ricerca e Cura a Carattere Scientifico of Candiolo, CANDIOLO, Prot. Profiling 001-IRCC-00IIS-10. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

CM and AS: conceptualization and funding acquisition. EB, LA, UM, CP, EP, EM, DB, PC, AP, IS, TV, and CM: methodology. EB, CM, and AS: formal analysis and investigation. EB and CM: writing—original draft preparation. CM, AS, LA, and TV: writing—review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.00173/full#supplementary-material

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SYSTEMATIC REVIEW
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Hippocampus—Related Cognitive and Affective Impairments in Patients With Breast Cancer—A Systematic Review

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Background: Although improvements in medical treatment lead to a steadily rising survival rate of breast cancer patients (BCP), it is associated with a decrease in cognitive and affective function. The hippocampus, a brain region with a high influence on both cognitive and affective function, is increasingly becoming the focus of current research because of its high vulnerability to adverse direct (chemotherapeutic agents, endocrine therapeutic agents, and radiation) or indirect (stress and other psycho-social factors) treatment-related effects.

Methods: This systematic review analyses current data from literature combining hippocampus-related brain changes due to breast cancer treatment with associated cancer-related cognitive and affective impairments (CRCI/CRAI). The seven studies that met the inclusion criteria consisted of six cross-sectional studies and one longitudinal study.

Results: The study results indicate hippocampal differences across all types of treatment. Those differences include volume loss, deformation, and changes in functional connectivity. They are associated with CRCI, revealing executive function as well as working memory, episodic memory, and prospective memory as the most affected domains. Although an interaction between hippocampus-related brain changes, CRCI, and CRAI can be hypothesized, CRAI are less reflected in current research.

Discussion: More research including longitudinal assessments with better overall methodology is needed to fully understand the interaction between hippocampal alterations and both CRCI and CRAI due to breast cancer treatment.

Keywords: breast cancer, cancer/cancer treatment-related side effects, hippocampus, cognitive impairments, affective impairments

INTRODUCTION

Rationale

The ongoing global demographic and related epidemiologic changes indicate an ever-increasing cancer burden with more than 20 million new cancer cases expected annually within the next decade (1). Breast cancer is the most frequent cancer type among women (2). Due to improvements in early detection and treatment (3, 4), breast cancer survivorship rates continue to rise steadily since the 1990s (5, 6). In this context, a focus on breast cancer survivors'quality of life seems to be of particular importance due to a vast body of literature reporting about lingering treatment–related side-effects cancer survivors have to deal with (7, 8). Those impairments can even persist up to 15 years after the end of treatment (9), inhibit occupational reintegration (10), and have a considerable influence on the quality of life of those affected (11).

Thereby, an increasing number of studies focused on measuring cancer–related cognitive impairments (CRCI) in those patients over the last ten years (12). They revealed attention, processing speed, executive function, and working memory as the most affected domains (9, 13, 14). Recent literature also provides evidence for various types of cancer-related affective impairments (CRAI) (15). A current study indicates an estimated prevalence of 48.6% for the development of anxiety and a prevalence of 15% for the development of depression in breast cancer patients (BCP) during and after the course of medical cancer treatment (16). Furthermore, an association between both anxiety and depression and CRCI in BCP undergoing chemotherapy and endocrine therapy seems to be present in current research (17, 18).

Trying to reflect the origin of these changes more precisely, both CRCI and CRAI have been associated with specific structural brain changes, including the temporal cortices (19). In this context, one of the most intensively studied brain regions, in both animal and human studies, is the hippocampus (20, 21). Due to its strong connection with other brain regions, including higher cortical brain structures and the limbic system, it is estimated that the hippocampal formation serves as a large integrating organ, which encodes and consolidates memory content by transforming new information, received from multiple brain regions (22). As a part of the posterior medial system, the posterior hippocampus is connected to the parahippocampal cortex, retrosplenial cortex, anterior thalamus, mammillary bodies and the pre- and parasubiculum. Moreover, it is connected with components of the default mode network, which plays a role in memory retrieval and spatial cognition (23, 24). Overall, the integration of processed information across the two cortical systems, supporting different kinds of memory-guided behavior, seems to depend on the dentate gyrus and cornu ammonis region (CA) 3 (23). Furthermore, the anterior hippocampal subregions are a part of the anterior temporal system, which are preferentially connected to the amygdala (25), the lateral orbitofrontal cortex as well as the ventral temporopolar cortex (23). It is involved in the hypothalamic-pituitary-adrenal axis (HPA), the major stress system in the body, as well as the limbic prefrontal circuit (26, 27).

The hippocampus generally consists of several subregions forming the so-called hippocampal formation, including the dentate gyrus, the subiculum, and the cornu ammonis regions with the CA1–CA4 fields (25, 28, 29). The dentate gyrus is thereby particularly involved in the process of generating new neurons in the hippocampus throughout life, a process called neurogenesis (30, 31). This mechanism mainly regulates the maintenance of brain plasticity, memory, and learning (32, 33). Studies provide evidence that the rate of maturation and survival of these cells are influenced by environmental conditions (34, 35).

For example, there is a lot of discussion on the key role of treatment-related structural brain changes in the hippocampus and its consequences for memory processes (36) but also for emotion-related processes (37). Among different forms of medical cancer treatment, chemotherapy is especially associated with hippocampal volume decrease (20), reduced neurogenesis (38–40), and has been linked to an array of experienced cognitive impairments (41). Especially hippocampal neurogenesis seems to be highly vulnerable to chemotherapeutic treatment as well as other types of cancer treatment (42, 43). For example, radiotherapy and endocrine therapy have been equally linked to volume loss and reduced hippocampal neurogenesis (42, 43). Interestingly, studies repeatedly documented a reduction in hippocampal volume in stress related psychiatric disorders like Major Depression (44, 45). Although it remains unclear whether hippocampus-related brain changes cause depression and anxiety or whether affective changes (for example caused by a cancer diagnosis) impact hippocampal structure and function as well as overall cognitive capabilities. Current research results indicate an association between CRAI and CRCI in BCP undergoing chemotherapy (17, 18).

Objectives

A comprehensive theory revealing the underlying mechanisms of hippocampus-related brain changes due to cancer and its treatment is not present, even though current research and implications from animal studies indicate its role in causing CRCI. The aim of this systematic review is to elucidate the current knowledge on the impact of medical cancer treatment on the hippocampus and potential associations with CRCI and CRAI in BCP, investigated through specific brain imaging as well as neuropsychological assessment.

Research Question

What influence do different forms of medical breast cancer treatment have on the hippocampus and how do these changes contribute to affective and cognitive impairments?

METHODS

Study Design

Due to the fact that the question forms an interface between the medical and psychological field of science, the two databases PubMed and PsycINFO were searched for relevant literature in November 2018, after registering the systematic review in PROSPERO (ID: 117173). The search string used to perform this review was designed following the PICO (population,

intervention, comparison, outcome) method (46). It is described as most effective for an overall comprehensive search (47, 48). In terms of formulating a well-focused question relevant to patient care as basis for any review (49), databases were searched regarding hippocampus-related brain changes induced by chemotherapy, endocrine therapy, and/or radiotherapy and their impact on CRCI and CRAI in BCP.

Search Strategy and Data Extraction

The following key words and MeSH terms were used: "chemotherapy," "cancer treatment," "radiotherapy," "hormone therapy," "endocrine therapy," AND "hippocampus," "hippocampal," "dentate gyrus," "neurogenesis," AND "breast cancer," "breast tumor," "breast carcinoma," "breast neoplasm," "mammary tumor," AND "depression," "mood," "fatigue," "affective," "cognition," "cognitive," "impairment." Additionally, the search was restricted to studies conducted within the last 10 years in order to appropriately represent the current status of research. Using this search string and the restriction concerning the publication date, 68 results were found after sorting out those studies occurring in both databases.

During the initial screening of titles and abstracts, studies were included which met the following inclusion criteria: (a) the studies involved BCP (no animal studies); (b) treated with chemotherapy, endocrine therapy, and/or radiotherapy; (c) measurement of hippocampus-related brain changes/differences compared to healthy controls (HC); (d) neuropsychological tests; and (e) abstracts written in English. The detailed literature search strategy is shown in **Figure 1**.

RESULTS

Study Selection and Characteristics

The initial screening showed that 54 studies had to be excluded in view of the fact that they did not match all inclusion criteria. In this process, animal studies have been deliberately excluded to ensure methodological comparability. Even though studies conducted with animals (40, 50) appropriately investigate crucial structural brain changes caused by the breast cancer treatment and are useful to discuss the study results conducted with humans, CRCI were not measured in a comparable way.

After full text screening of the remaining 14 studies, four studies were excluded because they did not meet all of the inclusion criteria. One study was excluded because it did not involve BCP, two studies because treatment was not specified and one study because measuring hippocampus-related brain changes was not involved. Moreover, three reviews were excluded from analysis after being screened for potential original data and one dissertation was excluded since studies indicate that dissertations rarely influence the conclusions of reviews (51).

Following this, data on authorship and publication year, study population, study design, hippocampus-related differences, parameters indicating differences in cognitive and affective function as well as correlations and additional findings were extracted from each study, as shown in **Table 1**.

As shown in **Figure 1**, six cross-sectional studies and one longitudinal study were chosen for further analysis due to fitting the inclusion criteria, comprising a total number of 381 individuals. Of these, 190 were BCP and 191 were HC. The selected studies were substantively sorted by collecting and comparing findings on hippocampal as well as cognitive and affective changes or differences between BCP and HC and their correlation, based on the extracted information shown in **Table 1**.

Synthesized Findings

Structure- and Connectivity-Related Hippocampal Changes or Differences in BCP Compared to HC

All seven studies illustrated structure- and connectivity-related hippocampal changes or differences compared to HC, including hippocampal volume loss (52–54, 56, 58), hippocampal deformation (58) as well as changes in bilateral hippocampal (57) and whole-brain functional connectivity (55). Reduced hippocampal volume was reported in most of the studies, referring mainly to the left hippocampus (53, 54) and posterior regions (52, 54). Bergouignan et al. (52) found a posterior hippocampal volume reduction of 11% for BCP in remission from "standard treatment," which included tumorectomy, chemotherapy and radiotherapy, compared to HC. By assessing hippocampal gray matter volume in BCP before (T1), 1 month after (T2), and 1 year after chemotherapeutic treatment with fluorouracil, epirubicin, cyclophosphamide and docetaxel (T3), Perrier et al. (56) also provided insides into time effects. Gray matter volume reduction was found in the left hippocampus 1 month but not 1 year after chemotherapeutic treatment. Perrier et al. (56) also linked hippocampal atrophy to anxiety and education by showing that larger anxiety in highly educated BCP at T2 was linked with a significant atrophy in the left posterior hippocampus but not in lower educated BCP. Additionally, findings of Chaddock-Heyman et al. (54) highlight the role of individual differences in cardiorespiratory fitness (CRF) on hippocampus-related brain changes. Even though large differences were measured for lower fit BCP compared to HC, higher fit BCP did not differ in hippocampal volume compared to HC. Kesler et al. (53) also associated reduced left hippocampal volume in BCP with lower levels of IL-6 and higher levels of TNFα.

In contrast to hippocampal volume reduction, hippocampal deformation (meaning specific morphological abnormalities and differences in shape compared to healthy controls) in BCP who underwent chemotherapy and endocrine therapy were mainly observed in the right hippocampus after controlling for age compared to HC (58). In addition to structural differences, two studies measured differences in functional connectivity, including whole brain functional connectivity (55) and bilateral hippocampal network connectivity (57). Thereby, Chen et al. (55) found decreased functional connectivity of the Dorsolateral prefrontal cortex (DLPFC) with the right hippocampus in BCP treated with tamoxifen compared to HC. Additionally, Cheng et al. (57) found right hippocampus in BCP treated with tamoxifen compared to HC. Additionally, Cheng et al. (57) found chemotherapy compared to HC, including not only the frontal and parietal cortex but also the precuneus, posterior cingulate cortex, and the cerebellum. Decreased hippocampal functional connectivity in the left hippocampal network was present

TABLE 1 | Human studies.

References	Groups	*n*	Age (year) Mean ± *SD*	Status of treatment	Study design	Hippocampus-related differences/changes (cHC)		Parameters indicating differences in cognitive/affective function (cHC)		Correlations and additional findings
Bergouignan et al. (52)	BCP HC	16 21	48.73 (4.95) 47.68 (5.31)	"Standard treatment", in remission since 18 months Ø 16.4 weeks of CT Ø 5.8 weeks of RT Ø 39.27 months in remission [*n* = 4 ET]	Cross-sectional study	Hippocampal volume Total Total anterior Left anterior Right anterior Total posterior Left posterior Right posterior	 (↓8%) (→) (→) (→) (↓11%) (↓) (→)	EAMR (TEMPau task) Depression (MADRS) [Exclusion criterion MADRS score >10]	(↓20%) (↑)	EAMR score was predicted by the group/volume of posterior hippocampus and interaction of group by volume of posterior hippocampus
Kesler et al. (53)	BCP HC	42 35	54.6 (6.5) 55.5 (9.3)	Surgery and CT Ø 4.8 years off-treatment [*n* = 29 RT] [*n* = 22 ET]	Cross-sectional study	Hippocampal volume Left Right [*N* = 5 *n* = 2 BCP *n* = 3HC]	 (↓) (→)	Verbal memory (HVLT-R) Subjective memory functioning (MMQ) Depression (CAD)	(↓) (↓) (→)	Association between cytokine levels and left hippocampal volume in BCP Association between verbal memory performance and cytokine levels/hippocampal volume in both groups
Chaddock-Heyman et al. (54)	BCP HC	29 27	55.55 (1.48) 55.44 (2.13)	Surgery and CT/RT Ø 17 months off-treatment [*n* = 11 RT *n* = 7 CT *n* = 11 RT and CT]	Cross-sectional study	Hippocampal volume Total Total anterior Left anterior Right anterior Total posterior Left posterior Right posterior	 (→) (→) (→) (→) (→) (↓) (→)	Spatial memory (memory "swap" errors) Cognitive function (MMSE) [Exclusion criterion MMSE score ≤ 23]	(→) (→)	Positive correlation between CRF and hippocampal volume in BCP No differences in hippocampal volume between higher fit BCP and HC Smaller left posterior hippocampal volume in lower fit BCP compared to lower fit HC Large effect for the difference in total hippocampal volume between lower fit BCP and HC
Chen et al. (55)	BCP HC	31 32	44.97 (4.56) 43.66 (4.66)	Treated with tamoxifen for at least 24 months Ø 40.45 months [*n* = 12 RT]	Cross-sectional study	Whole-brain FC FC of the right DLPFC with the right Hippocampus (↓)		General cognitive function (MoCa) Short term memory (DS) Processing speed (SCWT/TMT-A) General executive function (SIT/TMT-B) Working memory (2-back ACC/2-back RT) Depression (HAMD) Anxiety (HAMA)	(→) (→) (→) (↓) (↓) (→) (→)	Correlations between the functional connectivity strength of the right DLPFC with the right hippocampus and the ACC in the 1-back task, 2-back task/RT in the 2-back task
Perrier et al. (56)	BCP HC	20 27	53.95 (4.75) 56.44 (3.17)	T1: After surgery, before initiation of adjuvant therapy T2: One month after the end of chemotherapy T3: One year after the end of chemotherapy	Comparative longitudinal study	Gray matter volume at T2 in the left hippocampus	(↓)	Episodic Memory Verbal T1/T3 (ESR) Visual (BEM) Working memory (WAIS III) Executive function T3 (TMT/Verbal fluency)	 (↓) (→) (→) (↓)	Larger anxiety at T2 was linked with a significant atrophy in the left posterior hippocampus in P-high compared to P- low Performance on neuropsychological tests was not directly related to hippocampal atrophy

(Continued)

TABLE 1 | Continued

References	Groups	*n*	Age (year) Mean ± *SD*	Status of treatment	Study design	Hippocampus-related differences/changes (cHC)	Parameters indicating differences in cognitive/affective function (cHC)	Correlations and additional findings
							Processing speed (TMT) (→) Depression (BDI) (→) Anxiety (STAI-A and B) P-high > P-low at T2	
Cheng et al. (57)	BCP HC	34 34 31	- 52.00 (8.48) 50.61 (8.32)	Before CT (CB) After CT (CC)	Cross-sectional study	Bilateral hippocampal FC (↑) Hippocampal FC in the frontal and parietal cortex, precuneus, PCC, and cerebellum (↓) Hippocampal FC in the right parahippocampus and left temporal pole	cHC & (cCB) Cognitive function (MMSE) (↓) Executive function (VFT) (↓) Working memory and attention (DS) (↓) EBPM (↓) TBPM (↓)	Connectivity between right hippocampus and bilateral precuneus was negatively correlated with DS performance Connectivity between left hippocampus and PCC.B and left MOG was negatively correlated with VFT scores in CC patients Left hippocampus and left FFA connectivity were negatively correlated with EBPM scores Connectivity between left hippocampus and Cbm.R was negatively correlated with EBPM/TBPM scores in BCP
Apple et al. (58)	BCP HC	16 18	37.93 (5.20) 27.17 (4.08)	CT within 18 months prior to the study and ET Ø 14,43 months off-treatment	Cross-sectional study	Hippocampal deformation (↑) Total (↑) Right (→) Left (↓) Hippocampal volume Total	NIH Toolbox Cognition Battery Episodic memory (↓) Attention (→) Processing speed (→) Executive function (→) Language (→) Neuro-QoL General cognitive concerns (↑) Executive function concerns (→) Anxiety (→) Depression (→) Fatigue (→)	

BCP, Breast cancer patients; HC, Healthy controls; CT, Chemotherapy; RT, Radiotherapy; ET, Endocrine therapy; cHC, compared to healthy controls; →, no significance; EAMR, Episodic autobiographical memory retrieval; TEMPau task, Test Episodique de Mémoire du Passé autobiographique; MADRS, Montgomery-Åsberg Depression Rating Scale; HVLT-R, Hopkins Verbal Learning Test-Revised; MMQ, Multifactorial Memory Questionnaire Ability Scale; CAD, Clinical Assessment of Depression; MMSE, Mini-Mental-Status Examination; CRF, Cardiorespiratory fitness; CB, Before chemotherapy; CC, After chemotherapy; FC, Functional connectivity; PCC, Posterior cingulate cortex; cCB, compared to before chemotherapy; VFT, Verbal Fluency Test; DS, Digital span; EBPM, Event-based prospective memory; TBPM, Time-based prospective memory; PCC.B, Bilateral cingulate cortex; MOG, Middle occipital gyrus; FFA, Fusiform area; Cbm.R, Right cerebellum; Neuro- QoL, Quality of Life in Neurological Disorders; DLPFC, Dorsal lateral prefrontal lobe; MoCa, Montreal Cognitive Assessment Test; SCWT, Stroop Color and Word Test; TMT-A/B, Trailmaking Test A/B; SIT, Stroop Interference Test; ACC, Accuracy; RT, Reaction Time; HAMD, Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Rating Scale; ESR, Encoding Storage Retrieval; BEM, Batterie d'efficience mnésique; P-high/low, high/low level of education.

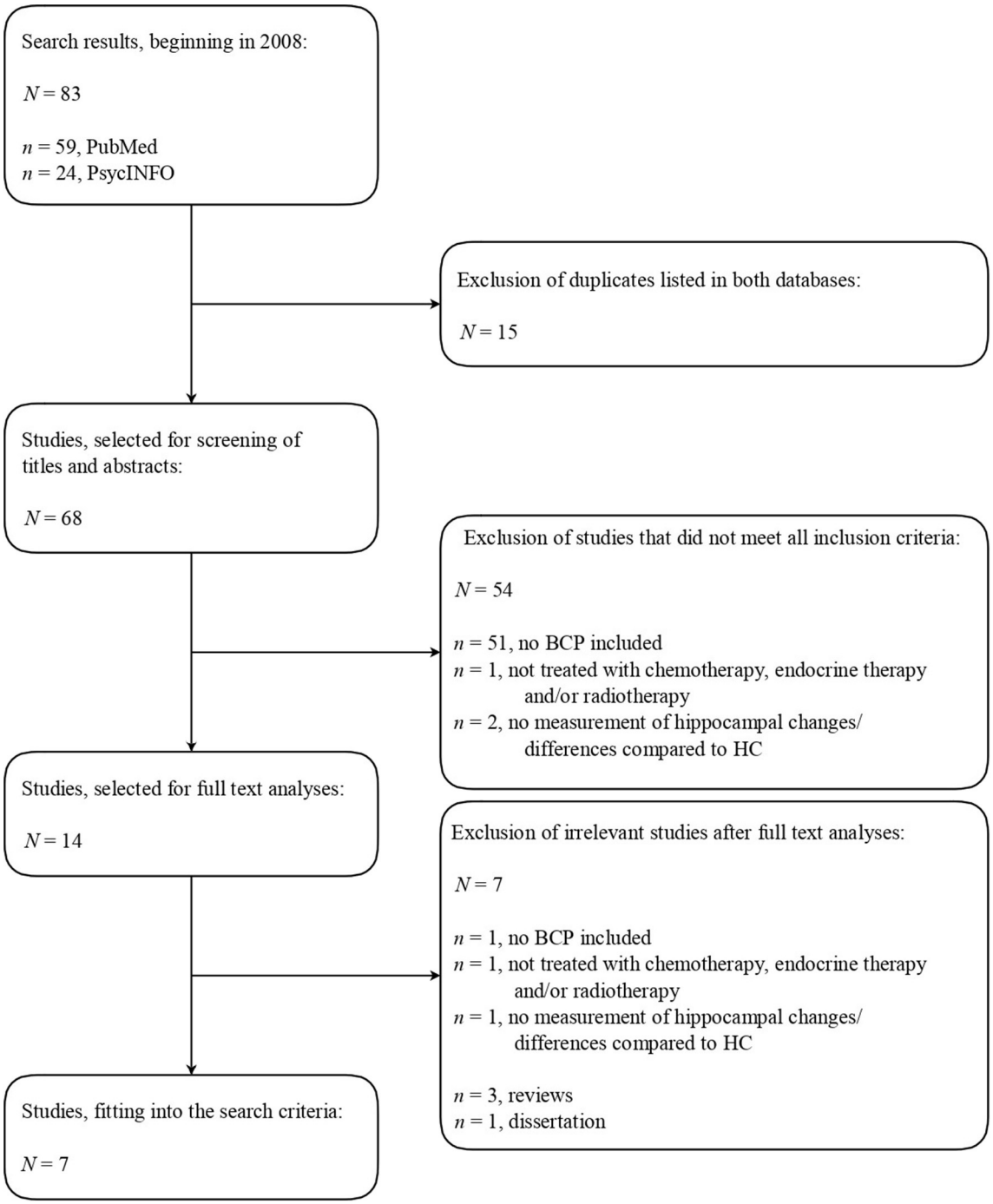

FIGURE 1 | Literature search strategy.

with the right parahippocampus and in the right hippocampal network with the left temporal pole.

Subjectively Experienced and Objectively Measured Differences in Cognitive Function

Within two studies, subjective assessments of cognitive function and memory ability was provided, including subjective memory function (53) and general cognitive concerns as well as executive function concerns (58). In this context, BCP, who underwent chemotherapy (cyclophosphamide or paclitaxel and doxorubicin; 5-fluorouracila and paclitaxel or methotrexate and cyclophosphamide; taxane, anthracycline, and cyclophosphamide) and endocrine therapy (tamoxifen), and HC did not differ regarding executive function concerns. However,

subjective memory function was decreased in BCP after surgery and chemotherapy (53) and global cognitive concerns were increased in BCP after being treated with chemotherapy and endocrine therapy (58). In addition to subjective assessments, six studies objectively recorded differences in cognitive function in BCP compared to HC, including worsened verbal memory (Hopkins Verbal Learning Test-Revised) (53) and episodic memory performance (Picture Sequence Memory Test) (58) as well as declines in episodic memory retrieval (Test Episodique de Mémoire du Passé autobiographique task) (52), following various forms of breast cancer treatment (52, 53, 58). Beyond that, BCP, who have been treated with tamoxifen, performed significantly worse in tests evaluating general executive function and working memory tasks (55).

Cheng et al. (57) compared cognitive deficits of BCP after chemotherapy to BCP before treatment. They found a decrease in cognitive function, using the Mini Mental Status Examination (MMSE), in executive function, using the Verbal Fluency Test (VFT), and in working memory and attention, measured by digital span (DS) performance as well as event-based and time-based prospective memory (EBPM/TBPM). Additionally, Perrier et al. (56) assessed changes in episodic memory, working memory, executive functions as well as processing speed longitudinally with differing results. BCP performed significantly worse in episodic verbal memory retrieval (Encoding Storage Retrieval) at T1 (after surgery but before initiation of adjuvant therapy) and T3 (1 year after the end of chemotherapy) and showed lower performances than HC at T3 regarding executive function tasks, using the Trail Making Test (TMT) and Verbal Fluency Test (VFT). No differences between HC and BCP were found for visual episodic memory, using the Batterie d'efficience mnésique (BEM) as well as processing speed, using the TMT.

Correlations Between CRCI and Hippocampus-Related Differences

Overall, findings indicate that the mentioned differences in measured cognitive function can be associated with hippocampal regions, differing between left and right hippocampus as well as anterior and posterior regions within the hippocampus.

Left hippocampus

With regards to the left hippocampus, Kesler et al. (53) found associations between hippocampal volume reduction and cytokine levels (IL-6 decreased and TNFα increased) and diminished verbal memory performance assessed with the HVLT. By measuring bilateral hippocampal connectivity, Cheng et al. (57) linked worsened test results in tests measuring executive function (VFT) as well as EBPM and TBPM to changes in bilateral hippocampal functional connectivity. Thereby, connectivity between the left hippocampus, bilateral cingulate cortex, and left middle occipital gyrus was negatively correlated with VFT scores. Moreover, the left hippocampus and left fusiform area were negatively correlated with EBPM scores and connectivity between the left hippocampus and right cerebellum was associated with declines in EBPM and TBPM scores.

Right hippocampus

Two studies (55, 57) indicate that the functional connectivity between the right hippocampus and other brain regions is associated with working memory performance. In this context, correlations between the functional connectivity strength of the right dorsal lateral prefrontal lobe (DLPFC) with the right hippocampus and accuracy in the 1-back and 2-back task of the DS test and the reaction time in the 2- back task were measured (55). Moreover, connectivity between the right hippocampus and bilateral precuneus was negatively correlated with DS performance (57).

Posterior hippocampal regions

Posterior regions within the hippocampus were mainly associated with changes in episodic memory retrieval and declines in spatial memory performance. Bergouignan et al. (52) examined significant differences in episodic autobiographical memory retrieval (measured with TEMPau task) between BCP after "standard treatment," including chemotherapy, radiotherapy and endocrine therapy, and HC, showing that BCP had significantly lower episodic memory retrieval than HC. Additionally, episodic memory score was predicted by the volume of the posterior hippocampus and the interaction of group by volume of the posterior hippocampus. Chaddock-Heyman et al. (54) compared higher and lower CRF in BCP to higher and lower CRF in HC but found no significant differences between BCP and HC in their self-constructed spatial memory task, even though the means indicate more "swaps," errors made during reconstructing the relative positions of objects, in BCP. However, memory "swap" errors were related to reduced left posterior hippocampal volume in BCP.

A contradictory picture was only drawn by Perrier et al. (56), who reported a decrease in gray matter volume 1 month after chemotherapeutic treatment (epirubicin, fluorouracil, docetaxel, and cyclophosphamide) but no direct relation between performances on neuropsychological tests (ESR, BEM, WAIS III, TMT) and hippocampal atrophy.

Changes in Affective Function in BCP and Differences Compared to HC

Generally, results on differences in affective function must be considered with caution due to the fact that in six out of seven studies, to avoid potential confounding, BCP with a current or past history of psychiatric disorders were excluded (52, 53, 55–58). However, five of seven studies (52, 53, 55, 56, 58) included measuring changes or differences in affective function compared to HC. Five of seven studies recording depression scores by using the Montgomery Åsberg Depression Rating Scale (52), Clinical Assessment of Depression (53), Neuro-QoL (58), Beck Depression Inventory (56) and Hamilton Depression Rating Scale (55), reported no significant differences between BCP and HC. Only one study (52) found significantly higher depression scores in BCP in remission after "standard treatment," including chemotherapy and radiotherapy, compared to HC. Besides, both studies measuring anxiety scores (55, 58) found no differences in BCP, treated either with endocrine therapy (55) or a combination of chemotherapy and endocrine therapy (58).

Additionally, using the Neuro QoL, Apple et al. (58) recorded self-reported impairment information surrounding fatigue but found no differences between BCP and HC.

DISCUSSION

Only few human studies with predominately methodological limitations focused on linking those two research topics.

Summary of Main Findings

The results on hippocampus-related consequences of cancer treatment are in line with the empirically substantiated assumption that the hippocampus is highly vulnerable during the course of cancer and its treatment (20, 21). In addition to chemotherapy, volume loss, and reduced hippocampal neurogenesis (42, 43) were present in studies equally focusing on radiotherapy (54), or endocrine therapy (55). Moreover, possible diverse CRCI due to hippocampus-related brain changes were recorded, reflecting the assumption that the effects of cancer treatment on the hippocampus result in declines in many tasks related to memory and learning processes (36, 52, 59, 60).

With regard to memory-related cognitive function, the studies indicate that impairments are evident in tasks measuring hippocampus-related executive function as well as working memory, episodic memory, and prospective memory tasks (52, 55–58). These results are particularly interesting since research results suggest that the memory processes involved are highly interrelated. For example, McCabe et al. (61) found out that correlations between episodic memory and either working memory capacity or executive function ranged between $r = 0.73$ and $r = 0.90$. They even advocate an underlying mechanism which they call executive attention. Impairments in executive function were measured in three out of four studies for BCP treated with chemotherapy, using the TMT-B/VFT56 and the VFT57, as well as for BCP treated with endocrine therapy, using the SIT and the TMT-B58, compared to HC. Thereby, Cheng et al. (57) found out that connectivity between the left hippocampus and bilateral cingulate cortex and the left middle occipital gyrus was negatively correlated with VFT scores in BCP.

Due to the fact that executive function generally includes control functions related to inhibiting prepotent responses, shifting mental sets, updating task demands, planning, working memory as well as cognitive flexibility (61), it is not surprising that a deterioration of the working memory was present in most of the studies. The study results further helped to provide an insight into the possible causes of impairments in working memory by linking impairments to changes in functional brain connectivity (55, 57). According to Cheng et al. (57), connectivity between the right hippocampus and bilateral precuneus was negatively correlated with DS performance, measuring working memory, and attention. As a part of the posterior medial system, the hippocampus is connected with the default mode network, which the precuneus is a part of (23), that plays an important role in memory retrieval processes (24). Additionally, Chen et al. (55) linked worsened working memory performance, measured by the 1-back and 2-back task, with decreased functional connectivity strength of the right DLPFC with the right hippocampus.

Attention processes are mainly related to the functioning of the working memory, being included in the study by Cheng et al. (57) and measured individually by Apple et al. (58), and the prefrontal cortex is especially related to attention-based processes (80). Contrary to the results of Cheng et al. (57), Apple et al. (58) found no differences in attention, included in the NIH Toolbox Cognition Battery, between BCP treated with chemotherapy and endocrine therapy and HC. Interestingly, BCP treated with tamoxifen and HC did not differ in DS performance (55). Also, measuring digital span performance longitudinally, Perrier et al. differ in DS performance (55). Also, measuring digital span performance longitudinally, Perrier et al. impairments in spatial memory were recorded by Chaddock-Heyman (54), focusing on BCP treated with radiotherapy and/or chemotherapy on average 17 months off-treatment, even though spatial cognition is equally linked to the default mode network in current research results (24).

The studies involved indicate that besides working memory, episodic memory is highly affected by breast cancer treatment. Two studies including episodic memory performance found that it was significantly worsened in BCP in remission from "standard treatment," containing all types of treatment (52), as well as in BCP treated with chemotherapy and endocrine therapy (58) compared to HC. Thereby, Bergouignan et al. (52) provided an insight into the extent and the causes of episodic memory impairments due to hippocampus-related brain changes. They did not only find out that the BCP group had 20% less access to episodic autobiographical memory retrieval than HC, but that episodic autobiographical memory retrieval score was predicted by the group and volume of the posterior hippocampus as well as the interaction of group by volume of the posterior hippocampus. These results can also be well-integrated into the current knowledge on the role of the posterior hippocampus in memory processes as it forms a part of the posterior medial system that plays a role in recollection and episodic memory (23). Because memory-related information from both systems is integrated in the dentate gyrus and the CA3 region (23), it is not surprising that those impairments have been linked to disrupted neurogenesis in mouse models (38, 40, 59). Furthermore, the study of Cheng et al. (57) included measuring both EBPM and TBPM. Thereby, BCP treated with chemotherapy performed significantly worse in tasks related to EBPM as well as TBPM compared to HC and compared to BCP before treatment. Reasons may be found in the connectivity of the left hippocampus with other brain regions. Thereby, the left hippocampus and left Fusiform area (FFA) connectivity were negatively correlated with EBPM scores and the connectivity between the left hippocampus and the right cerebellum was negatively correlated with both EBPM and TBPM scores in BCP (57). Interestingly, results by Perrier et al. (56) indicate, that declines in episodic memory in BCP compared to HC are already present before treatment since BCP showed lower performances in verbal memory retrieval, measured by Encoding Scoring Retrieval, both before initiation of adjuvant chemotherapy and 1 year after the end of chemotherapy. Also, even though differences between BCP and HC were found for verbal episodic memory, no significant differences were found for visual episodic memory, using the BEM (56). This suggests

that other factors may also have an impact on CRCI and that there may be a difference in the vulnerability of different types of episodic memory to treatment- related effects.

Recent research results also indicate that CRAI as a result of hippocampus- related impairments could represent one influential factor by pointing to the interacting effects between cognitive and affective functions and brain structural changes. For example, Perrier et al. (56) found out, that larger anxiety scores were linked to significant atrophy in the left posterior hippocampus in highly educated BCP but not in less educated BCP. Nevertheless, even though most of the studies included the recording of depression (52, 53, 55, 56, 58) and anxiety scores (55, 56, 58), CRAI did not play a central role in the studies involved. For instance, measuring fatigue, one of the most common symptoms that can even persist up to 10 years after the end of treatment (62, 63), was only included in one study (58). Despite one study (52), no differences in affective function were found between BCP and HC although different tests were used and different treatment types were included. These results correspond to the hippocampus-related results to the extent that the volume reduction due to the breast cancer treatment mainly seems to have an influence on posterior parts (52, 54). This is in line with the assumption of affective impairments being more closely related to changes in anterior parts of the hippocampus due to connections with the amygdala (25), HPA axis and the limbic prefrontal circuit (26, 27). However, current research results indicate that CRAI would have been likely since neurogenesis in the dentate gyrus plays a role in buffering stress responses and depressive behavior by modulating the HPA axis (64). Moreover, Kesler et al. (53) found out that there is an association between cytokine levels and left hippocampal volume and a link between inflammation and depression has been increasingly suggested (65, 66). This is in line with current research results linking pro-inflammatory cytokines to synaptic dysfunction and neuronal death, particularly focusing on neurogenesis impairment in the dentate gyrus due to several direct and indirect effects, including the death of neural progenitor cells as well as limiting effects on neuronal differentiation (67). An association between lower levels of IL-6 with lower left hippocampus volume reported by Kesler et al. (53) were in contrast to previous findings, suggesting an inverse relationship between IL-6 levels and hippocampus volume in healthy adults (53). As an explanation for this contradictory finding, Kesler et al. referred to an altered pattern of influence for IL-6 in patients with a history of various illnesses. Furthermore, IL-6 seems to fulfill a role as a pro- as well as an anti-inflammatory agent following brain injury, which is indicative of a complex mechanism regulating IL-6 levels in patients with cancer and during/after its treatment (53).

Comparing results of the evaluated studies with recent findings on depression and anxiety in breast cancer survivors (BCS), a systematic review from Carreira et al. (68) found 33 studies reporting more depression in BCS compared to women without cancer (with 19 studies being statistically significant) and 17 studies reporting more anxiety (with 11 studies being statistically significant). The reasons for increased depressive symptoms in BCS seemed to be comparable to those in the general female populations, including: lower rates of social and psychological support, lower socio-economic status as well as impact on lifestyle and relationship (68). Interestingly, a phenomenon called "posttraumatic stress growth," which goes along with feelings of improved empathy, closer relationships and great appreciation of life is reported in about 60% of BCS and might be a reason why symptoms of anxiety and depression are not persistent in some subgroups of BCS (68). Although this review provides compelling evidence of BCS being at increased risk for development of depression and anxiety, comparable to the studies used in our review, certain limitations were mentioned by the authors. Those limitations included the cross-sectional study design, low power, selection bias of participants, information bias, no control for confounding factors such as age and socio-economic status and methodological limitations of how depression and anxiety are assessed in various studies.

Methodological Limitations

First of all, the cross-sectional design of six of the seven studies has to be seen within its limitations. The fact that most of the studies focused on CRCI and brain structural changes after the end of treatment has to be discussed since current research results support the assumption that CRCI are already observed before treatment (56, 69). In this context it also has to be mentioned that most of studies did not precisely differentiate between the types of breast cancer treatment or the chemotherapeutic agents the BCP had been treated with (52–54, 56–58). Including pre-treatment baseline assessments, comparable to the study design by Perrier et al. (56), and a differentiation between the treatment types may contribute to a better understanding of side effects attributable to the treatment (70).

Using expensive imaging techniques to properly measure structural brain changes makes it difficult to guarantee high sample sizes. It can therefore not be excluded that the relatively small sample sizes may have reduced statistical power in detecting smaller effects (53). It also must be taken into account that BCP often belong to a relatively old population (71) and that the hippocampus and especially the dentate gyrus are influenced by aging processes during the course of life (72, 73).

Moreover, only two studies included subjective measures (53, 58) even though subjective complaints are considered to be the heart of the CRCI problem (74). Objective measures of CRCI ranged from focusing on a single task (52) to including test batteries covering multiple cognitive domains (58). Thereby, even though focusing on just a few or even just one facet of cognitive function contributes to providing concrete information on one point of interest, a comprehensive picture of the complex interrelationships may not be derivable. The influence of affective factors on cognitive function is also not fully understood, mainly because CRAI were often used as an exclusion criterion in the studies involved. This is not only problematic because CRAI seem to play a major role in the treatment-caused side effects (15) but also because latest findings indicate that psychological variables do contribute to CRCI and also to hippocampus-related brain changes BCP have to deal with (56, 75).

Limitations of This Review

The results should be considered within the context of the limitations of the present review. First of all, even though study selection was performed by two independent reviewer, selection bias cannot be fully precluded. Secondly, it was based on the overall data retrieved from the literature, so the stated limitations of the studies must therefore also be regarded as limitations of the present work. It cannot be completely ruled out that errors have occurred or wrong conclusions have been drawn trying to link and compare studies using different experimental designs, chemotherapeutic agents, patient populations (12), and tests measuring hippocampus-related changes and resulting CRCI and CRAI differently. Thereby, as already emphasized, comparisons, and connections between studies conducted with animals and those with humans must also be drawn with caution.

Future Need for Research and Implementation of Clinical Study Results in Medical Routine

The studies involved in this review indicate that a highly relevant but yet understudied field of research revolves around the question of how hippocampus-related brain changes due to breast cancer treatment result in CRCI and CRAI. In order to get a deeper understanding of the complex interrelationships further research is required. For this purpose, future research should include baseline assessment not only of the cognitive and affective abilities but of the condition of the hippocampus pre-treatment by using longitudinal assessments and adjusting for age and patients abilities. This is also supported by the fact that breast cancer treatment relies on a combined treatment approach (76) and a differentiation of the different types of treatment could provide information on whether they lead to different consequences and whether interaction effects occur.

Since both animal and human studies indicate that the dentate gyrus is of decisive relevance to both CRCI and CRAI in BCP (40, 59, 60, 64, 77), it would be interesting to include tasks in future research which address functions that are specifically associated with the dentate gyrus. The findings further plead for a focus on tasks measuring executive function, working memory, episodic memory as well as prospective memory because they seem to be those cognitive domains being particularly influenced. Therefore, research results could be replicated using larger sample sizes.

Besides measuring CRCI, a field of research that is not reflected in current literature is the effect of hippocampus-related brain changes on CRAI. Since it is not clear whether hippocampus-related brain changes cause depression and anxiety or whether affective changes are caused by the diagnosis, further research should focus on understanding the cause and interacting effects of various factors, such as inflammation or individual factors, CRAI, CRCI, and brain-structural changes in BCP. The detection of fatigue as an interface between CRCI and CRAI (78), for example, could provide an insight into the interaction of treatment-related consequences.

