BREAST CANCER IN YOUNG WOMEN

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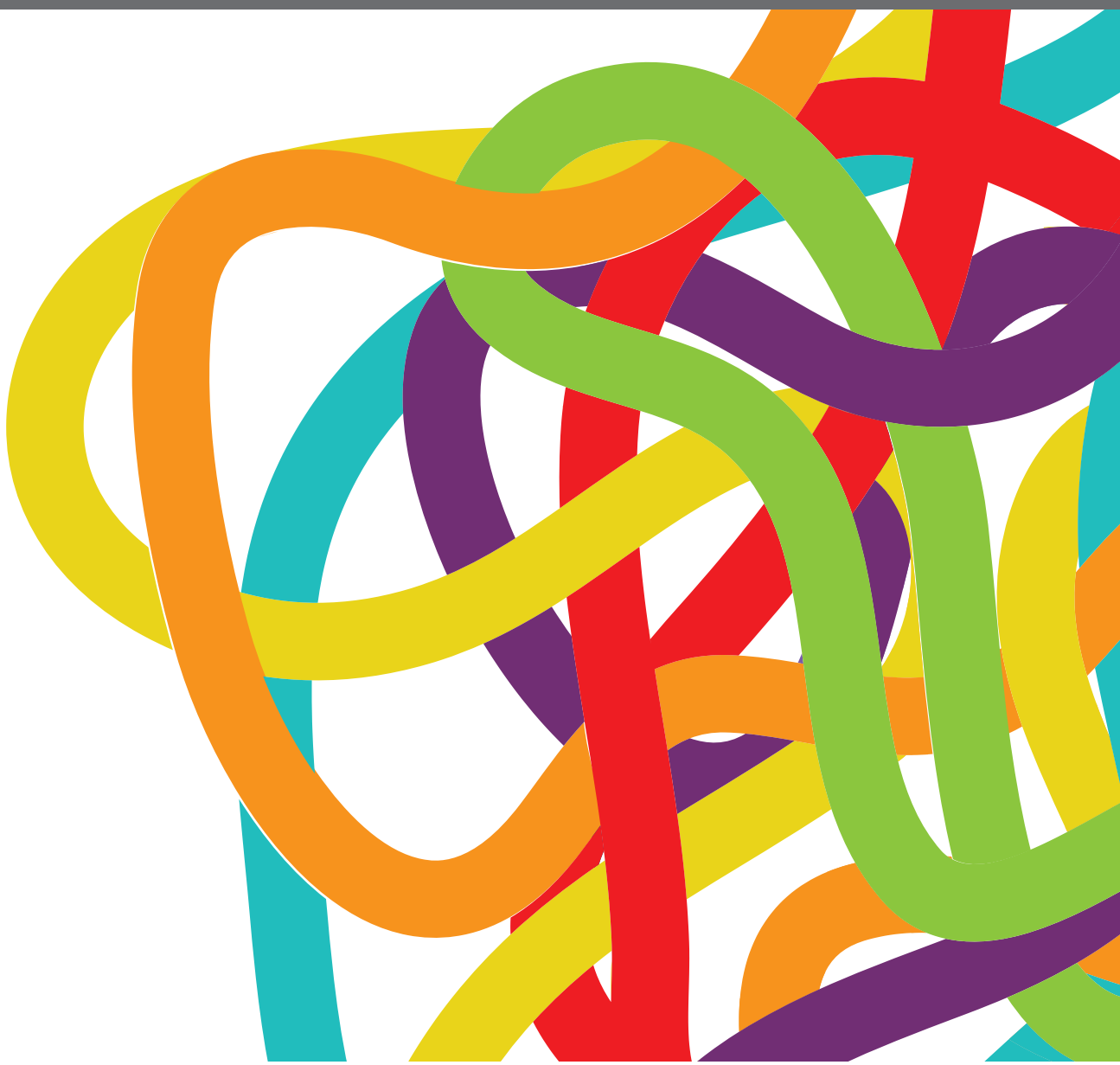

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BREAST CANCER IN YOUNG WOMEN

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EDITORIAL
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Editorial: Breast Cancer in Young Women: Dedicated Research Efforts Are Needed

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Keywords: breast cancer, young women, BRCA, fertility, pregnancy

Editorial on the Research Topic

Breast Cancer in Young Women

MANUSCRIPT

Caring for women with newly diagnosed breast cancer at a young age, defined according to international guidelines as ≤ 40 years, is particularly challenging due to its associated additional age-related issues (1). Breast cancer in young women represents approximately one third of the total cases of malignancies in women aged less than 40 years, being the most frequent malignancy and cause of cancer-related death in this group of patients (2) (Silva et al.). The negative prognostic implication of young age at diagnosis may be partially explained by both the lack of screening programs and the higher risk of unfavorable biological features as compared to breast cancer cases in older patients (3). While young age has long been considered a negative prognostic factor, recent data have shown that this seems to be restricted only to patients with hormone receptor-positive disease (4) (Cai et al.). The biological and clinical reasons behind these findings have not been elucidated yet, although suboptimal adjuvant endocrine treatment and lower therapeutic adherence may be considered among the potential explanations (5) (Lu et al.).

Breast cancer in young women is considered a public health problem considering its substantial morbidity and mortality as well as the burden of disparities existing in the care of these patients (6).

While a breast cancer diagnosis at any age can substantially impact on familial relationships and other domains, young women are at a life stage in which additional implications including career, employment and family issues are particularly important. Hence, the potential financial, psychosocial, and social impacts of a breast cancer diagnosis at a young age can be even more burdensome. Importantly, when managing young women with newly diagnosed breast cancer, specific age-related issues should be considered. Among them, genetic counseling, fertility preservation, management of long-term side effects, impact on social and couple relationships and employment are highly relevant. Therefore, the care of young women with breast cancer is particularly complex and a multidisciplinary approach is mandatory (1).

Young age at diagnosis is considered a criteria to refer patients for genetic testing (1). Among different breast cancer susceptibility genes (Wang et al.) and in addition to the implications in terms of screening and risk-reducing strategies (Shraga et al.), identifying a pathogenic variant in *BRCA1* or *BRCA2* genes has clear therapeutic implications in both the early and advanced settings (1).

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Therefore, more attention is needed to better understand the behavior and outcomes of breast cancer in young women carrying germline *BRCA* pathogenic variants (7). Moreover, the clinical implications of carrying this genetic defect beyond cancer risk require a special focus. Among them, the impact of germline *BRCA* pathogenic variants on women's ovarian function and fertility are acquiring importance considering their potential negative impact of these defects on the ovarian reserve (8). Considering the current and upcoming availability of new anticancer therapies for the care of these patients, these issues need to be urgently addressed.

Over the past years, increasing attention has been paid to the oncofertility care of young adult cancer patients. As advocated by all guidelines, proper counseling on the risk of anticancer treatment-induced gonadotoxicity is mandatory at diagnosis with all patients with any malignancy and stage diagnosed at reproductive age (9, 10). Being a hormonally-driven form of tumor, there were historically several concerns on the safety and feasibility of managing fertility and pregnancy-related issues in the specific cohort of breast cancer patients. Recent data have contributed to dispel these concerns supporting the safety of accessing fertility preservation strategies prior to starting (neo) adjuvant chemotherapy (11) (Rothé et al.) and of having a pregnancy in women with prior history of breast cancer (12). Nevertheless, some special considerations are needed to manage oncofertility care in young women with breast cancer. Specifically, there are barriers for proper onco-fertility counseling including patients' side of decision conflict, oncologists' preference of referral to fertility specialists and standardized protocols of fertility preservation for women with breast cancer, including the preference for adding letrozole a part of controlled ovarian stimulation in order to reduce the rise in estradiol levels during the procedure (9, 10) (Bonardi et al.). The implementation of special oncofertility programs requiring a well organized network between oncology and fertility units are crucial to properly deal with fertility care in young women with breast cancer (Blondeaux et al. and Hours et al.).

The possible diagnosis of breast cancer during pregnancy is another additional possible situation to be considered when caring for young patients (13). This is a challenging condition characterized by several unique medical and psychological needs that require special attention (Costa et al.). Several advances have been made over the past years to better understand the biology of breast cancer arising during pregnancy (Korakiti et al. and Allouch et al.) as well as on the clinical management of this difficult situation (14, 15). Considering the current trend in delaying childbearing, a growing attention is needed to the possible occurrence of breast cancer during pregnancy.