Even though a lot of research is still needed to assess the effects of breast cancer treatment on CRCI and CRAI, the research results clearly indicate that there is a great need for these side effects to be addressed in everyday medical practice. Healthcare professionals are in the position not only to give their patients advice about the most suitable treatment approach (43, 79) but to inform them about the affective and cognitive challenges resulting from the procedure. This includes providing information about the possibility of participating in interventions.

CONCLUSIONS

Overall, although improvements in the treatment of breast cancer cause survivorship rates to rise steadily (3, 4), treatment side effects due to hippocampus-related brain changes seem to be omnipresent across all types of therapy (52–58). CRCI due to hippocampus-related brain changes seem to be particularly relevant in cognitive domains including executive function as well as working memory, episodic memory, and prospective memory (52, 55–58). Although an interaction between hippocampus-related brain changes, CRCI, and CRAI can be hypothesized, CRAI are less reflected in current research. This is problematic because latest findings indicate that CRAI in BCP can contribute to hippocampus-related brain structural changes (56). Against this background, further hypothesis- and knowledge-based research on treatment side effects and interaction effects is required, especially by combining information received from imaging techniques and specific neuropsychological tests. Identifying the mechanisms by which hippocampus-related brain changes affect CRCI and CRAI may help to understand why those impairments even persist up to years after the end of treatment and substantiate the importance of integrating this knowledge into everyday medical practice. This could contribute to improve early detection, timely treatment, and informed therapeutic options (58) and lead to an improved quality of life, both during and after treatment, for the increasing number of women dealing with this life-threatening diagnosis.

AUTHOR CONTRIBUTIONS

XP, KS, PM, and PZ devised the main conceptual ideas, which have been further specified in consultation with the other authors. KS, PM, and PZ defined the databases to be searched and the inclusion and exclusion criteria for the studies. XP and AR searched the databases and performed the initial and full text screening. The studies were read by all authors and criteria for sorting and summarizing the content of the studies were provided, which were then extracted from the studies by XP and KS. The manuscript was written by XP and KS, with the help of AR. After all authors had discussed the results and commented on the manuscript, it was further compressed and specified in content by XP, AR, and SS.

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Effect of Tamoxifen on the Risk of Osteoporosis and Osteoporotic Fracture in Younger Breast Cancer Survivors: A Nationwide Study

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Background: Although international guidelines recommend bone screening for premenopausal breast cancer patients taking adjuvant tamoxifen, the effects of tamoxifen on osteoporosis and related risks remain controversial. The objective of this study was to investigate the incidence of and risk factors for osteoporosis and osteoporotic fractures in younger breast cancer patients.

Methods: A nationwide retrospective cohort study was conducted using South Korea Health Insurance Review and Assessment Service claims data. The rates of osteoporosis and osteoporotic fracture were calculated as incident cases per person-year and disease-free probability rates were analyzed with the Kaplan-Meier method. To identify risk factors for osteoporosis and osteoporotic fracture, a multivariable Cox proportional hazard regression model was applied.

Results: From January 2009 to December 2014, a total of 47,649 breast cancer patients were included. The incidence rates of osteoporosis and osteoporotic fracture were 23.59 and 2.40 per 1,000 person-years, respectively. In the overall population, tamoxifen was significantly associated with a decreased risk of osteoporosis and osteoporotic fractures 0.76). However, tamoxifen was not associated with the risk of osteoporosis (HR 1.24, CI 0.85–1.82) and osteoporotic fracture (HR 8.15, CI 0.36–186.70) in patients under age 40. In the 40–49 years subgroup, tamoxifen significantly decreased the risk of osteoporosis (HR 0.74, CI 0.65–0.84) and osteoporotic fracture (HR 0.49, CI 0.31–0.76).

Conclusions: Tamoxifen is not associated with an increased risk of osteoporosis and osteoporotic fracture in premenopausal breast cancer patients. Tailored screening strategies for breast cancer survivors with different osteoporosis risks are needed.

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Precis: Tamoxifen is not associated with an increased risk of osteoporosis and osteoporotic fracture in premenopausal breast cancer patients. Tailored screening strategies for breast cancer survivors who are at different risks of developing osteoporosis are needed.

Keywords: breast neoplasms, survivorship, osteoporosis, bone fractures, tamoxifen

INTRODUCTION

As the survival rate of breast cancer patients increases, optimal survivorship care has become an essential part of clinical practice (1, 2). One of the common long-term effects of breast cancer treatments is osteoporosis, with up to 80% of breast cancer patients experiencing bone loss (3, 4). Women with breast cancer, even in the absence of skeletal metastases, are known to have a higher incidence of fractures than women of the same age without breast cancer (5). Aromatase inhibitor (AI) is one of the well-known risk factors for osteoporosis in postmenopausal breast cancer patients (6, 7). Osteoporotic fractures impose an enormous health burden on individuals and take a substantial economic toll on society (8–10).

Tamoxifen is a known risk factor for osteoporosis in premenopausal breast cancer patients. In previous studies involving premenopausal breast cancer patients taking tamoxifen, bone mineral density decreased progressively over a 3-years follow-up period (11), and tamoxifen was associated with significant bone loss in patients who remained premenopausal after adjuvant chemotherapy (12). Based on these studies, the American Cancer Society/American Society of Clinical Oncology (ACS/ASCO) guidelines recommend bone screening every 2 years for premenopausal women receiving tamoxifen (13).

However, these previous studies have limitations. The sample sizes were small and only univariate analyses were performed to calculate the difference between the tamoxifen and placebo groups. Also, the primary outcome was the percent change in bone mineral density (BMD) rather than clinically meaningful outcomes such as osteoporosis or osteoporotic fractures, and the follow-up periods were short. For these reasons, the effect of tamoxifen on osteoporosis risks in premenopausal breast cancer patients remains controversial. Therefore, we conducted this nationwide retrospective cohort study using data from the Health Insurance Review and Assessment Service (HIRA), which archives data from nearly 98% of all citizens in South Korea (14). The objective of this study was to investigate the incidence of and risk factors for osteoporosis and osteoporotic fractures in breast cancer patients and to assess whether tamoxifen is a risk factor for osteoporosis and osteoporotic fractures in younger breast cancer patients.

MATERIALS AND METHODS

Data Source

The HIRA, a governmental organization in South Korea, assesses healthcare services and makes reimbursement decisions under the national healthcare insurance service. The HIRA collects nationwide claims data from healthcare providers (14). The HIRA data consists of six parts: (1) general information; (2) healthcare services; (3) diagnoses; (4) outpatient prescriptions; (5) medication file; and (6) provider information. The diagnostic information is based on the International Classification of Diseases 10th revision (ICD-10).

Study Population

We selected the study period of January 2007 to December 2017 because of data availability. Newly diagnosed breast cancer was defined by the C50 code (invasive breast cancer) in combination with the specialized V193 claim code, which is an identifier for reimbursement of cancer patients (15). Because we considered a 2-years period before breast cancer diagnosis as a washout period to exclude prevalent breast cancer and any cancer, subjects were excluded from the study if they received a C code within that period. Patients who did not undergo breast cancer surgery and those with a history of *in situ* carcinoma, presumed metastatic breast cancer, preexisting or recent (within 1 year after breast cancer diagnosis) osteoporosis, previous rheumatoid arthritis, or long-term corticosteroid treatment (more than 90 days) were excluded. Male patients or subjects who did not have follow-up claims data after breast cancer diagnosis were also excluded.

From January 2009 to December 2014, a total of 191,942 patients received C50 and V193 codes in the HIRA database. We excluded 118,820 who had *C* codes (any cancer) within the washout period. We excluded 2,531 patients with a previous history of *in situ* carcinoma, 5,801 with metastatic or recurrent breast cancer, 8,433 with preexisting or recently diagnosed osteoporosis, 134 with previous rheumatoid arthritis, 2,046 with long-term corticosteroid treatment, and 6,336 who did not undergo breast cancer surgery. One hundred eighty-seven male patients and 4 patients who did not have follow-up data after breast cancer diagnosis were also excluded (**Figure 1**).

Variables and Operational Definitions

Patients' characteristics such as age, type of insurance (health insurance vs. medical aid), and the Charlson Comorbidity Index (CCI) based on ICD-10 codes were analyzed (16). We defined the treatment groups based on claims data within 1 year after breast cancer diagnosis. Radiation therapy (either left and/or right), chemotherapy, ovarian function suppression (OFS), trastuzumab, and endocrine treatment (tamoxifen or AI) were reviewed. Regardless of whether they may have subsequently switched anti-hormonal medications, the patients were allocated into treatment groups according to the initially prescribed endocrine therapy. We defined osteoporosis as

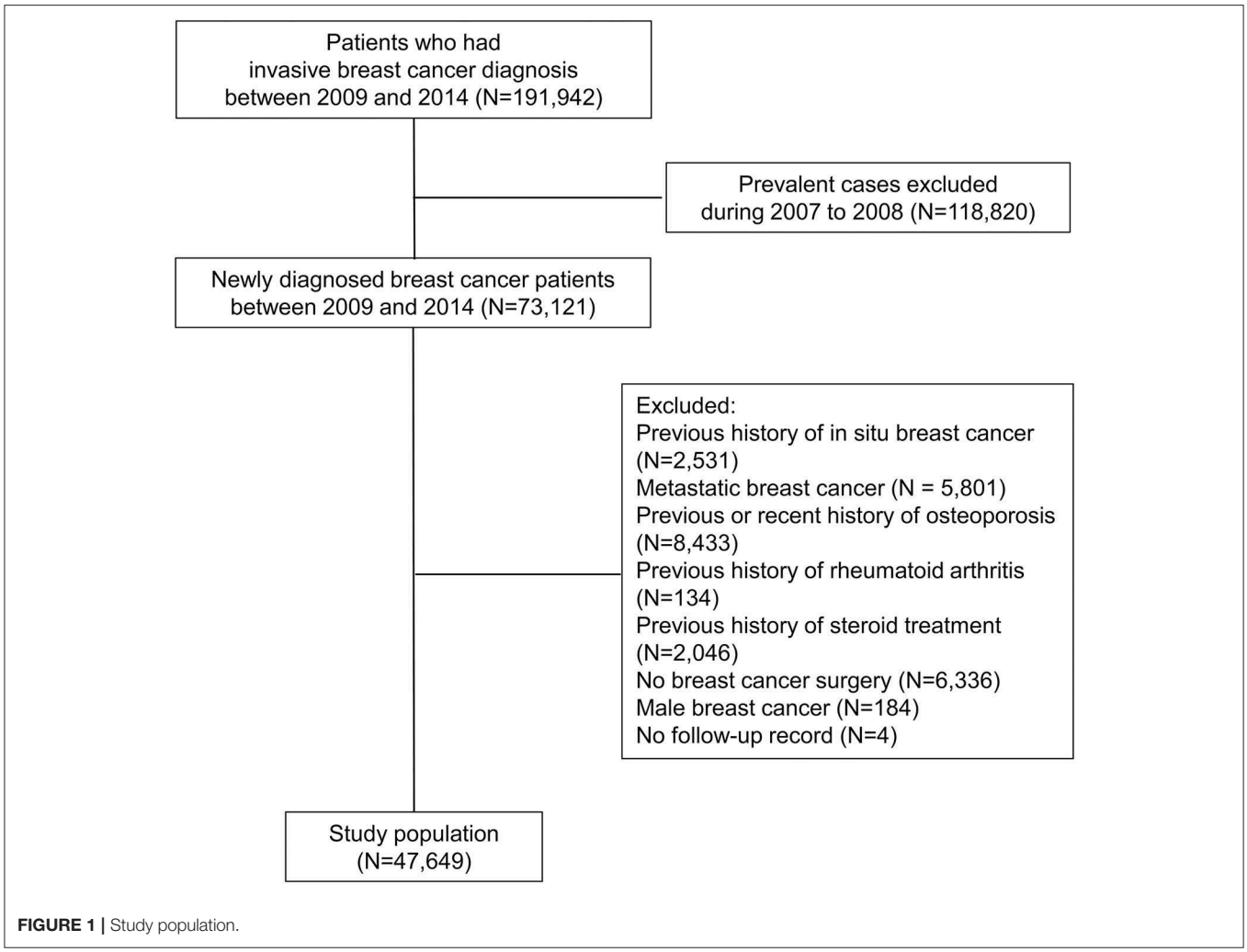

FIGURE 1 | Study population.

the newly claimed osteoporosis codes (M80, M81, M82) in conjunction with at least one of osteoporosis medications (pamidronate, alendronate, ibandronate, risedronate, tibolone, dienogest, estradiol hemihydrate, estradiol valerate, estropipate, conjugated equine estrogens, medroxyprogesterone acetate). The development of osteoporosis was defined using the newly claimed diagnosis in conjunction with medications. Osteoporotic fracture was defined as fracture-related codes (M80, osteoporosis with pathological fracture; S22, fracture of rib, sternum, and thoracic spine; S32, fracture of lumbar spine and pelvis; S52, fracture of forearm; S62, fracture at wrist and hand; S72, fracture of femur) or treatment of fractures and osteoporosis within 6 months before or after the fracture.

Statistical Analysis

The baseline characteristics of the included patients are presented as the number of patients (%) or mean ± SD. The incidence rates of osteoporosis and osteoporotic fracture were calculated by dividing the number of incident cases by the total follow-up period (person-years). The disease-free probability of osteoporosis and osteoporotic fracture were calculated by the Kaplan-Meier method, and the log-rank test was performed to confirm differences across risk factors.

For the identification of risk factors for osteoporosis and osteoporotic fracture, a multivariable Cox proportional hazard regression model was applied and adjusted hazard ratio (HR) and 95% confidence interval (CI) were estimated. Age, type of insurance, CCI, chemotherapy, endocrine therapy, OFS, radiotherapy, and trastuzumab were selected as covariates for regression models. Subgroup analyses were performed within age groups ($<$40, 40–49, 50–59, 60–69, and $\geq$70 years) to further clarify risk factors of osteoporosis and osteoporotic fracture.

Statistical analyses were performed with SAS software (version 9.4, SAS Institute, Cary, NC, USA). This study was approved by the Soonchunhyang University Seoul Hospital Institutional Review Board (IRB no. SCHUH 2018-11-011).

RESULTS

Baseline Characteristics

A total of 47,649 breast cancer survivors were included in this analysis. Among them, the proportion aged 40–49 years at the time of diagnosis was 42.04 % (**Table 1**). The proportion of breast cancer survivors who received any type of chemotherapy was 67.57% (n = 32,198). More than two-thirds of the survivors received endocrine treatment, and tamoxifen was the most

TABLE 1 | Baseline characteristics and adjuvant treatments in breast cancer survivors.

	Number (%)
Total	47,649
Age at diagnosis*	48.92 ± 9.82
<30	657 (1.38)
30–39	6,464 (13.57)
40–49	20,034 (42.04)
50–59	13,968 (29.31)
60–69	4,808 (10.09)
70–79	1,510 (3.17)
80-	208 (0.44)
Charlson comorbidity index*	1.34 ± 1.42
0	15,579 (32.70)
1	15,081 (31.65)
2	9,120 (19.14)
3	4,179 (8.77)
4	1,891 (3.97)
≥5	1,799 (3.76)
Reimbursement type	
National health insurance	46,871 (98.37)
Medical aid	777 (1.63)
Others	1 (0.00)
Chemotherapy(any)	
No	15,451 (32.43)
Yes	32,198 (67.57)
Endocrine treatment	
No	13,312 (27.94)
Tamoxifen	24,006 (50.38)
Aromatase inhibitor**	10,331 (21.68)
Ovarian function suppression***	
No	42,627 (89.46)
Yes	5,022 (10.54)
Radiotherapy	
No	13,301 (27.91)
Yes	34,348 (72.09)
Trastuzumab	
No	40,930 (85.90)
Yes	6,719 (14.10)

presented as Mean ± SD, measured at breast cancer diagnosis.
***letrozole, anastrozole, exemestane.*
****goserelin, leuprolide.*

frequently prescribed agent. OFS with goserelin or leuprolide was prescribed in 10.54% of the survivors. All the subjects underwent breast cancer surgeries, according to our operational definition.

Incidence of Osteoporosis and Osteoporotic Fracture

During the study period, 5,955 osteoporosis events were observed in 252,396 person-years. The incidence rate of osteoporosis in breast cancer survivors was 23.59 per 1000 person-years (95% CI, 23.00–24.20). Osteoporotic fracture incidence was assessed as 2.40 per 1000 person-years (95% CI, 2.23–2.60), with 647 events occurring in 269,075 person-years (**Table 2**). Age at diagnosis and CCI were significantly associated with development of osteoporosis ($p < 0.0001$) and osteoporotic fracture ($p < 0.0001$) in the univariate analysis. The incidence rate of osteoporosis was highest in patients aged 70–79 years. The risk of osteoporotic fracture was highest in patients older than 80 years (17.16 per 1,000 person-years) followed by patients aged 70–79 years. Event-free probability of osteoporosis and osteoporotic fracture after 1 year following breast cancer diagnosis is presented in **Supplementary Figure 1**.

Risk Factors for Osteoporosis and Osteoporotic Fracture According to Age at Diagnosis

Factors associated with the incidence of osteoporosis and osteoporotic fracture showed different patterns according to age subgroups (**Table 3**). In patients younger than 40 years, the use of OFS was significantly related to an increased incidence of osteoporosis. In the age 40–49 group, chemotherapy, AI and OFS were significantly associated with an increased risk of osteoporosis. In patients aged 50–69 years, AI significantly increased the risk of osteoporosis; in contrast, tamoxifen was associated with a decreased risk of osteoporosis.

There were only 9 cases of osteoporotic fracture in 7,121 patients younger than 40 years, and OFS was not associated with an increased risk of osteoporotic fracture in these patients (**Supplementary Table 1**). An increased risk of osteoporotic fracture was significantly associated with chemotherapy (HR, 1.75; 95% CI, 1.07–2.88) and AI (HR, 2.35; 95% CI, 1.34–4.12) in the age 40–49 subgroup.

Effect of Tamoxifen on Bone Health in Younger Breast Cancer Patients

In the total population, tamoxifen was significantly associated with a decreased risk of osteoporosis and osteoporotic fracture (**Figure 2**). The risk of osteoporosis (HR, 1.24; CI, 0.85–1.82) and osteoporotic fracture (HR, 8.15; CI, 0.36–186.70) was not associated with tamoxifen in patients younger than 40 years (**Table 3** and **Supplementary Table 1**). However, in the age 40–49 group, tamoxifen significantly decreased the risk of osteoporosis (HR, 0.74; CI, 0.65–0.84) and osteoporotic fracture (HR, 0.49; CI, 0.31–0.76).

DISCUSSION

The results of this large retrospective study show that the use of adjuvant tamoxifen does not increase the risk of osteoporosis and osteoporotic fracture in younger breast cancer survivors. In patients younger than 40 years at the time of breast cancer diagnosis, adjuvant tamoxifen was not associated with the development of osteoporosis and osteoporotic fracture. Furthermore, tamoxifen significantly decreased the risk of osteoporosis and osteoporotic fracture in breast cancer patients aged 40–49 years at the time of diagnosis. OFS was significantly associated with an increased risk of osteoporosis in patients younger than 50 years. Chemotherapy and AI were significantly

TABLE 2 | Incidence rates of osteoporosis and osteoporotic fracture according to age at diagnosis and comorbidities.

	N	Events (*n*)	Total person-year	Incidence rate (per 1,000 person-year)	HR (95% CI)	*p**
Osteoporosis						
Total	47,649	5,955	2,52,396	23.59 (23.00–24.20)		
Age at diagnosis					<0.0001	
<30	657	6	3,771	1.59 (0.71–3.54)	1 (Ref)	
30–39	6,464	161	37,017	4.35 (3.73–5.08)	2.73 (1.21–6.16)	
40–49	20,034	1521	1,10,010	13.83 (13.15–14.54)	8.67 (3.89–19.34)	
50–59	13,968	2,409	71,240	33.82 (32.49–35.19)	21.33 (9.57–47.52)	
60–69	4,808	1,360	22,766	59.74 (56.65–63.00)	37.97 (17.03–84.65)	
70–79	1,510	455	6,773	67.18 (61.28–73.64)	42.98 (19.21–96.18)	
80+	208	43	819	52.50 (38.94–70.79)	33.82 (14.39–79.45)	
CCI**						<0.0001
0	15,579	1,586	88,502	17.92 (17.06–18.82)	1 (Ref)	
1	15,081	1,799	80,528	22.34 (21.33–23.40)	1.24 (1.16–1.33)	
2	9,120	1,225	45,839	26.72 (25.27–28.26)	1.48 (1.38–1.60)	
3	4,179	682	20,203	33.76 (31.32–36.39)	1.88 (1.72–2.05)	
4	1,891	334	9,079	36.79 (33.05–40.95)	2.05 (1.82–2.30)	
≥5	1,799	329	8,245	39.90 (35.81–44.45)	2.22 (1.97–2.50)	
Osteoporotic fracture						
Total	47,649	647	2,69,075	2.40 (2.23–2.60)		<0.0001
Age at diagnosis						
<40	7,121	9	41,247	0.22 (0.11–0.42)	1 (Ref)	
40–49	20,034	110	1,14,136	0.96 (0.80–1.16)	4.45 (2.25–8.77)	
50–59	13,968	221	78,053	2.83 (2.48–3.23)	13.15 (6.75–25.61)	
60–69	4,808	180	26,762	6.73 (5.81–7.78)	31.20 (15.97–60.94)	
70–79	1,510	111	7,944	13.97 (11.60–16.83)	65.87 (33.40–129.94)	
80+	208	16	933	17.16 (10.51–28.00)	85.30 (37.69–193.08)	
CCI**						<0.0001
0	15,579	156	93,225	1.67 (1.43–1.96)	1 (Ref)	
1	15,081	178	85,669	2.08 (1.79–2.41)	1.27 (1.03–1.58)	
2	9,120	129	49,196	2.62 (2.21–3.12)	1.64 (1.30–2.08)	
3	4,179	83	22,033	3.77 (3.04–4.67)	2.38 (1.82–3.11)	
4	1,891	50	9,918	5.04 (3.82–6.65)	3.19 (2.32–4.39)	
≥5	1,799	51	9,034	5.65 (4.29–7.43)	3.63 (2.64–4.98)	

**p-value from log-rank test.*
***CCI, Charlson Comorbidity Index.*

related to the risk of osteoporosis and osteoporotic fracture in patients between the ages of 40 and 49 years.

The results of this study differ from those of previous studies. Previous studies showed an association between tamoxifen use and bone loss in premenopausal patients. One previous study showed an annual loss of BMD of 1.44% in premenopausal breast cancer patients on tamoxifen (11). However, only 125 premenopausal breast cancer patients were enrolled in that study and treatment variables that can influence BMD were not statistically adjusted. Another study showed a 4.6% decrease in BMD at the 3-years follow-up evaluation in premenopausal patients taking tamoxifen after adjuvant chemotherapy. The number of patients enrolled in that study was also small, and only univariate analyses were conducted (12). Recently, researchers from Germany reported that tamoxifen increased the risk of fracture in premenopausal breast cancer patients compared to control patients without cancer (17). However, the selection criteria for the non-cancer control patients were not able to demonstrate the effect of tamoxifen on fracture because adjuvant treatments such as OFS and chemotherapy could not be statistically adjusted. To address these drawbacks, we conducted this nationwide retrospective cohort study.

To our knowledge, this is the largest retrospective cohort study of the effect of tamoxifen on bone health in younger breast cancer patients. Approximately 27,000 breast cancer patients younger than 50 years were enrolled, comprising 150,798 person-years. The results of this study indicate that tamoxifen does not increase the risk of osteoporosis and osteoporotic fracture in younger breast cancer survivors. These results contradict the ASCO recommendation that primary clinicians should refer premenopausal breast cancer survivors who are taking tamoxifen for repeat bone screening every 2 years (13).

TABLE 3 | Factors associated with osteoporosis according to age subgroups in univariate and multivariate analysis.

	N	Events (*n*)	Total person-year	Incidence rate (per 1,000 person-year)	*p**	Crude HR (95% CI)	Adjusted HR (95% CI)
Age<40							
Reimbursement type					0.1178		
National health insurance	7,068	164	40,477	4.05 (3.48–4.72)		1 (Ref)	1 (Ref)
Medical aid	53	3	311	9.66 (3.11–29.94)		2.42 (0.77–7.57)	2.49 (0.85–7.25)
Chemotherapy(any)					0.5516		
No	1,878	41	10,837	3.78 (2.79–5.14)		1 (Ref)	1 (Ref)
Yes	5,243	126	29,951	4.21 (3.53–5.01)		1.11 (0.78–1.58)	1.39 (0.95–2.02)
Endocrine treatment					0.0363		
No	2,364	40	13,446	2.97 (2.18–4.06)		1 (Ref)	1 (Ref)
Tamoxifen	4,736	127	27,220	4.67 (3.92–5.55)		1.55 (1.08–2.21)	1.24 (0.85–1.82)
Aromatase inhibitor**	21	0	121	0.00		1.34 (0.08–22.02)	1.33 (0.08–22.34)
Ovarian function suppression* **				<0.0001			
No	5,632	108	32,433	3.33 (2.76–4.02)		1 (Ref)	1 (Ref)
Yes	1,489	59	8,355	7.06 (5.47–9.11)		2.12 (1.54–2.91)	2.15 (1.52–3.06)
Radiotherapy					0.859		
No	1,864	45	10,761	4.18 (3.12–5.60)		1 (Ref)	1 (Ref)
Yes	5,257	122	30,027	4.06 (3.40–4.85)		0.97 (0.69–1.36)	0.92 (0.65–1.30)
Trastuzumab					0.8198		
No	6,119	146	35,440	4.12 (3.50–4.85)		1 (Ref)	1 (Ref)
Yes	1,002	21	5,348	3.93 (2.56–6.02)		0.95 (0.60–1.50)	0.99 (0.62–1.59)
Age 40–49							
Reimbursement type					0.7624		
National health insurance	19,729	1,499	1,08,318	13.84 (13.16–14.56)		1 (Ref)	1 (Ref)
Medical aid	305	22	1,693	13.00 (8.56–19.74)		0.94 (0.62–1.43)	0.81 (0.53–1.24)
Chemotherapy(any)					<0.0001		
No	6,424	381	35,732	10.66 (9.64–11.79)		1 (Ref)	1 (Ref)
Yes	13,610	1,140	74,278	15.35 (14.48–16.27)		1.45 (1.29–1.62)	1.41 (1.24–1.60)
Endocrine treatment					<0.0001		
No	4,448	383	23,888	16.03 (14.51–17.72)		1 (Ref)	1 (Ref)
Tamoxifen	14,710	950	81,377	11.67 (10.95–12.44)		0.72 (0.64–0.82)	0.74 (0.65–0.84)
Aromatase inhibitor**	876	188	4,745	39.62 (34.34–45.71)		2.47 (2.08–2.94)	2.45 (2.05–2.92)
Ovarian function suppression* **				0.0113			
No	16,838	1,314	92,422	14.22 (13.47–15.01)		1 (Ref)	1 (Ref)
Yes	3,196	207	17,588	11.77 (10.27–13.49)		0.83 (0.71–0.96)	1.19 (1.01–1.40)
Radiotherapy					0.0548		
No	5,130	427	28,435	15.02 (13.66–16.51)		1 (Ref)	1 (Ref)
Yes	14,904	1,094	81,575	13.41 (12.64–14.23)		0.90 (0.80–1.00)	0.87 (0.77–0.97)
Trastuzumab					0.0282		
No	17,546	1,320	97,261	13.57 (12.86–14.32)		1 (Ref)	1 (Ref)
Yes	2,488	201	12,749	15.77 (13.73–18.10)		1.18 (1.02–1.37)	1.04 (0.89–1.21)
Age 50–59							
Reimbursement type					0.9962		
National health insurance	13,763	2,374	70,202	33.82 (32.48–35.20)		1 (Ref)	1 (Ref)
Medical aid	205	35	1,038	33.73 (24.22–46.98)		1.00 (0.72–1.40)	1.01 (0.72–1.41)
Chemotherapy(any)					<0.0001		
No	4,166	643	21,789	29.51 (27.32–31.88)		1 (Ref)	1 (Ref)
Yes	9,802	1,766	49,452	35.71 (34.08–37.42)		1.21 (1.11–1.33)	1.09 (0.99–1.20)
Endocrine treatment					<0.0001		
No	4,526	752	22,869	32.88 (30.61–35.32)		1 (Ref)	1 (Ref)
Tamoxifen	3,555	405	19,237	21.05 (19.10–23.21)		0.64 (0.56–0.72)	0.69 (0.61–0.78)

(Continued)

TABLE 3 | Continued

	N	Events (*n*)	Total person-year	Incidence rate (per 1,000 person-year)	*p**	Crude HR (95% CI)	Adjusted HR (95% CI)
Aromatase inhibitor**	5,887	1,252	29,134	42.97 (40.66–45.42)		1.31 (1.19–1.43)	1.34 (1.22–1.47)
Ovarian function suppression* **				<0.0001			
No	13,631	2,385	69,368	34.38 (33.03–35.79)		1 (Ref)	1 (Ref)
Yes	337	24	1,872	12.82 (8.59–19.13)		0.37 (0.25–0.56)	0.61 (0.40–0.92)
Radiotherapy					0.8216		
No	3,732	657	19,327	33.99 (31.49–36.69)		1 (Ref)	1 (Ref)
Yes	10,236	1,752	51,913	33.75 (32.21–35.37)		0.99 (0.90–1.08)	0.94 (0.86–1.03)
Trastuzumab					0.0158		
No	11,535	1,970	59,582	33.06 (31.64–34.56)		1 (Ref)	1 (Ref)
Yes	2,433	439	11,658	37.66 (34.29–41.35)		1.14 (1.02–1.26)	1.09 (0.98–1.22)
Age 60–69							
Reimbursement type					0.0231		
National health insurance	4,673	1,311	22,166	59.15 (56.03–62.44)		1 (Ref)	1 (Ref)
Medical aid	135	49	600	81.65 (61.71–108.03)		1.39 (1.05–1.85)	1.42 (1.06–1.89)
Chemotherapy(any)					0.0450		
No	1,778	483	8,679	55.65 (50.91–60.85)		1 (Ref)	1 (Ref)
Yes	3,030	877	14,087	62.25 (58.27–66.51)		1.12 (1.00–1.25)	1.11 (0.99–1.25)
Endocrine treatment					<0.0001		
No	1,463	389	6,818	57.05 (51.66–63.01)		1 (Ref)	1 (Ref)
Tamoxifen	651	151	3,357	44.98 (38.35–52.76)		0.79 (0.65–0.95)	0.81 (0.67–0.99)
Aromatase inhibitor**	2,694	820	12,591	65.13 (60.82–69.74)		1.14 (1.01–1.28)	1.18 (1.04–1.34)
Ovarian function suppression* **				NA			
No	4,808	1,360	22,766	59.74 (56.65–63.00)	NA	NA	NA
Yes	0	NA	NA	NA	NA	NA	NA
Radiotherapy					0.3174		
No	1,584	469	7,597	61.73 (56.39–67.58)		1 (Ref)	1 (Ref)
Yes	3,224	891	15,168	58.74 (55.01–62.73)		0.94 (0.84–1.06)	0.92 (0.83–1.04)
Trastuzumab					0.7159		
No	4,121	1,170	19,683	59.44 (56.13–62.95)		1 (Ref)	1 (Ref)
Yes	687	190	3,082	61.64 (53.47–71.06)		1.03 (0.88–1.20)	1.01 (0.86–1.19)
Age ≥70							
Reimbursement type					0.665		
National health insurance	1,638	476	7,225	65.88 (60.22–72.08)		1 (Ref)	1 (Ref)
Medical aid	80	22	367	59.90 (39.44–90.96)		0.91 (0.59–1.40)	0.91 (0.59–1.39)
Chemotherapy(any)					0.0289		
No	1,205	333	5,416	61.49 (55.22–68.46)		1 (Ref)	1 (Ref)
Yes	513	165	2,176	75.81 (65.08–88.31)		1.23 (1.02–1.48)	1.18 (0.96–1.45)
Endocrine treatment					0.0126		
No	511	139	2,132	65.19 (55.21–76.98)		1 (Ref)	1 (Ref)
Tamoxifen	354	86	1,713	50.22 (40.65–62.03)		0.77 (0.59–1.01)	0.83 (0.63–1.10)
Aromatase inhibitor**	853	273	3,747	72.85 (64.70–82.02)		1.11 (0.91–1.36)	1.19 (0.96–1.47)
Ovarian function suppression* **				NA			
No	1,718	498	7,592	65.59 (60.08–71.61)	NA	NA	NA
Yes	0	NA	NA	NA	NA	NA	NA
Radiotherapy					0.3657		
No	991	277	4,399	62.97 (55.97–70.84)		1 (Ref)	1 (Ref)
Yes	727	221	3,193	69.21 (60.66–78.97)		1.09 (0.91–1.30)	1.05 (0.87–1.25)

(Continued)

TABLE 3 | Continued

	N	Events (*n*)	Total person-year	Incidence rate (per 1,000 person-year)	*p**	Crude HR (95% CI)	Adjusted HR (95% CI)
Trastuzumab					0.0760		
No	1,609	460	7,157	64.27 (58.66–70.42)		1 (Ref)	1 (Ref)
Yes	109	38	435	87.33 (63.55–120.02)		1.35 (0.97–1.88)	1.22 (0.85–1.75)

NA, not available.
**log-rank test.*
***letrozole, anastrozole, exemestane.*
****goserelin, leuprolide.*

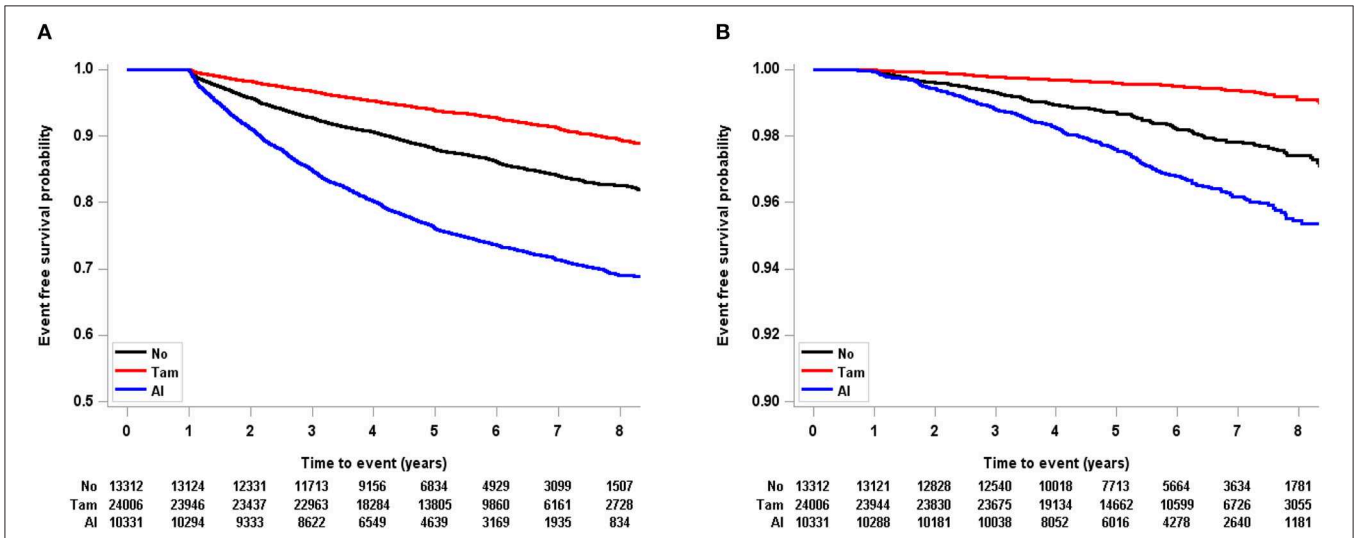

FIGURE 2 | Kaplan-Meier event free probability according to endocrine treatment in total population (**A**: osteoporosis, **B**: osteoporotic fracture). Tam, tamoxifen; AI, aromatase inhibitor.

One of the interesting findings of this study is that tamoxifen significantly decreased the risk of osteoporosis and osteoporotic fracture in breast cancer patients aged 40 to 49 years at the time of diagnosis. This can be explained by their perimenopausal status and long-term treatment with adjuvant tamoxifen. For patients with hormone receptor-positive breast cancers and lymph node metastasis, 10 years of adjuvant tamoxifen treatment are usually recommended (18). With the extended tamoxifen therapy, premenopausal women who are premenopausal at the time of breast cancer diagnosis might continue to take tamoxifen beyond the start of menopause, after which tamoxifen would show a protective effect on bone health.

In postmenopausal patients, AI increases the incidence of osteoporosis and osteoporotic fracture. The current study, in accordance with other reports (19, 20), demonstrated a marked increase in the risk of osteoporosis with AI, reflecting the near-complete estrogen depletion and subsequent disruption in bone homeostasis caused by these agents (21).

Multiple factors are related to the incidence of osteoporosis. The most common causes of bone loss in women are menopause and aging. Aging is associated with greater bone resorption and less bone formation, whereas menopause induces accelerated bone loss due to lowering levels of endogenous estrogen (22). In HIRA data study about the burden of osteoporosis in the general population, the prevalence of osteoporosis increased with age; the peak was at 70–79 years, with a rate of 5,253 diagnoses per 10,000 persons (23). Although we cannot directly compare this to the results of our study because of the different operational definitions, we similarly found an increasing incidence of osteoporosis in the older age group.

The limitations of this study should be noted. First, we were not able to perform survival analysis because the HIRA data is claims-based in accordance with the Personal Information Protection Act in Korea. Therefore, we could not assess the effect of osteoporosis and osteoporotic fracture on overall survival. Second, endocrine therapy treatment group allocation was based on claims data from the 1st year after diagnosis, and some patients may have subsequently switched from tamoxifen to AI. Third, as we defined osteoporosis as osteoporosis diagnosis codes in combination with osteoporosis medications, osteoporosis patients to whom osteoporosis medications were not prescribed due to other medical conditions were not included in this analysis. Lastly, due to the limitations of claims data, we were unable to gather information about diet, exercise, exposure to sunlight, and vitamin D supplementation, which are important factors for maintaining bone health.

The results of this study should be interpreted in the context of the study period. First, we did not analyze the use of denosumab

which has been reimbursed for osteoporosis treatment from 2018 in South Korea. The resulting change in clinical practice could potentially affect the study outcomes. Second, after the practice-changing report from the Suppression of Ovarian Function Trial in 2015, adjuvant ovarian suppression in premenopausal breast cancer patients who remain premenopausal after chemotherapy, especially young patients, is recommended (24). Although OFS was not associated with a significantly increased risk of osteoporotic fracture in patients younger than 40 years in this study, OFS is now more often prescribed, possibly affecting the incidence of osteoporosis and osteoporotic fractures.

In conclusion, tamoxifen is not associated with an increased risk of osteoporosis and osteoporotic fracture in premenopausal breast cancer patients. Risk factors for osteoporosis and osteoporotic fractures vary according to patient age. Tailored screening strategies for breast cancer survivors who are at different risks of developing osteoporosis are needed.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. The datasets are from the Korean National database which is not allowed to be extracted from the server.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Soonchunhyang University Seoul Hospital Institutional Review Board (2018-11-011). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JLe and HA: planned, wrote and revised the article. JK, MK, JWL, BK, BS, SA, JEL, SHa, ZK, and SHu: reviewed and edited the article. JSL: planned, analyzed data and statistics. IC: planned, wrote, revised, and supervised the concept of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.00366/full#supplementary-material

Supplementary Figure 1 | Kaplan-Meier event free probability in total population (**A**: osteoporosis, **B**: osteoporotic fracture).

Supplementary Table 1 | Factors associated with osteoporotic fracture according to age subgroups in univariate and multivariate analysis.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Reviewer MI is currently organizing a Research Topic with one of the authors JK, and confirms the absence of any other collaboration.

frontiers in Oncology

REVIEW
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Integrating Biological Advances Into the Clinical Management of Breast Cancer Related Lymphedema

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Breast cancer-related lymphedema (BCRL) occurs in a significant number of breast cancer survivors as a consequence of the axillary lymphatics' impairment after therapy (mainly axillary surgery and irradiation). Despite the recent achievements in the clinical management of these patients, BCRL is often diagnosed at its occurrence. In most cases, it remains a progressive and irreversible condition, with dramatic consequences in terms of quality of life and on sanitary costs. There are still no validated pre-surgical strategies to identify individuals that harbor an increased risk of BCRL. However, clinical, therapeutic, and tumor-specific traits are recurrent in these patients. Over the past few years, many studies have unraveled the complexity of the molecular and transcriptional events leading to the lymphatic system ontogenesis. Additionally, molecular insights are coming from the study of the germline alterations involved at variable levels in BCRL models. Regrettably, there is a substantial lack of predictive biomarkers for BCRL, given that our knowledge of its molecular milieu remains extremely puzzled. The purposes of this review were (i) to outline the biology underpinning the ontogenesis of the lymphatic system; (ii) to assess the current state of knowledge of the molecular alterations that can be involved in BCRL pathogenesis and progression; (iii) to discuss the present and short-term future perspectives in biomarker-based patients' risk stratification; and (iv) to provide practical information that can be employed to improve the quality of life of these patients.

Keywords: breast cancer related lymphedema, pathobiology, genetics, breast cancer, survivorship, quality of life

INTRODUCTION

Breast cancer-related lymphedema (BCRL) is a particular form of secondary lymphedema occurring after axillary surgical procedures and/or irradiation in 14–54% of breast cancer survivors (1). Its clinical signs are related to an augmented volume of the upper limb due to tissue swelling and subsequent fibrosis (2). These include impaired function and strength,

malaise, pain, comorbidities, and psychosocial frailty (3, 4). The diagnosis of BCRL is established by the measurement of the arm volume. Over the past decades, a wide variety of strategies have been proposed to identify and quantify alterations in the upper limb volume, including tape, perometry, bioimpedance, imaging (e.g., lymphography and magnetic resonance imaging), and augmented reality tools (5–9). BCRL prevention is centered on general healthcare suggestions, such as physical activity, body weight control, skincare, avoidance of infections (10). However, microsurgery-based primary prevention schemes, such as axillary reverse mapping and lymphatic-venous bypass, are showing promising results (11). For decades BCRL has been considered as an incurable condition but several therapeutic approaches are now available, both in the setting of physical therapy (e.g., complex decongestive therapy, manual lymph drainage, Qigong exercise, yoga, laser therapy, extracorporeal, shock wave therapy) and surgery (e.g., tissue excision, derivative microsurgery, microsurgical reconstruction, vascularized lymph node transfer, block of sympathetic innervation) (8, 12–15). Regrettably, the pre-surgical identification of high-risk individuals is extremely challenging.

Despite these insights, the multifaceted biology of BCRL remains poorly understood due to the substantial lack of molecular data. Therefore, tailored prevention and treatment schemes are not routinely performed in these patients. In this review article, we seek to outline the biological and genetic changes in the lymphatic system development and impairment in breast cancer survivors, focusing on possible biomarkers for its risk assessment, diagnosis, prognostication, and treatment.

PATHOPHYSIOLOGY

Ontogenesis of the Lymphatic System

The lymphatic system is composed of a complex network of vessels and organs complementary to the cardiovascular system (16). It plays a crucial role in several biological events, including immune response and homeostasis of interstitial fluids, cells, molecules, and tissue debris (17, 18). At early stages of embryogenesis, the lymphatic vessels develop from the embryonic veins through the stepwise expression of numerous molecules, including prospero-related homeobox domain 1 (PROX1) and nuclear receptor subfamily 2, group F, member 2 (NR2F2) (17, 19). Interestingly, the silencing of these two genes in mice prevents lymphangiogenesis (20, 21). The lymphatic sac, which is lined by lymphatic endothelial cells (LECs), represents the earliest lymphatic structure (22). The LECs express lymphatic-specific proteins, such as vascular endothelial growth factor C (*VEGFC*). The absence of this molecule in animal models results in diffuse and lethal tissue swelling (23). The separation of the lymphatic system from the blood vessels leads to the formation of the lymphatic plexus (24). This process is mediated by a signaling pathway in which podoplanin (PDPN), expressed by the LECs, interacts with its receptor on platelets, promoting their aggregation (25). Subsequently, platelet microthrombi form a physical barrier that interrupts the communication between lymphatic and blood vessels (26, 27). Inactivating mutations in *PDPN* are related to defects in vascular system separation, and subsequent abnormal shunts (24, 27, 28). The development of a contractile component (i.e., myoepithelial cells) coupled with that of a valve system allows for the unidirectional flow of the lymph fluid. This phase is characterized by the differential expression of PROX1, forkhead box protein C2 (FOXC2), GATA2, integrin α9 (ITGA9), and its ligand extra domain A fibronectin (29, 30). Their deficiency is associated with failure in valve formation and consequent lymphedema (31–33). The key molecular and transcriptional events in the lymphatic system ontogenesis are outlined in **Figure 1**.

Fluid Drainage and Anatomic Considerations

The lymph flow is determined by both intrinsic and extrinsic forces that promote lymph propulsion in the lymphatic conduct; intraluminal one-way valves minimize the backflow (34). Given the lack of a central pump for the lymph fluid, the flow is driven by rhythmic contractions of smooth muscle cells in the lymphatic vessels (35). Arterial pulsations, skeletal muscle compression, fluctuations of central venous pressure, gastrointestinal peristalsis, and respiration are also involved in this mechanism, representing the passive lymph pump. The entire interstitial drainage process is governed by the Starling equation (**Figure 2**). Three types of lymphatic channels are present, namely capillaries (also referred to as initial lymphatics), pre-collecting vessels, and collecting vessels (**Figure 3**). Capillaries are blind-ending vessels composed of a single layer of non-fenestrated LECs, with an incomplete basal lamina. These structures have specialized junctions and anchoring systems that act synergistically in promoting the passage of lymph from the interstitium to the lumen (36). Pre-collecting vessels are characterized by the alternation of propulsion segments (i.e., provided with muscular coat and intraluminal valves) and tracts with an absorbing architecture (i.e., irregularly-arranged of smooth muscle cells and discontinuous basal lamina) (37). These vessels converge into the collecting vessels, whose functional unit is represented by the lymphangion, defined as the segment between two valves (38). Lymphangions have zipper-like junctions between LECs, continuous basement membrane, well-represented muscular layer, and bi-leaflets one-way valves (39). It should be noted that the lymphatic network is asymmetric. Hence, the right lymphatic duct, which drains in the right subclavian vein, is present only in the right upper limb, the right side of the trunk, and the head and neck region (40), while all other territories are drained by the thoracic duct into the left subclavian vein (41).

Understanding the Tissue Milieu: Inflammation and Matrix Response

The soft tissue composition is a key factor in lymphatic homeostasis, as demonstrated by the increased risk of lymphedema related to fat accumulation (8, 42, 43). Importantly, the lymphatic fluid stasis regulates the expression of genes with regulatory functions in adipogenesis, such as peroxisome proliferator-activated receptor gamma (*PPARG*) and CCAAT/enhancer-binding protein alpha (*CEBPA*) (44). Another key factor is represented by the adiponectin, a protein hormone involved fatty acid breakdown, that contributes to the signaling between adipose and immune cells and regulates the chronic inflammatory response (44). This protein can be overexpressed

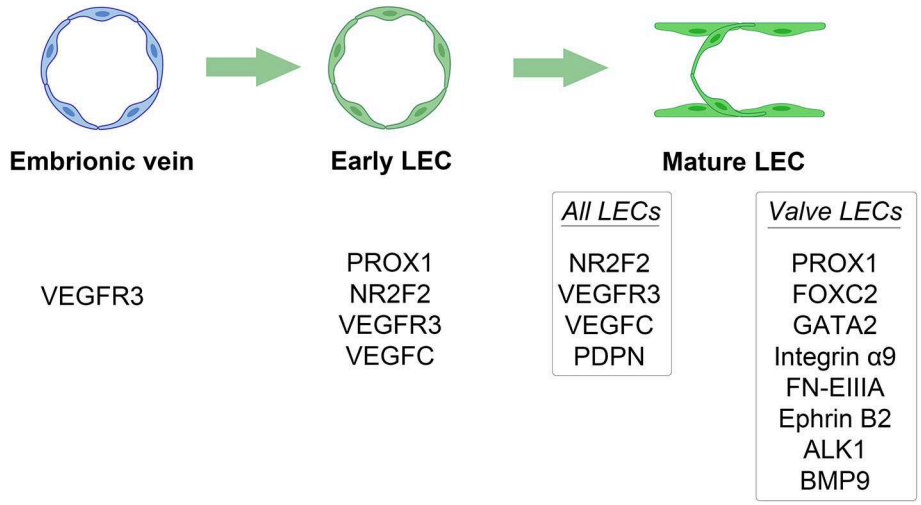

FIGURE 1 | Key molecular and transcriptional events in the lymphatic system ontogenesis. Different stages of lymphatic system development are outlined by their distinct stage-specific expression of different molecules. LEC, lymphatic endothelial cell.

in response to lymphatic fluid stasis, thus mediating the tolerance to proinflammatory stimuli in the case of obstruction (44, 45). Recently, adipose-derived stem cells co-cultured with human lymphatic endothelial cells have been shown to induce mRNA expression of lymphatic markers and proliferation/migration of lymphatic endothelial cells, without affecting tube formation (46). These data pave the way for possible engineering therapies to improve secondary lymphedema outcome.

Fibrosis and increased subcutaneous adipose tissue volume are the two main aspects of tissue remodeling which characterize late-stage BCRL (47). Therapeutic interventions designed to reduce their presence can increase the lymphatic function (48). In this respect, both cytokines and immune cells promote lymphangiogenesis, with a subsequent potential therapeutic role (49, 50). Interestingly, alternatively activated macrophages (M2) are often increased in lymphedema tissues, particularly in the setting of T helper 2 cell-mediated anti-inflammatory response in fibrotic phases (45). The macrophage infiltration in lymphedema decreases the overall inflammation and inhibits fibrosis (45). It has recently been proposed that a high capillary filtration coefficient coupled with increased plasma levels of VEGFC may constitute important biological traits of BCRL patients (51). Hence, a systemic increase in VEGFC promotes microvascular permeability, and an overload of the remaining lymphatic drainage capacity (52). On the other hand, the recovery of interstitial fluid drainage and the natural resolution of acute BCRL are not hindered by the administration of VEGF receptors blockers, suggesting that these processes are lymphangiogenesis independent. Taken together, the interstitial matrix plays a central role in the increase of lymph drainage (53).

RISK STRATIFICATION: WHO IS LIKELY TO DEVELOP BCRL?

Despite early detection can improve BCRL patients' outcome, the preventive options available to date are extremely limited (54). The physical disruption of the arm lymphatics, such as in case of axillary lymph node dissection (ALND), is a well-established determinant of BCRL (55). Of note, both the number of lymph nodes removed and the number of metastatic lymph nodes are associated with an increased risk (56, 57). It has been hypothesized that this could be due to the higher dose of radiations that these patients receive in the axilla (55, 57). Hence, radiation-induced necrosis is likely to be involved BCRL pathogenesis (58). A higher prevalence of BCRL has also been observed in patients treated with anti-tumor systemic drugs, such as taxanes and trastuzumab, probably due to diminished lymphatic contractility (59–61). The correlation between body max index (BMI) >25 kg/m^2, post-operative weight increase, dyslipidemia, and BCRL has been widely demonstrated (8). However, novel tumor-specific pathological features, such as peritumoral lymphovascular invasion and the extra-nodal extension of the metastatic deposits, have recently been proposed to improve BCRL risk stratification (56, 57). In general, there is a wide agreement that breast-conserving surgery is protective against long-term complications, including BCRL (62).

In addition to the classical mechanistic explanation, the study of the genetics underpinning BCRL has provided intriguing insights. Several germline alterations in genes involved at various levels in lymphangiogenesis have been documented in BCRL patients, suggesting a possible role for individual predisposition in the development of lymphedema following breast cancer therapy (**Table 1**). These genes include lymphocyte cytosolic protein 2 (*LCP2*), spleen associated tyrosine kinase (*SYK*), endothelial cell adhesion proteins (i.e., promoters, growth factors, and their receptors), interleukins, and K-channel genes (50, 63–72). Interestingly, these genes show recurrent somatic alterations in breast cancer, with a higher prevalence of gene copy-number alterations (CNAs) than somatic mutations (**Figure 4**). Despite these relevant observations, no tumor-specific recurrent molecular alterations have been identified in BCRL patients.

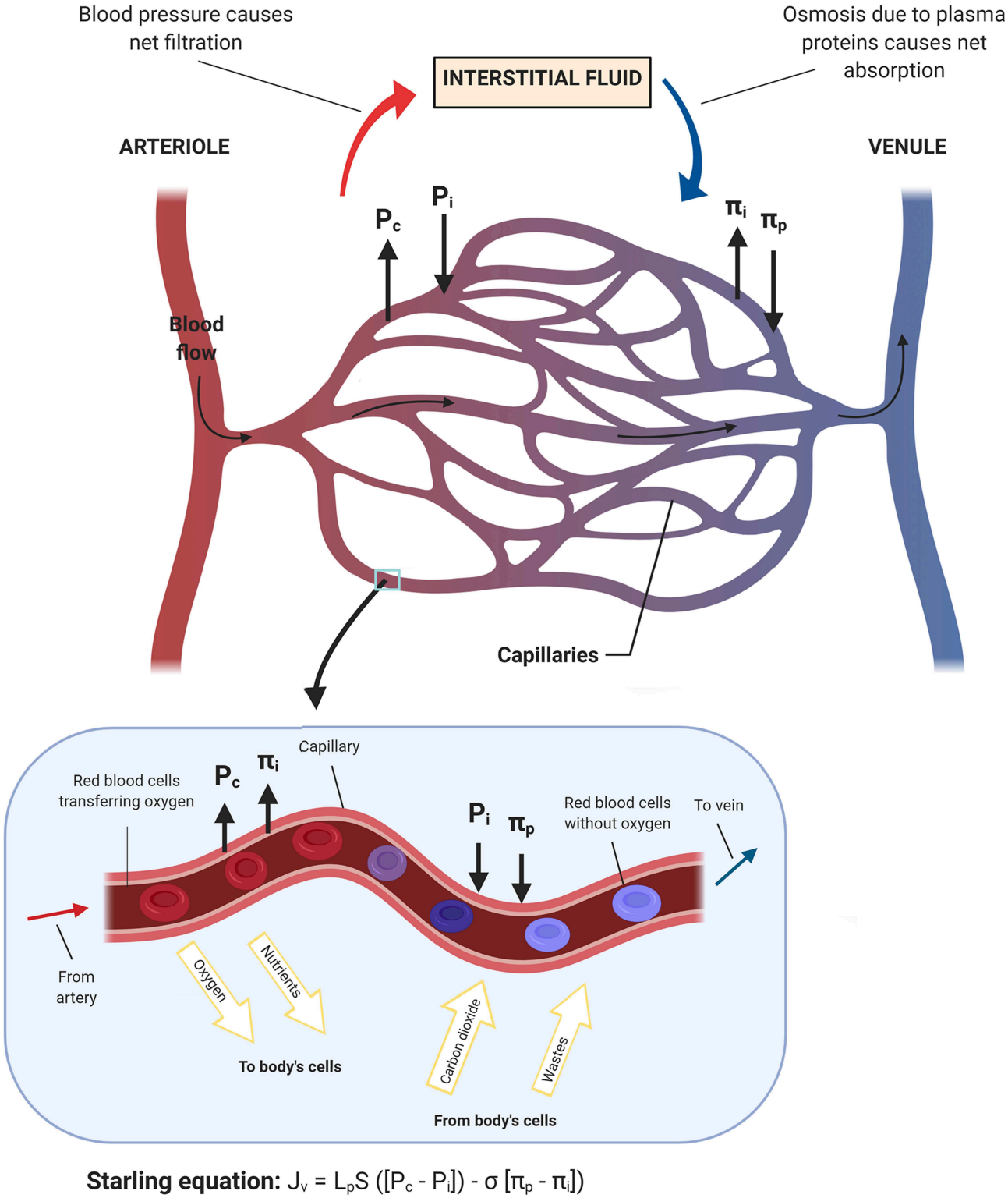

FIGURE 2 | Schematic representation of the fluid homeostasis based on the Starling equation. When the blood flow goes into the capillary, the capillary hydrostatic pressure (Pc) and the interstitial oncotic pressure (πi) drive oxygen and nutrients toward body's cells. Conversely, when blood moves toward venules, the interstitial fluid hydrostatic pressure (Pi) along with the plasma oncotic pressure (πp), which are mainly applied by the surrounding proteins, drive wastes and carbon dioxide into the capillary and subsequently out of the body.

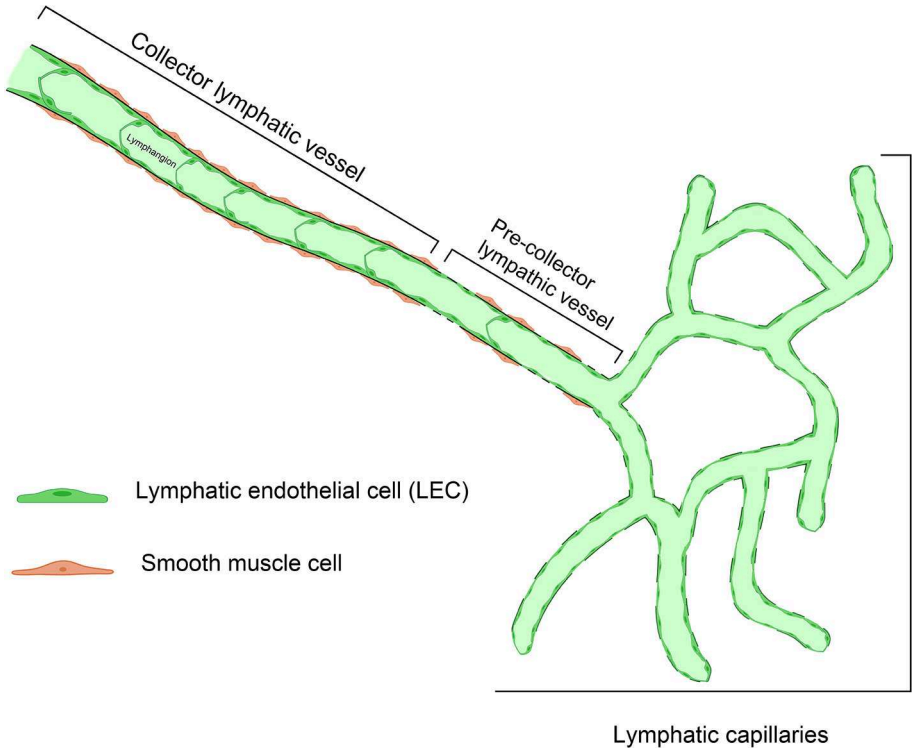

FIGURE 3 | Representative structure of the different types of lymphatic vessels. Small, branching lymphatic capillaries lined by a single file of lymphatic endothelial cells (LEC) are connected to pre-collector lymphatic vessels, showing tracts with a discontinuous basal lamina. The collecting vessels, whose functional unit is the lymphangion, are larger in diameter and have a prevalent propulsion function.

GENOMIC LANDSCAPE AND MOLECULAR HETEROGENEITY

Genetic Determinants and Putative Driver Alterations

It has been suggested that BCRL susceptibility might have individual determinants, raising the possibility that therapy-associated lymphatic injuries might heighten a pre-existing deficit in the lymphatic function (73). Hence, among patients with BCRL, those with the involvement of the whole arm and hand showed an impairment of lymphatic function also in the contralateral unaffected arm (74). Following this circumstantial evidence, the detection of recurrent genetic traits is strategic to achieve the goal of precision medicine in BCRL.

Lymphangiogenic and Angiogenic Genes

In the last decade, the presence of alterations in genes related to lymphangiogenesis, lymphatic function, and permeability has been unraveled in BCRL. One of the most studied genes is *LCP2*, which is involved in the immune response through the modulation of the T-cell signaling pathway (75). In addition, *LCP2* plays a central role in the lymphatic development, participating in the platelet-dependent mechanism of separation between blood and lymphatic vessels during embryogenesis (26, 76). Alterations in this gene are related to inherited lymphedema (77, 78). Copy-number alterations in *LCP2* occur in 1.4% of breast cancer patients (**Figure 4**). They show a strong tendency toward co-occurrence with alterations in other genes known to be implicated in BCRL, such as interleukins (i.e., *IL4*, *IL10*, *IL13*) and neuropilin 2 (*NRP2*), as detailed in **Table 2**. NRP2 is a transmembrane glycoprotein expressed in blood and LECs, which is upregulated in the presence of ischemia and/or hypoxia (79–81). This protein is considered an important mediator of angiogenesis and lymphangiogenesis, acting as a co-receptor with VEGFC. This a molecule is encoded by two genes, namely *VEGFC* and Fms-related tyrosine kinase 4 (*FLT4*) (82–84). Somatic alterations in *NRP2*, *FLT4*, and *VEGFC* have a strong tendency of co-occurrence in breast cancer (**Table 2**) and may predispose to secondary lymphedema (68, 69, 73). Vascular cell adhesion protein 1 (VCAM1) is an adhesion molecule that promotes lymphocyte trans-endothelial migration in cytokine activated endothelium (85, 86). This adhesion molecule fosters tissue inflammation and contributes to lymphedema progression. CNAs in *VCAM1* occur in ∼1% of breast cancer patients (**Figure 4**) and they are simultaneously present together with somatic alterations in other genes implicated in BCRL pathogenesis (**Table 2**). These include interleukins, nuclear kappa factor-beta 2 (*NFKB2*), *VEGFR/KDR*, as well as the hepatocyte growth factor (*HGF*) and its receptor *MET*. Six *HGF/MET* mutations in the sites of interaction and binding domain, respectively, were identified in secondary lymphedema, suggesting that altering this pathway can

TABLE 1 | Genes that have been related to BCRL predisposition.

Genes	Gene family	Function
LCP2	Signal-transducing adaptor protein	T-cell activation.
SYK	Spleen tyrosine kinase	Adaptive immune receptor signaling; Cell proliferation, differentiation, and phagocytosis; Separation of newly formed lymphatic vessels from the blood vasculature.
VCAM1	Cell adhesion promoters	Vascular endothelial cell adhesion and signal transduction.
HGF, HGFR/MET, VEGFC, FLT4, VEGFR2/KDR, NRP2	Growth factors and receptors	Mitogenesis and morphogenesis; Embryonic development; Myocardial development; Epithelial-mesenchymal transition; Liver regeneration. Cardiovascular development; Angiogenesis; Lymphangiogenesis; Endothelial cell growth; Permeability of blood vessels.
NFKB2, RORC, FOXC2	Transcription factor-coding	Inflammation and immune response Lymphoid organogenesis (in mice). Valves development.
GJC2, GJA4	Connexins	Arteriogenesis; Oocyte survival; Oligodendrocyte development.
IL1A, IL4, IL6, IL10, IL13	Interleukins	Apoptosis and cell proliferation; Immunoregulation and inflammation; Expressed also in endothelial cells.
KCNA1, KCNJ3, KCNJ6, KCNK3	K channel proteins	Electrochemical gradient across cell membranes; In the lymphatic system facilitate lymph flow.

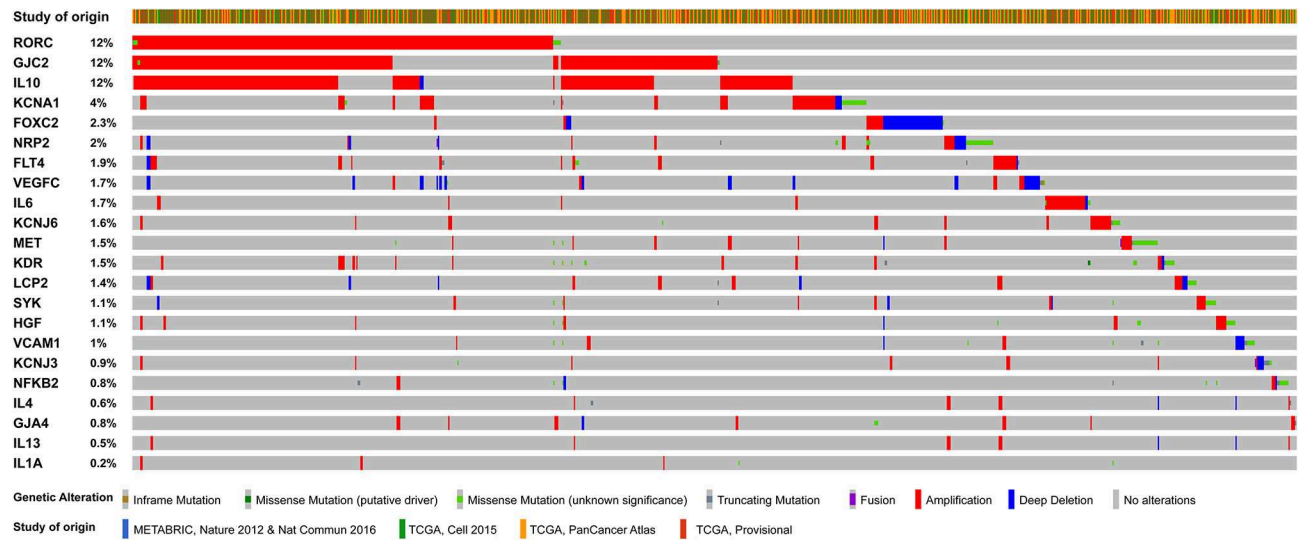

FIGURE 4 | Oncoprint visualization of the somatic molecular alterations in breast cancers ($n = 3,394$ samples) involving 22 genes with reported germline alterations in BCRL patients. Each column represents a sample, each row represents a gene, as reported on the left. The genes were sorted by alterations frequency (percentage on the left). Types of alterations and study of origin (publicly available at cBioportal.com) are color-coded on the basis of the legend on the bottom.

increase individual risk of developing lymphedema after breast surgery and thus providing a new potential therapeutic target (66). Another important gene in BCRL is represented by RAR-related orphan receptor gamma (*RORC*), which is known to be implicated in lymphangiogenesis, lymph node organogenesis, immune response, and cancer (87). Regrettably, the specific functions of this transcription factor in humans remain poorly understood. Interestingly, both somatic missense mutations and

TABLE 2 | Significant trends in co-occurrence between pairs within genes linked to BCRL in breast cancer public datasets available at cBioPortal.

A	B	Neither	A not B	B not A	Both	log_2 O.R.	*p*-value	*q*-value
LCP2	FLT4	2756	26	35	14	>3	<0.001	<0.001
LCP2	IL13	2785	34	6	6	>3	<0.001	<0.001
LCP2	IL4	2782	34	9	6	>3	<0.001	<0.001
LCP2	GJC2	2476	27	315	13	1.92	<0.001	0.003
LCP2	IL10	2490	30	301	10	1.463	0.009	0.034
LCP2	NRP2	2737	34	54	6	>3	<0.001	0.001
NRP2	KCNA1	2687	50	84	10	2.678	<0.001	<0.001
NRP2	IL13	2762	57	9	3	>3	0.002	0.01
NRP2	IL4	2759	57	12	3	>3	0.003	0.017
NRP2	KCNJ6	2739	56	32	4	2.612	0.006	0.028
NRP2	KCNJ3	2750	57	21	3	2.785	0.013	0.045
MET	NRP2	2733	38	56	4	2.361	0.011	0.039
VEGFC	NRP2	2727	44	53	7	>3	<0.001	<0.001
VEGFC	FLT4	2739	43	41	8	>3	<0.001	<0.001
VEGFC	RORC	2465	35	315	16	1.839	<0.001	0.001
VEGFC	IL10	2484	36	296	15	1.806	<0.001	0.002
SYK	VCAM1	2774	29	25	3	>3	0.004	0.017
VCAM1	NFKB2	2781	25	22	3	>3	0.002	0.01
VCAM1	GJA4	2784	25	19	3	>3	0.001	0.008
VCAM1	HGF	2774	25	29	3	>3	0.004	0.017
VCAM1	IL13	2793	26	10	2	>3	0.006	0.026
VCAM1	KDR	2763	25	40	3	>3	0.008	0.033
VCAM1	IL4	2790	26	13	2	>3	0.009	0.034
VCAM1	MET	2766	23	37	5	>3	<0.001	<0.001
MET	KDR	2753	35	36	7	>3	<0.001	<0.001
MET	KCNA1	2700	37	89	5	2.035	0.012	0.04
HGF	MET	2763	26	36	6	>3	<0.001	<0.001
SYK	MET	2760	29	39	3	2.872	0.011	0.039
RORC	GJC2	2377	126	123	205	>3	<0.001	<0.001
RORC	IL10	2372	148	128	183	>3	<0.001	<0.001
RORC	KCNA1	2432	305	68	26	1.608	<0.001	<0.001
RORC	GJA4	2485	324	15	7	1.84	0.01	0.036
KDR	RORC	2470	30	318	13	1.751	<0.001	0.006
FLT4	RORC	2467	33	315	16	1.925	<0.001	<0.001
NFKB2	GJA4	2787	22	19	3	>3	<0.001	0.006
NFKB2	IL10	2502	18	304	7	1.678	0.015	0.049
GJA4	IL10	2505	15	304	7	1.943	0.007	0.03
FLT4	GJA4	2763	46	19	3	>3	0.006	0.026
GJC2	IL10	2423	97	80	231	>3	<0.001	<0.001
FLT4	GJC2	2475	28	307	21	2.596	<0.001	<0.001

gene amplification in *RORC* are highly recurrent in breast cancers, being detected in up to 12% of patients (**Figure 4**). Alterations in this gene can be observed in patients that harbor alterations in other BCRL genes, such as *FLT4*, *IL10*, and *VEGFR2/KDR* (**Table 2**).

Immunomodulation and Inflammatory Response

Variations in pro-inflammatory (e.g., *IL1*, *IL2*, *IL8*, *IL17*, *NFKB2*) and anti-inflammatory (e.g., *IL4*, *IL10*, *IL13*) cytokines have been found in the circulating DNA of patients with BCRL (50). Among these, the single nucleotide polymorphisms (SNPs) significantly related to the development of unilateral arm swelling are those targeting *NFKB2*, *IL10*, and *IL4*. In particular, NFKB2 is a transcription factor involved in a multitude of biological processes, including (but not limited to) angiogenesis, cell proliferation, inflammation, tumorigenesis, and tumor progression (88). Alterations in this gene are relatively rare (∼0.8%) in breast cancers and display the strong propensity toward co-occurrence with those targeting IL10, that are highly recurrent (12%), as shown in **Figure 4** and **Table 2**. IL10 is an anti-inflammatory cytokine that acts downregulating the expression of Th1 cytokines, MHC class II antigen-presenting

molecules, and costimulatory molecules on macrophages (48). In particular, IL10 influences active transcription factor binding sites that are involved in lymphangiogenesis. Most importantly, this interleukin induces immunosuppression and tumor escape from immune surveillance, particularly in breast cancers lacking the expression of the estrogen receptor (89). Alterations in *IL4* have also been detected in the circulating DNA of BCRL patients. This pleiotropic cytokine is produced by CD4+ T-cells and it has an important role in B-cell immune response modulation (48). This pathway is thought to be involved in alterations observed in lymphedematous tissues, such as fibrosis, adipose deposition, and lymphatic dysfunction (48, 90). Interestingly, it has been recently observed that cyclooxygenase (COX)2 and its product prostaglandin (PG)E2 are overexpressed in breast cancer stroma, having a possible role in lymphangiogenesis and metastatic spread s through lymphatics (91). Specifically, PGE2 activates the EP4 receptor in cancer cells and macrophages, promoting local VEGF-C/D overexpression, and LECs proliferation (91). All this information opens new avenues in BCRL risk stratification, providing that further prospective clinical studies will be designed to investigate whether NFKB2, IL10, IL4, and EP4 can be employed as circulating biomarkers for pre-surgical risk assessment.

Transmembrane Diffusion and Inter-cellular Communication

Connexins are a family of specialized transmembrane proteins that form the gap junctions between cells (70). They are crucial for both blood and lymphatic vessel homeostasis (70). Many authors have suggested that connexins may be implicated in the initial development of the lymphatic system, particularly in the formation of the lymphatic valves and sac (92, 93). Mutations in genes encoding the connexins 47 and 37, namely gap junction protein gamma 2 (*GJC2*) and gap junction protein alpha 4 (*GJA4*), have been linked to both primary and secondary lymphedema (67, 72, 94). Intriguingly, *GJC2* CNAs are highly recurrent in breast cancer, being present in 12% of cases in the cBioPortal, as depicted in **Figure 4**. Furthermore, CNAs in *GJC2* and *GJA4* are significantly present together with somatic alterations in other BCRL genes, such as *RORC*, *IL10*, and *FLT4* (**Table 2**). So far, these gap junction proteins represent promising biomarkers in both breast cancer and BCRL prognostication.

Membrane Action Potential and Smooth Cell Contraction

Several potassium channel genes were found to be the target of SNPs in the setting of secondary lymphedema. These genes include potassium voltage-gated channel subfamilies A member 1 (*KCNA1*), J member 3 (*KCNJ3*), 2 (*KCNJ6*), and K member 3 (*KCNK3*) (71). In particular, KCNA1 is a transmembrane protein selective for potassium-positive ions; its functions are to shape the action potential and promote the return of the depolarized membrane to its resting state. KCJN3 and KCJN6 are inward rectifying channels that act in an opposite way to voltage gated-channels, supporting the flow of positively charged potassium ions into the cell and stabilizing the resting membrane of cells (38, 71). Finally, KCNK3 is another relevant tissue factor that contributes to the maintenance of the resting potential, giving rise to the background or outward leak potassium-positive currents (38). Despite the great efforts that have been made to determine the influence of genetic predisposition in BCRL pathophysiology, these analyses have several limitations. Larger sample sizes could reveal additional associations between polymorphisms and BCRL.

Biological Characteristics of the Primary Tumors

The possible existence of molecular indicators evaluable in a pre-operative/operative setting remains one of the key topics surrounding BCRL. For this aim, a search on the public genomic database cBioPortal has been conducted to determine whether genetic alterations associated with both congenital and postsurgical lymphedema occurred also in breast cancer. A correlation between lymphedema candidate genes and mutations in the primary tumor could be useful as an indicator of patients' individual susceptibility, along with the well-known treatment-related risk factors. A query was submitted in order to search genetic alterations of literature driven genes in 2,509 breast cancer samples from METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) project. Notably, in almost all cases genetic alterations found in candidate gene consist of gene amplification, while previous genetic studies individuated single nucleotide polymorphisms associated with BCRL. Most genes were altered in a small percentage of tumor samples, ranging from 0.1 to 2.5%. However, three of them were amplified in at least one-fifth of the breast cancer cases. Specifically, the RORC gene was amplified in 20%, GJC2 in 24% and IL10 in 25% of samples.

To date, the function of RORC's encoded protein in humans remains poorly understood. However, there are several lines of evidence to suggest that this gene may play a part in lymphoid organogenesis and thymopoiesis regulation (87). In addition, RORC protein plays a role in the expression of some clock genes and its expression has been linked to breast cancer survival outcomes (95). RORC overexpression seems to increase distant metastasis-free survival in breast cancer patients (96–98). However, given the lack of knowledge on its precise function and interactions in humans, it is not possible to speculate on the role of RORC in BCRL pathogenesis, preventing also any consideration of the correlation between its amplification and lymphedema occurrence. Connexins are widely expressed in the normal mammary glands, where gap junctions have distinct functions in development and homeostasis, such as modulation of cell proliferation and lactation (99). In advanced breast neoplasms, they are believed to increase the capacity of tumor cells to metastasize through enhancing their invasion and adhesion ability as well as by protecting tumor cells from hypoxia-induced death (100–102). Furthermore, some subtypes of connexins, namely Cx26, Cx32, and Cx43 are overexpressed in metastatic lymph nodes of ductal carcinomas (103, 104). These findings suggest that, in later stages, connexins facilitate the metastatic involvement of locoregional lymph nodes. However, further studies are required to support this hypothesis.

Immunoregulatory cytokines, such as IL10, are important actors in tumor microenvironment associated with breast cancer. Specifically, IL10 is a pleiotropic anti-inflammatory cytokine with

a dual role in breast cancer, exhibiting both pro- and anti-tumor activities (105). Its intricate molecular pattern of interactions has not been fully elucidated yet, however, this regulatory molecule is thought to take part in tumor initiation and progression, promoting immunosuppression and tumor immune evasion. IL10 predominantly displays a tumor-inhibiting activity through the activation of NK cells, enhancement in surface expression of MHC antigen and promoting tumor infiltration by neutrophil and macrophages (106). In the opposite way, IL10 may also reduce immune response against cancer, mainly decreasing the antigen presentation capacity and modulating the production of several cytokines. Hence, higher levels of IL10 may increase tumor immune escape and this hypothesis is consistent with the observation of increased IL10 concentration in serum of breast cancer patients, particularly in case of metastatic disease (89). Hypothesizing that gene amplification leads to an increase in protein expression, IL10 immunosuppressive properties could the metastatic potential of breast cancer, increasing the risk of lymph node involvement, which represents a well-known predisposing factor for BCLR. These assumptions on the possible prognostic value of IL10 amplification for lymphedema risk prediction remain largely speculative. However, some studies found higher IL10 levels in metastatic lymph nodes and IL10 polymorphisms associated with increased expression in patients with lymph node-positive breast cancer (107, 108). Interestingly, high IL10 levels were also found in inflammatory breast cancer, a particularly aggressive and highly metastatic form of breast cancer, in which this cytokine correlates with the presence of lymphovascular invasion (109). This parameter has been recently associated with an increased risk of BCRL in patients with left side localization.

In summary, there is no specific evidence to date that genetic alterations in primary tumor play a direct role in BCRL pathogenesis. However, the correlation between somatic mutations and higher rates of nodal involvement could indirectly lead to more aggressive therapeutic schemes, including ALND and axillary radiation, and thus increasing the odds of developing post-surgical lymphedema.

LYMPHANGIOGENESIS-RELATED MECHANISMS AS POTENTIALLY DRUGGABLE TARGETS

All these novel data suggest that novel individualized therapeutic strategies can be realistically implemented. In particular, the crucial role of VEGF and the observation of BCRL improvement in patients treated with anti-VEGF monotherapy provided evidence for the possible role of anti-angiogenic drugs in lymphedema treatment (110). In particular, a pilot study was conducted in order to evaluate the efficacy and safety of bevacizumab, a monoclonal antibody directed against VEGF, in patients with lymphedema following breast cancer treatment (110). The working hypothesis was that VEGF-inhibitors could significantly reduce interstitial fluid collection through the modulation of vascular permeability, resulting in an indirect improvement of lymphatic obstruction and drainage. Preliminary study results confirmed the hypothesis that Bevacizumab has a role in interstitial fluid pressure and extracellular fluid volume reduction (NCT00318513). However, many aspects limit its use in clinical practice for breast cancer patients. To date, Bevacizumab is no longer approved for breast cancer treatment and there is only partial evidence regarding the use of VEGF-inhibitors in subjects without active cancer. Lymph fluid collection represents the starting point of BCRL, which is worsened by chronic inflammatory tissue response to protein-rich fluid accumulation. The modulation of immune signal molecules, such as interleukins, could reduce inflammation and tissue reaction, preventing lymphedema chronicization. In this setting, a trial is ongoing to test the efficacy of peripheral intravenous injections of a combination of two monoclonal antibodies that neutralize the biologic activity of IL4 and IL13 (NCT02494206). Further clinical studies are needed to develop targeted therapies directed to improve lymphatic regeneration and function, together with the modulation of inflammatory pathways. An appropriate medical treatment combining physical and molecularly targeted drugs administered early on after surgery in high-risk individuals could become the key strategy to prevent lymphedema formation.

CONCLUSIONS

BCRL is a complex and underdiagnosed condition, with potentially devastating consequences on the quality of life of breast cancer survivors. Several genetic, anatomical, biological, and clinical factors might intervene in its development, supporting the hypothesis of a multifactorial etiopathogenesis. Impairment of the lymphatic system embryogenetic differentiation mechanisms, anatomical variations, alterations of the lymphatic pacemaking system, mechanisms of phasic contractions of the lymphatic vessel, and systemic inflammation might act synergistically. In addition, mutations in genes encoding inter-cellular communication have been linked to both primary and secondary lymphedema. There is no evidence that genetic alterations related to the different molecular subtypes of breast cancer could influence BCRL pathogenesis. On the other hand, medical, surgical, and radiation therapies are crucial factors in its development and progression. Further research is needed in order to clarify, according to a novel multidisciplinary approach, the strict correlation between clinical and biological aspects of BCRL. The identification of specific molecular targets, novel biomarkers, and validated risk stratification tools could prove significantly crucial, bringing us closer to achieving the goal of precision medicine for BCRL.

AUTHOR CONTRIBUTIONS

MI and NF: study concept and design. MI, RB, and NF: supervision. GL, AM, AS, and LR: manuscript writing (first draft). AM: bibliography. GL, KV, and ES: iconography. LD and MG: first draft revision. All authors: revision and approval of the final draft.