In addition to the potential impact of anticancer therapies on fertility and chances of a subsequent pregnancy, other additional survivorship issues should be considered when caring for young women with breast cancer. Among them, the side effects of endocrine therapy (particularly for the need to administer ovarian function suppression in most of these patients) can be particularly impactful and require dedicated pharmacological and non-pharmacological approaches to counteract them (16) (Choi et al.). Indeed, survivorship is becoming an area of crucial importance in the care of patients with cancer and *ad hoc* programs should be implemented for improving the quality of life of young survivors (17).

With a special series focused on breast cancer in young women (https://www.frontiersin.org/research-topics/13438/breast-cancer-in-young-women), *Frontiers in Oncology* aims at providing updates and news in this field with topics spanning from epidemiology to treatment and its long-term consequences in order to contribute in improving the care of these patients. Further dedicated research efforts are needed to support young women with breast cancer.

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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SYSTEMATIC REVIEW
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The Genomic Profile of Pregnancy-Associated Breast Cancer: A Systematic Review

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Breast cancer is the most common malignancy diagnosed during pregnancy. Strong data on the genomic profile of pregnancy-associated breast cancer are lacking. This systematic review aims to integrate and analyze all existing data from the literature regarding the genomic background and the gene mutational patterns of pregnancy-associated breast cancer. Using various genomic analysis methods, multiple differentially expressed genes and numerous non-silent mutations have been detected. More particularly, our review demonstrates the aberrant expression of several oncogenes (e.g., *MYC*, *SRC*, *FOS*), tumor suppressor genes (e.g., *TP53*, *PTEN*, *CAV1*), apoptosis regulators (e.g., *PDCD4*, *BCL2*, *BIRC5*), transcription regulators (e.g., *JUN*, *KLF1*, *SP110*), genes involved in DNA repair mechanisms (e.g., Sig20, *BRCA1*, *BRCA2*, *FEN1*), in cell proliferation (e.g., *AURKA*, *MKI67*), in the immune response (e.g., *PD1*, *PDL1*), and in other significant biological processes (e.g., protein modification, internal cell motility). Further research on the genomic profile of pregnancy-associated breast cancer is urgently required in order to identify potential biomarkers facilitating early-stage diagnosis and individualized therapy.

Keywords: pregnancy, breast, cancer, gene, mutation

INTRODUCTION

Breast cancer is the most common type of malignancy diagnosed in women. Its incidence is notably rising with increasing age (1, 2). Breast cancer represents a heterogeneous disease with fundamental histological variations among patients of different age, sex, and in certain conditions such as gestation. Pregnancy-associated breast cancer (PABC) is generally defined as breast cancer diagnosed anytime during gestation, lactation or within 1 year after delivery (2–4). Several other PABC definitions with minor modifications regarding the postpartum period exist in the literature (2, 5). Along with melanoma and cervical cancer, they are the most frequent types of pregnancy related cancer (3, 6, 7). Every year, 1 in 3,000–10,000 women is diagnosed with breast cancer during pregnancy, representing only 0.2–3.8% of overall breast cancer cases (3, 4). As women postpone childbearing to a later age in our society, PABC rate is expected to increase significantly (2, 3).

PABC management consists a real challenge for physicians as both the mother and the fetus may be critically damaged (3, 6). Due to the rarity of the disease, strong data regarding PABC treatment are lacking and current guidelines are based on small retrospective studies and systematic meta-analyses. Cancer diagnosis in a period of hope and joy is an unendurable situation that may trigger symptoms of psychological distress such as depression, anxiety, social isolation and self-blame. On the one hand, patients face a life-threatening disease and an uncertain pregnancy. On the other hand, medical professionals face an ethical dilemma involving the future mother and her unborn child; what is best for the mother in terms of aggressive chemotherapy may be fatal for the fetus and vice versa, delaying therapy and protecting the fetus may have a negative impact on the mother as the tumor progresses (8).

The molecular nature of PABC remains an unknown field and considerable controversy exists in the literature regarding the influence of pregnancy on breast cancer prognosis (3). PABC exhibits particularly aggressive behavior and its poor outcome is largely attributed to tumor characteristics; advanced T stage in diagnosis, nodal involvement, high histologic grade, negative estrogen (ER) and progesterone (PR) status and *HER-2* overexpression (4, 9). Despite the substantial efforts in managing breast cancer during pregnancy, yet there has been little progress in explaining PABC biological characteristics.

This review aims to synthesize all existing data from the literature regarding gene expression in PABC. Genomic profiling studies identify both the spectrum of somatic mutational patterns and the genomic heterogeneity of the disease. A deeper understanding of PABC underlying mechanism may potentially explain its rather aggressive clinical behavior and may lead to individualized therapies.

METHODS

All eligible articles included in this literature review were identified in the Medline/PubMed bibliographical database and the research was conducted according to the PRISMA guidelines (10); the end-of-search date was June 10, 2020. The search strategy consisted of the following keywords: [breast AND (neoplasm OR neoplasms OR cancer OR cancers OR carcinoma OR carcinomas)] AND (pregnancy OR pregnant OR gestation) AND (genomics OR genomic OR gene OR genes OR mutation OR mutations). Furthermore, in order to identify any additional eligible articles, reference lists were also meticulously examined resulting in a total of 9 articles to be included as shown in **Figure 1**.

While working separately, two researchers (AMK and MM) searched the literature and another pair of investigators (AMK and EZ) independently extracted data from each eligible study. In case of disagreement between the members of each pair, team consensus was obtained after consulting the principal designers of the study (FZ and MAD). The articles included in this systematic review had to meet certain inclusion criteria: (1) studies highlighting the genomic profile of PABC, including mutational patterns, (2) studies based on the analysis of biological samples and/or bioinformatic approaches or computational algorithms with data originating from databases, (3) articles written in the English language. Publications were excluded if they met one or more of the following criteria: (1) animal studies without subsequent validation in human specimens, (2) reviews of literature, comments, letters or duplicate publications.

Abbreviations: PABC, pregnancy-associated breast cancer; ER, estrogen receptors; PR, progesterone receptors; HER-2, human epidermal growth factor receptor-2; FFPE, formalin-fixed paraffin embedded; CNA, copy number alteration; GPCR, G-protein-coupled receptor; DFS, disease-free survival; DEG; differentially expressed gene; OS, overall survival; PTM, post-translational modification.

RESULTS

The search strategy retrieved 23 articles. Of these, 16 were omitted based on the exclusion criteria and 7 were eligible (11–17). While examining the references of eligible articles, 2 more articles were included (18, 19). A summary of the studies describing the genomic profile of pregnancy-associated breast cancer is demonstrated in **Table 1**.

As far as the genomic analysis approach is concerned, four studies were based on formalin-fixed paraffin embedded (FFPE) tissue analysis (11–13, 18), one study examined fresh frozen tissue (14), three studies were associated with bioinformatic analysis and microarray profile datasets (15, 16, 19), and one study did not provide precise information regarding the methodology steps (17). Importantly, all studies included in the review were case-control studies; thus, the groups compared in each study exhibited similar clinicopathological characteristics.

According to the literature, the most frequently up-regulated genes encountered in PABC are several oncogenes: *MYC* (11, 16), *FOS* (16), *MUC1* (19), and gene sets related to *SRC* (12); multiple apoptosis regulators: *BIRC5* (14), *TRIM69* (15); transcription regulators: *JUN* (16), *KLF1*, *SP110* (19); genes involved in the immune response: *PD1*, *PDL1* (12), *IL18, CD274* (16); in DNA repair mechanisms: *BRCA1* (12), *FEN1* (19); in DNA replication: *RRM2* (14); in cellular growth and proliferation: *IGF1* (12), *MKI6*, *PRC1*, *MKI67*, *KIF2C*, *AURKA* (14); in protein modification: *KLHL3*, *ASB6* (15); in collagen degradation: *MMP11* (14), *MMP9* (15); in cell adhesion: *β-catenin* (12), *PXN* (15); and in internal cell motility: *ACTA2* (16). Additionally, high expression of the G-protein coupled receptor (GPCR) pathway and the serotonin receptor pathway is demonstrated (12).

In contrast, the most commonly down-regulated genes detected in PABC are numerous tumor suppressor genes: *TP53* (11), *PTEN* (14, 18), *CAV1* (14); cell cycle regulators: *AKTmTOR* (12), *GAS1* (14); apoptosis regulators: *PDCD4, BCL2* (18), *p63* (14), *SIAH1* (15); transcription regulators: *HOX* genes (14), *CREB1* (15); ribosomal genes (14); ECM-encoding genes (14); genes involved in DNA repair mechanisms: *BRCA2* (13); in protein modification: *UBA5, HECTD1, MEX3C, UBE2Q2, FBXO22* (15); in protein transport: *ARF3* (15); and in mRNA processing: *EIF4A3* (15).