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SYSTEMATIC REVIEW
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Effect of Tai Chi Chuan in Breast Cancer Patients: A Systematic Review and Meta-Analysis

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Background: Tai Chi Chuan(TCC), as a mind-body exercise, may have a positive impact on physical function and psychological well-being in breast cancer patients. The latest systematic review and meta-analysis of TCC for breast cancer was made 4 years ago and some new clinical trials about it were published. We remade a systematic review and meta-analysis to evaluate the effect of TCC in breast cancer patients.

Methods: In this systematic review and meta-analysis, we searched MEDLINE (via PubMed), EMBASE (via embase.com), CENTRAL, CNKI, COVIP, Wanfang, Chaoxing, CiNii, J-SSTAGE, DBpia, and ThaiJO with no language restrictions from inception to December 31, 2018 (updated on February 16, 2020), for randomized clinical trials comparing TCC with non-exercised therapy in breast cancer patients. The primary outcome was quality of life in patients with breast cancer and data pooled by a random-effects model. Subgroup analyses were conducted to estimate the effect of different durations of TCC for breast cancer patients. This study was registered in PROSPERO, number CRD 4201810326.

Results: Fifteen articles involving a total of 885 breast cancer participants were included in this review. Compared with non-exercised therapy, TCC had a significant effect on quality of life in breast cancer patients (SMD = 0.37, 95% CI 0.15–0.59, $p = 0.001$), and subgroup analysis found that TCC showed beneficial effect in 12 weeks and 25 weeks (12 weeks: SMD = 0.40, 95% CI 0.19–0.62, $p = 0.0003$; 25 weeks: SMD = 0.38, 95% CI 0.15–0.62, $p = 0.002$). Meta-analyses of secondary outcomes showed that 3 weeks TCC increased shoulder function (SMD = 1.08, 95% CI 0.28–1.87, $p = 0.008$), 12 weeks TCC improved pain (SMD = 0.30, 95% CI 0.08–0.51, $p = 0.007$), shoulder function (SMD = 1.34, 95% CI 0.43–2.25, $p = 0.004$), strength of arm (SMD = 0.44, 95% CI 0.20–0.68, $p = 0.0004$), and anxiety (MD = −4.90, 95% CI −7.83 to −1.98, $p = 0.001$) in breast cancer patients compared with the control group.

Conclusions: TCC appears to be effective on some physical and psychological symptoms and improves the quality of life in patients with breast cancer. Additional randomized controlled trials with a rigorous methodology and low risk of bias are needed to provide more reliable evidence.

Keywords: Tai Chi Chuan, breast cancer, physical and psychological symptoms, quality of life, meta-analysis

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INTRODUCTION

Although breast cancer is the most commonly diagnosed cancer and being the leading cause of cancer-related mortality in female (1–3), the long-term survival rates after a diagnosis of breast cancer are steadily rising in recent years (4–6). In the meantime, more patients with breast cancer are facing persistent symptoms and side-effects after diagnosis and treatment, such as fatigue (7–10), cognitive limitations (7, 9), depression (7, 9), anxiety (9), sleep problems (7, 9), and pain (9, 11). To address the persistent symptoms, complementary and alternative medicine (CAM), a non-mainstream medicine used together with or in place of conventional medicine, is recommended as supportive care strategies during and following the treatment (12).

Complementary therapies, especially mind-body practices, are effective approaches to manage breast cancer symptoms, and side-effects of treatment (12). For breast cancer patients, physical and psychosocial therapies improve physical function, and emotional disorders (13–15), and moreover, post-diagnostic physical activity reduces cancer-related mortality (16–18). Tai Chi Chuan (TCC) and Qigong are complementary therapies involving physical and psychological aspects (19). They are ancient Chinese mind-body exercises that both combine meditation, breathing, relaxation, and physical activity (20–22). Traditional TCC is usually a series of elaborate, lengthy, and complex movements, while Qigong is a simpler and more repetitive exercise (22, 23). TCC is a martial art that was gradually simplified and made into a common sport in 1950s (24). Nowadays, TCC as a sport focuses more on body environment and mind-body interaction (25). When TCC is performed for health and energy enhancement, it is a form of Qigong (26). Qigong produces more than a dozen forms to keep people healthy (22). Because other forms of Qigong are different from the way of practicing TCC, this review only focused on the effects of TCC on breast cancer patients.

TCC with slow, supple, graceful, curved, spiral and sequential motions, is a mild-to-moderate intensive whole-body exercise incorporating meditation into breathing control (20, 27, 28). TCC improves pain and anxiety in patients with fibromyalgia (29), exercise self-efficacy and mood disturbance in patients with chronic heart failure (30), depression and physical function in patients with osteoarthritis (31), and balance and tumble in patients with Parkinson's disease (32). TCC for more than 8 weeks reduces cancer-related fatigue, especially in breast or lung cancer patients (33, 34). In senior female cancer survivors, TCC affects systolic blood pressure and cortisol area-under-curve which may regulate the endocrine system (35). In general, TCC plays a good role in improving the quality of life (QOL) in cancer patients (36). TCC is recommended to patients with chronic conditions for multi-effects, easily learning, good safety, and low-cost (28).

Breast cancer is a chronical clinical setting with persistent symptoms of body and mind, and TCC may have a positive effect on it. Several clinical trials found TCC reduced inflammatory responses and improved quality of life, muscle strength, shoulder function, bone formation, and insomnia in breast cancer patients (37–41). Two early reviews published in 2007 and 2010, respectively, both found that the effect of TCC for breast cancer patients to improve QOL and symptoms was not definite (42, 43). In a systematic review and meta-analysis published in 2015, Pan and colleagues considered TCC had a significant effect on improving handgrip strength and limb elbow flexion, but failed to improve QOL, physical and emotional well-being, pain, and body mass index (44).

Previous reviews pooled studies ignoring the influence of diverse exercise durations and control therapies, and found limited evidence that TCC was beneficial on physical and psychosocial capacity in breast cancer patients. Additionally, some new clinical trials explored TCC for breast cancer have been published. Therefore, a systematic review and meta-analysis including the latest randomized clinical trials of TCC for breast cancer is necessary to be done. This systematic review and meta-analysis are performed to evaluate the effect of TCC on QOL and psychosomatic outcomes in breast cancer patients.

METHODS

Search Strategy

The report of this systematic review and meta-analysis is followed by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (45). The checklist of PRISMA is in **Supplement Table 1**. We searched English databases (MEDLINE, EMBASE, and CENTRAL), Chinese databases (CNKI, CQVIP, Wanfang, Chaoxing), Japanese databases (CiNii, and J-SSTAGE), Korean database (DBpia), and Thai database (ThaiJO) from inception to December 31, 2018 (updated on February 16, 2020). MEDLINE and EMBASE were available for consultation through PubMed and embase.com, respectively. The following search terms in various relevant combinations were used to screen potential studies: tai*ji*, tai*chi*, breast cancer, breast tumor, breast neoplasm, breast carcinoma. Example of search strategy is in **Supplement Table 2**. Language restrictions were not part of data searches. The reference lists of identified original or review studies were searched manually for further articles.

Selection Criteria

The following inclusive selection criteria were applied: (I) participants were adult female patients who were diagnosed breast cancer through pathology with any tumor stage; (II) intervention measure was TCC, such as Yang-style TCC, Chen-style TCC, Wu-style TCC, Sun-style TCC, 24 simplified TCC, or movements of TCC; (III) compared intervention was non-exercised treatment, such as standard support therapy (a psychotherapy for education and peer discussion on nutrition, exercise, stress, cancer risk, and fatigue) (46, 47), usual health

Abbreviations: CAM, Complementary and alternative medicine; CON, Control; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue, FACT-B, Functional Assessment of Cancer Therapy–Breast; GRADE, Grades of Recommendations Assessment, Development and Evaluation; MOS SF-36, Medical Outcomes Study 36-Item Short-Form Health Survey; QOL, Quality of life; RRT, Routine rehabilitation training; TCC, Tai Chi Chuan; WHOQOL-BREF, World Health Organization Quality of Life Brief Questionnaire.

care (regular check-ups, medication, and health education by health care workers), or blank control; (IV) primary outcome was QOL and the measurement was not limited [e.g., the Medical Outcomes Study 36-Item Short-Form Health Survey (MOS SF-36), the World Health Organization Quality of Life Brief Questionnaire (WHOQOL-BREF), the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT–F), the Functional Assessment of Cancer Therapy–Breast (FACT-B), and the Generic Quality of life Inventory 74 (GQOLL 74)]; secondary outcomes were pain, shoulder function, strength of arm, anxiety, and other clinical outcomes; (V) only randomized controlled trial (RCT) was included. Exclusive selection criteria: (I) participants were not only breast cancer patients; (II) experimental intervention was TCC combining other exercise; (III) control intervention included other exercise methods, such as yoga, physical activity, aerobics; (IV) data of outcomes couldn't be acquired; (V) non-randomized clinical trial.

Data Extraction and Quality Assessment

Information extracted from each eligible study through electronic form containing the first author, year of publication, country of origin, number of participants, age, status of cancer, current treatment, experimental intervention, duration and frequency of TCC, controlled intervention, and outcomes. Data from eligible studies were extracted independently by two investigators (XL, LS). The risk of bias was assessed by the Cochrane Collaboration's tool with seven items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias (48). We used the Grades of Recommendations Assessment, Development and Evaluation (GRADE) system to evaluate the certainty of outcomes (49). Any disagreements were resolved by discussion and consensus.

Data Analysis

We tried to make meta-analysis when the number of eligible studies was more than one in each outcome and data were combined by Review Manager (version 5.3, Cochrane Library). We selected a random-effect model to pool data (50–53). Mean difference (MD)/standardized mean difference (SMD) and 95% confidence interval (CI) were calculated. If outcome with the same measurement method, we selected MD, otherwise chosen SMD. Heterogeneity across studies was tested by the I^2 statistic. I^2 is regarded of 25, 50, and 75% as low, moderate, and high amount of heterogeneity, respectively (54). In the Cochrane Handbook, more than 50% of I^2 are regarded as may material heterogeneity (55). We did subgroup analysis based on different TCC durations. A two-tailed $p \leq 0.05$ was considered as a criterion for statistical significance.

Registration

This study was registered in PROSPERO, number CRD 4201810326.

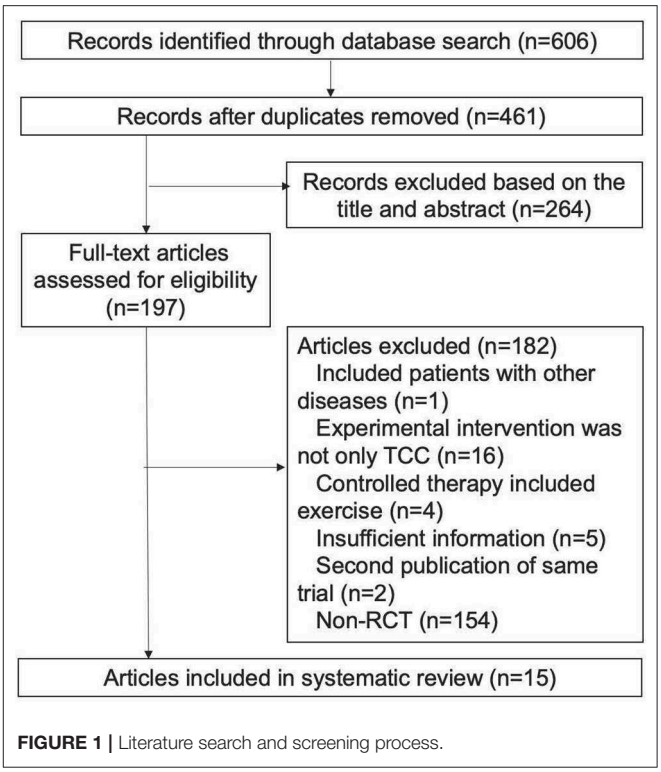

FIGURE 1 | Literature search and screening process.

RESULTS

Study Selection and Characteristics

By searching English, Chinese, Japanese, Korean, and Thai databases, we obtained 606 records, of which 409 records were excluded for irrelevance, or duplication through the titles and abstracts. The full-text of the remaining 197 articles were retrieved for more detailed evaluation, and 182 articles were excluded (**Figure 1**). Fifteen articles (40, 41, 56–68) met the inclusion criteria containing 885 participants (447 in the TCC group, 438 in the control group). Six articles (40, 41, 65–68) came from the USA, eight (56–61, 63, 64) from China and one (62) from Thailand. The types of TCC were 15-move short-form of Yang-style TCC (40, 65–68), Chen-style TCC (58, 60), Tai Chi Yunshou (63), 18-form TCC (62), 24-form TCC (57, 59, 61, 64), 8-form TCC (56), and the other article (41) didn't mention the specific form of TCC. Duration of TCC was from 12 weeks to 6 months. The frequency of TCC was 120 min per week (41), 40–60 min per session and three sessions a week (40, 61, 62, 65–68), 20–30 min per session and two sessions per day (57–60, 63, 64), and at least 40 min per session for twice a day (56). TCC was performed during chemotherapy or after conventional therapy. Some trials (56–58, 61, 63, 64) used TCC intervention after surgery to alleviate the side effects of operation. The interventions of control groups were cognitive behavioral therapy (41), standard or psychosocial support therapy (40, 65–68), usual care (56, 62), and routine rehabilitation training (57–61, 63, 64). The interest control treatments had cognitive behavioral therapy (41), standard or psychosocial support therapy (40, 65–68), and

TABLE 1 | Characteristics of the eligible articles.

Study	Country	Patients (*N*)		Mean age(y)		Status	Treatment condition	TCC intervention	Controlled intervention	Frequency Duration	Outcomes
		TCC	CON	TCC	CON						
Han et al. (56)	China	23	21	46.39	45.52	I-III	After modified radical mastectomy and chemotherapy	Usual care+8-form TCC	Usual care	At least 40 min/session and 2 sessions /day, 5 days/week, 12 weeks	1. Fatigue; 2. Adverse event
Irwin et al. (41)	USA	45	45	59·6	60·0	–	Treatment at least 6 months before enrollment	Tai Chi Chih	Cognitive behavioral therapy	120 min/session, 3 sessions/week, 3 months	1. Insomnia treatment response; 2. Insomnia remission; 3. Sleep quality; 4. Sleep continuity
Wang et al. (57)	China	45	41	50·5		I-III	10 days after modified radical mastectomy	RRT + 24-form TCC	RRT	20 min/session, every morning and evening, 6 months	1. QOL(WHOQOLBREF); 2. Fatigue; 3. Sleep quality; 4. Anxiety; 5. Depression
Wang et al. (58)	China	44	46	53·64	51·74	–	10 days after modified radical mastectomy	RRT + Chen-style TCC	RRT	20 min/session, every morning and evening, 3 months	1. QOL(FACT-B); 2. Neer shoulder function score
Zhu et al. (59)	China	47	48	45·51		–	After modified radical mastectomy	RRT +24-form simplified TCC	RRT	20 min/session, every morning and evening , 3 months	1. Pain; 2. Activities of daily living; 3. Range of motion; 4. Strength of arm
Wang et al. (60)	China	75	74	51·4	50·58	–	10 days after modified radical mastectomy	RRT + Chen-style TCC	RRT	20 min/session, every morning and evening, 6 months	Self-rating anxiety scale
Lv et al. (61)	China	50	49	48·61		–	After modified radical mastectomy and chemotherapy	RRT + 24-form simplified TCC	RRT	90 min/session, 3 sessions/week, 6 months	1. QOL(MOSSF-36); 2. Pain; 3. Activities of daily living; 4. Range of motion; 5. Strength of arm
Thongteratham et al. (62)	Thailand	15	15	–		0-IIIb	Completion of treatment at least 1 year before enrollment	Usual care + 18-form TCC	Usual care	60 min/session, 3 sessions/week, 12 weeks	1. QOL(FACT-B); 2. Rosenberg Self-esteem; 3. Fatigue Symptom Inventory; 4. Cortisol
Li et al. (63)	China	29	28	47·56		0-IIIb	7 days after modified radical mastectomy	RRT + Tai Chi Yunshou	RRT	30 min/session, at 7:00 and 17:00, 6 months	1. QOL(WHOQOLBREF); 2. Edema of upper extremity; 3. Function of shoulder joint; 4. Muscle strength
Wang et al. (64)	China	63	71	47·19		I-III	10 days after modified radical mastectomy	RRT + 24-form simplified TCC	RRT	20 min/session, every morning and evening, 180 days	1. QOL(WHOQOLBREF); 2. Pain; 3. Activities of daily living; 4. Range of motion; 5. Strength of arm

(Continued)

TABLE 1 | Continued

Study	Country	Patients (*N*)		Mean age(y)		Status	Treatment condition	TCC intervention	Controlled intervention	Frequency Duration	Outcomes
		TCC	CON	TCC	CON						
Sprod et al. (65)	USA	11	10	54·33	52·70	0–IIIb	Chemotherapy, radiotherapy, hormone therapy, or none	A 15-move short-form of Yang-style TCC	Standard support therapy	60 min/session, 3 sessions/week, 12 weeks	1. QOL(MOSSF-36); 2. Pain; 3. Biomarkers (IL-6, IL-8, IGF-1, IGFBP-1, IGFBP-3, glucose, insulin, cortisol)
Janelsins et al. (66)	USA	9	10	54·33	52·70	0–IIIb	Chemotherapy, radiotherapy, hormone therapy, or none	A 15-move short-form of Yang-style TCC	Psychosocial support therapy	60 min/session, 3 sessions/week, 12 weeks	1. Biomarkers (insulin, IGF-1, IGFBP-1, IGFBP-3, IL-6, IL-2, IFN-γ); 2. BMI; 3. Body composition
Peppone et al. (40)	USA	7	9	53·8	52·6	0–IIIb	Treatment completed from 1 to 30 months before enrollment	A 15-move short-form of Yang-style TCC	Standard support therapy	60 min/session, 3 sessions/week, 12 weeks	1. Bone-specific alkaline phosphatase; 2. N-telopeptides of type I collagen; 3. Bone Remodeling Index; 4. Biomarkers (IGFBP-1, IGFBP-3, cortisol, IL-2, IL-6, IL-8)
Mustian et al. (67)	USA	11	10	52		0–IIIb	Chemotherapy, radiotherapy, hormone therapy, or none	A 15-move short-form of Yang-style TCC	Psychosocial support therapy	60 min/session, 3 sessions/week, 12 weeks	1. QOL(FACIT-F); 2. Strength; 3. Flexibility; 4. Aerobic capacity
Mustian et al. (68)	USA	11	10	52		0–IIIb	Chemotherapy, radiotherapy, hormone therapy, or none	A 15-move short-form of Yang-style TCC	Psychosocial support therapy	60 min/session, 3 sessions/week, 12 weeks	1. Aerobic capacity; 2. Strength; 3. Flexibility; 4. Weight; 5. BMI;6. Body composition

TCC, Tai Chi Chuan; CON, control; SD, standard deviation; QOL, quality of life; MOSSF-36, Medical Outcomes Study 36-Item Short-Form Health Survey; IL-6, interleukin-6; IL-8, interleukin-8; IGF-1, insulin-like growth factor-1; IGFBP-1, insulin-like growth factor-binding protein-1; IGFBP-3, insulin-like growth factor-binding protein-3; IL-2, interleukin-2; IFN-γ, interferon- γ; BMI, body mass index; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; RRT, routine rehabilitation training; WHOQOLBREF, World Health Organization Quality of Life Brief Questionnaire; FACT-B, Functional Assessment of Cancer Therapy–Breast.

blank control (56–64). The cognitive behavioral therapy involves re-establishing a consistent sleep-wake schedule, sleep restriction, relaxation, sleep hygiene education and cognitive procedures (69). The extracted information of eligible studies is in **Table 1**.

Risk of Bias

According to the Cochrane Collaboration's tool, risk of bias analysis was assessed to evaluate the quality of included studies. For eligible articles, 80% had low risk of random sequence generation and 54% had low risk of allocation concealment in selection bias; 73% had unclear risk of blinding both in performance and detection bias; 87% had low risk of incomplete outcome data in attrition bias; reporting bias was unclear because the proposals of these trials were not available (**Figure 2**). Publication bias was not analyzed because the number of eligible studies in each meta-analysis was <10 and conceivable publication bias existed in each meta-analysis.

Meta-Analysis of TCC for QOL in Breast Cancer Patients

QOL were evaluated with different scales in studies that performed a meta-analysis. One study (65) used the MOS SF-36 to assess general QOL, and three studies (57, 63, 64) chosen the WHOQOL-BREF. One study (67) used the FACIT–F to assess QOL in chronic disease, while another study (62) measured cancer-special QOL with the FACT-B. In this meta-analysis, SMD were calculated for different measures of QOL. TCC had a positive effect on QOL in breast cancer patients compared with the non-exercised therapy (SMD = 0.37, 95% CI 0.15–0.59, p = 0.001, I^2 = 0%) (**Figure 3**). Subgroup meta-analyses of 12 and 25 weeks durations showed that TCC improved QOL in breast cancer patients (12 weeks: SMD = 0.40, 95% CI 0.19–0.62, p = 0.0003, I^2 = 0%; 25 weeks: SMD = 0.38, 95% CI 0.15–0.62, p = 0.002, I^2 = 0%), but meta-analyses of 3 and 6 weeks both were no statistical significance (3 weeks: SMD = 0.23, 95% CI −0.01 to 0.47, p = 0.06, I^2 = 0%; 6 weeks: SMD = 0.04, 95% CI −0.52 to 0.60, p = 0.89, I^2 = 0%).

Meta-Analysis of TCC for Pain, Shoulder Function, Strength of Arm, Anxiety, and Fatigue in Breast Cancer Patients

For secondary outcomes, we did meta-analyses of pain, shoulder function, strength of arm, anxiety, and fatigue, respectively. The heterogeneity was high among the eligible studies in meta-analysis of TCC for shoulder function, even though we did sensitive analysis trying to delete one of the three included studies successively. In the meta-analyses of shoulder function and anxiety, the included studies were placed on one side of the no effect line, respectively. Therefore, we did meta-analyses of shoulder function and anxiety overlooking the heterogeneity. Meta-analyses showed that TCC was beneficial to alleviating pain (SMD = 0.30, 95% CI 0.08–0.51, p = 0.007, I^2 = 0%) (**Figure 4**), recovering shoulder function (SMD = 1.34, 95% CI 0.43–2.25, p = 0.004, I^2 = 92%) (**Figure 5**), increasing strength of arm (SMD = 0.44, 95% CI 0.20–0.68, p = 0.0004, I^2 = 16%) (**Figure 6**), easing anxiety (MD = −4.25, 95% CI −5.87 to −2.63, p < 0.00001, I^2 = 80%) (**Figure 7**), and relieving fatigue (SMD = −1.11, 95% CI −1.53 to −0.69, p < 0.00001, I^2 = 30%) (**Figure 8**) compared with non-exercised therapy in breast cancer patients. TCC increased shoulder function in 3 weeks duration, but improved pain, strength of arm, and anxiety in 12 weeks in breast cancer patients.

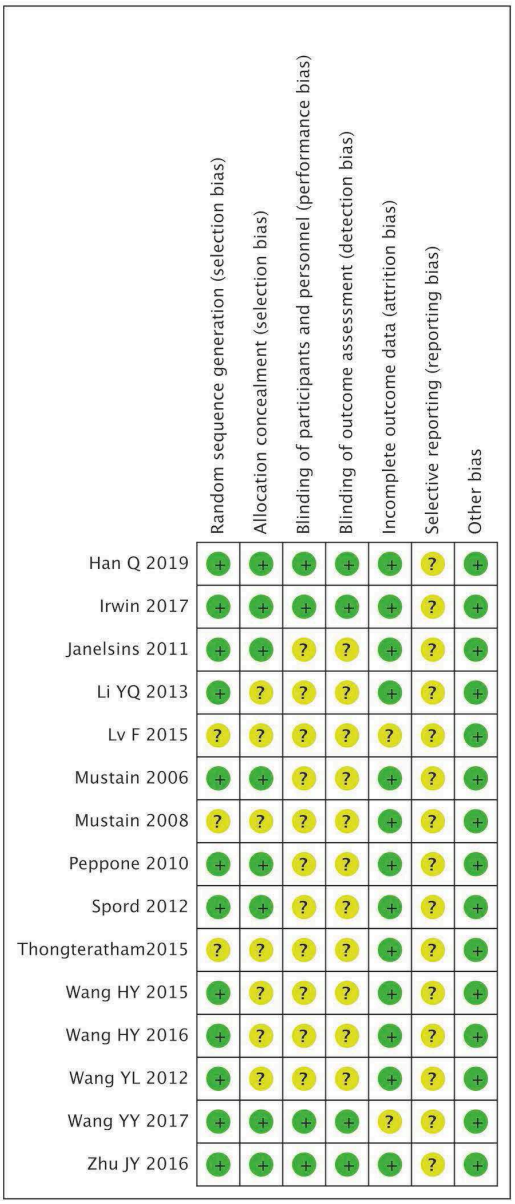

FIGURE 2 | Risk of bias graph summary.

GRADE Evidence of Outcomes

GRADE system was used to assess the evidence quality of outcomes in this review. Risk of bias might exist because method of randomization, allocation concealment, and blinding were not definite. Outcomes measured by different methods might

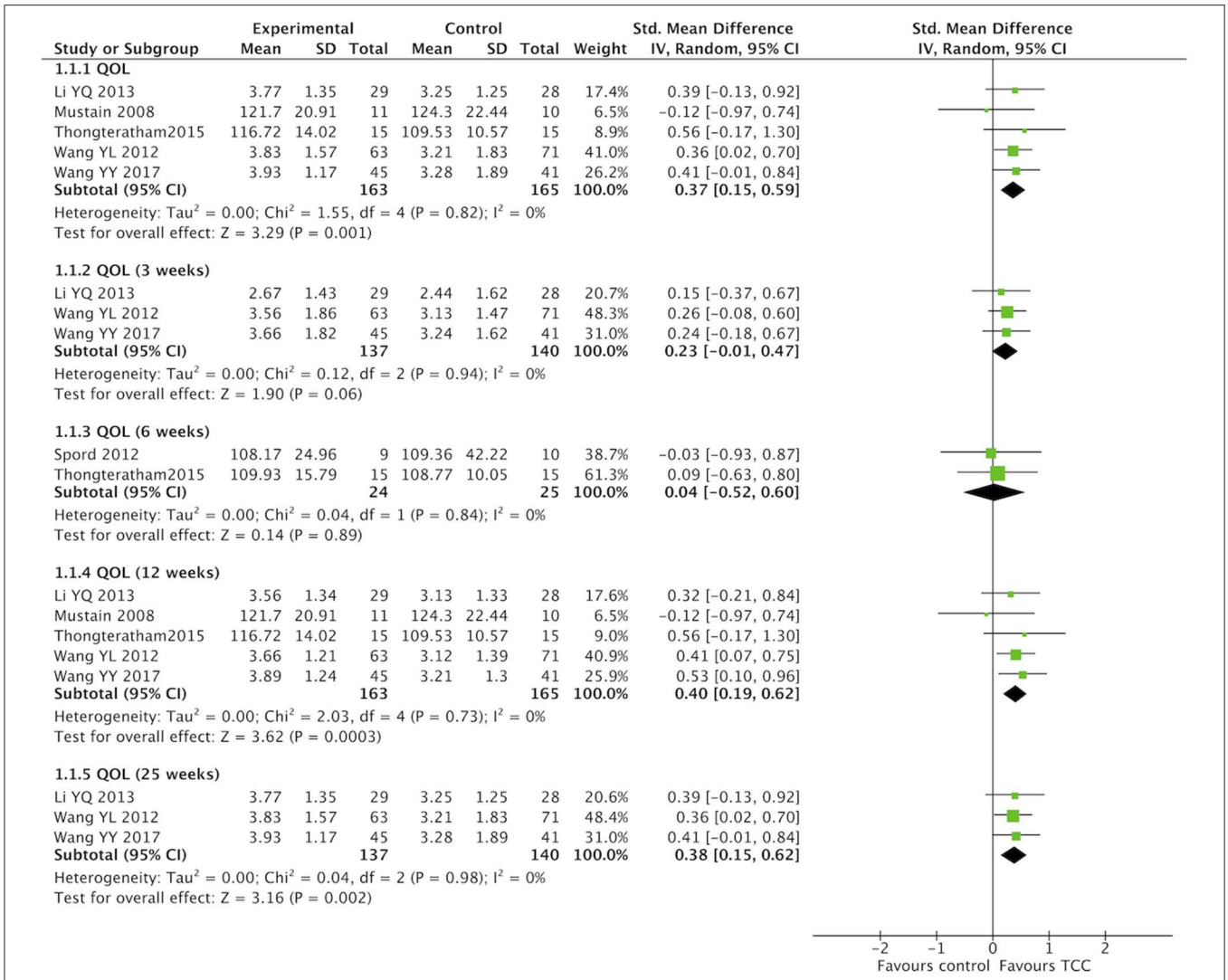

FIGURE 3 | Meta-analyses of Tai Chi Chun for quality of life in breast cancer patients compared with non-exercised therapy. Standardized mean difference and 95% confidence interval are calculated.

produce inconsistency. Although the heterogeneity in shoulder function and anxiety outcomes was high, inconsistency might not be downgraded because all the eligible studies in meta-analyses were on one side of the no effect line. The eligible studies and participants of each meta-analysis were small that imprecision and publication bias might exist. In general, the certainty of the six outcomes was very low (**Table 2**).

DISCUSSION

This systematic review and meta-analysis concentrated on the QOL and psychosomatic symptoms in women breast cancer patients comparing TCC with non-exercise therapy.

Compared with the prior reviews (43, 44), this study searched more different language databases, included more RCTs, and made subgroup analysis on the basis of TCC duration. Lee et al. (43) and Pan et al. (44) made a systematic review of TCC for breast cancer in 2010 and 2015, respectively. Lee retrieved English, Chinese, and Korean databases, and Pan searched four English databases neglecting the different types of control intervention. In this review, we searched English, Chinese, Japanese, Korean, and Thai databases setting non-exercised therapy as the control treatment. Lee's review included seven studies, of which three were RCTs and four were non-RCTs, and only one meta-analysis of two RCTs was conducted; nine RCTs met the inclusion criteria for the Pan's review, and two to six studies were included in the meta-analyses; 15 RCTs were eligible in this review, but no more than five RCTs included in each meta-analysis. This review included 10 new RCTs (41, 56–64) which did not presented by Pan et al. (44) and might affect the results of meta-analysis. Pan did not search Chinese databases, while we searched four Chinese databases and found

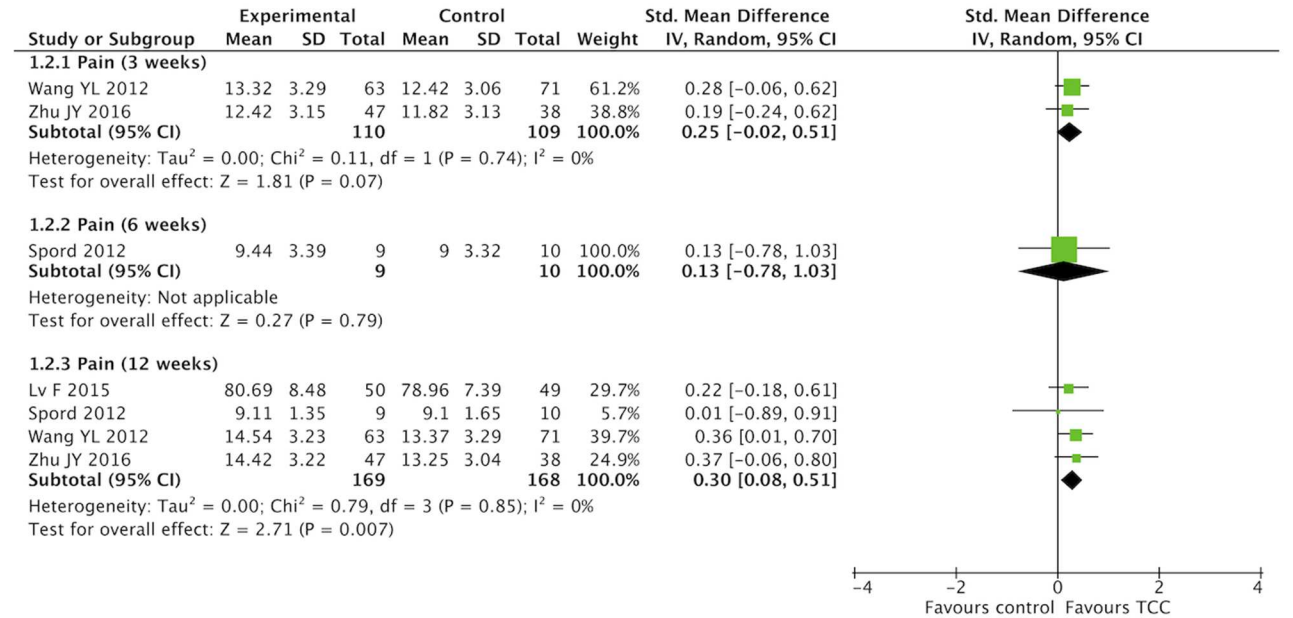

FIGURE 4 | Meta-analyses of Tai Chi Chun for pain in breast cancer patients compared with non-exercised therapy. Standardized mean difference and 95% confidence interval are calculated.

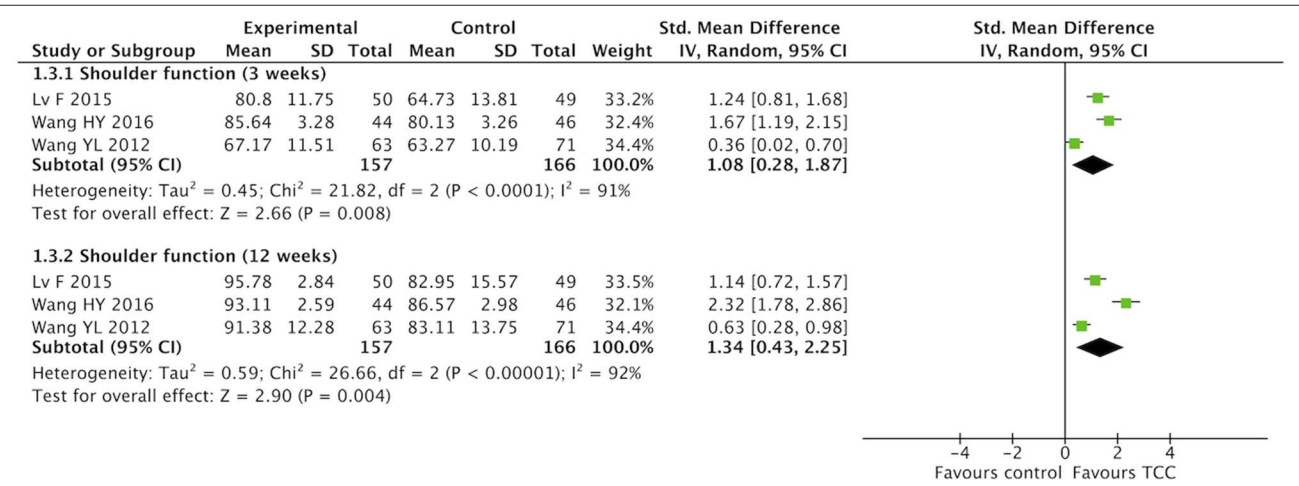

FIGURE 5 | Meta-analyses of Tai Chi Chun for shoulder function in breast cancer patients compared with non-exercised therapy. Standardized mean difference and 95% confidence interval are calculated.

eight new Chinese articles (56–61, 63, 64) that met the inclusion criteria. The other two new studies (41, 62) were published in English from Thai and English databases, respectively. We made subgroup analysis according to the exercise time of TCC, and found that 12 weeks TCC was more effective than 3 or 6 weeks TCC in improving QOL, pain, and strength of arm in breast cancer patients. However, the two previous reviews did not conduct subgroup analysis according to the characteristics of TCC.

The findings of these two published reviews are quite different from those of our study. Lee et al. (43) found that TCC had no significant effect on QOL, physical outcomes (fatigue, body mass index, heart rate, and blood pressure) and psychological variables (self-esteem and depression) in patients with breast cancer. Pan et al. (44) discovered that TCC had a positive effect on handgrip dynamometer strength and limb elbow flexion, but had no significant difference in general health-related QOL, pain, and body mass index in breast cancer patients. In this review, TCC had an improvement on QOL in breast cancer patients. In the meta-analysis of TCC for QOL, Lee's review included two small sample trials, and Pan's review involved studies reporting the same trials, while we pooled data from five RCTs. Pan's review

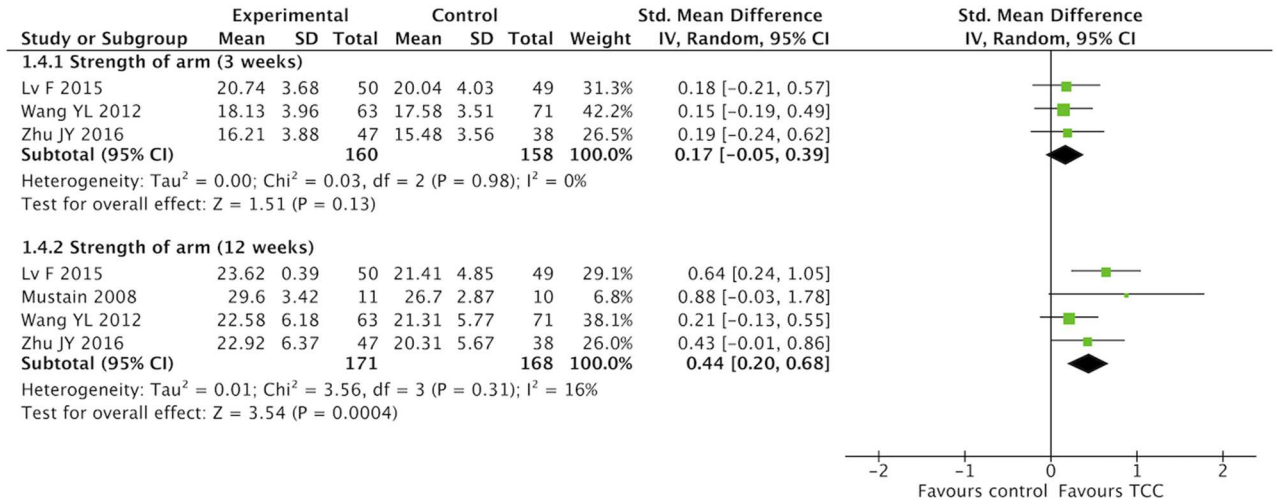

FIGURE 6 | Meta-analyses of Tai Chi Chun for strength of arm in breast cancer patients compared with non-exercised therapy. Standardized mean difference and 95% confidence interval are calculated.

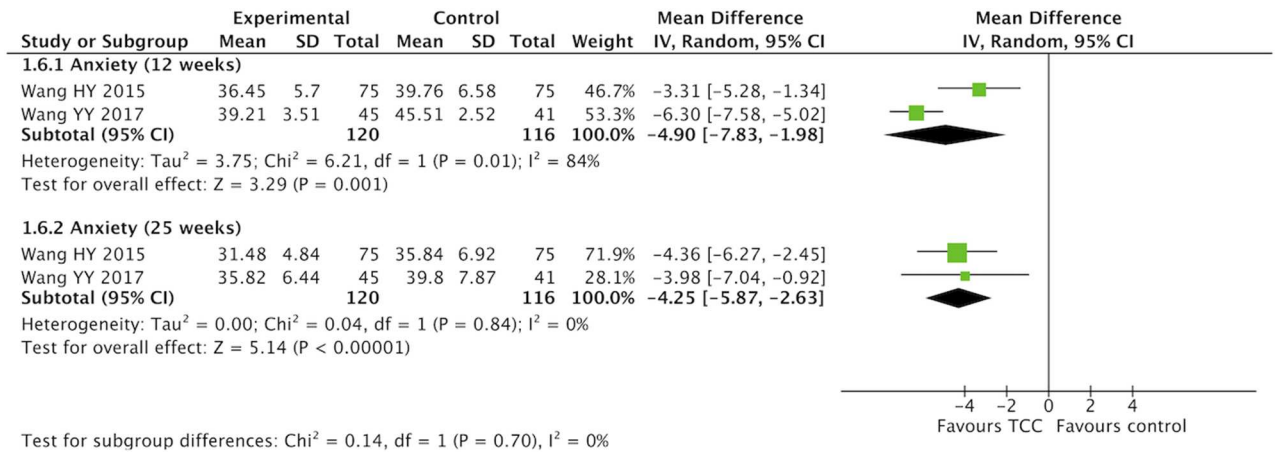

FIGURE 7 | Meta-analyses of Tai Chi Chun for anxiety in breast cancer patients compared with non-exercised therapy. Mean difference and 95% confidence interval are calculated.

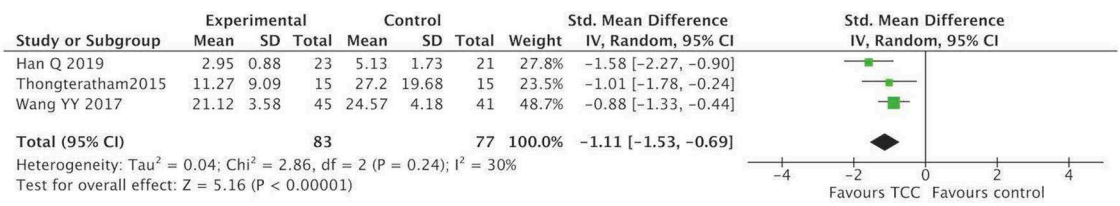

FIGURE 8 | Meta-analyses of Tai Chi Chun for fatigue in breast cancer patients compared with non-exercised therapy. Standardized mean difference and 95% confidence interval are calculated.

discovered that TCC failed to alleviate pain in breast cancer patients, whereas TCC had an inverse result in this systematic review. The meta-analysis of pain in this review included some new studies which influenced the result. Meta-analyses of fatigue and anxiety in this review were changed between the previous two reviews. Although this study discovered that TCC could reduce fatigue and anxiety in breast cancer patients compared with the non-exercised group, the result is uncertain because of a small number of eligible studies and high risk of selection bias, performance bias, detection bias, and publication bias. TCC was associated with positive effects on handgrip dynamometer strength and limb elbow flexion in Pan's review, and in this review

TABLE 2 | GRADE evidence profile of outcomes.

	Certainty assessment							No. of patients		Effect	Certainty
Outcomes	**No. of studies**	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**TCC**	**CON**	**Absolute (95% CI)**	
QOL	5	RT	Serious*	Serious†	Not serious	Serious‡	Publication bias strongly suspected§	163	165	SMD 0·37 higher (0·15 higher to 0·59 higher)	⊕◯◯◯◯ VERY LOW
Pain	4	RT	Serious¶	Not serious	Not serious	Serious‡	Publication bias strongly suspected§	169	168	SMD 0·3 higher (0·08 higher to 0·51 higher)	⊕◯◯◯◯ VERY LOW
Shoulder function	3	RT	Serious ‖	Not serious	Not serious	Serious‡	Publication bias strongly suspected§	157	166	SMD 1·34 higher (0·43 higher to 2·25 higher)	⊕◯◯◯◯ VERY LOW
Strength of arm	4	RT	Serious**	Serious†	Not serious	Serious‡	Publication bias strongly suspected§	171	168	SMD 0·44 higher (0·20 higher to 0·68 higher)	⊕◯◯◯◯ VERY LOW
Fatigue	3	RT	Serious††	Serious	Not serious	Serious‡	Publication bias strongly suspected§	83	77	SMD 1.11 lower (1.53 lower to 0.69 lower)	⊕◯◯◯◯ VERY LOW
Anxiety	2	RT	Serious‡‡	Not serious	Not serious	Serious‡	Publication bias strongly suspected§	120	116	MD 4·25 lower (5·87 lower to 2·63 lower)	⊕◯◯◯◯ VERY LOW

TCC, Tai Chi Chuan; CON, control; CI, confidence interval; QOL, quality of life; RT, randomized trials; SMD, standardized mean difference; MD, mean difference.

**2/5 studies didn't mention the method of randomization, 4/5 studies didn't mention allocation concealment and blinding.*

†*Measured by different scales.*

‡*The sample size was small.*

§*Number of eligible studies was small.*

¶*1/4 studies didn't mention the method of randomization, 2/4 studies didn't mention allocation concealment, 3/4 studies didn't mention blinding.*

‖*1/3 studies didn't mention the method of randomization, 2/3 studies didn't mention allocation concealment and blinding.*

***2/4 study didn't mention the method of randomization, 2/4 studies didn't mention allocation concealment and blinding.*

††*1/3 study didn't mention the method of randomization.*

‡‡*1/2 study didn't mention allocation concealment and blinding.*

also revealed that TCC improved strength of arm and shoulder function including range of motion.

QOL, a common gauge of cancer treatment effect, is widely used to measure the health of breast cancer patients (70) QOL is a multidimensional assessment with physical (including function/disability and symptoms/complications concepts), mental (including emotional distress, psychological well-being, perceived cognitive functioning, and spiritual/existential concerns concepts) and social (including socioeconomic challenges, role/relationship changes, perceived support/satisfaction, and social participation concepts) domains (70). Yan's and Tao's systematic reviews found TCC failed to improve QOL in breast cancer survivors (71, 72), but this study showed 12 weeks TCC had a positive influence on QOL. Three studies in Yan's systematic review were not included in this review because the intervention therapy of two studies (73, 74) was Tai Chi combining aerobics, and another study (75) was a prospective longitudinal study from a larger randomized controlled trial. We included some new studies that might produce the different pooled result. TCC improved the physical and mental health domains of QOL in cancer survivors at a low-level evidence in a recent review (76). Due to the limited number of included studies in the meta-analysis of QOL, we conducted a meta-analysis of overall QOL total score rather than a meta-analysis of each QOL domain in breast cancer patients, but obtained a very low-level evidence. Heather Greenlee and colleagues (12) recommends Qigong as C-graded therapy to improve QOL in breast cancer patients. TCC is one kind of Qigong and can be considered as a complementary health approach for QOL in breast cancer patients.

In this review, TCC also improved pain, shoulder function, strength of arm, anxiety, and fatigue in breast cancer patients. Pain is one of the most common symptoms in cancer patients and can be caused by tumors, surgery, chemotherapy, radiation therapy, targeted therapy, supportive care therapies, and diagnostic procedures (77). Half of breast cancer patients have mild pain and 16% have moderate to severe pain in 1 year after surgery (78). Pain has a psychological effect making patients product anxiety, nervousness, and anger (79). Control of surgery-related and non-operative pain in patients with breast cancer is essential. In addition to pain, side-effects of surgery include depressed shoulder function and arm strength (80). Decreased arm muscle strength in breast cancer patients after chemotherapy is also measured (81). Breast cancer patients have suffered psychological disorders as well as physical symptoms. About one-third of breast cancer survivors feel fatigue (82), and 40% patients are anxious in the year after diagnosis (83). Fatigue correlates with depression and sleep disturbance in breast cancer patients (84). Breast cancer patients experience persistent physical and mental disorders which influence QOL after or during cancer diagnosis and treatment. Three months exercise reduces fatigue, anxiety, and depression in breast cancer patients (85). American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline recommends that primary care clinicians should counsel breast cancer patients to engage in regular physical activity to reduce cancer-related fatigue, musculoskeletal symptoms, pain, and obesity (86). Previous meta-analyses discovered that TCC improved psychological well-being including reduced stress, anxiety, depression and mood disturbance, and physical function in people with cancer (36, 87). In this review, TCC improved pain, shoulder function, strength of arm, and anxiety in breast cancer patients in 12 weeks, and also increased QOL in 12 and 25 weeks. We recommend breast cancer patients for more than 12 weeks TCC to manage symptoms and enhance QOL finally.

TCC is not only active in breast cancer, but also in other cancers. TCC improves vigor in patients with lung cancer undergoing chemotherapy (88). In addition, TCC can alleviate fatigue, enhance neck and shoulder joints mobility, and improves sleep in nasopharyngeal carcinoma patients (89, 90). For cancer survivors undergoing chemotherapy, TCC improves cancer-related fatigue, self-efficacy, and QOL (91).

As an exercise of body and mind, the mechanism of TCC is complex and evidence from basic research is lacking, thus we roughly discuss it. Resting-state functional magnetic resonance images of TCC experts found that TCC practitioners had greater functional homogeneity in the right post-central gyrus (PosCG), and less functional homogeneity in the left anterior cingulate cortex (ACC) and the right dorsal lateral prefrontal cortex (DLPFC) (92). The PosCG affects pain that its partial resection reliefs of severe limb pain (93). The posterior cingulate cortex is activated by visuospatial imagery (94). The caudal ACC is associated with the complex social interactions (95). The left ACC regulates the hypothalamic-pituitary-adrenal (HPA) axis (96). The prefrontal cortex is particularly important for cognitive control, and the DLPFC may reflect the expression of task goals (97, 98). In breast cancer patients, TCC may establish visuospatial imagery of the movements to posterior cingulate cortex, set task goals through the DLPFC, and reduce pain through the ACC. TCC may also mediate the HPA axis (99). The HPA axis plays a key role in suppressing and shaping immune responses (100, 101). Dysregulation of the HPA axis and the increased levels of pro-inflammatory cytokines [interleukin (IL)-6 and tumor necrosis factor (TNF-α)] could produce fatigue (102). TCC decreases cortisol, IL-6 and TNF-α in cancer survivors that might reduce cancer-related fatigue (35, 37, 103). TCC increases oxygen intake that might improve shoulder function and strength of in patients with breast cancer (104).

Limitations were also in this systematic review and meta-analyses. First, TCC interventions had Yang-style, Chen-style, simplified 24-action and some movements of TCC with different session length, weekly frequency and duration. The eligible studies had clinical heterogeneity because of different TCC interventions. Second, treatments such as chemotherapy, radiation therapy, hormone therapy, and surgery for breast cancer unlimited in this review might restrict the activity of patients to complete TCC. Third, the small quantity and low quality of eligible studies in each meta-analysis might produce risk of bias, inconsistency, imprecision, and publication bias. Finally, although main English, Chinese, Japanese, Korean, and Thai databases were searched, some published or gray literatures might have been missed and all the meta-analyses included <10 studies that publication bias might exist.

CONCLUSION

In this systematic review and meta-analysis, TCC had positive effects on QOL, pain, shoulder function, strength of arm, anxiety, and fatigue in breast cancer patients compared with the non-exercise therapy. TCC may have an improvement on QOL, physical function and psychological health in breast cancer patients. Due to the risk of bias in each study, further additional randomized controlled trials with a rigorous methodology and low risk of bias are needed to provide more reliable evidence.

AUTHOR CONTRIBUTIONS

YT designed the study, interpreted data, and wrote the manuscript. X-CL, JL, JF, and H-YY contributed to literature search, figures, data collection, data analysis, data interpretation, and draft manuscript. LS, M-LL, LL, JY, X-LQ, and C-ZT contributed to data analysis and data interpretation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.00607/full#supplementary-material

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The Reliability and Validity of Quality of Life Questionnaire Upper Limb Lymphedema (ULL-27) Turkish Patient With Breast Cancer Related Lymphedema

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Purpose: Breast cancer is the most common cancer amongst women both in Turkey and in the world. Lymphedema, which negatively affects the quality of life, is one of the most prevalent problems reported by breast cancer survivors. Upper Limb Lymphedama 27 (*ULL-27*) questionnaire is a valid and reliable tool that assesses the quality of life in patients with breast cancer-related lymphedema. Until now, a Turkish-language version was lacking. The aim of this study was to perform a cross-cultural validation and reliability of the Turkish version of the ULL-27 questionnaire.

Methods: This cross-sectional study involved forward- backward translation, and cross-cultural adaptation. 81 women (mean age and body mass index 54.96 ± 11.35 years and 29.50 ± 5.74 kg/m^2) who had breast cancer related-upper extremity lymphedema were evaluated using the ULL-27 Quality of life questionnaire-Turkish version. Assessment of limb size was quantified by using circumferential limb measurements. European Organization for Research and Treatment of Cancer (EORTC) 30-item Quality of Life Questionnaire and Quality of Life Questionnaire breast cancer-23 (QLQ-BR23) were analyzed by Pearson's correlation analysis with the ULL-27 Turkish Version to indicate the convergent validity. Cronbach's alpha (internal consistency) and exploratory factor analysis were used to assess the questionnaire's reliability.

Results: The mean of lymphedema duration and severity were 23.12 ± 30.88 months. Mild lymphedema was reported in 42% (34 people) of the cases included in the study. It was observed that 33.3% (27 people) had moderate lymphedema and 24.7% (20 people) had severe lymphedema. The alpha coefficient (internal consistency) for the Turkish *ULL-27* total score was high (alpha = 0.93). Content validity was good because all questions were understandable for all participants (The alpha coefficient for the subgroups of the scale of physical, psychological, social scores, were 0.90, 0.87, and 0.75, respectively). External construct validity was highly confirmed by expected correlations with comparator scales, EORTC-30, and QLQ-BR23 ($p < 0.01$).

Conclusions: The Turkish version of the ULL-27 Questionnaire is a valid and reliable tool for evaluating QoL in women with upper limb lymphedema related to breast cancer.

Keywords: ULL-27 quality of life questionnaire, breast cancer, lymphedema, quality of life, eortc30

INTRODUCTION

Breast cancer is still the most common type of cancer among women in the world (1). Its incidence rates have been increasing mostly in developing countries, including Turkey (2). But breast cancer survival rates have also increased worldwide. The recent decline in breast cancer mortality in many countries might be due to early diagnosis and improved treatment protocols (3, 4). Among the many symptoms, lymphedema is one of the most common side effects of breast cancer treatment.

A recent meta-analysis of women with breast cancer, the lymphedema rate was 21.4%. The risk of developing lymphedema is especially high during the first two years of the surgery (5). Many sources indicate the likelihood of lymphedema development between 2 and 50% (6–9). Lymphedema is a chronic and progressive condition resulting from an abnormality of, or damage to, the lymphatic system. Any reduction in the capacity of the lymphatic system to drain fluid from the interstitium and return it to the blood circulation will cause fluid to build up in the skin and subcutaneous tissues of the affected part of the body. It is known to negatively affect the quality of life (QoL) in breast cancer survivors due to limb swelling, heaviness, pain, pitting of skin, tightness or hardness in the limb, inflammation, and reduced mobility in the shoulder and arm (10–14).

There is a widespread awareness among researchers on the importance of assessing the specific quality of life related to lymphedema. On the other hand, very few specific questionnaires have been developed on upper extremity lymphedema. Upper Limb Lymphedema 27 (ULL-27), introduced by Launois et al. (15) is a scale that can describe all symptoms in one form, can provide a holistic approach, is easy to use, and can evaluate their ability to perform common functional activities in patients with Breast Cancer Related Lymphedema (BCRL). However, The ULL-27 has been validated in very few countries. Therefore, the aims of this study were: (1) to perform a translation and cross-cultural adaptation of the ULL-27 among patients with breast cancer related-upper extremity lymphedema, to investigate the scale's validity, and to conduct exploratory factor analysis (confirmatory factor analysis has been done previously in other languages) with responsiveness within a Turkish-speaking population sample; and (2) to assess quality of life in Turkish patients with breast cancer related-upper extremity lymphedema.

MATERIALS AND METHODS

Study Design and Participants

This study was performed on 81 women who had developed upper extremity lymphedema after breast cancer treatment. Participants who were referred to Dokuz Eylul University (DEU) Hospital, Department of Medical Oncology in Izmir, Turkey between June 2016 and May 2017 were assessed in the School of Physical Therapy. All participants were informed about the purpose and the procedures of the study and signed an informed consent form according to guidelines approved by the university hospital ethical committee. Ethical protocol number was 2543-GOA and decision number was 2016/07-23.

To meet the inclusion criteria, patients had to: (a) be aged 18 and over; (b) have received no local and systemic treatment (colorectal surgery, chemotherapy, radiotherapy) in the last 6 months; (c) able to read, write, and understand Turkish; (d) have mild–moderate-severe degreed lymphedema; (e) be willing and able to attend the study. Women were ruled ineligible according to the following exclusion criteria: malignant lymphedema; recurrent cancer or infection in the arms; severe disorders related to cognition, muscles, or joints.

Assessment

A complete medical history was obtained from each participant, including demographic information (i.e., age, gender, height, weight, body mass index [BMI], occupation, dominant hand, and affected hand) and disease characteristics (i.e., type and side). In addition, the type of operation, the number of excised lymph nodes, radiotherapy session received, other treatments, lymphedema duration, and previous infection attacks were also recorded.

Circumferential Measurement

Edema was assessed by circumferential measurement (CM). CM were taken with participants in a supine position and the arm abducted at 30°C. The circumference of both limbs was measured every 5 cm, starting at the nail bottom of 3rd fingers and continuing 50 cm proximally. The difference between both arms were recorded in cm. All patients were evaluated with the same standard tape measure (150 cm length, 7 mm width). The severity of the edema was done according to the criteria set by the American Physical Therapy Association. According to this, the difference between both limbs is slightly less than 3 cm, the middle 3–5 cm, anything over 5 cm was recorded as severe lymphedema (16).

Design

This cross-sectional methodological study involved translation, back translation, and cross-cultural adaptation, that is, localization. To assess the questionnaire's reliability, Cronbach's alpha (for internal consistency) and exploratory factor analysis were conducted. To indicate the convergent validity, Pearson's correlation analysis was performed with the European Organization for Research and Treatment of Cancer Quality of Life Cancer module (EORTC QLQ-C30) and the European Organization for Research and Treatment of Cancer Quality

of Life—Breast Cancer Module (EORTC BR-23) for which reliability and validity studies have been conducted in the Turkish-speaking population.

Women participating in the research were evaluated by the same researchers; information was given about the purpose and methods of the study. All measurements were carried out face to face with the participants. All evaluations lasted about 45-60 min.

Quality of Life

EORTC QLQ-C30, EORTC BR-23 and ULL-27 were used to measure QoL.

The EORTC QLQ-C30 is composed of 30 items assessing global perceived health status and QoL (QL2). These items are grouped into five functional scales (physical-PF2, role-RF2, cognitive-CF, emotional-EF, and social functioning-SF); three symptom scales (fatigue-FA, nausea & vomiting-NV, and pain-FA); six single item scales—dyspnea-DY, insomnia-SL, appetite loss-AP, constipation-CO, diarrhea-DI, and financial difficulties-FI (17).

QLQ-BR23 has 23 items to assess functional scales (Body Image-BRBI, Sexual Functioning-BRSEF, Sexual Enjoyment-BRSEE, and Future Perspective-BRFU); symptom scales (systemic therapy side effects -BRST, breast symptoms -BRBS, arm symptoms -BRAS, and upset by hair loss-BRHL) (17).

The QLQ scores vary from 0 (worst) to 100 (best) for the functional and global health status (GHS) parameters and from 0 (best) to 100 (worst) for symptoms parameters. A five-point difference in QoL scores is considered the minimum clinically significant difference. Both questionnaires were cross-culturally adapted to Turkish by Demirci et al. (18).

The original ULL-27 was created by Launois et al. (15). It is a questionnaire that evaluates the quality of life in three dimensions in subjects with upper limb lymphedema. The scale consists of 27 questions with physical, psychological, and social dimensions. 5-point Likert scoring scale (1 = strongly disagree, 5 = strongly agree) is used. The first 15 questions are on the physical dimension (min 15 and max 75 points), the questions between 16 and 22 on social dimension (min 7 and max 35 points), and the questions between 23 and 27 evaluate the social dimension (min 5 and max 25 points) of the individual. The total score of 27 questions is calculated for the global score. The lowest score is 27 and the highest score is 135 points. The high score of the scale shows that it affects the quality of life of the individual badly (15).

Convergent Validity

Convergent validity is the principle that measures theoretically similar constructs that should be highly intercorrelated. The convergent validity of two similar constructs can be estimated using correlation coefficients. To test the hypothesis for convergent validity for the ULL-27, we used the EORTC QLQ-C30, QLQ-BR23. Convergent validity in subjects with upper extremity lymphedema after breast cancer treatment was evaluated by investigating correlations between the scale's psychometric parameters and the commonly used assessments EORTC QLQ-C30, QLQ-BR23.

Translational and Cross-Cultural Adaptation

The process of translation and cross-cultural adaptation, that is, localization, was carried out according to Beaton's guidelines (19, 20).

(a) Translation into Turkish: the ULL-27 was translated from English into Turkish in accordance with Newmark's concept of "communicative translation" to achieve a dynamic equivalence between the source and target texts. "Communicative translation attempts to produce in its readers an effect as close as possible to that obtained on the readers of the original." The text was independently translated by two native Turkish speakers, one of whom was a linguist and the other a health care professional who knew English as a second language. Finally, both target texts were compared for equivalent effect, and a single version was agreed upon. (b) Back translation into English: two bilingual translators with English as a first language back translated the agreed Turkish version into English taking into account cultural adaptation, that is, the localization process. They compared the two versions and agreed on a single version. (c) Review committee: the final version was submitted to a bilingual committee consisting of clinicians and translators. The text was checked for semantic and idiomatic equivalence acceptable for dynamic equivalence. Step 3 ended with a final approval. (d) Test of the prefinal version: the prefinal version was sent to the authors of the original form, and their comments were taken into consideration. Then, the final version was piloted with 15 women by testing what was meant by each item and response chosen in order to verify whether the formulation of the item was clear or not. All of the findings were reevaluated by the expert committee. Finally, the back translation of the scale was approved by the author who composed the original form.

Statistical Analysis

Data analysis was made with Statistical Package for Social Science (SPSS) version 20. All categorical data frequency and percentage were calculated. Descriptive statistics on the demographics of patients were used to show information about cancer and lymphedema. Statistical significance level was regarded as 0.05 for all tests. Confirmatory factor analysis was used to test the construct validity of the questionnaire. In light of the assumptions set forth in the multiple regression analysis to examine a dependent variable, Path analysis was performed on all arguments. Quality of life survey to measure the reliability and Cronbach's alpha coefficients were calculated to measure the internal consistency. Cronbach's alpha was determined to be an acceptable level of reliability above 0.7. A poll of the *Kolmogorov-Smirnov test* to measure compliance that conforms to a normal distribution was made. Three major scores of the questionnaire (physical, psychological, and social) and the correlation between the content in question was examined by *Spearman correlation* test. ULL27 life-selected EORTC QLQ-C30 and QLQ-BR23 questionnaires quality of parallel survey evaluated concurrent validity by calculating the *Pearson correlation.*

Ethical Considerations

The study was approved by the local University Medical Ethics Committee, and the patients gave their written informed consent to take part in the research prior to the study. R. Launois, the creator of the original ULL-27, was asked for permission to apply the scale in a convergent validity study for the Turkish language. In addition, during the ethical considerations, the Head of the Dokuz Eylul University Faculty of Medicine, Department of Oncology, approved the study to be held in their department.

RESULTS

Patients' compliance during evaluation was good. The EORT-C30 and BR23 questionnaires were handed out to the patients and they were requested to fill in the forms. Eighty-one patients diagnosed BCRL with a mean age of 54.96 ± 11.35 years were enrolled in the study. Demographic and clinical data related to the patients are given in **Table 1**.

TABLE 1 | Demographic characteristics of the participants ($n = 81$).

Age (years) (X ± SD)	54.96 ± 11.35
BMI (kg/m^2) (X ± SD)	29.50 ± 5.74
Waist circumference (cm) (X ± SD)	95.39 ± 10.50
Hip circumference (cm) (X ± SD)	109.73 ± 10.34
Occupation (%)	
Housewife	59.3
Worker	24.7
Retired	16
Dominant arm (%)	
Right	92.6
Left	7.4
Effected arm (%)	
Right	43.2
Left	56.8
First observed part of lymphedema in arm (%)	
Hand	21
Forearm	19.8
Upper arm	29.6
Severity of lymphedema (%)	
Mild	42
Moderate	33.3
Severe	24.7
Type of Operation (%)	
Lumpectomy	47
Total mastectomy	53
Treatments (%)	
ET+CT+RT	41.98
CT+RT	44.44
RT	13.58
Lymph nodes removed (number) (X ± SD)	15.94 ± 8.36
History of recurrent lymphangitis (%)	
Yes	24.7
No	75.3
Duration of lymphedema (months) (X ± SD)	23.12 ± 30.88

SD, Standard Deviation; BMI, Body Mass Index; ET, endocrine therapy; CT, chemotherapy; RT, radiotherapy.

Reliability of ULL-27 Questionnaire

The reliability of the scale, internal consistency, and item scores were investigated in terms of correlation and invariance. ULL-27 internal consistency of the quality of life questionnaire (reliability) was assessed by Cronbach's alpha score. Analysis of the internal consistency of all cases related to the scale of its response to the ULL-27 quality of life questionnaire was out of the total score. Croncbach alpha coefficient of *0.93* was found. Subgroups of the scale of physical scores had an alpha coefficient of *0.90*, psychological *0.87,* and social score *0.75* identified. Accordingly, the survey revealed that the degree of internal consistency was good. According to this model, when we look at the reliability analysis, all questions are consistent and valid for the Turkish people, without removing any items from the original survey (**Table 2**). Agent scale correlation (inter correlation) was rated on the same answer and substance-test are displayed by calculating the correlation coefficient. The obtained substance-test coefficients of correlation $r = 0.43$ *and* $r = 0.87$ was found to take values from **Table 3**. Test-retest analysis scale was determined by inter class correlation method. The test was applied twice to the last 15 patients at 20-day intervals. In both applications, the reliability coefficient taken according to the total scores was $r = 0.40$ ($p > 0.05$). Calculating a consistency coefficient, item-correlation coefficients and the number of test-retest times were used to show statistical significance. These results scale internal consistency, substance-test shows that the correlation coefficient is high and test-retest reliability examined for the test.

ULL-27 Validity of Questionnaire

The validity of the ULL-27; parallel forms (concurrent) were analyzed in two ways: validity and construct validity. ULL-27 was used in order to determine the construct validity of the questionnaire survey according to the applied confirmatory factor analysis. It was first seen in the value of RMSEA confirmatory factor analysis. The RMSEA value of our study was found to be 0.074. According to the Path diagram, the first 15 questions were on the physical score in the Turkish version, which gives the item distribution as in the original form of the UL-27 quality of life questionnaire. Questions between 16 and 22 give the psychological score and questions between 23-27 give the social score ($x^2 = 463.20$) ($p = 0.000$) (**Table 4**, **Figure 1**). Whether the relationship between the variables-assumed absence model that the difference *Comperative Fit Index (CFI)* according to close to the minimum *(0.97)* and *Incremental Fit Index (IFI)* based on "acceptable harmony" *(0.97)* was detected. Goodness of Fit Index measured the sample covariance matrix of the model *(GFI),* what is viewed as "acceptable harmony" was determined to be in the group (0.96). With the ULL-27, scoring a minimum of 0 (27) and a maximum of 100 (135) points formula used to be;$(total\ score - minscore)/(max\ score - min\ score)\ x100$

Accordingly, 81 individuals participated in the study and the ULL-27 global score for quality of life was found to be 42.54 ± 19.71 (**Table 5**). ULL-27 quality of life questionnaire of

TABLE 2 | ULL-27 quality of life questionnaire of physical, psychological, social, and global measures of reliability test.

	Cronbach's alpha	Number of questions
Physical Score	0.90	15
Psychological Score	0.87	7
Social Score	0.75	5
Global Score	0.93	27

TABLE 3 | Reliability of each question in ULL-27.

	Mean ± SD	α	*r*
Difficulties grasping high objects	3.17 ± 1.31	0.90	0.47
Difficulties maintaining certain positions	3.07 ± 1.33	0.90	0.46
Arm feels heavy	3.30 ± 1.38	0.89	0.69
Arm feels swollen	3.54 ± 1.30	0.90	0.57
Difficulties getting dressed	2.72 ± 1.32	0.89	0.74
Having trouble getting to sleep	2.74 ± 1.38	0.90	0.45
Having trouble sleeping	2.81 ± 1.33	0.90	0.50
Difficulties grasping objects	2.75 ± 1.26	0.89	0.68
Difficulties holding objects	2.96 ± 1.35	0.90	0.64
Difficulties walking heavy arm	2.53 ± 1.33	0.89	0.77
Difficulties washing	2.32 ± 1.30	0.89	0.73
Difficulties taking public transport	2.49 ± 1.34	0.89	0.68
Tingling, burning feelings	2.67 ± 1.36	0.90	0.43
Feelings of swollen, hard, tense skin	3.15 ± 1.33	0.90	0.53
Difficulties in working relationship and tasks	2.75 ± 1.19	0.90	0.60
Feeling sad	2.61 ± 1.25	0.73	0.73
Feeling discouraged	2.41 ± 1.25	0.87	0.87
Feeling lack of self-confidence	2.45 ± 1.27	0.67	0.67
Feeling distressed	2.79 ± 1.22	0.81	0.81
Feeling well in oneself	2.82 ± 1.14	0.26	0.26
Feeling a wish to be angry	2.50 ± 1.25	0.54	0.54
Having confidence in the future	2.61 ± 1.30	0.74	0.74
Difficulties taking advantage of good weather, in life outside the house	2.36 ± 1.31	0.55	0.55
Difficulty with personal projects holidays and hobbies	2.89 ± 1.32	0.60	0.60
Difficulties in emotional life with spouse or partner	2.33 ± 1.11	0.53	0.53
Difficulty in social life	2.63 ± 1.13	0.55	0.55
Fearful of looking in a mirror	1.52 ± 0.838	0.34	0.34

TABLE 4 | ULL-27 Quality of Life questionnaire indices of confirmatory factor analysis.

Index	RMSEA	CFI	IFI	GFI
ULL-27 Life Quality Questionnaire	0.074	0.97	0.97	0.96

RMSEA Index (Root Mean Square Error of Approximation), CFI (Comperative Fit Index) and IFI (Incremental Fit Index).

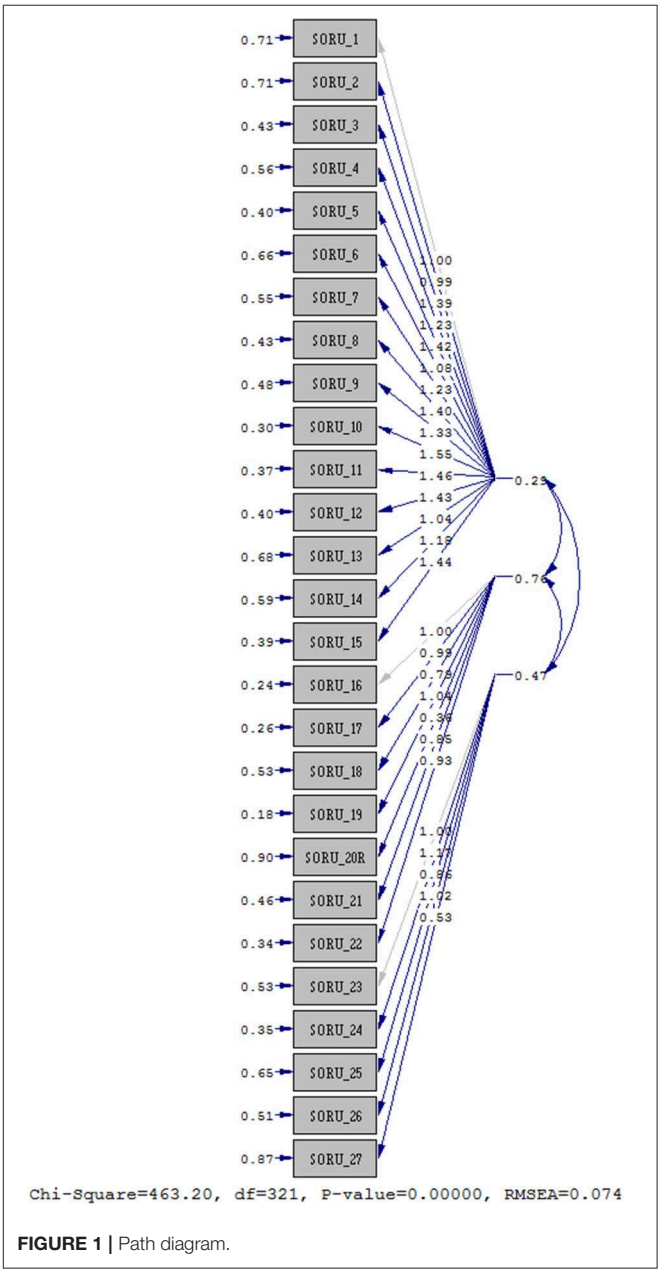


FIGURE 1 | Path diagram.

the *Kolmogorov-Smirnov and Shapiro-Wilk test* was performed to examine whether they fit a normal distribution. The test does not conform to a normal distribution ($p < 0.05$). ULL-27 questionnaire of physical, psychological, social, and global relationship between the score and the questions were analyzed with Pearson's correlation coefficient. It was found to be statistically significant in itself ($p < 0.01$) ($p < 0.05$). With the Turkish version of ULL-27 quality of life questionnaire, a parallel score was found within the scope of EORTC-QLQ-C30 and BR23 related validity. The correlation between the vertex of all cases of this survey were analyzed by Spearman correlation coefficient. Accordingly, the global score of the ULL-27 questionnaire and the correlation coefficient between BR23 and C30 and the scores of the scale were found to be significantly similar ($p < 0.05$) (**Table 5**). A statistically significant difference was found between the psychological items of the ULL-27 questionnaire between

TABLE 5 | Correlations between ULL-27 and EORT QLQ C30; BR-23 parameters.

	Physical score (46.64 ± 21.90)	Psychological score (40.12 ± 23.65)	Social score (33.64 ± 20.52)	Global score (42.54 ± 19.71)
QL2	−0.208	**−0.472****	**−0.324****	**−0.337****
(60.79 ± 18.93)	0.062	**0.000**	**0.003**	**0.002**
PF2	**−0.564****	**−0.333****	**−0.459****	**−0.546****
(62.88 ± 21.51)	**0.000**	**0.002**	**0.000**	**0.000**
RF2	**−0.410****	**−0.349****	**−0.449****	**−0.437****
(63.87 ± 31.94)	**0.000**	**0.001**	**0.000**	**0.000**
EF	−0.177	**−0.526****	**−0.362****	**−0.328****
(70.23 ± 24.88)	0.115	**0.000**	**0.001**	**0.003**
CF	−0.101	**−0.323****	**−0.269***	−0.204
(69.95 ± 24.91)	0.372	**0.003**	**0.015**	0.068
SF	−0.151	**−0.266***	**−0.297****	**−0.221***
(75.71 ± 26.88)	0.178	**0.016**	**0.007**	**0.047**
FA	**0.362****	**0.356****	**0.421****	**0.417****
(44.57 ± 26.43)	**0.001**	**0.001**	**0.000**	**0.000**
NV	**0.347****	**0.364****	**0.406****	**0.369****
(12.75 ± 24.33)	**0.001**	**0.001**	**0.000**	**0.000**
PA	**0.450****	**0.417****	**0.519****	**0.503****
(40.32 ± 28.11)	**0.000**	**0.000**	**0.000**	**0.000**
DY	**0.384****	**0.297****	**0.343****	**0.403****
(23.24 ± 29.77)	**0.000**	**0.007**	**0.002**	**0.000**
SL	**0.398****	**0.465****	**0.488****	**0.467****
(37.72 ± 33.58)	**0.000**	**0.000**	**0.000**	**0.000**
AP	**0.266***	**0.402****	**0.379****	**0.355****
(8.64 ± 18.08)	**0.016**	**0.000**	**0.000**	**0.001**
CO	0.081	**0.244***	0.170	0.149
(24.48 ± 31.07)	0.474	**0.028**	0.130	0.186
DI	0.147	0.212	**0.221***	0.207
(11.52 ± 25.90)	0.189	0.057	**0.047**	0.064
FI	0.122	**0.273***	0.161	0.177
(22.83 ± 29.39)	0.277	**0.014**	0.152	0.113
BRBI	−0.183	**−0.321****	**−0.239***	**−0.248***
(71.19 ± 25.10)	0.102	**0.004**	**0.032**	**0.025**
BRSEF	0.065	0.019	0.081	0.068
(81.89 ± 24.60)	0.566	0.866	0.472	0.545
BRSEE	0.045	0.015	0.088	0.049
(78.59 ± 30.41)	0.688	0.891	0.433	0.662
BRFU	**−0.241***	**−0.423****	**−0.351****	**−0.343****
(48.13 ± 30.27)	**0.030**	**0.000**	**0.001**	**0.002**
BRST	**0.268***	**0.381****	**0.389****	**0.348****
(28.59 ± 18.12)	**0.015**	**0.000**	**0.000**	**0.001**
BRBS	**0.228***	0.202	0.215	0.225*
(29.82 ± 24.11)	**0.041**	0.071	0.054	0.044
BRAS	0.606**	0.313**	0.504**	0.562**
(49.84 ± 25.52)	0.000	0.004	0.000	0.000
BRHL	0.176	0.064	0.012	0.122
(15.21 ± 28.87)	0.115	0.569	0.918	0.278

***p < 0.001, *p < 0.005.*

physical-PF2, role-RF2, cognitive-CF, emotional-EF, social functioning-SF, fatigue-FA, nausea & vomiting-NV, pain-FA, dyspnea-DY, insomnia-SL, appetite loss-AP, constipation-CO, diarrhea-DI, financial difficulties-FI, Body Image-BRBI, Sexual Functioning-BRSEF, Sexual Enjoyment-BRSEE, Future Perspective-BRFU, systemic therapy side effects -BRST, breast symptoms -BRBS, arm symptoms –BRAS, upset by hair loss-BRHL.

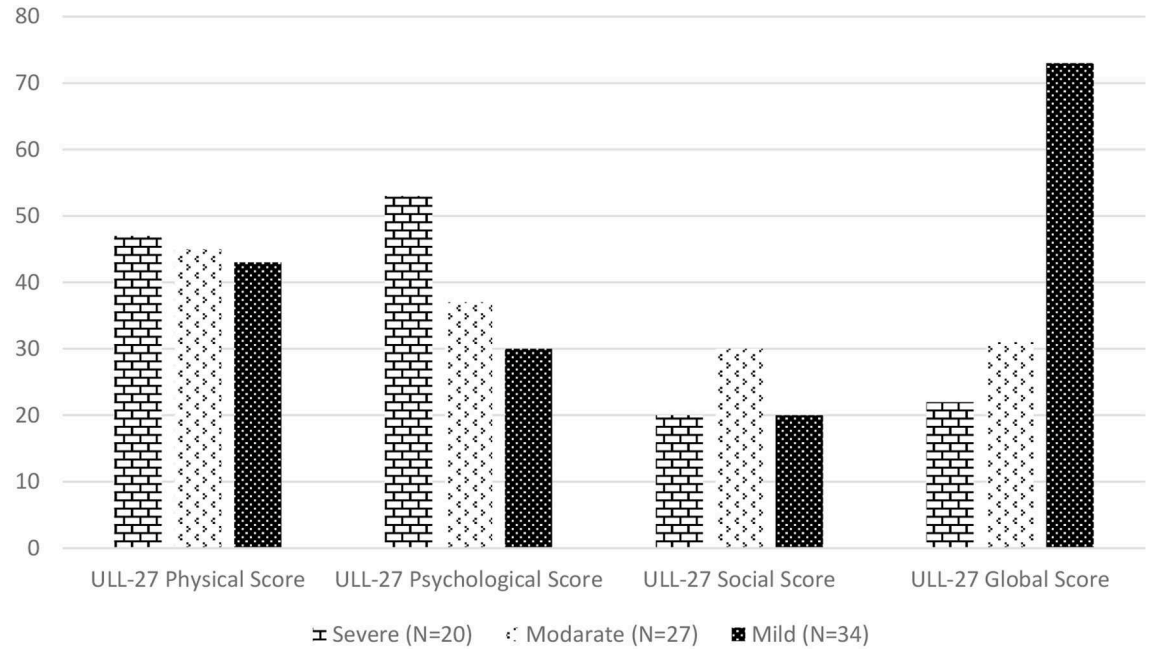
The values in the table show that the ones with negative sign are negative meaningful and those without sign have positive meaning.

diarrhea, sexual function, sexual pleasure, breast symptoms, and sadness that caused hair loss ($p < 0.05$). Shortly, we found that symptom scores worsened as the severity of lymphedema increased. Accordingly, we saw that the quality of life decreased (**Graph 1**). We have demonstrated that the Turkish version of the ULL-27 quality of life questionnaire we evaluated was a valid and reliable test battery for the use of patients to evaluate the condition.