To conclude, non-silent mutations characterizing PABC are most frequently enriched in the tumor suppressor gene *TP53* and

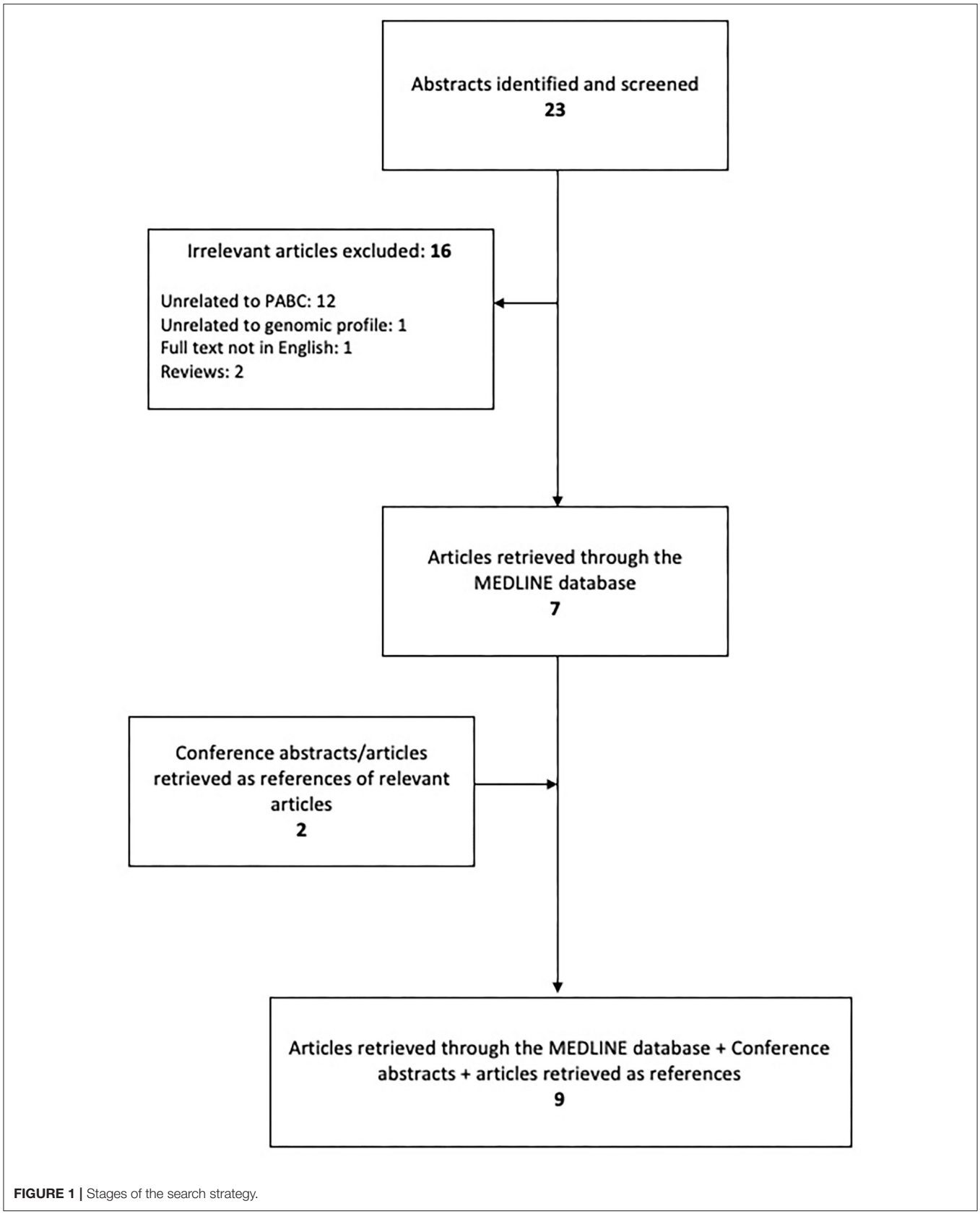

FIGURE 1 | Stages of the search strategy.

TABLE 1 | Summary of studies describing the genomic profile of pregnancy-associated breast cancer.

References	Sample	Technique	Groups	Up-regulated DEGs in PABC	Down-regulated DEGs in PABC	PABC enriched mutations
Nguyen et al. (11)	FFPE	WGS Microarray assay	54 PABC -vs-113 non-PABC	MYC*	TP53*	TP53*, PIK3CA* Mucin gene family Sig1, Sig20
Azim et al. (12)	FFPE	Multiplex PCR Microarray assay	54 PABC -vs-113 non-PABC	PD1, PDL1, BRCA1, Gene sets related to SRC, IGF1, β-catenin GPCR pathway Serotonin receptor pathway	AKTmTOR	TP53*, PIK3CA*
Walter et al. (18)	FFPE	RT-PCR IHC	25 PABC -vs.-adjacent normal breast tissue		PTEN, PDCD4, BCL2	
Shen et al. (13)	FFPE	PCR-based LOH assay	12 PABC -vs-15 non-PABC		BRCA2	
Johansson et al. (17)	N/A	N/A	14 sporadic PABC -vs-10 hereditary PABC			BRCA1
Harvell et al. (14)	Fresh frozen tissue & FFPE	LCM Microarray assay	6 PABC epithelia -vs-7 normal adjacent epithelia	MKI6, BIRC5, MMP11, RRM2, PRC1, MKI67, KIF2C, AURKA	PTEN, CAV1, GAS1, p63, Ribosomal genes	
Harvell et al. (14)	Fresh frozen tissue & FFPE	LCM Microarray assay	6 PABC stroma -vs-4 non-PABC stroma		HOX genes ECM-encoding genes	
Zhang et al. (15)	Microarray profile datasets (12, 14)	Bioinformatic analysis	74 PABC -vs −126 non PABC	KLHL3, MMP9, TRIM69, ASB6, PXN	CREB1, ARF3, UBA5, SIAH1, HECTD1, MEX3C, UBE2Q2, FBXO22, EIF4A3,	
Zhou et al. (16)	Microarray profile dataset (14)	Bioinformatic analysis	7 PABC stroma -vs-4 normal adjacent stroma	JUN, FOS, MYC, ACTA2, IL18, CD274		
Thanmalagan et al. (19)	Microarray profile dataset (14)	Bioinformatic analysis	20 PABC -vs −13 non PABC	KLF1, FEN1, SP110, MUC1		

PABC, pregnancy-associated breast cancer, FFPE, formalin-fixed paraffin embedded tissue, WGS, whole genome sequencing, PCR, polymerase chain reaction, RT-PCR, real time PCR, IHC, immunohistochemistry, LOH, loss of heterozygosity, LCM, laser capture microdissection.

**Up-regulated/Down-regulated/Mutationally enriched: when compared to normal tissue (no statistically significant difference between PABC and non-PABC groups).*

the cell cycle regulator *PIK3CA*, the mucin gene family involved in glycosylation (*MUC17, MUC2, MUC4, MUC12, MUC20*) and the *BRCA1* gene (11, 12, 17). Sig1 and Sig20 are the most common single-base alterations highlighted in PABC patients (11). A more detailed analysis of the results is presented in the following discussion.

DISCUSSION

This review aims to systematically summarize all existing data from the literature regarding the molecular nature of pregnancy-associated breast cancer. Multiple differentially expressed genes and numerous non-silent mutations have been detected. More particularly, our review demonstrates the aberrant expression of several oncogenes (e.g., *MYC*, *SRC*, *FOS*), tumor suppressor genes (e.g., *TP53*, *PTEN*, *CAV1*), apoptosis regulators (e.g., *PDCD4*, *BCL2*, *BIRC5*), transcription regulators (e.g., *JUN*, *KLF1*, *SP110*), genes involved in DNA repair mechanisms (e.g., Sig20, *BRCA1*, *BRCA2*, *FEN1*), in cell proliferation (e.g., *AURKA*, *MKI67*), in the immune response (e.g., *PD1*, *PDL1*) and in other significant biological processes (e.g., protein modification, internal cell motility). The most significant studies on the genomic profile of PABC are shortly presented.

In one of the most recent studies, Nguyen et al. analyzed retrospectively 167 breast cancer patients, 54 of whom were diagnosed during pregnancy, in order to identify specific molecular alterations characterizing PABC (11). No significant differences were found among PABC and non-PABC subgroups in terms of the copy number alteration (CNA) profiles. Of note, *MYC* oncogene was the most commonly amplified, whereas *TP53* tumor suppressor gene was the most frequently deleted gene in both subgroups. The study demonstrated that PABC group had a significantly higher number of non-silent mutations. Across the whole cohort, *TP53* and *PIK3CA* were the most frequently mutated genes. PABC group though was associated with a higher frequency of mutations in the mucin gene family that plays a major role in the mechanism of glycosylation (*MUC17, MUC2, MUC4, MUC12,* and *MUC20*); of note, alterations in the biological functions of glycosylation are correlated with breast carcinogenesis and metastasis (20). While investigating particular patterns of mutations on cancer genomes termed signatures, the researchers proved that the base-substitution mutational Signature 1 (Sig1) and Signature 20 (Sig20) predominated in PABC subgroup. As it is well established in the literature, Sig1 is associated with age at diagnosis. Sig20 was proven to be related to DNA mismatch repair (MMR) deficiency due to copy number loss of *MSH2* allele (21). In addition, Sig-20-positive patients were highly associated with PR negative status and a shorter disease-free survival (DFS) rate when compared to Sig20-negative patients.

Azim et al. also evaluated the biological pathways of PABC aiming to define the prognostic value of the different molecular aberrations (12). Even though the study found no significant differences in somatic mutations among pregnant and non-pregnant breast cancer patients, *TP53* and *PIK3CA* were the most commonly mutated genes in both subgroups, similarly to the aforementioned study. Moreover, Azim et al. demonstrated that the expression of two particular pathways was significantly enriched in breast tumors diagnosed during pregnancy when compared to non-pregnancy related cases; the G-protein coupled receptor pathway (GPCR) and the serotonin receptor signaling pathway (22, 23). Using transcriptomic profiling methods, the study also revealed that PABC tumors had a higher expression of *PD1, PDL1, BRCA1,* and gene sets related to *SRC, IGF1* and *β-catenin* and a lower expression of the *AKTmTOR* gene set. None of the above differentially expressed genes (DEGs) was statistically associated with DFS in the multivariate model.

A few years ago, Walter et al. focused on the expression of the tumor suppressor gene *PTEN* and on the levels of the apoptosis regulators *PDCD4* and *BCL2* in PABC, and on their role as potential markers of poor prognosis (18). In the analysis, protein levels of the aforementioned genes were lower in PABC tumors when compared to adjacent normal breast tissue. A statistically significant correlation was found in PABC group between *PTEN* gene downregulation and *miR-21* overexpression. Overexpression of *miR-21* was demonstrated in the PABC subgroup and it was correlated positively with lymph node involvement and negatively with prognosis.

Two studies regarding *BRCA1* and *BRCA2* mutations were retrieved from the literature. On the one hand, Shen et al. studied retrospectively 12 archival samples from PABC patients and demonstrated high frequency of loss of heterozygosity (LOH) at the *BRCA2* gene when compared to non-PABC cases, suggesting an initial genetic event in the pathogenesis of PABC (13). On the other hand, Johansson et al. investigated the influence of pregnancy on the risk of developing breast cancer in *BRCA1* and *BRCA2* mutation carriers (17). The statistical analysis proved that more women with *BRCA1* mutations developed PABC and implied a close monitoring of women with *BRCA1* familial mutations during and after pregnancy.

One of the largest studies on the genomic signatures of PABC was conducted by Harvell et al. who meticulously examined breast epithelial and stromal cells gene regulation by estrogens and progesterone (14). Both epithelia and tumor-associated stroma of PABC were characterized by enhanced expression of genes related to the immune response and the cell cycle regulation, many of which were hormone regulated. Tumor microenvironment influenced by the several-fold increased gestational hormones had a pivotal role in tumor aggressiveness (24, 25). In addition, the study revealed decreased expression of extracellular matrix (ECM)-encoding genes in PABC-associated stroma that is correlated with cancer invasion and metastasis (26).

Zhang et al. recently published an analysis on core genes and their clinical roles in PABC. Their research was based on two microarray profile datasets that derived from studies previously described in our review (12, 14), but instead focused on the identification of molecular biomarkers using the collective data (15). A total of 239 DEGs were detected in PABC, including 101 up-regulated and 138 down-regulated genes. The up-regulated DEGs were mainly enriched in the immune response, the fatty acid activation and the fibroblast growth factor signaling pathway, whereas the down-regulated DEGs were primarily

involved in the activation of DNA fragmentation factor and the apoptosis-induced DNA fragmentation. The 14 most significant identified node degree genes given by the number of links in the protein interaction network were the following: *CREB1, ARF3, UBA5, SIAH1, KLHL3, HECTD1, MMP9, TRIM69, MEX3C, ASB6, UBE2Q2, FBXO22, EIF4A3, PXN*. The node degree can be used to define groups of genes that are co-regulated and consequently may serve similar functions. Interestingly, the up regulation of *ASB6* was the only highly associated with worse overall survival (OS) rate in PABC, particularly in triple negative molecular subtype and pre-menopausal status. The researchers indicated that *ASB6* may have an essential role as a prognostic biomarker and a therapeutic target in PABC management (27).

Zhou et al. also investigated the genomic pathways of PABC through bioinformatic analysis of a microarray dataset (14). This study was differentiated by detecting DEGs in tumor-associated stroma of PABC (16). A total of 480 DEGs were identified among tumor-related and normal stromal cells in PABC patients. The node degree genes *JUN, FOS, MYC* and *ACTA2*, including the up-regulated DEGs *IL18* and *CD274* that were associated with the immune response, were primarily enriched in carcinogenesis pathways and should be further validated as potential anti-cancer targets.

Last but not least, Thanmalagan et al. attempted to explain the biological profile of the disease and to improve the diagnostic and therapeutic tools by analyzing the microarray profile dataset by Harvell et al. (14, 19). In this case, the researchers thoroughly studied the post-translational modification (PTMs) pattern of the DEGs in PABC patients in comparison to non-PABC cases. The researchers evaluated multiple up-regulated and down-regulated DEGs, appraised their corresponding PTMs (phosphorylation, ubiquitylation etc.) and proved that four particular genes (*KLF1, FEN1, SP110, MUC1*) may be recognized as promising therapeutic targets ensuring no harm to pregnancy progress and fetal development.

Among the limitations of this review, it should be stressed that our conclusions are based on studies that utilized heterogeneous genomic approaches (tissue or bioinformatic analysis), different sample preparation methods and sample types (FFPE, fresh frozen tissue). Additionally, no correlation among the genomic profile and the clinicopathological characteristics of PABC was examined in the majority of the studies included in our review. Furthermore, the number of eligible articles was limited due to the rarity of the disease. Thus, we are not allowed to draw definite conclusions and formulate recommendations; further studies should be conducted to confirm the abovementioned observations.

CONCLUSION

In conclusion, several studies on PABC indicate an adverse prognostic outcome for the disease that is correlated with its unexplained molecular nature. Highlighting the genomic background of PABC and analyzing all the biological pathways will further facilitate the identification of novel biomarkers defining women among the general population who are at high-risk of developing PABC. Our review systematically summarizes all available data on the distinct genomic profile of PABC offering valuable insight into PABC biological characteristics; this approach may eventually serve as a significant resource for further research in the field and elucidate PABC underlying mechanisms for the disclosure of new diagnostic, prognostic and therapeutic targets. Further research in the field of pregnancy-associated breast cancer is highly recommended as its rate is expected to increase substantially in the upcoming years.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

AMK and EZ were the writers of the article. MM, EZ, and AMK performed the literature search and data extraction from all studies examined. FZ and MAD contributed to the conception and design of the study and to the revision of the manuscript. All authors have read and approved the final manuscript.

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The Prognostic Impact of Age at Diagnosis Upon Breast Cancer of Different Immunohistochemical Subtypes: A Surveillance, Epidemiology, and End Results (SEER) Population-Based Analysis

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Background and Objectives: The influence of age at diagnosis of breast cancer upon the prognosis of patients with different immunohistochemical (IHC)-defined subtypes is still incompletely defined. Our study aimed at examining the association of age at diagnosis and risk of breast cancer-specific mortality (BCSM).

Methods: 172,179 eligible breast cancer patients were obtained for our study cohort using the Surveillance, Epidemiology, and End Results database from 2010 to 2015. Patients were classified into four IHC-defined subtypes according to their ER, PgR, and HER2 status. Kaplan–Meier plots were used to describe BCSM among patients in different age groups. A Cox proportional hazards model was used for multivariate analysis. A multivariable fractional polynomial model within the Cox proportional hazards model was used to evaluate the relationship between age at diagnosis and the risk of BCSM.