DISCUSSION

After breast cancer treatment approaches, people face several problems. These problems affect the quality of life of the individual. Lymphedema is one of these problems. Therefore, it is important to measure the degree to which the quality of life in people with BCRL is affected. In our study, we assessed reliability and validity of the Turkish version of ULL-27 in the upper limb lymphedema. The ULL-27 was found to be a valid and reliable measure in Turkish patients with BCRL. Previous studies stated that upper limb lymphedema effects patient's lives in different ways and there were many symptoms which were specific like heaviness and swollen limbs. The SF-36, EORTC QLQ C-30, and BR-23 are the most preferable scales for patients having upper limb lymphedema. Lymphedema specific questionnaires such as Lymph ICF, LyQLI, and LYMQOL have started to be used to assess patients by professionals (21–23). EORT QLQ C-30 and BR-23 are the most frequently used parameters to assess the disease-specific quality of life in people who have had breast cancer in Turkey. So, EORT QLQ C-30, BR-23, and ULL-27 was the main assessment parameters in this study. The EORT QLQ C-30 involved all breast cancer symptoms, and only four out of nine specific questions were about arm symptoms (18, 24, 25). Its manual scoring take long time, whereas ULL-27 is only about upper limb lymphedema symptoms and quickly calculates the score. Pusic et al. showed that according to COSMIN criteria the ULL-27 was the only scale that could be used with patients that left no doubt on the results (18). ULL-27 physical, psychological, and social scores of Cronbach's alpha values were supported by Launois and Viehoff. Launois et al. were calculated in the same way (15). Similarly, Viehoff et al. reported that the Dutch version of the questionnaire was a valid and reliable study, Cronbach's alpha values were found to be close (26). Our values showed parallel values. In this study we found physical, psychological, and social Cronbach's alpha values that were relatively high. Global score Croncbach's alpha was found by calculating the high reliability of the questionnaire. ULL-27 and EORT QLQ C-30, BR-23 sub parameters were found to be highly correlated. In order to be able to compare our results with those of the original questionnaire, the tests were performed in a similar attitude. A factor analysis was done with RMSEA, Comparative Fit Index (CFI), and Incremental Fit Index (IFI). Goodness of Fit Index (GFI) was evaluated for covariance and we found that ULL-27 questionnaire is suitable for Turkish BCRL patients. Structural equation of the questionnaire showed high adaptation.

Physical scores of individuals (ULL-27) was found to be an average. We considered this a score that increases an individual's



GRAPH 1 | According to lymphedema severity quality of life scores of ULL-27.

quality of life deteriorated. The EORTC C30 is consistent with the scores of the quality of life, physical function, role function, emotional function, cognitive function and social function parameters and the physical score of the ULL 27 quality of life questionnaire. The higher the ULL-27 quality of life score, the higher the other parameters. According to the analysis of ULL-27 in individuals with high physical score points, we saw a low score of the role and function scale in EORT C-30. We have seen that pain, weakness, nausea-vomiting, insomnia, dyspnea, anorexia, constipation, diarrhea, and financial parameters decrease the quality of life. We found that when individuals' ULL-27 physical score increased, fatigue, nausea, vomiting, pain, shortness of breath, insomnia, and loss of appetite worsened. We have also seen that high ULL-27 physical score has a negative impact on BR-23 body image, sexual function, sexual satisfaction, and future opinion parameters. Likewise, we found that patients with the highest physical score had higher breast and arm symptoms.

Psychological and social dimensions also affect individuals' quality of life. The high points of sexual function and sexual pleasure have a negative impact on an individual's quality of life. Our study is high in these two parameters. We observed that the psychological score of ULL-27 worsened as the hair loss symptom score increased. One of the side effects of chemotherapy is hair loss. Although time has passed, this causes us to think that the effect of this situation continues. In this study, we found that the physical and cosmetic effects of treatments generally affect the social and psychological state of those with BCRL.

One study limitation was that there were not enough participants. This lack of participants might have affected the results of our study. One strength of our work was that all patients were women. Lymphedema after breast cancer in women is very high so we think that our results are close to the general population.

In conclusion, the ULL-27 questionnaire seems to be a reliable and valid scale for assessing the quality of life in Turkish upper limb lymphedema patients. It is available for use in clinical practice and research.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This article does not contain any studies involving animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

AUTHOR'S NOTE

The ULL27 was developed by Professor Robert Launois with an educational grant from REES France. Any person who wishes to use the questionnaire should contact Professor Robert Launois (reesfrance@wanadoo.fr).

AUTHOR CONTRIBUTIONS

AK and DK conceived of the presented idea. TY developed the theory and performed the computations. AK and DK verified the analytical methods. DK encouraged AK to investigate (a specific aspect) and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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MINI REVIEW
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Breast Cancer Survivorship, Quality of Life, and Late Toxicities

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Breast cancer is the most frequent cancer in women: in 2018, almost two million cases have been diagnosed all over the world and it represents the principal cause of death from a neoplastic disease in women. In the past years, breast cancer prognosis has significantly improved over time: currently 5-year survival rates are in the range of 90%, and 10-year survival is about 80%. This improvement has been mostly observed in western countries, due to high coverage and compliance with screening programs, leading to early diagnosis, i.e., when the disease is at a subclinical level, and to an improvement in tumor molecular characterization and innovative systemic treatments. Yet the identification of different biological breast cancer subtypes prompted the development of innovative targeted agents and improved treatment personalization. On the other hand, longer survival rates and increasing proportions of cured patients require dedicated strategies to manage long-term sequelae of breast cancer treatments, with particular attention to quality of life. This review analyzes the most important issues, potentially occurring with cancer treatments, concerning long-term sequelae and quality of life, to define a global approach to breast cancer survivorship.

Keywords: adjuvant therapy (AT), osteoporosis, cardiotoxicity, fertility, lifestyle

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BACKGROUND

It has been estimated that in the twenty-first century, cancer will be the most frequent cause of death in western countries: in fact, according to the 2015 World Health Organization (WHO) statement, cancer accounts for the most important cause of death before the age of 70, in a great proportion of countries (1).

The most frequently diagnosed neoplasm is lung cancer (12% overall), and it also represents the first cause of death by cancer (18%); in women, breast cancer is the most diagnosed one and the most represented cause of death. In 2018, almost two million breast cancer cases have been diagnosed in women (**Figures 1**, **2**), with one out of four cancer cases due to breast cancer (2).

In 2019, in Italy, 53,000 new cases of breast cancer have been reported, representing the most commonly diagnosed cancer in women: it can be estimated that about one in three cancer cases in women is represented by breast cancer. Overall, there are more than 800,000 women who have been diagnosed with breast cancer, accounting for 44% of all women living with a previous breast cancer diagnosis.

Even in 2016, breast cancer represented the major oncological reason of death in women, with more than 12,000 deaths (3). It also is the primary cause of mortality among all ages, 28% before 50 years, 21% between 50 and 69 years, and 14% after 70 years. The overall 5-year survival in Italy is in the range of 87%, without significant differences by age or region; 10-year overall survival is about 80% (4).

The recent improvements in breast cancer prognosis are mainly due to two distinct factors:

(1) Early diagnosis, i.e., when the disease is at a subclinical level, and (2) improvements in treatment personalization. The availability of large-scale population screening programs, by routine mammography, produced a significant reduction in breast cancer–related mortality in western countries; a decline in breast cancer mortality, together with an increased incidence, was recently noticed in Italy as well (5). Even if efficacy of screening mammography is still debated, usefulness of structured population programs was demonstrated by several studies (5, 6), such as the importance of an early-stage diagnosis (7). Parallel to the increased compliance to screening programs, innovative technological assays allowed an improved subtype classification that allowed innovative targeted therapies, in endocrine-sensitive and HER2-positive diseases.

It has been estimated that in 2014, 14,000 metastatic breast cancers were diagnosed in Italy, accounting for an actual prevalence in the range of 37,000 cases (8). Today, the median overall survival in patients with HER2-positive metastatic breast cancer is the range of 50 months (9–11); the recent introduction of CDK 4/6 inhibitors in the clinic also significantly improved survival in ER+ patients (12, 13). These data indicate that even in the case of metastatic disease, in a large proportion of women, breast cancer may be considered a chronic disease with an increasing proportion of long-term survivors, which can be estimated in the range of 70%.

As a consequence, there is an increasing need to develop strategies to manage breast cancer survivors with dedicated resources.

In particular, there is an urgent need to systematically approach the possible sequelae of medical treatments emerging with longer follow-up.

These include late occurring toxicities, such as chemotherapy-induced toxicity, fertility preservation in pre-menopausal women, endocrine-related bone health, and quality of life. Finally and not strictly related to toxicity sequelae or quality of life, a global approach to lifestyle interventions in breast cancer survivors, should be implemented.

CARDIOTOXICITY AFTER ADJUVANT THERAPY

Nowadays, breast cancer prognosis, whether early or advanced setting, has improved noticeably. This is in part due to the availability and large-scale use of new treatment solutions (14). However, some of these agents may cause short-term and long-term side effects that can sometime be life threatening. One of the most important side effects of breast cancer adjuvant treatments is cardiac toxicity. In particular, late cardiotoxicity might occur years after the administration of adjuvant therapies and is mainly related to the use of adjuvant anthracyclines and trastuzumab; endocrine therapy (12) and chest wall radiotherapy, especially when left breast is involved, can also have an impact on cardiac toxicity. Cardiotoxicity is mainly due to a direct effect on cardiomyocytes, leading to cell death and permanent or transient left ventricular ejection fraction reduction, resulting in symptomatic congestive heart failure in some cases (15). It is noteworthy to remember that cardiotoxicity induced by breast cancer treatments also includes vascular disorders, arrhythmias, and ischemia (16, 17).

For these reasons, the assessment of baseline risk of potential cardiotoxicity is really crucial before starting treatment. It is important to investigate and check the existence of risk factors related to lifestyle habits (smoking, alcohol intake, obesity, sedentary attitude), demographic features (age, family history, hypertension, diabetes mellitus, hypercholesterolemia), previous cardiotoxic therapy, or any event related to heart disease (heart failure, asymptomatic left ventricular systolic dysfunction, cardiomyopathy, or coronary artery disease). In fact, their presence can increase the risk of symptomatic cardiac dysfunction (18).

Heart examinations during follow-up are recommended at definite intervals after adjuvant anthracyclines and trastuzumab, although no clear indication on how long this approach should be maintained is provided.

OVARIAN FAILURE AND FERTILITY PRESERVATION

Among mid-term toxicities with a strong impact on quality of life, fertility impairment is one of the most important factors in younger patients who are candidates for systemic adjuvant therapies. This issue is of particular importance due to the substantial increase in the incidence of breast cancer in European women in their 20s and 30s (19). According to individual disease characteristics, a relevant proportion of these young women will require adjuvant treatments, including chemotherapy, and will receive drugs associated with different magnitudes of gonadotoxicity. Increased risk of premature ovarian failure is mainly related to the use of alkylating agents such as cyclophosphamide, while anthracyclines and taxanes have shown an intermediate risk. Methotrexate and 5-fluorouracil have been associated with a low risk of ovarian damage (20). As a consequence, fertility preservation techniques should be discussed with all young women requiring adjuvant chemotherapy. In this setting, the following options are available: oocyte cryopreservation, embryo cryopreservation, ovarian tissue cryopreservation, and ovarian suppression mediated by gonadotropin releasing hormone analogs (GnRHa).

Cryopreservation of embryos and oocytes is considered the standard approach and is currently recommended by consensus of experts and international literature (21). In the past years, embryo cryopreservation represented the

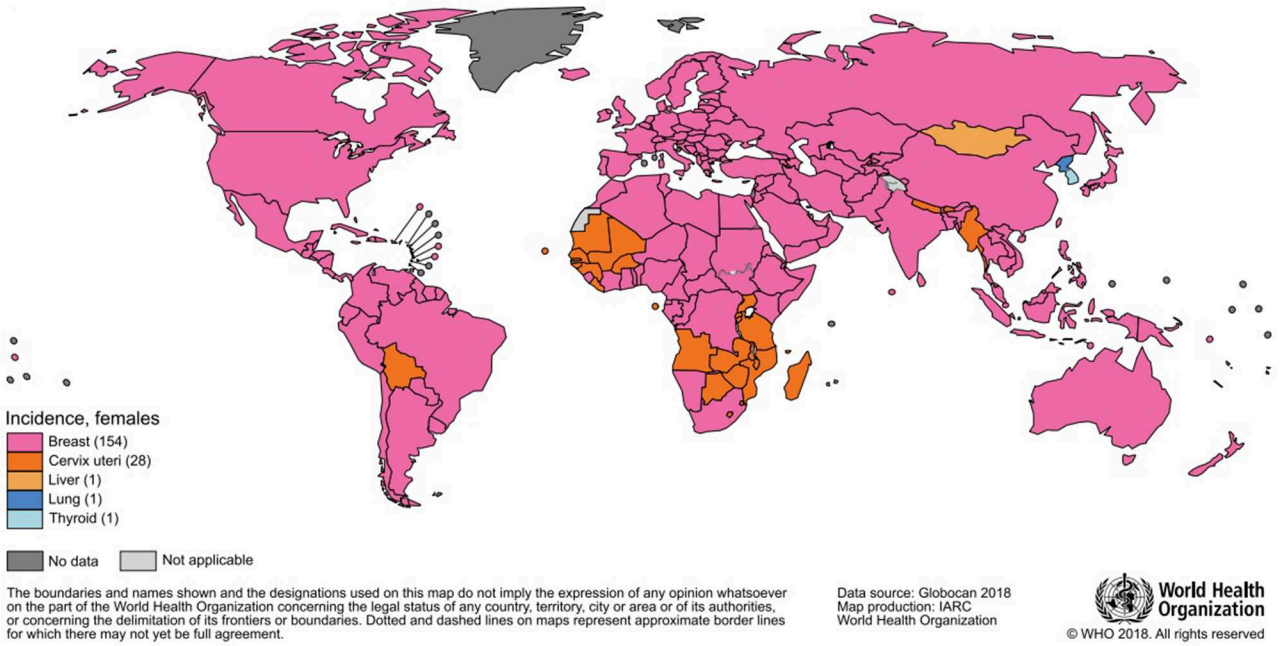

FIGURE 1 | Most common type of cancer incidence in 2018 among women.

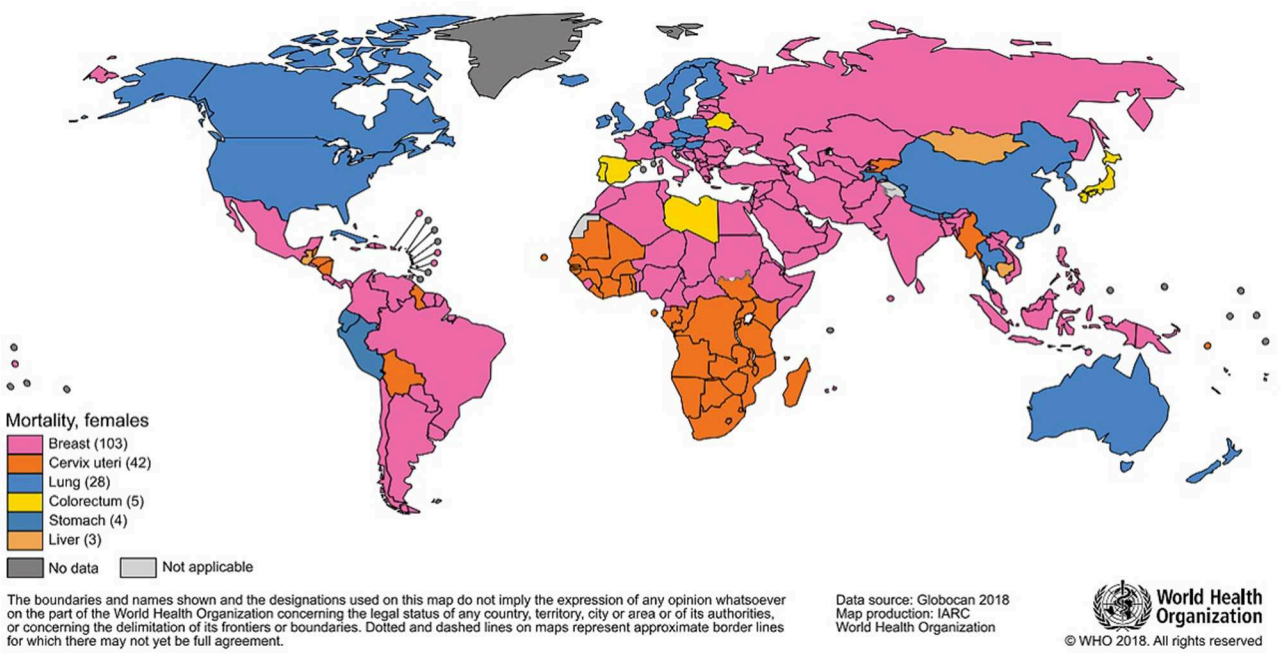

FIGURE 2 | Most common type of cancer mortality in 2018 among women.

most widely approved procedure for fertility preservation; however, since 2013, cryopreservation of oocytes is no longer considered experimental and is currently recommended to the majority of young women (22). The most important benefit of oocyte cryopreservation over embryo cryopreservation is the potential use in patients without a partner and feasibility in countries where embryo cryopreservation is not allowed.

Ovarian tissue cryopreservation is an experimental method of freezing and transplantation. Ovarian tissue can be stored as entire ovary, fragments of ovarian cortex, or isolated follicles; however, when the tissue is re-implanted, concerns have been

raised on the potential hypoxia-induced damage, leading to loss of primordial follicles and increased risk of implanting malignant cells (23).

Finally, the concomitant administration of GnRHa has been reported to reduce gonadal toxicity mediated by chemotherapy (24). Recently, a systematic review conducted on individual patient data evaluated the efficacy of this approach in patients affected by early breast cancer. In this study, 873 patients were included from five clinical trials comparing adjuvant chemotherapy with adjuvant chemotherapy plus synthetic GnRHa. Among the 873 patients, 37 (10.3%) women had at least one post-treatment pregnancy in the GnRHa group vs. 20 (5.5%) in the control group (HR, 1.83; 95% CI, 1.06–3.15; $P = 0.03$). No significant differences in disease-free survival and overall survival were observed. These data confirmed the efficacy and safety of temporary ovarian suppression with GnRHa during chemotherapy. After these data, ovarian function suppression achieved by the administration of GnRHa during adjuvant chemotherapy in fertile women currently represents the most prescribed approach to decrease the likelihood of chemotherapy-induced premature ovarian failure and preserve fertility in premenopausal women (25).

DISTRESS, BODY IMAGE, SELF-ESTEEM, AND SEXUALITY

Breast cancer experience is often associated with relevant physical and psychosocial changes in affected women. The impact of breast cancer diagnosis is different across the lifespan, since younger patients have increased risk of depression, anxiety, and intrusive thoughts (26). High degree of psychosocial adaptation, family relationship, and support networks represent protective factors for distress, because of their role in counteracting experience of loneliness and sense of isolation. The experience of anxiety and fear of the future is common in 20–30% of patients, in a metaphorical "sword of Damocles," related to the perceived risk of disease recurrence and death. Distress due to the disruption of body image in breast cancer, linked to hair loss, paleness, weight gain, and discontent for aesthetical outcomes of surgery, is also reported (27).

In this perspective, hospital-based programs of beauty care intervention can have beneficial effect in patients with breast cancer. A group makeup workshop is a low-cost intervention with patient-reported outcomes of distress reduction and amelioration of quality of life; moreover, this approach has been shown to immediately build confidence and self-esteem, with short-term and midterm benefic effects (28).

Furthermore, a low self-esteem affects self-perceived attractiveness and consequently intimacy and sex life. Sexuality is a complex area, including psychosocial, sociocultural, and biological aspects. Satisfaction in one's sex life can be a critical issue for the quality of life in breast cancer patients and should be included in individual patient assessment. Sexual dysfunction is more frequently observed in patients with breast cancer than in healthy women, and treatment-related adverse effects can have a prolonged, social negative impact (29). With these factors in mind, sexual satisfaction in breast cancer survivors deserves more attention, particularly in pre/peri-menopausal patients (30). Sexuality should be included in assessment (alone or with partner). Additionally, women prefer being informed by professional figures (preferably nurse or primary doctor) about potential issues in sex health and related solutions (31); this should also be taken into account. In conclusion, sexual counseling can be useful to patients and to their partners, to help improve quality of life during the cancer experience.

OSTEOPOROSIS AND LONG-TERM SURVIVAL

Osteoporosis is by far the most common problem in terms of bone health in the aging female population in most industrialized countries. The lifetime risk of fractures among US and European women at the age of 50 is about 40% with a risk of hip fracture in the range of 15–20%.

Being an estrogen-dependent tissue, bone is strongly affected by its circulating levels. Breast cancer patients with endocrine sensitive disease are candidate to receive adjuvant endocrine therapy with aromatase inhibitors (AIs) for 5–10 years according to the individual risk of recurrence; this treatment currently represents the standard of care for postmenopausal women and for high-risk premenopausal patients. Yet, the decrease in circulating estrogen levels associated to AIs can produce a rapid increase in the potential risk of fractures (32). Data from adjuvant clinical trials do not comprehensively represent the true impact of the related increased risk of fractures, especially in women with no baseline osteoporosis. At the same time, the long-term risk for factures in premenopausal women at the time of breast cancer diagnosis is still poorly recognized (33, 34). In the clinical practice, a baseline evaluation of fracture risk in postmenopausal and premenopausal women with early disease, candidate to AIs, should be regularly performed and repeated on a 2-year basis, in the absence of bone-related symptoms or events. The adoption of pharmacologic interventions to prevent bone loss is supported by a number of randomized clinical trials showing that bisphosphonates may be active also in women with a high risk of fracture following cancer treatment. Based on these results, guidelines recommend treatment in women with a T-score $\leq$-2 or those with at least two clinical risk factors.

Recently,1 denosumab, an anti-RANK ligand antibody, also approved for fracture prevention in the healthy postmenopausal woman, has been shown to extend the time to first fracture in breast cancer postmenopausal women treated with AIs. These benefits have led clinicians to consider denosumab as a key therapeutic option in the prevention of AI-induced bone loss. However, several issues still need to be addressed regarding the use of these different agents in an adjuvant setting (35). It is also worth mentioning that women receiving AIs are at higher risk of developing periodontal disease, with a possible impact on quality of life (36). In our clinical setting, we have implemented a separate consultation, where all women are regularly (every year) supervised by dental hygienists: this dedicated approach was appreciated by patients, and the incidence of periodontal disease was reduced (unpublished data).

HOST METABOLISM AND LIFESTYLE IN BREAST CANCER SURVIVORS

In addition to all the aspects previously described, when dealing with long-term survivorship, particular attention should be dedicated to metabolic aspects including weight control and management of physical inactivity, through lifestyle interventions. This issue, although not strictly related to long-term toxicity from adjuvant treatments or quality of life, might become the leading survivorship emergency in the short period, due to the well-known and increasingly proven interactions between altered metabolism and breast cancer prognosis (37–40). A possible approach should include innovative care strategies, non-hospital based, to overcome the risk of excessive medicalization in breast cancer survivorship.

CONCLUSIONS

Breast cancer survivorship represents one of the most challenging aspects to be approached in dedicated clinical follow-up settings. This is mainly due to improvements in survival that have occurred over the past 20 years, leading to disease chronicization in advanced stages and cure in early stages. In this review, we have discussed the most important treatment sequelae occurring with cancer treatments that require appropriate management and dedicated resources. This aspect is particularly important since today we interrupt breast-specific follow-up 5–10 years after breast cancer diagnosis. In the perspective of ameliorating the overall quality of life of breast cancer survivors, however, additional resources must be allocated to manage "breast cancer survivorship."

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript. SN, AG, FD'A, and SR wrote the manuscript. AG provided final revision.

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Role of Kinematics Assessment and Multimodal Sensorimotor Training for Motion Deficits in Breast Cancer Chemotherapy-Induced Polyneuropathy: A Perspective on Virtual Reality Avatars

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Chemotherapy-induced polyneuropathy (CIPN), one of the most severe and incapacitating side effects of chemotherapeutic drugs, is a serious concern in breast cancer therapy leading to dose diminution, delay, or cessation. The reversibility of CIPN is of increasing importance since active chemotherapies prolong survival. Clinical assessment tools show that patients experiencing sensorimotor CIPN symptoms not only do they have to cope with loss in autonomy and life quality, but CIPN has become a key restricting factor in treatment. CIPN incidence poses a clinical challenge and has lacked established and efficient therapeutic options up to now. Complementary, non-opioid therapies are sought for both prevention and management of CIPN. In this perspective, we explore the potential that digital interventions have for sensorimotor CIPN rehabilitation in breast cancer patients. Our primary goal is to emphasize the benefits and impact that Virtual Reality (VR) avatars and Machine Learning have in combination in a digital intervention aiming at (1) assessing the complete kinematics of deficits through learning underlying patient sensorimotor parameters, and (2) parameterize a multimodal VR simulation to drive personalized deficit compensation. We support our perspective by evaluating sensorimotor effects of chemotherapy, the metrics to assess sensorimotor deficits, and relevant clinical studies. We subsequently analyse the neurological substrate of VR sensorimotor rehabilitation, with multisensory integration acting as a key element. Finally, we propose a closed-loop patient-centered design recommendation for CIPN sensorimotor rehabilitation. Our aim is to provoke the scientific community toward the development and use of such digital interventions for more efficient and targeted rehabilitation.

Keywords: breast cancer, chemotherapy-induced peripheral neuropathy, virtual reality, machine learning, sensorimotor rehabilitation, body sensors

1. INTRODUCTION

Breast cancer remains the most prevalent chronic disease in women, with a predicted 5 million new cases reported annually and among those with higher survival rates (i.e., 5-years median survival in most developed countries is more than 80%). Following the initial diagnosis, the increasing number of survivors for relatively long periods highlights the need for adequate supporting rehabilitation interventions. Chemotherapy-induced polyneuropathy(CIPN) is among the most severe side effects of many regularly used chemotherapy drugs with a direct impact on the autonomy and quality of life of patients (1–3) with incidence varying between 12 and 96% in the case of taxane- and platinum-based chemotherapy (4–6). In both the prevention and treatment of CIPN, innovative rehabilitation approaches that can be implemented as an alternative to traditional pharmacological treatments are absolutely necessary (7). The current landscape of solutions is broad and diverse comprising:

Intervention	Study
Wearable sensors	(8)
Sensorimotor balance training	(9)
Specialized physical exercise training	(10–13)
Physiotherapy	(14, 15)
Joint stabilizers and orthotics	(16)
Impact and vibration training	(17, 18)
Closed kinematic chain exercise	(19)
Visual computer-feedback balance training	(20, 21)
Transcutaneous electrical stimulation	(22)

These studies have laid the foundation for using technology in assessing, monitoring and controlling sensorimotor deficits associated with CIPN. Yet, none of them considered whole-body kinematic assessment of deficits and closed-loop deficit compensation. Our perspective sheds the light on the possibility to use commodity digital technologies, such as Virtual Reality (VR) and Machine Learning (ML), in combination, for personalized CIPN sensorimotor rehabilitation. We discuss the applicability of such a digital intervention facilitating motor deficits (e.g., gait and posture) in breast cancer patients.

We start our perspective by evaluating the impact chemotherapy has upon patient's sensorimotor system. We briefly introduce the typical metrics in sensorimotor deficits assessment and motivate the need kinematics assessment. Afterwards, we analyse the rehabilitation facilitating effects that VR offers, highlighting aspects, such as validity, multisensory integration, and neuroplasticity. Finally, we consolidate this thesis and propose a patient-centered design recommendation for CIPN sensorimotor rehabilitation.

2. ANALYSIS OF SENSORIMOTOR EFFECTS OF CIPN

CIPN defines the harm to the peripheral nervous system experienced by a patient who has been administered a neurotoxic chemotherapeutic agent. The substances that most frequently cause CIPN in breast cancer are: vinca alkaloids, taxanes, and platin derivates.

2.1. Sensorimotor Deficit Assessment

Independent of the mechanisms of action, the targeted impact of such agents is on axonal transmission (1, 23, 24) with consequences leading up to neuronal apoptosis (25). CIPN generates sensory (26) as well as motor symptoms (27), with a high prevalence between 30 and 83% of the patients reporting persistent neuropathy, with 68.1% in <30 days following termination of the chemotherapy (6). Considering wider timescales, the average occurrence of CIPN was shown to be up to 28.7% in the first year after diagnosis, with more than 80% of the patients presenting symptoms after 6 months (28).

Joint deficits and dysfunction in the sensorimotor domain due to neurotoxicity can influence everyday activities, including gait, posture, and induce falls (29, 30). Any such deterioration in the sensory and motor information available can affect cortical integration of sensory and motor streams and the learning of internal models (31–33). Thus, any contradiction or inconsistency in sensorimotor input, or a decline in the reliability of perceptual information, facilitates the formation of sensorimotor aberrations, further hindering motor planning and execution during biomechanical processes, such as gait and postural control (34, 35).

In rehabilitation, the severity of such sensorimotor effects induced by CIPN is traditionally measured with subjective scales, such as the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group Neurotoxicity (FACT/GOG-Ntx) and the European Organization for Research and Treatment in Cancer Quality of Life Quest CIPN 20/30 (EORTC QLQ—CIPN 20/30) (36). Their ability to ascertain deficiencies in body structure/function and restrictions in tasks during the therapy is confined and tied to the subjectivity of the patient (37). Typically, to support rehabilitation, such scales are complemented by quantitative scales of neurotoxic impact on perception [e.g., Fullerton Advanced Balance Scale (FABS), the Balance Evaluation Systems Test (BESTest)] (38). These standardized measures enrich whole-body assessment of sensorimotor deficits and provide patient motion context information (39), but lack the kinematic assessment. This is crucial, especially in the light of closed-loop training, where perceptual information needs to drive compensation of the deficits. In the next section, we analyse existing sensorimotor rehabilitation techniques used in CIPN with respect to kinematics assessment and sensorimotor training.

2.2. Sensorimotor Rehabilitation

Continuous monitoring after initiation of a neurotoxic intervention is critical for the formation and advancement of CIPN related symptoms (40). A large number of interventions have been developed to cope with the prevention or management of CIPN. With up to 26 identified complementary therapies for the typical neurotoxic agents used in typical chemotherapy schemes (i.e., Oxaliplatin, Cisplatin, Platinum/Taxane combination, Taxanes), Kalisch et al. emphasized the rather

scarce space of efficient solutions in supportive therapies (7). We now analyse the most relevant interventions, highlighting the need for kinematics assessment in sensorimotor training.

2.2.1. Primary Rehabilitation Strategies

Physical training can attenuate CIPN symptoms and reduce deficits manifested in both sensory and motor domains. Streckmann et al. supports such hypothesis by evaluating the adaptations of the neuromuscular system through EORCT-QLQ-CIPN20, FACT/COG-Ntx and a batch of sensorimotor exercises (41). Moreover, McCrary et al. (10) argued about the importance and the impact of multimodal exercise interventions on CIPN symptoms by emphasizing that neuromuscular system structural changes are sophisticated, and depend on the amount and vigor of the exercise. Interestingly, most relevant studies employing physical training (10–13) only evaluate a subset of motion parameters and pay the price for the high variability between interventions, such as the variety and length of exercises. In addition, the measurement procedures of the investigations were not compatible; for example, balance control was measured with different scales (9).

Providing a richer depiction of patient's motion parameters, the next level in CIPN sensorimotor rehabilitation focuses on the use of wearable sensors in physical training (8, 17, 19). Such an approach is an inexpensive, robust, and efficient method to screen motor performance deterioration by assessing spatio-temporal parameters of gait and balance [as shown by (8, 18)]. Extending the range of wearable rehabilitation modalities, whole-body vibration (WBV) training, has proven to attenuate sensorimotor deficits by de-conditioning skeletal muscles in order to reduce fall frequency (9). Our perspective is that such sensor-based rehabilitation systems have the potential to capture patient motion peculiarities, mainly in an open-loop configuration, but without compensating for deficits thorough closed-loop sensory feedback control (42).

2.2.2. Advanced Rehabilitation Strategies

Targeting a closed-loop approach, the most advanced rehabilitation systems to date look at interactive motor adaptation training programs based on wearable sensors and visual animation (18). Such approaches exploit the benefit of the interactive joint movement feedback (43) and tap into error-dependent cortical learning rules between reference/desired motor action and the measured/executed motor action. Such techniques trigger adaptation of the neural internal model through closed-loop stimulation (32). Such systems promote the role of interactivity for motor rehabilitation through significant reduction of deficits (e.g., center of mass sway, disturbed postural stability, and coordination) (21). As vision dominates perception, visual computer-feedback balance training systems have the potential to improve balance in patients with CIPN (20), as measured by static-dynamic posture and balance scales and kinematic chains (19). Such rehabilitation methods emphasize the role of precise kinematic assessment and the potential that wearable sensors and interactive visual feedback have in compensating deficits, such as impaired joint proprioception (21, 44, 45).

3. ANALYSIS OF VIRTUAL REALITY FOR SENSORIMOTOR REHABILITATION

3.1. Virtual-Real Feedback Loop

VR enables a rich patient-centered and patient-tailored interaction with the virtual environment via tools, such as head mounted devices which require less set-up and effort than would be needed to train a patient for a rehabilitation routine in the real environment (46). Moreover, VR technology can be used to deliver rich and targeted stimulation to a patient's nervous system and thereby take advantage of the plasticity of the brain to promote learning and re-learning and, hence, support sensorimotor rehabilitation (47). Significantly important and relevant for the adoption of VR in rehabilitation is the assessment of the consistency and resemblance of responses within a virtual environment to those in the corresponding physical environment. Transferring such responses to the real world after training would trigger perceptual-motor adaptation and motor re-learning (46, 48, 49). Hence brain's adaptation to VR changing environment circumstances could be similar to those in the physical world (50). The validity of the sensorimotor stimulation in VR is crucial, to make sure that there is a consistent match between the real and the virtual world, especially when assessing deficits. Supporting our hypothesis, Mellet et al. proved, through fMRI, that the neural correlates of recalling patterns acquired when moving in VR were equivalent to those evoked when walking in a physical environment, while ensuring movement quality and parameters (51). In CIPN rehabilitation the goal is improving movement quality through deficit compensation and we hypothesize that a digital intervention should guarantee that movements made in VR are equivalent in spatial and temporal structure to those which have to be reacquired in the physical environment. But the nervous system's underlying redundancy might eventually restrict the degree of motor recuperation that can be attained (52).

3.2. Compensation Through Adaptive Multisensory Integration

Human perception of motion and bodily self involves multisensory integration, with vision playing an especially important role (53–58). The flexibility of VR allows for a deliberately vivid visual experience that can be "mismatched" from the bodily signals (57, 59). Such a context allows to investigate how far would the brain perform multimodal integration and solve sensorimotor inconsistencies (60, 61). Such multisensory processes describe the impact that VR has upon the compensation of sensorimotor deficits, by guiding patients' perception toward correcting for the inconsistencies that the real deficits determine. Information from afferents in joints, muscles, and tendons as well as visual, vestibular, and auditory signals reach cortical integration areas in the frontal, parietal, and temporal lobes, where the fusion of these body signals occurs (55, 62–65). Thus, it appears likely that the self-identification of body motion and underlying kinematics is achieved in a similar manner, by dynamic multisensory integration processes known to be elicited in VR. After VR immersion, the brain is biased to estimate self-motion from visual cues in a vestibular cue

conflicting process (66, 67). Our perspective is that such error correction mechanism, at cortical level, or the underlying re-cueing needs to occur in order to decorrelate the current percept from the adapted stimuli in VR. Thus, a subsequent re-weighting of sensory signals from the different modalities is necessary for optimal sensory integration (68, 69). Our hypothesis is that the stimulation environment richness modulates multisensory integration to compensate for sensorimotor deficits. Moreover, we believe that a digital intervention tapping into motion deficits should target the underlying correlates of cortical multisensory integration. VR has the potential to offer such targeted multimodal stimulation and elicit re-weighting and adaptation processes driving compensation. In order to drive compensation self-presence is vital. In VR self-presence is the feeling that my avatar is me. Using an interactive virtual environment that requires the use of avatars adds the component of self-presence, which turns the intervention into a personal experience (70) given the many factors that determine the cognitive connection or sense of identification between user and avatar (71). Our belief is that the precise mapping of one's motion peculiarities, the quality of the multimodal perception, and the consistency and validity of the internal model predictions can support a successful intervention (72, 73).

4. RECOMMENDATION FOR A PATIENT-CENTERED VIRTUAL REALITY INTERVENTION

In this final section, given the generous frame we already unfolded, we provide a concept VR system for CIPN sensorimotor rehabilitation. Our aim is to provoke the scientific community to develop and use digital technologies for efficient patient-centered rehabilitation interventions.

The conceptual architecture depicted in **Figure 1** looks at kinematics assessment and multimodal sensorimotor training from a closed-loop control system perspective [as we previously shown in (74)]. Such a system would simultaneously update two models, a world model (i.e., avatar rendered in the training lab through VR system) and one self-motion model (i.e., obtained through wearable sensors). Such models can extract through machine learning algorithms the particularities of each patient's kinematics (i.e., joint angles and translation velocities ranges) and the world (i.e., peripersonal space and distal obstacles) using efficient correlation learning previously developed in Axenie et al. (75). Basically, by extracting the correlations among the various sensory streams describing the 3D motion of the patient (e.g., joint angles), the machine learning algorithms are able to regress the underlying dependencies in high-dimensional spaces among the motion variables (e.g., forearm w.r.t shoulder). The machine learning algorithms will, hence, use more complex features (e.g., trend detection, frequency, amplitude) and patterns underlying the sensory streams to extract motion peculiarities (e.g., drift, offset). The world model would hence describe the spatio-temporal aspects (i.e., patient executing a task: heading to an object and grasping), whereas the self-motion model would describe the kinematics (i.e., how to change trunk center of mass to move and arm joint angles to grasp). During the execution of the task each patient's brain will update the internal models of the world and the self (i.e., marked as neural processing in **Figure 1**). The mismatch between the internal neural models updates during the task and the external assessment of the rehabilitation system would then be used to parametrize the VR stimulation through the avatar. Preliminary experiments in Axenie et al. (76) confirm this hypothesis. The parametrization assumes the particular rendering of the patient avatar (i.e., with deficits) against a healthy patient avatar (i.e., no deficit). This way, the patient visually perceives the mismatch and will try to compensate for it.

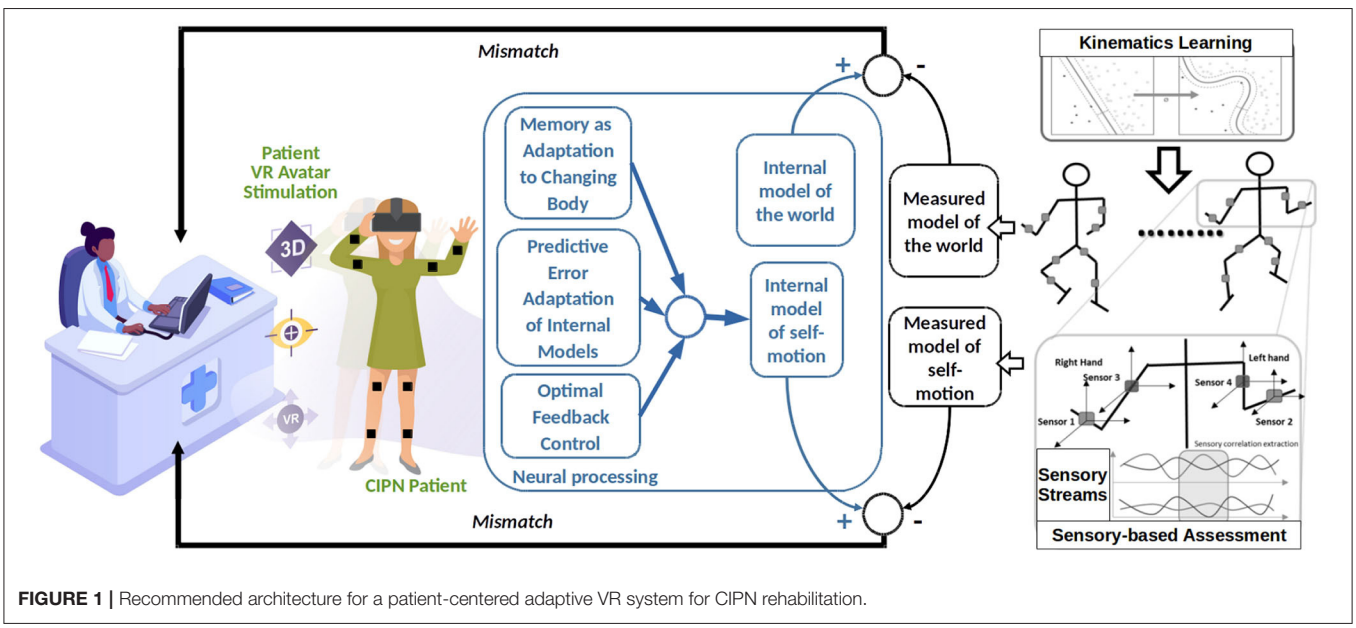

FIGURE 1 | Recommended architecture for a patient-centered adaptive VR system for CIPN rehabilitation.

Such an architecture has the potential to influence multisensory processes in the brain that account for both the perception of the surrounding space and self-motion. We hypothesize that this is possible through a series of processes that can also account as effects of VR stimulation, namely: the predictive adaptation of internal models, motion memory as adaptation to a changing body and optimal feedback control, respectively.

4.1. Predictive Error Adaptation of Internal Models

Under CIPN, peripheral nerves controlling muscles and tendons stiffness change, altering the kinematic and dynamic coupling between motor commands (e.g., torques) and motion of the limb (i.e., position and velocity). Along such progressive variations, the patient's body dynamics change. Hence in order to maintain a desired level of performance, the brain needs to be "robust" to such changes. This robustness may be attained through continuous update, or adaptation, of the internal kinematic model that predicts the sensory consequences of motor commands.

4.2. Memory as Adaptation to a Changing Body

Human brains exhibit patterns of learning and forgetting. Such processes unfold over different timescales where motion memory is consolidated as a consequence of the adaptation to a changing body. Chemotherapy neurotoxic effects, induces such changes in both sensory and motor systems. When considering sensorimotor rehabilitation, if a patient performs a task and observes an error, the brain tries to estimate the source of the error and to evaluate if the sensory encoding is still valid or exposed to aberrations.

4.3. Optimal Feedback Control

Our perspective is that under VR stimulation the patient's brain can act as a feedback compensator for sensorimotor deficits. This view taps into a core postulate of sensorimotor learning, namely optimal feedback control. In this framework, based on previously acquired experience, the brain computes a time-varying mapping from its internal states (i.e., internal models) into actions that minimize a total expected cost of responding to the incoming sensory input. Such learnt mappings/associations are essential to tune future motor responses to sensory information and, implicitly, compensate for eventual deficits.

5. LOOKING AHEAD

Virtual environments facilitate the transfer and generalization of sensorimotor learning into the physical environment. Transfer is promoted if the avatar engagement with the virtual environments is equivalent to that expected in the physical world and the patient cognitive processing involved in task completion similar to the one required in the physical instantiation. Validating this hypothesis yields kinematics assessment and at the same time a rich multimodal stimulation.

Such a system could bring unique advantages through cost reduction for the clinics and an easy deployment for home-based rehabilitation. This offers an important advantage to the patients that will not need to visit the clinic often, rather focus on their daily lives allowing the system to non-intrusively monitor and learn their deficits and adaptively compensate for them. Our belief is that such a digital intervention holds the promise of offering truly personalized rehabilitation for life after cancer through a unique combination of technologies, such as VR and ML.

AUTHOR CONTRIBUTIONS

CA and DK contributed the conception and design of the perspective. CA wrote the first draft of the manuscript. DK wrote the sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Machine Learning-Based Radiomics Nomogram Using Magnetic Resonance Images for Prediction of Neoadjuvant Chemotherapy Efficacy in Breast Cancer Patients

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Purpose: The construction and validation of a radiomics nomogram based on machine learning using magnetic resonance image (MRI) for predicting the efficacy of neoadjuvant chemotherapy (NACT) in patients with breast cancer (BCa).

Methods: This retrospective investigation consisted of 158 patients who were diagnosed with BCa and underwent MRI before NACT, of which 33 patients experienced pathological complete response (pCR) by the postoperative pathological examination. The patients with BCa were divided into the training set ($n = 110$) and test set ($n = 48$) randomly. The features were selected by the maximum relevance minimum redundancy (mRMR) and absolute shrinkage and selection operator (LASSO) algorithm in the training set. In return, the radiomics signature was established using machine learning. The predictive score of each patient was calculated using the radiomics signature formula. Finally, the predictive scores and clinical factors were used to perform the multivariate logistic regression and construct the nomogram. Receiver operating characteristics (ROC) analyses were used to assess and validate the diagnostic accuracy of the nomogram in the test set. Lastly, the usefulness of the nomogram was confirmed via decision curve analysis (DCA).

Results: The radiomics signature was well-discriminated in the training set [AUC 0.835, specificity 71.32%, and sensitivity 82.61%], and test set (AUC 0.834, specificity 73.21%, and sensitivity 80%). Containing the radiomics signature and hormone status, the radiomics nomogram showed good calibration and discrimination in the training set [AUC 0.888, specificity 79.31%, and sensitivity 86.96%] and test set (AUC 0.879,

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specificity 82.19%, and sensitivity 83.57%). The decision curve indicated the clinical usefulness of our nomogram.

Conclusion: Our radiomics nomogram showed good discrimination in patients with BCa who experience pCR after NACT. The model may aid physicians in predicting how specific patients may respond to BCa treatments in the future.

Keywords: radiomics, nomogram, breast cancer, neoadjuvant chemotherapy, pathological complete response, machine learning

INTRODUCTION

Currently, breast cancer (BCa) is the most commonly diagnosed malignancy in females worldwide as of 2018, accounting for ∼25% of all new diagnoses and nearly 15% of cancer-associated deaths in females (1). This translates to 2.1 million new cases of BCa in 2018, along with more than 600,000 BCa-related deaths. Globally, women have a 5% cumulative risk of being diagnosed with BCa by the age of 75, although the risk varies substantially by country.

Neoadjuvant chemotherapy (NACT) is a central component of BCa therapy (2). In previous studies, NACT has been associated with lower disease stages, increased sensitivity of chemotherapy drugs, improved resection and breast preservation rates, and increased pathological complete response (pCR) in some patients with BCa (3, 4). The disease-free survival (DFS) and overall survival (OS) of BCa patients with pCR are significantly longer than those of patients without pCR (5), suggesting that pCR may be a potential prognostic factor and target of NACT. However, even with the latest advances in chemotherapy regimens, the number of patients with pCR remains low at 12–28% worldwide (6). In non-pCR patients, NACT may fail to produce a full therapeutic effect, which can delay surgical intervention (7). Accordingly, the rapid and effective screening of patients is critically important to identify patients more likely to respond to NACT, which may lead to improved patient outcomes.

In recent years, there has been extensive cross integration of radiation medicine with bioengineering, which has produced the field of radiomics (8, 9). Radiomics is a method for the extraction of high-dimensional data from radiographic medical images using data-characterization algorithms. Recent studies have shown that radiomics features of magnetic resonance imaging (MRI) may allow for the prediction of NACT and radiotherapy response in patients with rectal cancer (10). In addition, the radiomic features from MRI have been used to predict how patients with BCa would respond to NACT before the treatment was initiated (11). Recently, a new multicenter study was conducted to assess a multiparameter prediction model using MRI data that could accurately predict which patients would have pCR before undergoing treatment (12). While radiomics may be used to predict the efficacy and potential benefits of NACT, no studies have evaluated the potential impact of different machine learning techniques on radiomics.

Machine learning involves the building of data-derived computational models and methods to improve the accuracy, performance, or predictive abilities of the model, which is an important part of radiomics (13, 14). Accordingly, machine learning strategies have high prognostic and predictive power, along with excellent stability, all of which are desired for radiomics-based analyses. In this study, our aim was to use a highly predictive and stable machine learning strategy for constructing a radiomics nomogram that could be used to predict pCR in patients with BCa. The radiomics nomogram provides a noninvasive, convenient, and low-cost strategy that may improve the treatment of BCa in the future.

MATERIALS AND METHODS

Patient Information

This study was approved by the Ethics Committee of Zhejiang Cancer Hospital (Gongshu, P.R. China). The requirement for informed consent was waived due to the retrospective nature of this study. The patients were enrolled in our hospital from June 2017 to December 2019. All the patients underwent MRI within 1–2 weeks prior to NACT. The inclusion criteria for this study were as follows: (a) invasive BCa confirmed by biopsy without distant metastasis; (b) complete NACT was initiated with no prior history of treatment; and (c) surgery was performed after NACT, and pathological evaluation was performed after the operation. The exclusion criteria for this study were as follows: (a) NACT was not completed; (b) surgery was not performed, or postoperative pathology was not evaluated; and (c) the MRI data were unavailable. In addition, the study population was group by 7:3 according to the diagnosis time. The patients from June 2017 to January 2019 were part of the training set (n = 110). During the training period, the robustness of the radiomic features was tested, and the model was constructed. The patients from February 2019 to December 2019 were part of the test set (n = 48) to verify the reliability of the constructed model. The recruitment path of the subjects and research design of this study are shown in **Figure 1**.

MRI Scanning Process and Immunohistochemical Evaluation

All breast MRI scans were performed at a local hospital using the 3.0 Tesla MRI scanner (MAGNETOM Verio A Tim System; Siemens Healthcare, Erlangen, Germany). During the scan, an axial fat-suppressed T2WI sequence and axial diffusion weight imaging (DWI) images were obtained using the two b-values of 0 and 1,000 s/mm^2 before the contrast agent was administered. Initially, a fat-saturated

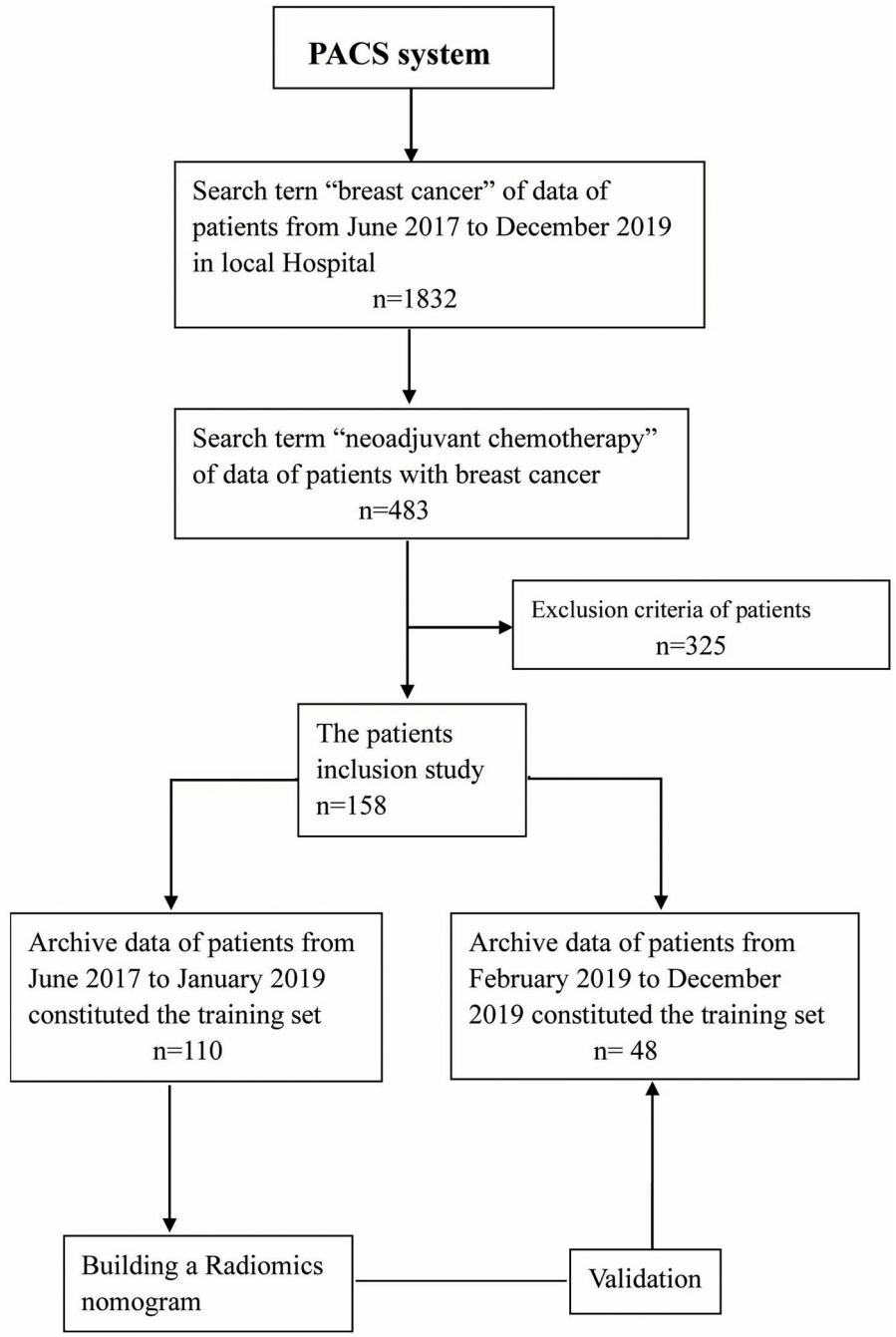

FIGURE 1 | Flowchart showing the recruitment of patients and the overall design of this retrospective study.

T1WI scan was recorded before injection of the contrast agent or dynamic contrast-enhanced (DCE) scanning were performed. Next, DCE images were acquired as six post-injection scans with intervals of 38 s following the intravenous injection of Magnevist. Next, 0.2 mL/kg of body weight of the gadolinium-based agent (Magnevist; Bayer Healthcare, Berlin, Germany) was injected using an MRI compatible power injector (rate = 2 mL/s), which was flushed with 20-mL of saline using the high-pressure injector. Additional details about the MRI parameters can be found in the **Supporting Information**.

Immunohistochemistry (IHC) was used to assess the expression of several receptors and antigens commonly associated with BCa from biopsy in the pre NACT, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and antigen

Ki67 (Ki-67). Tumors with <1% of nuclear staining were denoted as ER/PR-negative, while ≥1% was denoted as ER/PR-positive. Next, 20% was established as the cutoff value for Ki-67 expression. In terms of HER2 expression, IHC scores of 0 or 1+ were denoted as HER2-negative, and 3+ was denoted as HER2-positive. An IHC score of 2+ required further investigation using *in situ* hybridization (ISH), with non-amplified results being denoted as HER2-negative and amplified results being HER2-positive.

NACT and the Pathological Response of Treatment

The patients included in this study underwent four or six cycles of NACT before undergoing breast surgery. The National Comprehensive Cancer Network (NCCN) guidelines were used to establish the therapy timeline and procedures (15). In this study, 85% of patients ($n = 121$) were given the taxane-based NACT regimen, while the remaining 15% of patients ($n = 21$) were given the anthracycline and taxane-based NACT regimen. Additionally, the patients found to be HER2-positive were prescribed trastuzumab (loading dose = 8 mg/kg; maintenance dose = 6 mg/kg). All of the patients underwent surgery after NACT, and pathological specimens were evaluated using the Miller-Payne system (16) for the pathological assessment of NACT response. The histopathological examination and analysis were performed by a dedicated breast pathologist, who was blinded to the MRI data, with more than 12 years of experience in the field of breast pathology. Next, the efficacy of NACT was determined by examination and comparison of the specimens from the initial biopsies with those obtained from the radical resection specimens. The Miller-Payne system is divided into five grades. In the current study, grade 5 and the absence of lymph node invasion in the ipsilateral sentinel node or lymph nodes removed during axillary dissection (ypT0/isN0), while the remaining grades were denoted as the non-pCR group. Additional details about the pathological grades can be found in the **Supporting Information**.

Segmentation and Preprocessing of MR Images

The DCE-MRI images were imported into the itk-snap software. The third phase, which showed the most apparent enhancement, was selected for sketching tumor boundaries. The largest layer of the tumor was selected for sketching, and areas of necrosis, calcification, and bleeding were avoided. The region of interest (ROI) of the tumor was saved and imported into the DWI images ($b = 1{,}000$ s/mm^2) and T2WI images using the replication function. Next, the ROIs of tumors from three different MRI sequences were saved. In addition, manual corrections were further performed to prevent small deviations in delineating the ROI boundary. All tumor sketches were completed by two senior radiologists independently without knowing the pathological results. Radiologist A had ten years of experience, and radiologist B had ∼15 years of experience in the study of breast radiology.

The ROIs from the three MRI sequences were loaded into the AK analysis software for feature extraction. The images were initially processed before the feature extraction was performed. The initial processing of images required the resampling of voxels to $1 \times 1 \times 1$ mm^3 and standardization of gray levels to the 1–256 scale. This eliminated the potential influences of different imaging sequences on the extracted features (17).

Extraction of Radiomic Features

AK software was used to extract the radiomic features, including the histogram, FormFactor, gray level co-occurrence matrix (GLCM), and run-length matrix (RLM). These features can characterize the heterogeneity of cancer and reflect changes in the tumor microstructure (17). The most robust features were used for manual correction purposes to improve the usefulness of the model (18). The Spearman's rank test was used to assess the correlation coefficients between features of set-A (Radiologist A) and set-B (Radiologist B). Any features that had correlation coefficients > 0.8 were denoted as having "robust" features (19). Three sets of robust features corresponding to T2WI, DWI, and DCE sequences were obtained. The feature values in this study were the average values of feature set-A and feature set-B.

Establishment of an Optimal Radiomics Signature Based on Machine Learning

The maximum-relevance minimum redundancy (mRMR) algorithm was used to extract the robust features in the training set. Maximum relevance allowed for the selection of features most associated with pCRs (20), while minimum redundancy allowed for the selection of features with minimal redundancy among the others. Optimal features set with high correlation and low redundancy were obtained using the mRMR algorithm. Next, the typical absolute shrinkage and selection operator (LASSO) algorithm allowed for the reduction of dimensions and construction of the radiomics signature through machine learning techniques (21).

The five machine learning classifiers utilized in this study included Support Vector Machine (SVM), Bayes, *k*-Nearest Neighbor (KNN), Random Forest, and Decision Tree. The machine learning models were constructed using five-fold cross-validation. In short, this required that 20% of data be used to test the model, while the other 80% of data were used to create the model. After a total of 10 repeats, the average values were used to estimate the performance of the model. To demonstrate the correlation between the radiomics signature and pCR status, the signature model was used to score the training set in terms of pCR probability. The score was defined as the rad score, and was used to determine the effectiveness of the signature models for differentiating between pCR and non-pCR patients. The formula of the model used in the training set was employed to calculate the scores for the test set. Lastly, the accuracy of the radiomics signature from the training and test sets was evaluated with area under curve (AUC) value of receiver operating characteristic (ROC) curve. In addition, we selected the machine learning method with the largest AUC and the smallest difference between the training and the verification sets as the model construction method of this study. Detailed information about the dimensionality reduction and radiomics signature can be found in the **Supporting Information**.

Development and Evaluation of the Radiomics Nomogram

For the training group, univariate logistic regression analyses were performed to select independent predictors of pCR for each potential predictive variable, including clinical factors (i.e., gender, age, and menstrual status), clinical stage of the tumor, biomarker expression (i.e., ER, PR, HER2, and Ki-67), and the rad score. Multivariable logistic regression analyses that combined the independent predictors were applied to develop a pCR prediction model. Next, multivariate logistic regression was used to create the radiomics nomogram.

The variance inflation factor (VIF) was used to diagnose the collinearity of each variable (22) with VIF values >10, indicating severe multicollinearity (23). The calibration performance was evaluated with the calibration curve, and fitness was analyzed by the Hosmer–Lemeshow test. The ROC curve allowed for the estimation of diagnostic accuracy using the nomogram. The probability score for pCR was determined for the patients included in the study using the nomogram, and all patients were divided into high or low probability groups according to the ROC curve cut-off value. The clinical effect of the nomogram was determined using the actual patients with pCR from the different probability groups. The net benefit of the nomogram was determined using the DCA curve (24).

Statistical Analysis

SPSS 17.0 software (IBM, Chicago, IL, USA) was used to perform the Kolmogorov–Smirnov test for evaluating the normality of the distribution of the data, and the chi-square test for the categorical data. The likelihood ratio test with backward step-down selection was applied to the multivariate logistic regression model. VIFs were calculated using the SPSS 17.0 software. The MedCalc15.8 software (MedCalc, Ostend, Belgium) was used to assess the ROC curves, and differences between various AUCs were compared with the DeLong test. The R statistical software Version 3.4.1 was used for all other statistical analyses. The "mRMRe" and "glmnet" packages were used for mRMR and LASSO analyses. Calibration plots and the radiomics nomogram were established with the "rms" package, and DCA with the "dca.R" package. Two-sided $p < 0.05$ were considered as being statistically significant.

RESULTS

Characteristics of Patients in This Study

A flowchart of participant recruitment is presented in **Figure 1**. No significant differences were detected in the age, menstrual status, clinical stage of the tumor, or biomarker expression (i.e., ER, PR, HER2, and Ki-67) between patients in the training and test sets, as shown in **Table 1**. However, significant differences in ER expression, PR expression, and the radiomics signature were detected between pCR and non-pCR (all $p < 0.05$). As shown in **Table 2**, the other differences were insignificant.

Development of the Radiomics Signature and Assessment of Its Accuracy

The radiomics workflow is shown in **Figure 2**. A total of 328 radiomics features were obtained from the T2WI, DWI, and DCE sequence images. Accordingly, 984 radiomics features were extracted from each patient. The optimal combination of texture features from T2WI and DWI were chosen, and the random forest method was used to construct the radiomics signature. First, 396 features were obtained from the combination of radiomic features through the detection of robustness and reproducibility. Next, 35 features with the highest mRMR

TABLE 1 | Clinical characteristics of patients in the primary and internal validation cohorts.

Variables		Training set ($n = 110$)		Test set ($n = 48$)		*P*-value
		n	%	*n*	%	
Age (years, mean ± SD)		49.85 ± 8.78		52.96 ± 8.97		0.442
Menstrual status	Premenopausal	68	61.8	38	79.2	0.051
	Postmenopausal	42	38.2	10	20.8	
Histologic type	NST invasive carcinoma	89	80.9	39	81.3	0.96
	Other	21	19.1	9	18.7	
Clinical stage	I	16	14.5	7	14.6	0.954
	II	64	58.2	29	60.4	
	III	30	27.3	12	25	
ER status	Negative	49	44.5	22	45.8	0.881
	Positive	61	55.5	26	54.2	
PR status	Negative	43	39.1	18	37.5	0.85
	Positive	67	60.9	30	62.5	
HER2 status	Negative	51	46.4	15	31.3	0.076
	Positive	59	53.6	33	68.7	
Ki-67	Low	19	17.3	8	16.7	0.926
	High	91	82.7	40	83.3	

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, antigen Ki67.

TABLE 2 | Clinical characteristics of the training and validation sets of breast cancer (BCa) patients with and without pCR.

Variable		Training set (n = 110)			Test set (n = 48)		
		pCR (n = 23)	Non-pCR (n = 87)		pCR (n = 10)	Non-pCR (n = 38)	
		n (%)	n (%)	P-value	n (%)	n (%)	P-value
Age (years, mean ± SD)		48.9 ± 10.6	50.1 ± 8.3	0.583	48.8 ± 8.7	54.1 ± 8.8	0.112
Menstrual status	Premenopausal	15 (62.2)	53 (60.9)	0.706	9 (90)	28 (73.7)	0.275
	Postmenopausal	8 (34.8)	34 (39.1)		1 (10)	10 (26.3)	
Histologic type	NST invasive carcinoma	16 (69.6)	73 (83.9)	0.12	8 (80)	31 (81.6)	0.909
	Other	7 (30.4)	14 (16.1)		2 (20)	7 (18.4)	
Clinical stage	I	3 (13)	13 (14.9)	0.812	2 (20)	5 (13.2)	0.993
	II	13 (56.5)	51(58.6)		7 (70)	21 (55.3)	
	III	7 (30.5)	23 (26.5)		1 (10)	12 (31.5)	
ER status	Negative	16 (69.6)	33 (37.9)	0.007*	8 (80)	14 (36.8)	0.015*
	Positive	7 (30.4)	54 (62.1)		2 (20)	24 (63.2)	
PR status	Negative	16 (69.6)	27 (31)	0.001*	6(60)	9 (23.7)	0.027*
	Positive	7 (30.4)	60 (69)		4 (40)	29 (76.3)	
HER2 status	Negative	14 (60.9)	37 (42.5)	0.117	6 (60)	12 (31.6)	0.099
	Positive	9 (39.1)	50 (57.5)		4 (40)	26 (68.4)	
Ki-67	Low	5 (21.7)	14 (16.1)	0.524	2 (20)	6 (15.8)	0.751
	High	18 (78.3)	73 (83.9)		8 (80)	32 (84.2)	
Radiomics model score	0.773 ± 1.934	−2.244 ± 2.43	0.001*	0.349 ± 1.587		−2.629 ± 1.912	0.001*

*ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, antigen Ki67. *p < 0.05, with significant differences for clinical characteristics of pCR group and Non-pCR group.*

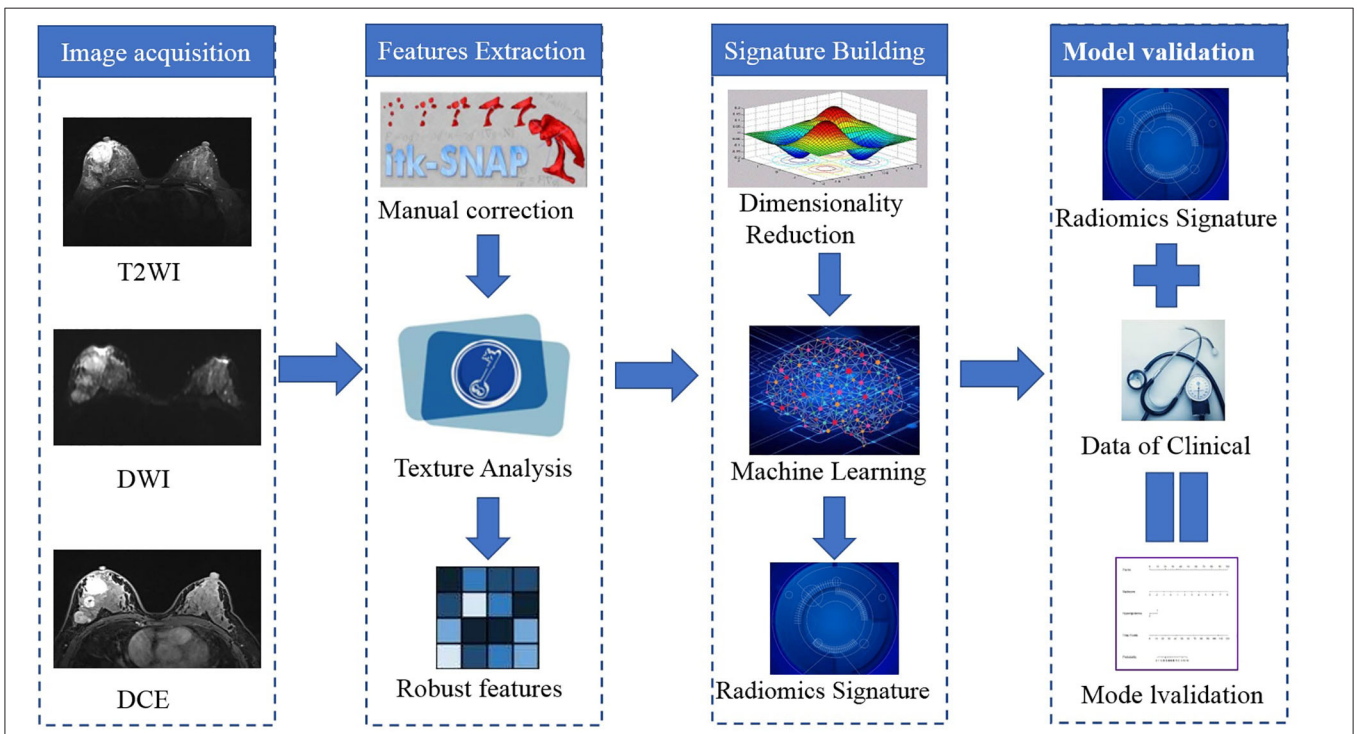

FIGURE 2 | Workflow for building the radiomics signature and creating the model. T2WI, T2-weighted image; DWI, diffusion-weighted imaging; DCE, dynamic contrast enhancement.

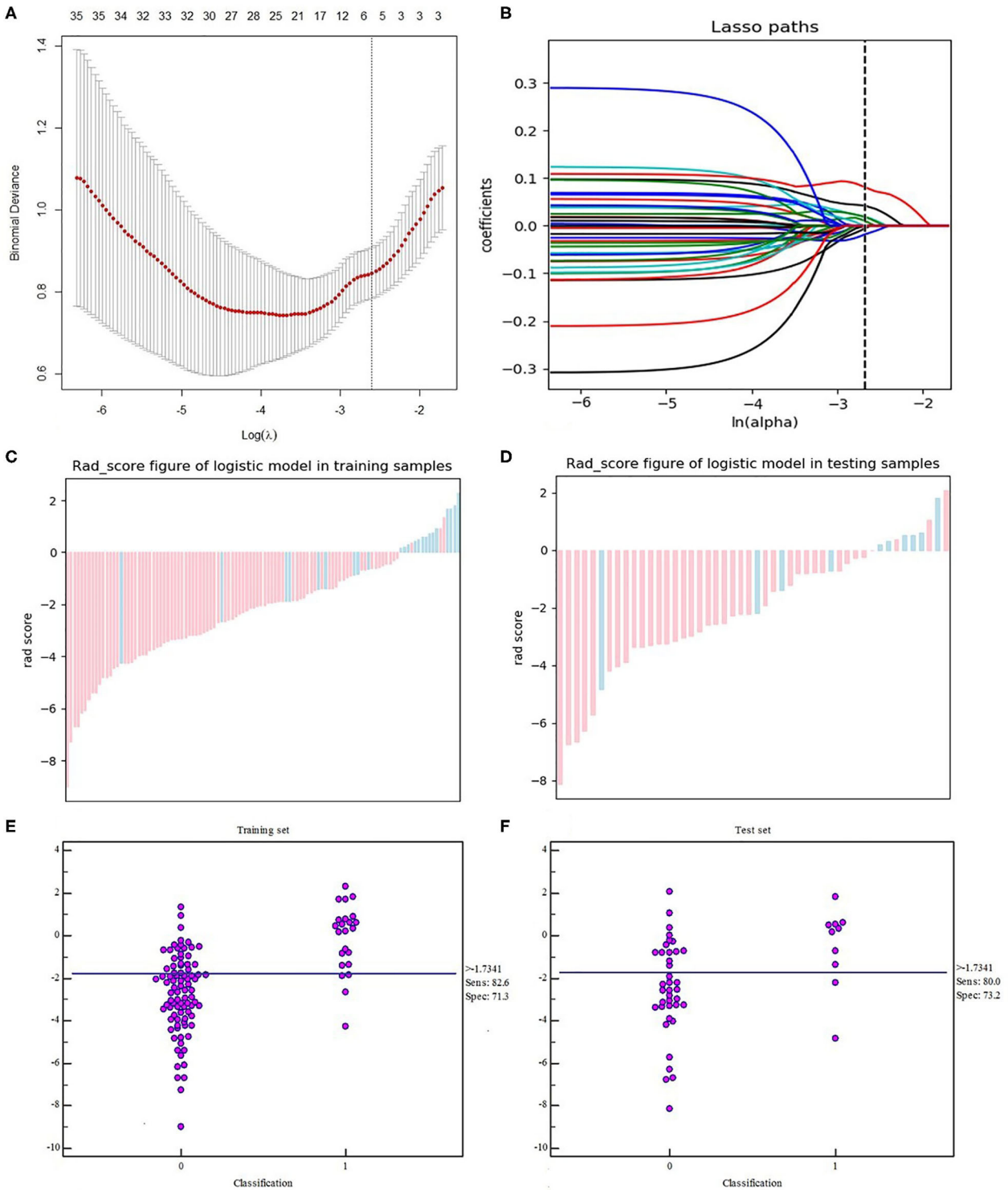

FIGURE 3 | Texture feature selection using the LASSO logistic regression. **(A)** The tuning parameter (λ) selection in the LASSO model used 10-fold cross-validation via the minimum criteria. Partial likelihood deviance was plotted vs. log (λ). The dotted vertical lines were drawn at the optimal values using the minimum criteria and the 1-SE criteria. **(B)** The LASSO coefficient profiles of the 35 texture features. The vertical line was drawn at the value selected using 10-fold cross-validation in the log (λ) sequence, and six features with non-zero coefficients are indicated. Score diagrams of the radiomics signature in the **(C)** training set and **(D)** test set. Red represents non-pCR and blue represents pCR. A score >0 indicates pCR, and a score <0 indicates non-CR. Both panels **(C,D)** show interactive dot diagrams revealing the accuracy of the radiomics signature for predicting pCR in patients of the **(E)** training set and **(F)** test set. Zero represents non-pCR, and 1 represents pCR. The horizontal line indicates the best threshold point to distinguish patients with pCR from patients with non-pCR.

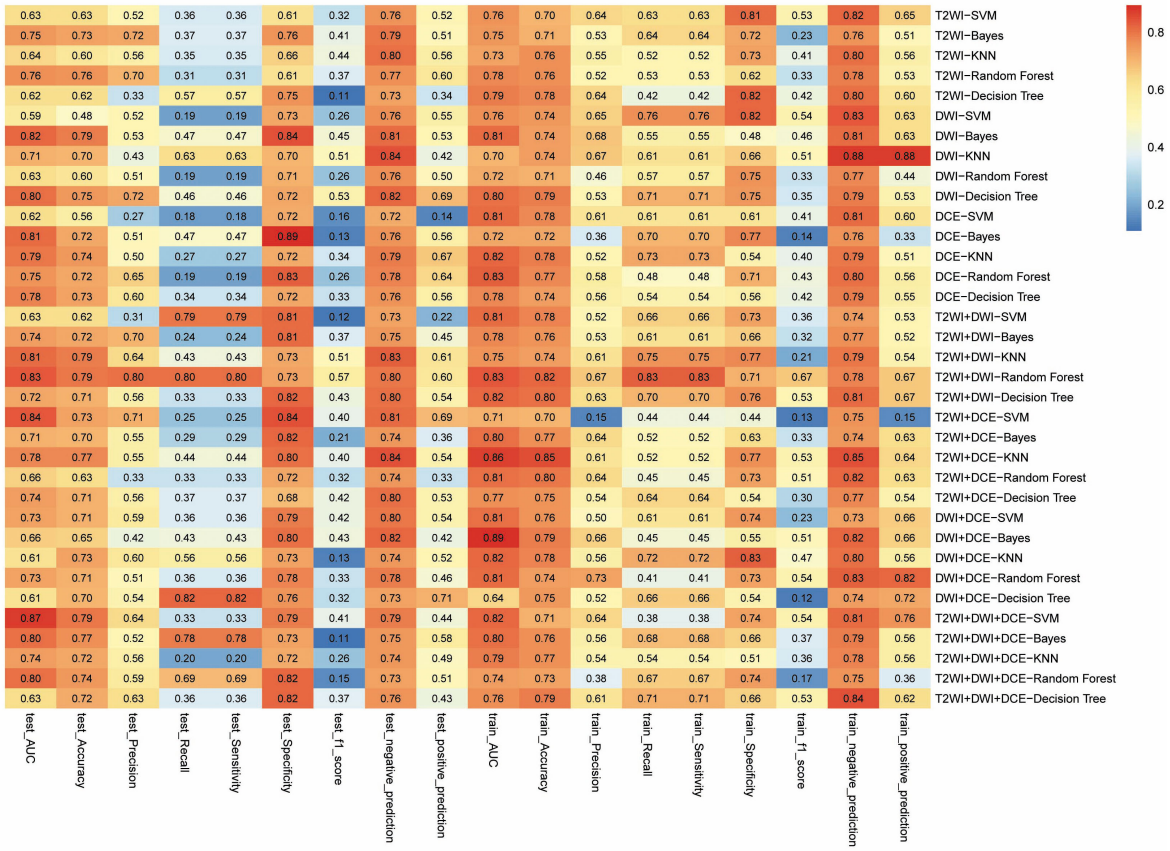

FIGURE 4 | The evaluation results of radiomics signature combined features from different imaging sequences and machine learning methods. The right column of the figure shows the combination of different sequences. The horizontal coordinate shows different items to be evaluated in the training set and the test set, and the value in each frame represents the evaluated result of the items of the corresponding sequence combination. The closer the color is to red, the greater the value. In this study, the larger the AUC value represents the better combined model built by machine learning.

rankings were selected to establish the optimal subset, and LASSO was used to reduce the dimensions of the optimal subset to obtain a total of six features, among which three of the features were from T2WI images, and three were from DWI images. Finally, the six features were used to construct the radiomics signature model. In this study, the diagnostic accuracy of the radiomic signatures constructed by the random forest showed good prediction performance in both the training set and the test set, while the difference between the two was the smallest. The radiomic signature showed favorable predictive efficacy in the two sets with AUC values of 0.835 and 0.834, specificity of 71.32 and 73.21%, and sensitivity of 82.61 and 80%, respectively. Rad-scores, which were calculated using the radiomics signature formula, were significantly different between the pCR and non-pCR in training and test sets, indicating that radiomics signature has a good correlation with pCR clinical outcome, as shown in **Figure 3**. The evaluation results of other sequence feature combinations and different machine learning techniques used to construct the radiomics signature are shown in **Figure 4.** Information about the dimensionality reduction process and LASSO are shown in the **Supporting Information**.

Development and Performance of the Radiomics Nomogram

Univariate logistic regression analyses revealed that ER status, PR status, and the radiomics signature were independent predictors of pCR. Based on the independent predictors, multiple logistic regression was utilized to construct prediction models and the nomogram, as shown in **Table 3** and **Figure 5**. The VIFs of ER status, PR status, and the radiomics signature were 1.017, 1.011, and 1.02, respectively.

The calibration curves showed excellent consistency between the predicted and actual pCR probabilities in the radiomics nomogram of both patient sets. The accuracy, specificity, and sensitivity of the nomogram for predicting pCR were 0.888, 79.31, and 86.96% in the training set and 0.879, 82.19, and 83.57% in the test set. The DeLong test showed AUCs of ER and PR were significantly different from that of nomogram in the training and test sets, as shown in **Table 4**. Therefore, the nomogram was found to perform well in both sets. Next, the Hosmer-Lemeshow test found no statistical differences between the training and test sets ($p > 0.05$), verifying the superior diagnostic accuracy of the nomogram. The pCR probability

TABLE 3 | Logistic regression analysis for predicting pCR in breast cancer (BCa) patients.