Results: For the whole cohort, the median follow-up time was 43 months. Patients younger than 40 years and those older than 79 years presented with the worst BCSM (hazard ratio [HR] 1.13, 95% confidence interval [CI] 1.03–1.23, and HR 3.52, 95% CI 3.23–3.83, respectively, $p < 0.01$, with age 40–49 years as the reference). The log hazard ratios of hormone receptor (HoR)(+)/HER2(–) patients formed a quadratic relationship between age at diagnosis and BCSM, but not in the other three subtypes of breast cancer. In the HoR(+)/HER2(–) subtype, patients younger than 40 years had worse BCSM than those aged at 40–49 years (HR 1.26, 95% CI 1.10–1.45, and $p < 0.01$).

Conclusions: Women diagnosed with HoR(+)/HER2(–) breast cancer younger than 40 years or older than 79 years of age suffer higher rates of cancer-specific mortality. Young age at diagnosis may be particularly prognostic in HoR(+)/HER2(–) breast cancer.

Keywords: breast cancer, mortality, immunohistochemical subtype, age at diagnosis, prognosis

INTRODUCTION

Breast cancer is the most common cancer in females. In 2020, it is estimated that 276,480 new breast cancer cases and 42,170 breast cancer deaths will occur in the United States alone (1). Breast cancer is a heterogeneous disease, which the 2013 St. Gallen Consensus classified into four main molecular subtypes: luminal A, luminal B, HER2-enriched, and basal-like breast cancer (2–4). Molecular subtypes play an important role in guiding the clinical treatment of breast cancer, and many studies have been conducted upon the differences between different tumor subtypes. For example, luminal B tumor is more likely to express genes associated with high tumor proliferation compared to luminal A tumors (5). The different molecular subtypes of breast cancer have diverse biological phenotypes and varying degrees of response toward systemic treatments (2, 3, 5), thus showing different patterns of relapse and long-term prognosis (6, 7). Though the molecular classification of breast cancer requires using Gene Expression Profiling (GEP) and DNA microarrays to identify distinct subtypes, the use of GEP in routine clinical diagnosis is neither economically feasible nor practical. Therefore, immunohistochemical staining of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor-2 (HER2) can be used as surrogate to roughly determine four main subtypes for clinical application: "luminal A" (ER and/or PgR positive and HER2 negative), "luminal B" (ER and/or PgR positive and HER2 positive), "HER2-overexpressed" (ER and PgR negative and HER2 positive), and "Triple-negative" (ER, PgR, and HER2 negative).

Age at diagnosis has been reported to be an independent prognostic factor for breast cancer in several studies (8–11). The influence of age upon the prognosis of patients with different tumor subtypes is still incompletely defined. Young age seems to be a significant prognostic factor in women with luminal subtype breast cancers (12–14). In addition, studies have also indicated that in triple-negative breast cancer, age group of <40 years is significantly associated with poor prognosis (15, 16). However, many of these previous studies were of limited sample size. Therefore, our study aimed at examining the relationship between age at diagnosis and the risk of breast cancer-specific mortality (BCSM) using the largest study population possible from the Surveillance, Epidemiology, and End Results (SEER) database.

MATERIALS AND METHODS

Data Source and Study Population

Data were obtained from the SEER database (www.seer.cancer.gov), which incorporates 18 population-based cancer registries (November 2016 submission). We enrolled eligible patients based on the following inclusion criteria (**Supplementary Figure 1**): female sex, unilateral breast cancer, only one primary breast cancer, year of diagnosis from 2010 to 2015, diagnosis not obtained from a death certificate or autopsy, age at diagnosis ≥20 years old, American Joint Committee on Cancer stages I–III, pathologic confirmation of invasive ductal carcinoma, and the known ER, PgR, and HER2 statuses. Due to the fact that HER2 status was not registered in the SEER database until 2010, only patients with breast cancer diagnosed after 2010 were included.

The status of ER, PgR, and HER2 was used to classify patients into four immunohistochemical (IHC)-defined breast cancer subtypes: hormonal receptor (HoR)(+)/HER2(−) group (ER-positive and/or PgR-positive and HER2-negative), HoR(+)/HER2(+) group (ER-positive and/or PgR-positive and HER2-positive), HoR(−)/HER2(+) group (ER-negative, PgR-negative, and HER2-positive), and triple-negative group (ER-negative, PgR-negative, and HER2-negative).

For this study, the follow-up time was calculated from the time of first diagnosis for breast cancer. The primary study outcome was BCSM, and it was defined as the time from the initial breast cancer diagnosis to the death of the patient from breast cancer. Patients who died of other causes were censored upon their date of death.

Statistical Analysis

The patient demographics and tumor characteristics of this study are provided in **Table 1**. Variables classified by IHC-defined breast cancer subtype were compared using the χ^2 test. The reverse Kaplan–Meier method was used to calculate median follow-up time. Breast cancer-specific survival in the different age groups was described using Kaplan–Meier. Age at diagnosis was treated as a categorical variable classified into the following age groups: <40, 40–49, 50–59, 60–69, 70–79, and >79 years. The association of age group with the risk of BCSM was evaluated using the Cox proportional hazards model. Variables shown to be significantly associated with BCSM in the univariate analysis were included in the multivariate analysis. Adjusted hazard ratio (HR) with 95% confidence interval (CI) was calculated using the multivariable Cox proportional hazards model, while simultaneously controlling for clinical prognostic risk. To further determine whether there was a significant interaction between age at diagnosis and IHC-defined breast cancer subtype for predicting BCSM, we used an interaction term (i.e., age × subtype) and performed pairwise comparisons using different combinations of age and subtype. Age was treated as a continuous variable, and a multivariable fractional polynomial model within the Cox proportional hazards model was used to examine a potential nonlinear relationship between age at diagnosis and BCSM. The difference between the nonlinear and linear models was assessed using a likelihood ratio test to test for nonlinearity. A two-sided p-value of <0.05 was considered to indicate statistical significance. All analyses were performed in STATA 15 (StataCorp, College Station, Texas, USA).

RESULTS

Patient Demographics and Tumor Characteristics

We identified 172,179 eligible patients from the SEER database according to the aforementioned inclusion criteria. Patient demographics, pathology, and clinical characteristics according to molecular subtype are summarized in **Table 1**. For the whole cohort, the median follow-up time was 43 months (interquartile range, 26–62 months). Significant differences ($p < 0.001$) were

TABLE 1 | Demographic and tumor characteristics of the patients.