Variable	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (per one increase)	0.955 (0.884–1.031)	0.238	NA	NA
Menopausal (No vs. Yes)	0.621 (0.145–2.656)	0.52	NA	NA
Histologic type (NST invasive carcinoma vs. Other)	2.345 (0.508–9.811)	0.118	NA	NA
ER status (Negative vs. Positive)	0.255 (0.068–0.958)	0.043*	0.215 (0.062–0.745)	0.015*
PR status (Negative vs. Positive)	0.174 (0.045–0.675)	0.011*	0.214 (0.064–0.715)	0.012*
HER2 status (Negative vs. Positive)	0.736 (0.195–2.783)	0.651	NA	NA
Clinical stage (I vs. II)	0.758 (0.167–3.446)	0.72	NA	NA
Clinical stage (I vs. III)	0.838 (0.295–2.375)	0.739	NA	NA
Ki-67 (Low vs. High)	0.913 (0.149–5.577)	0.921	NA	NA
Radiomics score (per 0.1 increase)	2.575 (1.591–4.168)	0.001*	2.408 (1.56–3.715)	0.001*

*ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, antigen Ki67. *p < 0.05.*

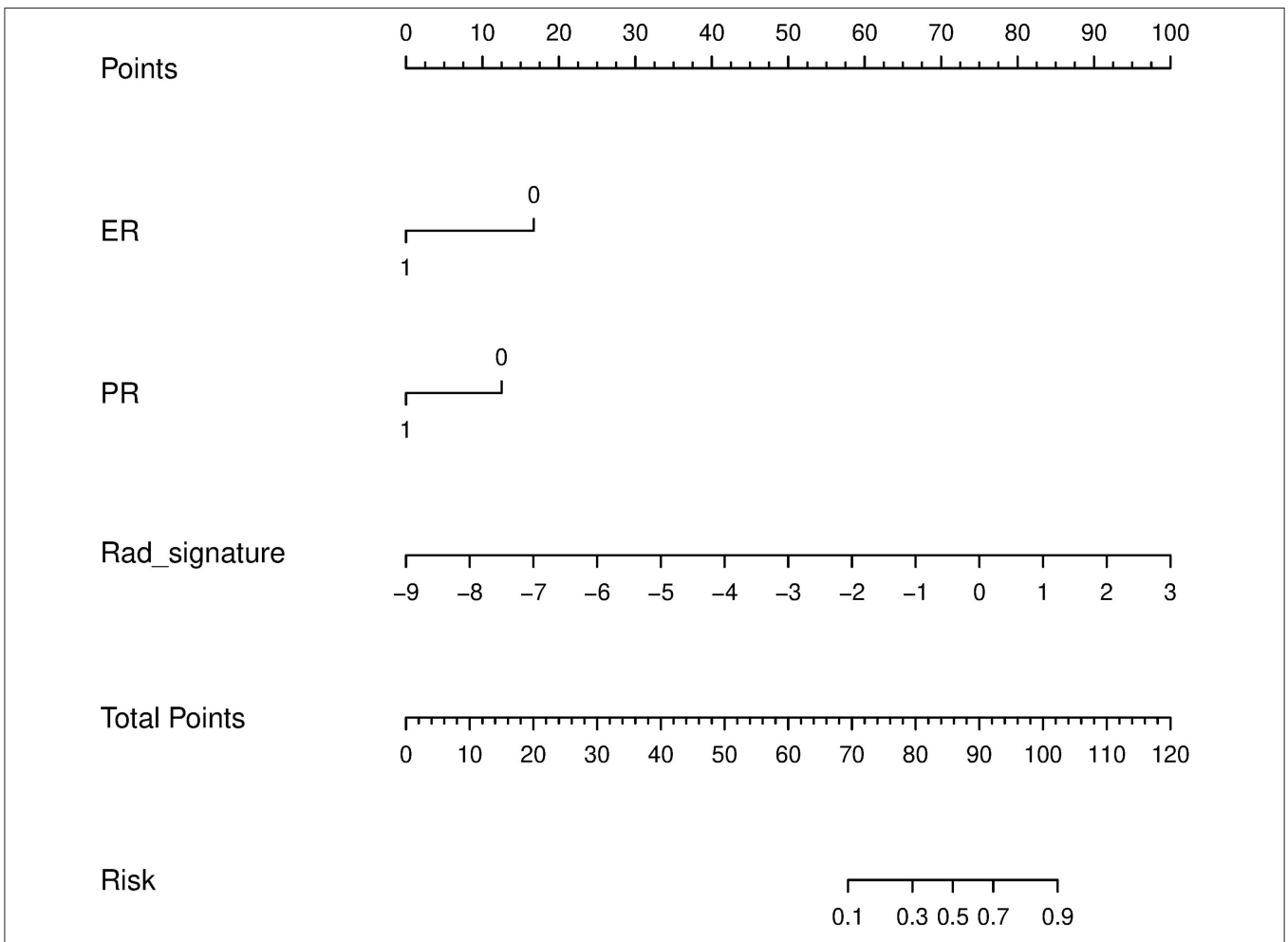

FIGURE 5 | Radiomics nomogram to predict the patient with pCR. The radiomics nomogram was developed in the training set, with the rad-score, ER status, and PR status.

scores were estimated using the nomogram, and patients were classified into the high and low probability groups according to the Yonden index (cut-off: 0.3371), which was based on the nomogram constructed by the training set. The number of pCR cases was significantly different between the high and low probability groups ($p < 0.0001$). In addition, DCA curves showed

TABLE 4 | AUCs of Nomogram, radiomics Signature, ER status, and PR status for pCR prediction in Trarining and Test sets.

	Nomogram(95% CI)	Signature (95% CI)	ER status(95% CI)	PR status(95% CI)
Training set	0.888 (0.814–0.94)	0.835 (0.753–0.899)	0.658 (0.562–0.746)	0.693 (0.598–0.777)
Nomogram vs. other metrics		0.0956	<0.0001*	0.0015*
Signature vs. other metrics			0.0133#	0.075
ER status vs. PR status				0.06392
Test set	0.879 (0.752–0.955)	0.834 (0.699–0.926)	0.716 (0.567–0.837)	0.642 (0.491–0.775)
Nomogram vs. other metrics		0.1446	0.0384*	0.0066*
Signature vs. other metrics			0.2239	0.0366#
ER status vs. PR status				0.5265

*p-value refers to Delong test for the differences of AUCs between different metrics in different cohorts, *$p < 0.05$, with significant differences for AUCs of Signature, ER status, and PR status compared with that of nomogram. #$p < 0.05$, with significant differences for AUCs of ER status and PR status compared with that of Signature.*

excelled net benefits of patients in training and test sets, as shown in **Figure 6**.

DISCUSSION

In this retrospective study, we have quantified the prognostic abilities of different machine-learning techniques for predicting the pCR status of patients with BCa. Considering the stability and prognostic performance together, two feature selection sequences (i.e., T2WI and DWI) and the random forest classification method should be preferred for the prediction of NACT response, as they display higher prognostic abilities and stability when compared with the other models. In addition, the nomogram constructed by the radiomics signature and pathological indexes could effectively predict the patients with pCR. In total, these findings indicate that imaging-based heterogeneity using machine learning and "big data" can provide complementary prognostic information about existing risk predictors.

In recent years, several research groups have searched for clinical or molecular markers that can predict the effect of NACT for the screening of patients who can benefit from the treatment (25). However, up to now, no factor has been found that can accurately predict the efficacy of NACT. The main reason is that the accuracy of single factor prediction is limited, yet a multifactor prognostic model may overcome this limitation for BCa. Based on this principle, a multifactor pCR prediction model was developed in this study. At the same time, we also extracted radiomic features from multiparameter images, including T2WI, DWI, and DCE. In the past, the features were extracted from multiparameter images to predict the prognosis of patients by merely distinguishing features of a single and joint sequence (26, 27). However, in this study, we created a pairwise sorting combination of three sequences (T2WI, DWI, and DCE), which further expanded the scope of this type of research. In addition, our result is consistent with Yoon's results that the features most associated with the clinical outcome of pCR mainly include Inverse difference moment and short-run emphasis features (28), which indicated these two types of feature had significant difference between non-pCR patients and pCR patients with breast cancer experiencing NACT. Although the remaining features also represent the degree of heterogeneity, the exact calculation method of each feature value varies depending on the parameters. Therefore, it is difficult to explain the subtle differences of various heterogeneous parameters caused by the mathematical equations. Besides, the unique biological mechanism that may cause heterogeneity parameters still remains unclear, which may require further research to figure out its mechanism.

The combination of features from T2WI and DWI sequences showed excellent performance of diagnosis for structure radiomics signature, suggesting that a conventional sequence can be used to predict the status of pCR without requiring enhanced imaging techniques in the future, such as DCE. Although recent studies have shown that DCE-MRI is the best sequence to predict NACT response so far (29, 30). Unfortunately, in this study, we found that the prediction efficacy of the radiomics signature was not optimal after combining the features extracted from DCE sequences with those of T2WI or DWI. This is mainly due to the instability of the model performance. Although the performance of the DCE-T2WI combination or the DCE- DWI combination model in the training group is higher than that of the T2WI-DWI combination model, the performance of the model with DCE features in the test set is significantly lower to that of the training group, which indicates that the model stability is not favorable. It may be possible that we only analyzed one 2D slice in a tumor other than a whole 3D tumor in this study, which may cause the instability of the model, we will conduct a further study using 3D tumor to validate the model stability in future research.

This may be due to the different amounts of contrast agent, which can affect the selection of features. Since patients receive different amounts of contrast agent, the enhancement effects of images can affect the permeability of tissue microvascular (31). This reflects the distribution of pixels and further affects the stability of the overall construction model. DWI was previously found to be useful as a tool to measure the efficacy of NACT in patients with BCa (32). However, the diffusion characteristics of water in tissue reflect the heterogeneity of tumors, rather than some external factors, such as contrast agents, which reflect the heterogeneity of tumor by imaging features. Accordingly, it should be demonstrated that T2WI and DWI sequences provide a suitable combination for feature extraction.

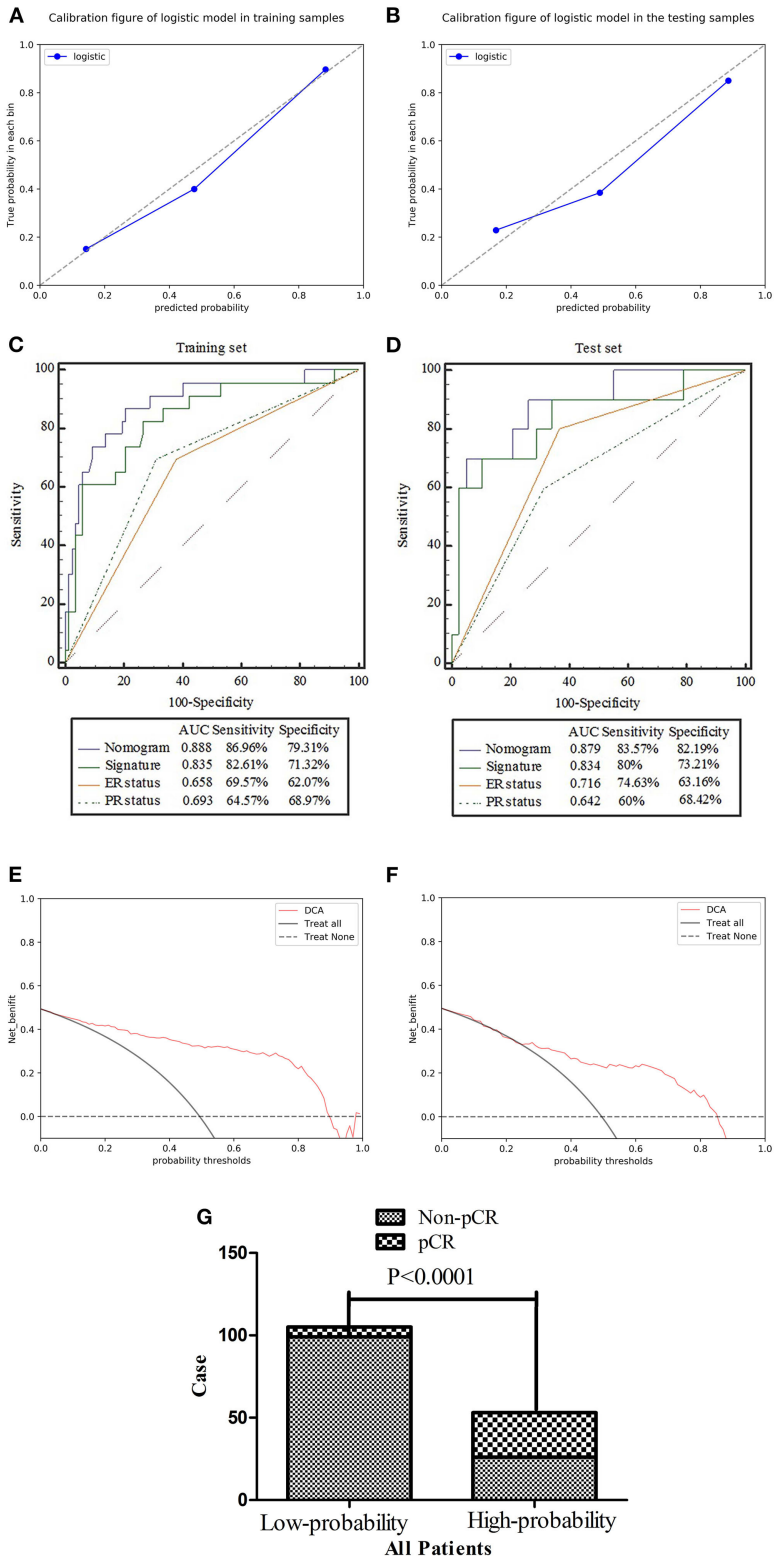

FIGURE 6 | (A,B) Calibration of the radiomics nomogram for predicting pCR in the training and test sets. Dashed line is the reference line where an ideal nomogram would lie, while the solid line corrects for any bias in the hybrid nomogram. **(C,D)** Evaluation of the accuracy of the ER status, PR status, radiomics signature, and nomogram for predicting pCR in the training and test sets. **(E,F)** Decision curve analysis was used to show the clinical effect of the nomogram in the prediction of pCR in breast cancer (BCa) patients in training and test sets. **(G)** Probability of pCR in the high-probability group was significantly higher than that in the low-probability group ($p < 0.0001$).

The development of statistical models that use the tumor and treatment data from a single patient are better prognostic indicators than human experts (33). Hence, the radiomics-based machine-learning models may be a viable tool for clinical decision support. In this study, we use different machine learning methods to construct a radiomics signature for the extended sequence combination and obtained the highest AUC of 0.835. In another study, Braman et al. (34) also used radiomics to predict pCR status based on different machine learning methods, yet their accuracy was significantly lower than the results of this study with an AUC of 0.74. This may be due to our radiomic features having higher dimensions in our study, which can better reflect the heterogeneity of tumors (35, 36). On the other hand, this study uses a variety of potential factors combined with the radiomics signature to build the prediction model, which also improved the accuracy of this model. In this study, we found that ER and PR expression were independent predictors of patients with pCR, which is similar to other studies (37, 38). The diagnostic efficacy of the nomogram was higher than the signature after adding pathological indicators, which further demonstrates the important role of hormone receptors in the prediction of pCR status. The relationship between texture-based heterogeneity indices and pathologic prognostic factors in breast cancer was confirmed as well. Tumor heterogeneity measured on FDG PET was higher in LABC with poor prognostic pathologic features, such as hormone receptor negativity, nuclear grade 3, and triple negativity (39).

Several features differentiate our work in this study from other radiomic-based studies. First, only features with high repeatability were used in this study, making this approach less prone to the risk of overfitting. The traditional repeatability test is to test the consistency of all the extracted features among the observers, which may allow for some features with poor repeatability to be included in the study. For this reason, some of the features with low repeatability may not accurately reflect the degree of tumor heterogeneity. On the other hand, we also reduced the dimensions of the features from the MRI sequences, including mRMR of the emerging method and LASSO of the traditional dimension reduction method, which may also explain why the diagnostic efficiency of our model is better than other prediction models constructed from LASSO alone. The superiority of this model is also related to the optimal machine learning techniques used to build the model. Therefore, the optimization of the classifier of this model is another important aspect of the study, which differs from traditional radiomics studies. Lastly, our nomogram was created for clinical research. A nomogram is a statistical tool that may be utilized to assess the probability or risk of a specific clinical outcome. For clinical practice, nomograms can be used to provide detailed risk assessments for the patient, aiding in clinical decision-making for clinicians (40). In our study, we built a radiomics nomogram that combines multi-dimensional information, which dramatically improved the accuracy and effectiveness of our model. These technical advances have contributed to the improved reproducibility found in our current study.

We acknowledge several shortcomings of the current study. First, this is a study representing only one medical center, which may not be applicable to populations in other centers. However, this study utilized a contemporary cohort of patients with BCa, allowing for the derivation of a hybrid nomogram that may be used to assess more extensive and diverse populations in the future. Secondly, a single slice of the tumor was sampled for the radiomics analysis, and volumetric assessments were not performed. In a previous study, data from a single slice was found to be sufficient for this type of analysis (41). Finally, our study population was relatively small. As this is a first proof-of-principle study, future studies should employ larger patient populations.

The potential prognostic abilities of radiomics models have been highlighted in other studies. However, with the expanded use of radiomics and feature dimensions, along with machine learning techniques, higher prognostic performance could be achieved in patients with BCa. This is also reflected in our nomogram, which showed high accuracy in the prediction of patients with pCR. As a non-invasive prediction tool, it can broaden the scope in the application of genomics for cancer treatment.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Zhejiang Cancer Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.01410/full#supplementary-material

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Impact of Rehabilitation on Breast Cancer Related Fatigue: A Pilot Study

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Breast cancer fatigue (BCF) is a complex and multidimensional condition characterized by a persistent sense of physical and/or mental stiffness, resulting in a substantial impairment of health-related quality of life in breast cancer survivors. Aim of this prospective cohort study was to evaluate the feasibility and the effectiveness of a 4-week rehabilitation protocol on BCF, muscle mass, strength, physical performance, and quality of life in breast cancer (BC) survivors. We recruited adult BC women with a diagnosis of BCF, according to the International Classification of Diseases 10 criteria, referred to the Outpatient Service for Oncological Rehabilitation of a University Hospital. All participants performed a specific physical exercise rehabilitative protocol consisting of 60-min sessions repeated 2 times/week for 4 weeks. All outcomes were evaluated at the baseline (T0), at the end of the 4-week rehabilitation treatment (T1), and at 2 months follow up (T2). The primary outcome measure was the Brief Fatigue Inventory (BFI); secondary outcomes included: Fat-Free Mass and Fat Mass, assessed by Bioelectrical Impedance Analysis (BIA); Hand Grip Strength Test (HGS); Short Physical Performance Battery (SPPB); 10-meter walking test (10 MWT); 6-min walking test (6 MWT); European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ–C30). Thirty-six women (mean age: 55.17 ± 7.76 years) were enrolled in the study. Significant reduction of BCF was observed both after the 4-week rehabilitation treatment (T1) (BFI: 5.4 ± 1.6 vs. 4.2 ± 1.7; $p = 0.004$) and at the follow-up visit (T2) (BFI: 5.4 ± 1.6 vs. 4.4 ± 1.6; $p = 0.004$). Moreover, significant differences ($p < 0.001$) HGS, SPPB, 10 MWT, 6 MWT, and EORTC QLQ-C30 were found at T1, while at T2 all the outcome measures were significantly different ($p < 0.05$) from the baseline. The rehabilitation protocol seemed to be feasible, safe, and effective in reducing BCF, improving muscle mass and function, and improving HRQoL in a cohort of BC survivors. The results of this study could improve awareness of this underestimated disease, suggesting the definition of a specific therapeutic exercise protocol to reduce BCF.

Keywords: breast cancer, quality of life, rehabilitation, fatigue, muscle strength, muscle performance, precision medicine

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INTRODUCTION

Breast cancer is the most common cancer in women and one of the leading causes of cancer-related death worldwide (1). Owing to the advances in the clinical management of this tumor, the number of long-term survivors has progressively increased during the past four decades (1). In this scenario, health-related quality of life has become more and more important in the overall patients' outcome evaluation (2–6).

Cancer-related fatigue, also known as cancer fatigue, is a highly prevalent long-term side effect among breast cancer survivors (7, 8). This complex and multidimensional condition is clinically characterized by a persistent sense of physical, emotional, and/or cognitive stiffness, resulting in a substantial impairment of health-related quality of life (7, 9). The etiology of breast cancer fatigue (BCF) is poorly understood and probably related to mitochondrial dysfunction, inflammation, and increased reactive oxygen species production (8, 10, 11). However, the wide subjectivity of BCF hinders further research to explain its pathogenesis. Several risk factors have been identified so far, including low socioeconomic status, sleep disturbance, emotional stress, anxiety, physical inactivity, high body mass index (BMI), radical surgery, chemotherapy, and radiotherapy (8, 9). According to the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) guidelines, specific screening programs for BCF should be performed (7, 12). In this respect, the gold standard for the evaluation of BCF is self-reporting using scales, questionnaires, and/or inventories (13). Regrettably, the great heterogeneity in these diagnostic methods, coupled with the lack of widely adopted guidelines, represents a major limitation in the clinical management of BCF (13, 14).

Several types of interventions have been proposed to treat or reduce BCF, including counseling, psycho-education, physical and mind-body activity, massage therapy, acupuncture, music therapy, supplements (e.g., ginseng, vitamin D, psychostimulants), and physical exercise (13). Among these, the supervised physical exercise is supported by the strongest evidence of safety and effectiveness in reducing BCF (15–19). Nevertheless, the optimal exercise interventions scheme (i.e., type, combination, frequency, intensity, and duration) to reduce BCF remains controversial. The aim of this study was to evaluate the feasibility and effectiveness of a 4-week rehabilitation protocol on BCF reduction.

MATERIALS AND METHODS

Patients

This prospective cohort study involved a consecutive series of breast cancer survivors suffering from BCF. All patients referred to the Outpatient Service for the Oncological Rehabilitation of the Physical Medicine and Rehabilitation Unit of University Hospital "Maggiore della Carità" in Novara, Italy over a 24-month period, from January 2018 to December 2019. The inclusion criteria were the following: (1) diagnosis of invasive breast cancer (2) surgery performed at least 12 months earlier; (3) diagnosis of cancer fatigue according to the International Classification of Diseases Tenth Revision (ICD-10) criteria. The exclusion criteria were the following: (1) anemia, defined as hemoglobin <9 g/dl; (2) severe thrombocytopenia, defined as platelets $<100{,}000/mm^3$; (3) history of bleeding; (4) hypothyroidism without replacement therapy; (5) persistent insomnia; (6) central nervous system primary and/or metastatic tumors. Inclusion and exclusion criteria are summarized in **Table 1**. The study protocol was approved by the local Institutional Review Board and was compliant with the ethical guidelines of the responsible governmental agency. At the enrollment, all the participants were asked to carefully read and sign an informed written consent. The investigators provided to protect the privacy and the study procedures according to the Declaration of Helsinki.

Intervention

All participants were subjected to a specific physical exercise rehabilitative protocol consisting of 10 min of warm-up, 40 min of aerobic exercise (e.g., walking, cycling, rowing) and strength training (e.g., light weightlifting), and 10 min of cool-down. Each session was repeated 2 times/week with at least 2 days of rest for 4 weeks, under the supervision of an experienced physical therapist. The study flow chart is shown in **Figure 1**. At the end of the rehabilitation treatment, a booklet encompassing the pictures and instructions of the previously performed exercises was provided to the patients. To maintain the benefits obtained during the hospital treatment, all patients were trained and strongly encouraged to continue the exercises at home. In the case of BCF evolution and/or worsening of general clinical conditions, the rehabilitation treatment was stopped.

Fatigue and Physical Performance Evaluation

At the baseline (T0), demographic and anthropometric characteristics, cancer location and staging, as well as pharmacologic history, have been assessed. All outcomes were also evaluated at the end of the 4-week rehabilitation treatment (T1), and at 2 months follow-up (T2).

Primary Outcome

The primary outcome measure was the Brief Fatigue Inventory (BFI), a multidimensional self-report scale that assesses the effects of fatigue on health-related quality of life originally reported by Mendoza et al. (20, 21). This survey is composed of nine questions scored on a 0–10 point scale. The BFI is presented as two parts. Specifically, the first three questions rate the current, usual, and worst levels of fatigue over the last 24 h, while the remaining six questions are related to the impact of fatigue on activity, mood, walking, work, relationships, and enjoyment of life. A total BFI score is then calculated by the mean of the nine scores, where scores 1–3 indicate slight fatigue, scores 4–6 moderate fatigue, and scores 7–10 severe fatigue.

Secondary Outcomes

The secondary outcomes were the following. (1) Body composition in terms of fat-free mass (FFM) and fat mass (FM) by bioelectrical impedance analysis (BIA). For this study,

TABLE 1 | Eligibility criteria of the study population.

Inclusion criteria	
(1) Diagnosis of invasive breast cancer	Patients with a diagnosis of breast cancer with cancer cells that have grown through the lining of the ducts into the surrounding breast tissue.
(2) Surgery at least 12 months earlier	All patients underwent breast surgery (conservative or mastectomy) at least 12 months earlier.
(3) Diagnosis of cancer fatigue according to the International Classification of Diseases Tenth Revision (ICD-10) criteria	The ICD-10 criteria define cancer-related fatigue (CRF) as diminished energy, an increasing need for rest, limb heaviness, diminished ability to concentrate decreased interest in engaging in normal activities, sleep disorder, inertia, emotional liability, perceived problems with short-term memory, and post-exertional malaise exceeding several hours.
Exclusion criteria	
(1) Severe anemia	A severe decrease in hemoglobin blood levels defined by the threshold of <9 g/dl.
(2) Severe thrombocytopenia	A severe decrease of thrombocyte blood levels defined by the threshold of <100,000/mm^3.
(3) History of bleeding	Patients that have a history of bleeding during cancer evolution.
(4) Hypothyroidism without replacement therapy	An endocrine system disorders, where the thyroid produces insufficient levels of thyroid hormone, leading to several symptoms, including fatigue.
(5) Persistent insomnia	Insomnia lasting more than 1 month, that might result in increased fatigue.
(6) Central nervous system primary and/or metastatic tumors	Other tumors that might affect patients.

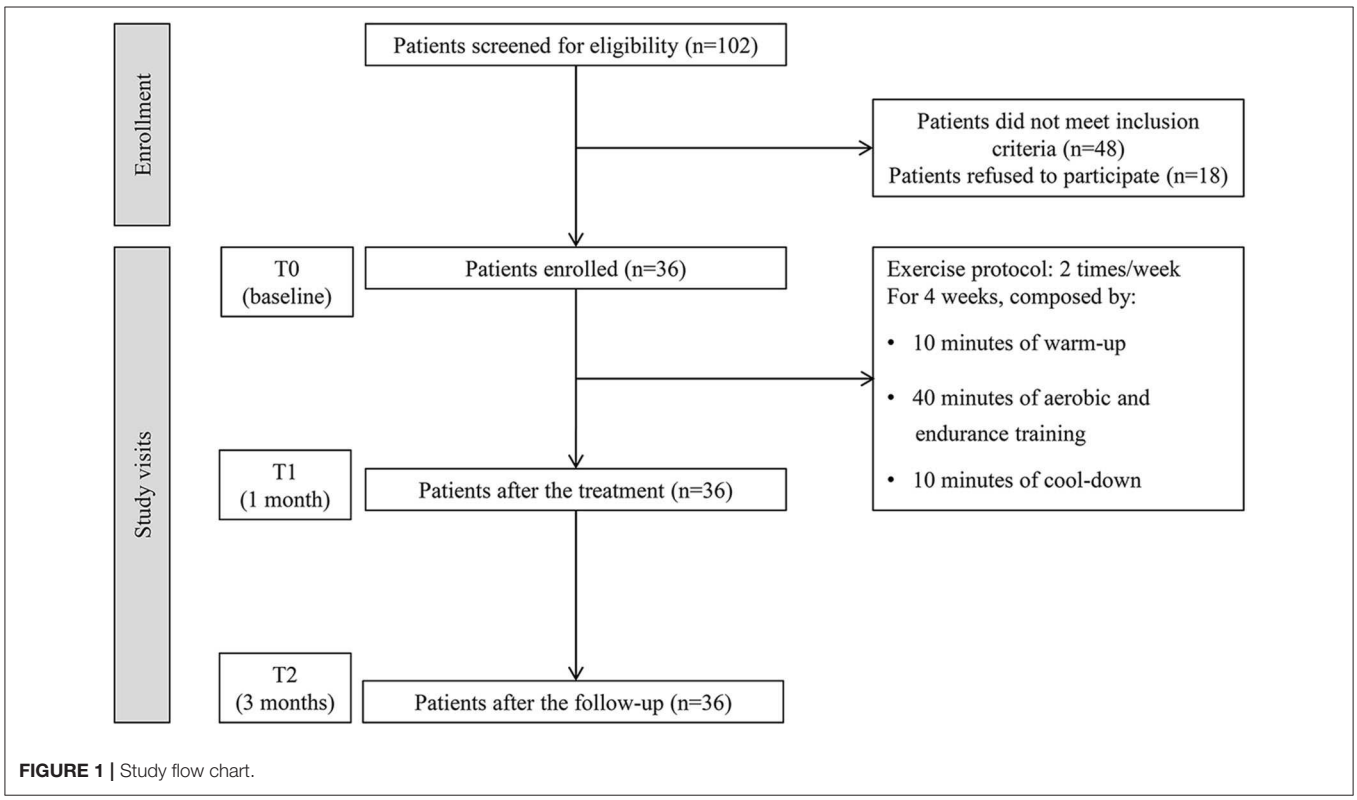

FIGURE 1 | Study flow chart.

the BIA101 Anniversary (Akern Srl, Pontassieve, Florence, Italy) was used. BIA evaluations were performed with patients in a supine position, with the upper and lower limbs abducted by about 30 and 45 degrees, respectively. The electrodes were placed on hands and feet at a minimum distance of 5 cm and connected to the cable with the red insulated tweezers (distal) and black (proximal). FFM and FM were determined according to the equation elaborated by Kyle et al. (22). (2) Handgrip strength test (HGS), using the Jamar® hydraulic hand dynamometer (Sammons Preston, Rolyon, Bolingbrook, IL, USA) to assess the isometric grip strength of the hand, according to the American College of Sports Medicine recommendations (23). This measure strongly correlates with global muscle strength (24). Briefly, the test was conducted with the participant seated on a chair, the shoulder adducted and neutral for rotation, with the elbow flexed at 90°, the forearm neutral for prono-supination and wrist extension between 0 and 30° with 0–15 degrees of ulnar deviation. The test was repeated three times to obtain the mean. (3) Short physical performance battery (SPPB), a composite scale ranging from 0 to 12, assessing walking speed, standing balance,

and sit-to-stand performance (25, 26). (4) Ten-meter walking test (10MWT) to assess walking speed (27). (5) 6-min walking test (6 MWT) for the integrated response of cardiopulmonary and musculoskeletal systems (28). (6) European Organization for Research and Treatment of Cancer 30-item quality of life questionnaire (EORTC QLQ–C30), a unidimensional scale that assesses the severity of symptoms related to cancer and its treatment, consisting of functional scales (i.e., physical, role, cognitive, emotional, and social functioning), a global quality of life scale, symptom scales (i.e., fatigue, nausea and vomiting, and pain), and global health (i.e., appetite loss, diarrhea, dyspnea, constipation, insomnia, financial impact) (29). Furthermore, at T1, both the enrolled patients and the physical therapist expressed their satisfaction regarding this treatment, which was assessed using the Global perceived effect (GPE) Scale, ranging from 1 (best satisfaction) to 7 (unsatisfaction) (30).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism®, version 7.00 (GraphPad Software, La Jolla California USA). Due to the low numerosity of the sample, we assumed a non-gaussian distribution of the considered variables, as previously described (31). Differences between single variables at different time-points were assessed by the two-way Friedman Analysis of Variance (ANOVA) for repeated measure and Dunn's *post hoc* test. A type I error level of 0.05 was chosen. A p-value lower than 0.05 was considered statistically significant.

RESULTS

A total of 102 women with BCF were assessed. Among them, 48 (47%) did not meet the eligibility criteria and 18 (18%) refused to sign the informed consent. Taken together, 36 patients were enrolled in the study. The study flowchart is depicted in **Figure 1**.

Demographic and General Characteristics of BCF Patients

The mean age at diagnosis of the 36 patients included in this study was 55.17 ± 7.76 years. Most of the women were of normal weight or borderline overweight (BMI = 25.15 ± 5.52 kg/m^2). The rate of smokers was similar to that of the general women population ($n = 8$, 22.2%). All of them underwent breast surgery, with equal distribution between conservative and radical surgery ($n = 19$, 52.7%, and $n = 17$, 47.3%, respectively). The en bloc axillary dissection was performed in 16 (44.4%) patients, while 21 (58.3%) were subjected to radiotherapy, either in the supraclavicular fossa or in the chest wall. Breast cancer related lymphedema was present in 12 (33.3) patients. The baseline characteristics along with the therapeutic information are listed in **Table 2**.

Reduction of BCF After Rehabilitation Treatment

We observed a statistically significant reduction of the BCF score after the 4-week rehabilitation treatment (T1) compared to T0 (4.2 ± 1.7 vs. 5.4 ± 1.6; $p = 0.004$). Despite the small sample size, the significance was substantially maintained at the follow-up visit (T2) (4.4 ± 1.6; $p = 0.004$), as showed in **Figure 2** and **Table 3**. However, no statistical significance was observed between the T1 and theT2 stage. Furthermore, we found significant differences at T1 in terms of HGS (20.1 ± 5.8 vs. 22.5 ± 5.2: $p < 0.001$), SPPB (9.3 ± 2.0 vs. 11.3 ± 1.2; $p < 0.001$), 10 MWT (1.5 ± 0.3 vs. 1.8 ± 0.3; $p < 0.001$), 6 MWT (464.5 ± 62.9 vs. 554.1 ± 71.6; $p < 0.001$), EORTC QLQ-C30 Functional score (69.2 ± 14.9 vs. 76.9 ± 15.7; $p < 0.001$), EORTC QLQ-C30 Symptoms score (29.2 ± 14.9 vs. 21.2 ± 16.0: $p < 0.001$), and EORTC QLQ-C30 Global Health score (40.7 ± 12.5 vs. 67.6 ± 14.8; $p < 0.001$). At 2 months (T2), all the outcome measures significantly differ from the baseline ($p < 0.05$), including FFM (43.2 ± 6.4 vs. 45.5 ± 6.6; $p < 0.001$) and FM (24.0 ± 10.6 vs. 21.7 ± 10.0; $p < 0.001$), as showed by **Table 3**. Moreover, the GPE score measured at T1 was 2.20 considering patients' perspective and 2.40 considering physical therapists' perspective.

TABLE 2 | Clinicopathologic and demographic characteristics of the patients included in this study.

	Patients ($n = 36$)
Age	55.17 ± 7.76 years
Body mass index (BMI)	25.15 ± 5.52 kg/m^2
Smoke (*n*, %)	8 (22.2)
Breast surgery	
Conservative (*n*, %)	19 (52.7)
Mastectomy (*n*, %)	17 (47.3)
Axillary surgery	
Sentinel lymph node (n, %)	20 (55.6)
En bloc dissection (n, %)	16 (44.4)
Radiotherapy (*n*, %)	21 (58.3)
Chemotherapy (*n*, %)	26 (72.2)
Hormone therapy (*n*, %)	29 (80.6)
Trastuzumab (*n*, %)	10 (27.7)
Upper limb lymphedema (*n*, %)	12 (33.3)

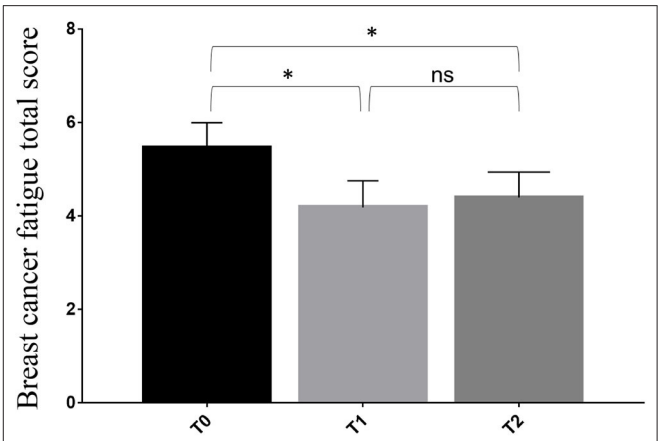

FIGURE 2 | Differences in primary outcome measure from the baseline (T0) to the end of 4-week rehabilitation treatment (T1) and the follow-up assessment at 3 months from the baseline (T2). * $p < 0.05$; ns, non significant.

TABLE 3 | Differences in outcome measures from baseline (T0) to the end of 4-week rehabilitation treatment (T1) and the follow-up assessment at 3 months from the baseline (T2).

	T0	T1	T0-T1 *P*-value	T2	T0-T2 *P*-value
BFI	5.4 ± 1.6	4.2 ± 1.7	0.004	4.4 ± 1.6	0.004
FFM (kg)	43.2 ± 6.4	44.4 ± 6.2	0.231	45.5 ± 6.6	<0.001
FM (kg)	24.0 ± 10.6	22.9 ± 10.2	0.297	21.7 ± 10.0	<0.001
HGS (kg)	20.1 ± 5.8	22.5 ± 5.2	<0.001	21.7 ± 6.0	0.012
SPPB	9.3 ± 2.0	11.3 ± 1.2	<0.001	11.7 ± 0.5	<0.001
10 MWT (m/s)	1.5 ± 0.3	1.8 ± 0.3	<0.001	1.9 ± 0.3	<0.001
6 MWT (m)	464.5 ± 62.9	554.1 ± 71.6	<0.001	567.1 ± 82.7	<0.001
EORTC QLQ-C30					
Functional score	69.2 ± 14.9	76.9 ± 15.7	<0.001	75.0 ± 17.1	0.005
Symptoms score	29.2 ± 14.9	21.2 ± 16.0	<0.001	21.9 ± 18.5	<0.001
Global Health score	40.7 ± 12.5	67.6 ± 14.8	<0.001	65.2 ± 20.0	<0.001

BFI, Brief Fatigue Inventory; FFM, fat-free mass; FM, fat mass; HGS, hand-grip strength test; SPPB, Short Physical Performance Battery; 10 MWT, 10-min walking test; 6 MWT, 6-min walking test; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire.

DISCUSSION

Several exercise programs have been proposed to reduce BCF (17, 32–36). However, the choice of the most appropriate intervention to offer remains troubled in real-life clinical practice. Here, we subjected an exploratory cohort of breast cancer survivors suffering from BCF to a physical exercise rehabilitative protocol consisting of 10 min of warm-up, 40 min of aerobic exercise and strength training, and 10 min of cool-down, twice a week for 4 weeks. Taken together, a significant decrease of BCF was observed at the end of the program and was maintained at the follow-up visits.

To date, there is still little evidence about the multifactorial mechanisms underpinning BCF pathogenesis. Historically, the loss of muscle mass, metabolism disorders and ATP production impairment have been viewed as founder events (37). In our study, we noticed a significant improvement in the FFM and a significant reduction of the FM at a 2-months follow-up but not at T1, suggesting that muscle mass modifications need more time to manifest compared to the relatively fast improvement of all the functional outcomes assessed. These results highlight that rehabilitative physical exercise counters the main mechanisms underpinning BCF and might be considered as an effective and reliable treatment option. This notion, however, should be considered in the context of the small sample size investigated in the present work.

A recent randomized controlled trial investigated the effects of a specific training program to modulate systemic inflammation (38). After this intervention, serum levels of TNF-α, IL-6, and IL-10 were significantly lower in the intervention group, confirming the anti-inflammatory properties of physical exercise previously demonstrated in several pathological conditions (39–42) and suggesting a possible mechanism through which it intervenes in countering BCF clinical manifestations. Of note, given the lack of adverse events in our study group, we confirm the excellent safety profile of this physical exercise intervention. This approach proved to be feasible, considering the high treatment adherence (i.e., no dropouts) and the high GPE scores obtained by both patients and physical therapists.

The skeletal muscle system has been recently hypothesized to have a key role in fatigue pathogenesis (43, 44). Furthermore, there are multiple examples in literature on the direct mitochondria damage, inducing a dysfunction characterized by an increased intracellular oxidative stress and low energy supply (8, 45–48). Noteworthy, exercise training could remodel the mitochondrial network, influencing the mitochondria intrinsic plasticity through different mechanisms and modulating their shape in response to fission and fusion events (45, 47, 49–51). Furthermore, it has been recently proved that physical exercise is able to improve mitochondrial function and dynamics in fragile patients (51). Similarly, an endurance exercise protocol could have a key role in the prevention of muscle wasting by stimulating mitochondrial dynamics. Taken together, all these findings, coupled with our preliminary observations, could suggest that exercise therapy might have a crucial impact not only in the clinical and therapeutic management of BCF, but also interfering directly in its pathogenesis.

This study has several limitations. First, the relatively small sample size of women with BCF included in the study could have limited the clinical impact of our conclusions. It should be noted, however, that our pilot prospective study provides for the first time in literature evidence on the possible clinical application of a specific physical exercise rehabilitative treatment in this setting. Further prospective studies embracing larger cohorts of patients are warranted to define the implications of our observations. Second, due to the study design, we did not collect any data on bone mineral density, falls, and fracture rate. Indeed, a high prevalence (80.6%) of patients treated with aromatase inhibitor therapy, a well-known risk factor for osteoporosis (52), has been recruited in the present study. Considering the beneficial effects of physical exercise on bone mineral density in premenopausal and postmenopausal women (53), the improvement of all functional parameters that we observed after a 1-month protocol might constitute the basis for a possible role in contrasting

osteoporosis, reducing the risk of falling and consequently the risk of fragility fractures. On the other hand, given that these women are at high risk of osteoporosis, it is mandatory to underline the role of an adequate therapeutic exercise to prevent fractures and all the disabling consequences. Third, the lack of a control group limits the translational relevance of our hypothesis. However, this study should be considered a proof-of-principle that rehabilitation interventions can be safety and effectively performed in breast cancer survivors. Lastly, we did not provide any data on long-term outcomes because all of the patients enrolled in this prospective study are still followed up by our multidisciplinary team.

Despite these limitations, we provide preliminary and previously unavailable evidence on the feasibility, reliability, and safety of a 1-month specific physical exercise rehabilitative protocol in reducing BCF, improving muscle mass, muscular-skeletal function, and health-related quality of life in breast cancer survivors. Our results advocate the need to define tailored physical exercise interventions that could be performed in common clinical practice as a first-line rehabilitative treatment to reduce BCF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Interaziendale Novara. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MI and CCi: study concept and design. MI, CCi, and NF: supervision. MI and AS: manuscript writing (first draft). AS, LL, KV, FG, and AG: bibliography. MI: iconography. KV, ES, CCr, and NF: critical revision. All authors contributed to the article and approved the submitted version.

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