Characteristic	Total	HoR(+)/HER2(–)	HoR(+)/HER2(+)	HoR(–)/HER2(+)	Triple-negative	*p*-value[a]
	(*n* = 172,179) *n* (%)	(*n* = 120,408) *n* (%)	(*n* = 20,643) *n* (%)	(*n* = 8,974) *n* (%)	(*n* = 22,154) *n* (%)	
Median follow-up: months (IQR)	43 (26–62)	43 (26–62)	41 (24–61)	42 (24–61)	45 (27–63)	
Age at diagnosis						$p < 0.001$
<40	10,615 (6.2)	5,597 (4.7)	2,041 (9.9)	766 (8.5)	2,211 (10.0)	
40–49	31,831 (18.5)	20,901 (17.4)	4,594 (22.3)	1,754 (19.6)	4,582 (20.7)	
50–59	44,722 (26.0)	29,888 (24.8)	5,932 (28.7)	2,879 (32.1)	6,023 (27.2)	
60–69	45,487 (26.4)	33,574 (27.9)	4,679 (22.7)	2,087 (23.3)	5,147 (23.2)	
70–79	26,557 (15.4)	20,512 (17.0)	2,294 (11.1)	1,003 (11.2)	2,748 (12.4)	
>79	12,967 (7.5)	9,936 (8.2)	1,103 (5.3)	485 (5.4)	1,443 (6.5)	
Race						$p < 0.001$
White	134,203 (78.0)	96,409 (80.1)	15,657 (75.9)	6,407 (71.4)	15,730 (71.0)	
Black	19,326 (11.2)	11,038 (9.2)	2,441 (11.8)	1,270 (14.2)	4,577 (20.7)	
Other[b]	18,650 (10.8)	12,961 (10.7)	2,545 (12.3)	1,297 (14.4)	1,847 (8.3)	
Marital status						$p < 0.001$
Married	97,347 (56.6)	68,117 (56.6)	11,961 (57.9)	5,185 (57.8)	12,084 (54.6)	
Unmarried	66,529 (38.6)	46,482 (38.6)	7,742 (37.5)	3,368 (37.5)	8,937 (49.3)	
Unknown	8,303 (4.8)	5,809 (4.8)	940 (4.6)	421 (4.7)	1,133 (5.1)	
Year of diagnosis						$p < 0.001$
2010	25,387 (14.7)	17,592 (14.6)	2,931 (14.2)	1,354 (15.1)	3,510 (15.8)	
2011	27,250 (15.8)	19,209 (16.0)	2,991 (14.5)	1,352 (15.1)	3,698 (16.7)	
2012	28,336 (16.5)	19,866 (16.5)	3,342 (16.2)	1,448 (16.1)	3,680 (16.6)	
2013	29,434 (17.1)	20,745 (17.2)	3,564 (17.3)	1,467 (16.4)	3,658 (16.5)	
2014	30,205 (17.6)	21,097 (17.5)	3,768 (18.2)	1,602 (17.8)	3,738 (16.9)	
2015	31,567 (18.3)	21,899 (18.2)	4,047 (19.6)	1,751 (19.5)	3,870 (17.5)	
Laterality						$p < 0.001$
Left	87,132 (50.6)	60,537 (50.3)	10,532 (51.0)	4,664 (52.0)	11,399 (51.4)	
Right	85,047 (49.4)	59,871 (49.7)	10,111 (49.0)	4,310 (48.0)	10,755 (48.6)	
Grade						$p < 0.001$
I	36,312 (21.1)	34,687 (28.8)	1,203 (5.8)	123 (1.4)	299 (1.4)	
II	71,479 (41.5)	57,862 (48.1)	8,205 (39.8)	1,994 (22.2)	3,418 (15.4)	
III	64,388 (37.4)	27,859 (23.1)	11,235 (54.4)	6,857 (76.4)	18,437 (83.2)	
Tumor size						$p < 0.001$
≤2 cm	107,059 (62.2)	82,378 (68.4)	10.644 (51.6)	4,098 (45.7)	9,939 (44.9)	
2–5 cm	55,146 (32.0)	33,023 (27.4)	8,317 (40.3)	3,792 (42.2)	10,014 (45.2)	
>5 cm	9,974 (5.8)	5,007 (4.2)	1,682 (8.1)	1,084 (12.1)	2,201 (9.9)	
Regional nodes						$p < 0.001$
Negative	118,127 (68.6)	85,924 (71.4)	12,561 (60.9)	5,078 (56.6)	14,564 (65.7)	
Positive	54,052 (31.4)	34,484 (28.6)	8,081 (39.1)	3,896 (43.4)	7,590 (34.3)	
Chemotherapy						$p < 0.001$
NO	95,129 (55.2)	82,944 (68.9)	5,218 (25.3)	1,917 (21.4)	5,050 (22.8)	
YES	77,050 (44.8)	37,464 (31.1)	15,425 (74.7)	7,057 (78.6)	17,104 (77.2)	
Radiation						$p < 0.001$
NO	71,202 (41.3)	47,502 (39.5)	9,472 (45.9)	4,442 (49.5)	9,786 (44.2)	
YES	94,620 (55.0)	69,167 (57.4)	10,094 (48.9)	4,076 (45.4)	11,283 (50.9)	
Unknown	6,357 (3.7)	3,739 (3.1)	1,077 (5.2)	456 (5.1)	1,085 (4.9)	

IQR, interquartile range. [a]p-value of chi-square test comparing the different subtype groups. [b]Other: including Asian or Pacific Islander and American Indian/Alaska Native and Unknown.

observed in all variables in each of the four different IHC-defined breast cancer subtypes. Elderly patients were a larger component in the HoR(+)/HER2(–) group than in the HoR(+)/HER2(+), HoR(–)/HER2(+), and triple-negative groups (25.2 vs. 16.9, 16.6, and 18.9% of age ≥ 70 years). However, the proportion of patients diagnosed at an earlier age (age < 40 years) was smaller in the HoR(+)/HER2(–) group than in the HoR(+)/HER2(+), HoR(–)/HER2(+), and triple-negative groups (4.7 vs. 9.9, 8.5,

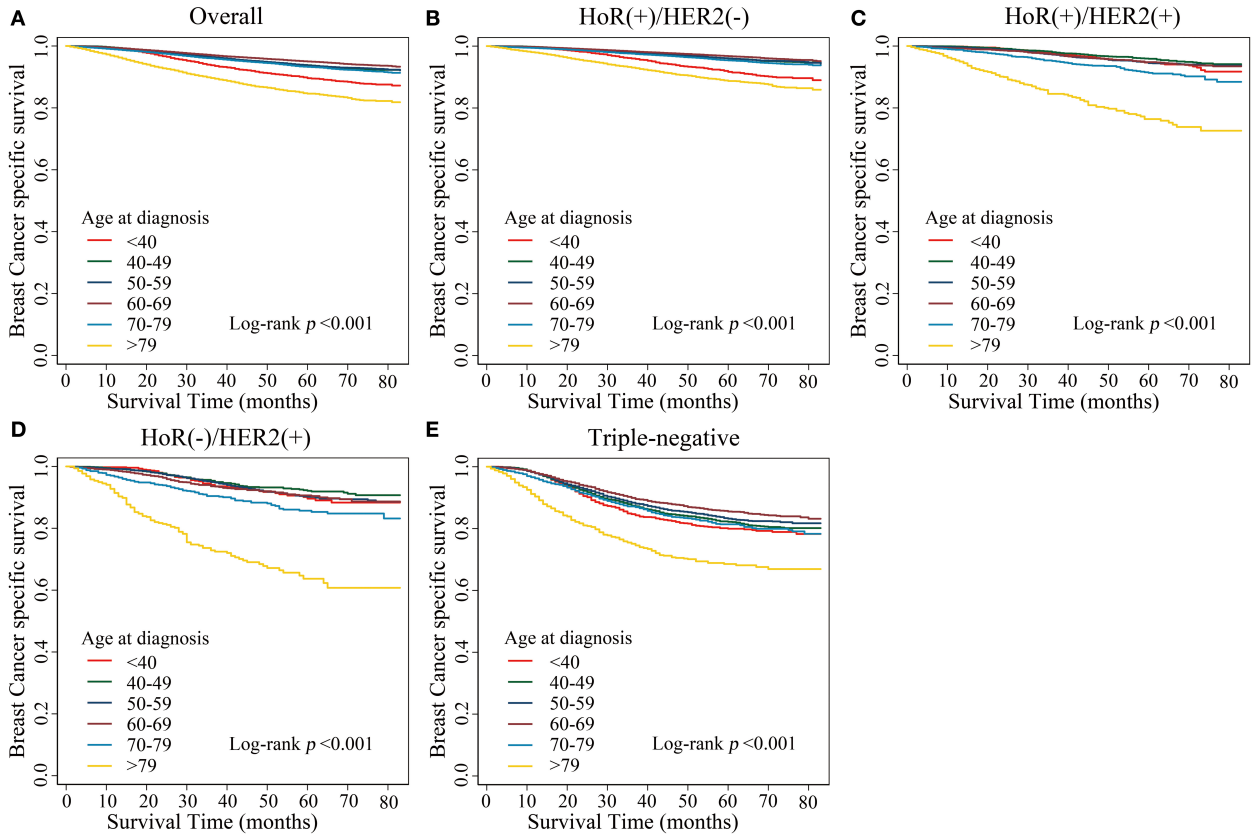

FIGURE 1 | Kaplan–Meier estimates of breast cancer-specific survival in different age groups. **(A)** Overall, **(B)** HoR(+)/HER2(–) subtype, **(C)** HoR(+)/HER2(+) subtype, **(D)** HoR(–)/HER2(+) subtype, and **(E)** Triple-negative subtype.

and 10.0% were age <40 years, respectively). In terms of tumor characteristics, the HoR(+)/HER2(–) group was associated with lower grade (for grade 1: 28.8 vs. 5.8, 1.4, and 1.4%), smaller tumor sizes (for size ≤2 cm: 68.4 vs. 51.6, 45.7, and 44.9%), fewer positive lymph nodes (for positive nodes: 28.6 vs. 39.1, 43.4, and 34.3%), and a lower chemotherapy proportion (31.1 vs. 74.7, 78.6, and 77.2%).

Survival Analysis of Different Age Groups

Kaplan–Meier estimates of breast cancer-specific survival in the different age groups showed that patients aged <40 years and patients aged >79 years presented with the worst survival rates ($p < 0.001$; **Figure 1A**). We further analyzed breast cancer-specific survival in each subtype and observed that the tendencies of the survival curves differed between patients of different subtypes. In the HoR(+)/HER2(–) subtype, patients aged <40 years showed poor survival rates, similar to that of patients aged >79 years, while the other age groups showed a flatter survival curve (**Figure 1B**). However, in the HoR(+)/HER2(+), HoR(–)/HER2(+), and triple-negative subtypes, patients aged >79 years showed poor survival rates, while the remaining age groups showed similar survival curves (**Figures 1C–E**).

Univariate analysis revealed that subtype, age at diagnosis, race, marital status, year of diagnosis, tumor laterality, grade, tumor size, regional lymph node status, and application of chemotherapy and radiotherapy were factors significantly associated with BCSM (**Table 2**, $p < 0.01$). The group aged 40–49 years presented with the best survival result and was subsequently used as the reference for other age groups in both univariate and multivariate analyses. In the multivariate analysis, the HR of BCSM was 1.13 (95% CI, 1.03–1.23; $p < 0.01$) in the group aged <40 years and was lowest in the group aged 40–49 years. Afterwards, the HR of BCSM began to increase alongside patient age, with the highest HR of 3.52 (95% CI, 3.23–3.83; $p < 0.01$) observed in the eldest age group (aged >79years). The results were consistent with the previous Kaplan–Meier plot analysis.

Comparison of Survival Between Age and IHC-Defined Subtype

To investigate whether there was significant interaction between age at diagnosis and IHC-defined breast cancer subtype in predicting BCSM, we utilized an interaction term (i.e., age × subtype). Pairwise comparison between the different combinations of age and subtype showed that in the HoR(+)/HER2(–) subtype, patients aged <40 years had worse BCSM than those aged 40–49 years (HR 1.26; 95% CI, 1.10–1.45; and $p < 0.01$) (**Table 3**). Similarly, the log hazard

TABLE 2 | Cox proportional hazards regression model analysis of breast cancer-specific mortality.

Variable	Univariate analysis		Multivariate analysis[a]	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**
Subtype				
HoR(+)/HER2(–)	Reference	–	Reference	–
HoR(+)/HER2(+)	1.34 (1.25–1.45)	$p < 0.01$	0.83 (0.77–0.90)	$p < 0.01$
HoR(–)/HER2(+)	2.55 (2.35–2.76)	$p < 0.01$	1.24 (1.13–1.35)	$p < 0.01$
Triple-negative	4.48 (4.27–4.70)	$p < 0.01$	2.42 (2.29–2.55)	$p < 0.01$
Age at diagnosis				
<40	1.65 (1.51–1.80)	$p < 0.01$	1.13 (1.03–1.23)	$p < 0.01$
40–49	Reference	–	Reference	–
50–59	0.98 (0.91–1.05)	$p = 0.51$	1.12 (1.05–1.20)	$p < 0.01$
60–69	0.80 (0.75–0.87)	$p < 0.01$	1.19 (1.11–1.28)	$p < 0.01$
70–79	1.10 (1.02–1.19)	$p = 0.02$	1.76 (1.63–1.91)	$p < 0.01$
>79	2.82 (2.61–3.04)	$p < 0.01$	3.52 (3.23–3.83)	$p < 0.01$
Race				
White	Reference	–	Reference	–
Black	2.04 (1.93–2.16)	$p < 0.01$	1.34 (1.27–1.42)	$p < 0.01$
Other[b]	0.70 (0.65–0.77)	$p < 0.01$	0.70 (0.64–0.77)	$p < 0.01$
Marital status				
Married	Reference	–	Reference	–
Unmarried	1.75 (1.67–1.83)	$p < 0.01$	1.25 (1.20–1.31)	$p < 0.01$
Unknown	1.35 (1.22–1.49)	$p < 0.01$	1.16 (1.05–1.29)	$p < 0.01$
Year of diagnosis				
2010	Reference	–	Reference	–
2011	0.97 (0.91–1.03)	$p = 0.29$	0.97 (0.91–1.04)	$p = 0.42$
2012	0.93 (0.87–1.00)	$p = 0.05$	0.96 (0.89–1.02)	$p = 0.20$
2013	0.90 (0.83–0.97)	$p < 0.01$	0.92 (0.86–0.99)	$p = 0.03$
2014	0.96 (0.88–1.04)	$p = 0.30$	1.01 (0.93–1.10)	$p = 0.30$
2015	0.94 (0.84–1.04)	$p = 0.23$	0.98 (0.88–1.09)	$p = 0.23$
Laterality				
Left	Reference	–	Reference	–
Right	0.95 (0.91–1.00)	$p = 0.03$	0.97 (0.92–1.01)	$p = 0.11$
Grade				
I	Reference	–	Reference	–
II	3.63 (3.21–4.11)	$p < 0.01$	2.31 (2.03–2.62)	$p < 0.01$
III	12.21 (10.84–13.76)	$p < 0.01$	4.58 (4.04–5.20)	$p < 0.01$
Tumor size				
≤2 cm	Reference	–	Reference	–
2–5 cm	4.51 (4.28–4.76)	$p < 0.01$	2.31 (2.18–2.44)	$p < 0.01$
>5 cm	12.05 (11.31–12.83)	$p < 0.01$	4.92 (4.59–5.20)	$p < 0.01$
Regional nodes				
Negative	Reference	–	Reference	–
Positive	4.24 (4.05–4.44)	$p < 0.01$	2.83 (2.69–2.97)	$p < 0.01$
Chemotherapy				
No	Reference	–	Reference	–
Yes	2.14 (2.05–2.24)	$p < 0.01$	0.93 (0.87–0.98)	$p < 0.01$
Radiation				
No	Reference	–	Reference	–
Yes	0.56 (0.54–0.59)	$p < 0.01$	0.61 (0.58–0.64)	$p < 0.01$
Unknown	0.94 (0.84–1.05)	$p = 0.25$	0.71 (0.63–0.79)	$p < 0.01$

HR, hazard ratio; CI, confidential interval. [a]Adjusted by Cox proportional hazards models including all factors, as categorized in [b]Other: including Asian or Pacific Islander and American Indian/Alaska Native and Unknown.

ratios for the HoR(+)/HER2(−) patients formed a quadratic relationship between age at diagnosis and BCSM; the lowest risk was approximately around 50 years of age (**Figure 2B**). Interestingly, the plot in the HoR(+)/HER2(+) subtype seemed to show a U-shaped curve, but the 95% CI was too wide to have a statistical significance (**Figure 2C**). We observed that in the HoR(−)/HER2(+) subtype, the risk of BCSM was the lowest in patients aged <40 years and increased gradually with age (**Figure 2D**). However, in the HoR(+)/HER2(+) and HoR(−)/HER2(+) subtypes, patients aged <60 years (including patient aged <40, 40–49, and 50–59 years) had the similar BCSM and exhibited no statistical significant differences (**Table 3**). HR in the triple-negative subtype was similar between different age groups in patients aged <70 years, but the HR showed significant increase in patients aged over 70 years (**Table 3** and **Figure 2E**).

DISCUSSION

The aim of this study was to explore the relationship between age at diagnosis and BCSM according to IHC-defined breast cancer subtype. Our results suggest that the impact of age upon survival may be more complex than we initially realized. Our study confirmed that the risk of BCSM was lower for patients aged 40–49 years old compared to those aged <40 years, but the risk of BCSM would increase significantly with patients aged ≥50 years.

The association of age at diagnosis with survival in breast cancer has been widely analyzed. Younger age at diagnosis has been reported to be a factor for poor prognosis and is associated with more aggressive disease (17, 18). Previous analyses have found a quadratic relationship between age at diagnosis and BCSM in different subsets (19, 20). Johnson et al. (19) proposed a quadratic relationship between age and the risk of BCSM. Liu et al. (20) proposed a U-shaped relationship between age and the risk of BCSM in the hormone receptor-positive subgroup, which was consistent with our result.

Recent studies have attempted to investigate the influence of age at diagnosis upon prognosis according to different molecular subtypes (12–16). It has been reported that for luminal breast cancer, patients younger than 40 years are more likely to suffer from a significant increase in the risk of BCSM compared with older patients (12). In addition, in the luminal A and luminal B-HER2-negative subtypes, age group younger than 40 years was found to be an independent prognostic factor (13). Liu et al. (14) reported that in the luminal A subtype, patients younger than 40 years had a lower 5-year disease-free survival (DFS) and distant metastasis-free survival (DMFS) compared with the 41–60 years age group, while no significant association of DFS or DMFS with age was found in the other three molecular subtypes. Dai et al. (16) divided patients into the younger group (<40 years) and the older group (≥40 years) and found that the younger group had poorer survival than the older group in the triple negative breast cancer (TNBC) subtype. Despite these conflicting findings, numerous studies support that age at diagnosis is an independent prognostic factor in breast cancer.

In this study, we included the largest number of cases possible from the SEER database in order to determine the effects of age upon survival. However, because the SEER database only provides ER, PgR, and HER2 expression status, we were unable to correctly classify patients into molecular subtypes such as luminal A and luminal B according to current guidelines (4). Therefore, in this study, patients were classified into four IHC-defined breast cancer subtypes, which may cause some disparity between our results and those garnered from studies using molecular subtyping. Our results confirmed that the relationship between age at diagnosis and BCSM showed a quadratic U-shaped pattern only for the HoR(+)/HER2(−) subtype but not for the other IHC-defined breast cancer subtypes.

Different studies have presented several varying ages at diagnosis (such as 45, 50, and 55) as a prognostic factor for the lowest risk (19, 21, 22). Liu et al. (20) took age as a categorical variable and observed that patients aged 40–49 years had the lowest risk of BCSM. In this study, we first treated age at diagnosis as a continuous variable in the fitting model, and we estimated that the minimum risk of BCSM in the HoR(+)/HER2(−) subtype was approximately at the age of 50 years old.

Our results showed that younger HoR(+)/HER2(−) patients aged <40 years had poorer survival rates than patients in the perimenopausal age group. The underlying mechanism is still unclear, but there are several hypotheses to explain this result. For example, premenopausal patients may underestimate the risk of breast cancer at their age, which could lead to a delay in diagnosis and result in later stage disease at initial diagnosis. Another possible explanation is that more aggressive disease may manifest in younger patients, as previous studies have found that patients <40 years of age have a higher histological grade, higher tumor stage, and poorer biological behavior (23). Liu et al. (14) identified 374 differentially expressed genes (DEGs) in the luminal A subtype when divided into two age groups (≤40 and >40 years), which were related to breast cancer progression and metastasis, but in the non-luminal A subtypes no age group-specific DEGs were identified. Azim et al. (24) discovered that patients aged ≤ 40 years had a higher expression of RANK-ligand, c-kit, mammary stem cell markers, luminal progenitor markers, and BRCA1 mutation signatures, independent of tumor subtype, grade, and stage. In addition, Morrison et al. (25) reported that in luminal breast cancer subtype patients aged ≤40 years, the expression of p53 was significantly higher than in patients aged ≥50 years.

Younger patients with luminal A breast cancer have been shown to have a higher incidence of endocrine resistance (26–28). Even when treated with endocrine therapy, they still may have a poor prognosis due to tamoxifen resistance (26). Young age retains a negative prognostic value particularly in the luminal A subtype (12). A lower incidence and shorter duration of chemotherapy-induced amenorrhea is reported in younger patients and may result in a worse prognosis for hormone receptor-positive breast cancer (29–31). Younger age is also reported to be a predictor of decreased adherence to adjuvant endocrine therapy, associated with increased mortality (32–34). Hershman et al. (32) reported that women <40 years were 40% more likely to be non-adherent to their endocrine treatment than patients aged 50–65 years old ($p < 0.001$). These finding are supported by the results from the SOFT and TEXT trials,

TABLE 3 | Pairwise comparisons between different combinations of age and subtype for breast cancer-specific mortality[a].

Age at diagnosis	HoR(+)/HER2(–)		HoR(+)/HER2(+)		HoR(–)/HER2(+)		Triple-negative	
	HR (95% CI)	*p* value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
<40	1.26 (1.10–1.45)	$p < 0.01$	1.12 (0.83–1.50)	$p = 0.45$	1.07 (0.75–1.51)	$p = 0.71$	1.03 (0.90–1.18)	$p = 0.68$
40–49	Reference	–	Reference	–	Reference	–	Reference	–
50–59	1.16 (1.04–1.29)	$p < 0.01$	1.34 (1.07–1.68)	$p = 0.01$	1.23 (0.96–1.59)	$p = 0.10$	1.05 (0.94–1.17)	$p = 0.39$
60–69	1.26 (1.12–1.40)	$p < 0.01$	1.48 (1.17–1.88)	$p < 0.01$	1.47 (1.12–1.91)	$p < 0.01$	1.03 (0.92–1.16)	$p = 0.57$
70–79	1.94 (1.73–2.19)	$p < 0.01$	2.27 (1.76–2.92)	$p < 0.01$	2.01 (1.51–2.69)	$p < 0.01$	1.43 (1.25–1.63)	$p < 0.01$
>79	4.19 (3.69–4.75)	$p < 0.01$	4.61 (3.56–5.96)	$p < 0.01$	4.60 (3.41–6.21)	$p < 0.01$	2.21 (1.90–2.57)	$p < 0.01$

HR, hazard ratio; CI, confidential interval. [a]The results of different combinations of age (rows) and subtype (columns) are presented in the cross-points of the rows and columns. All results are adjusted by Cox proportional hazards models including race, marital status, year of diagnosis, laterality, grade, tumor size, regional nodes, chemotherapy, and radiation.

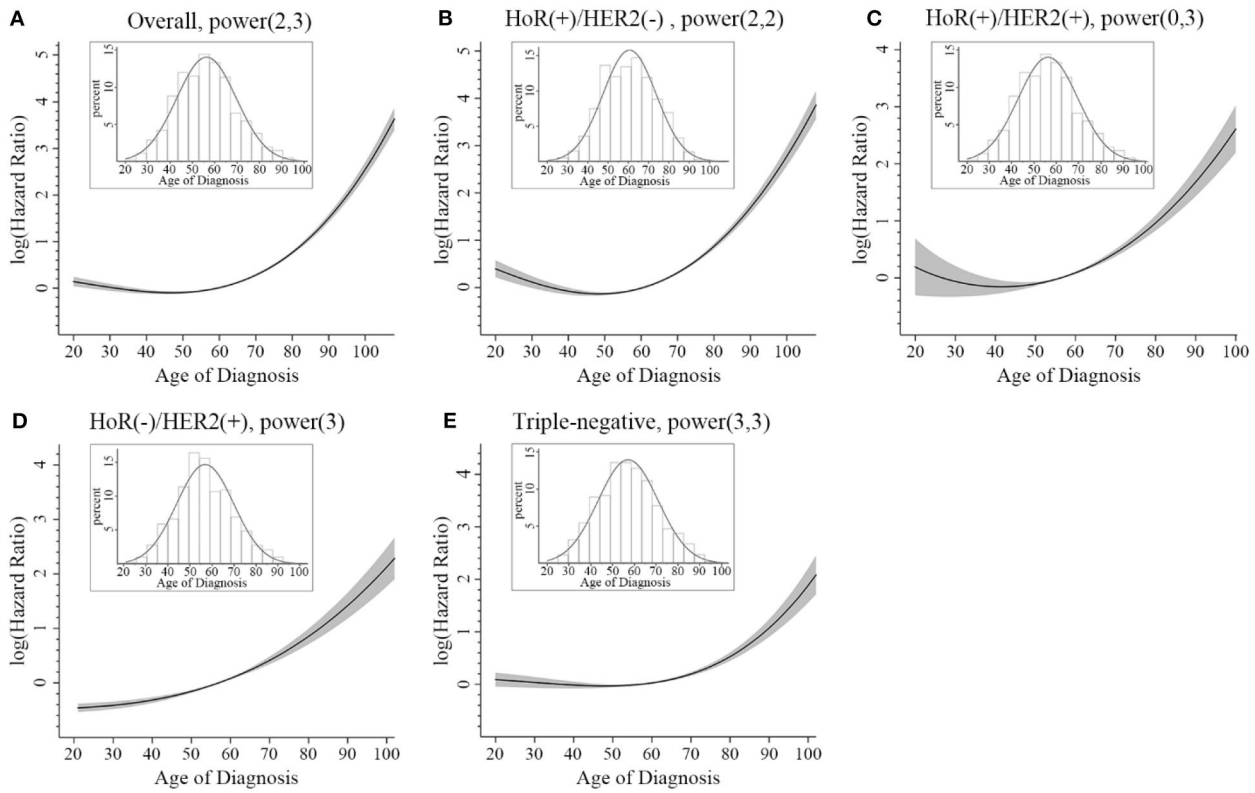

FIGURE 2 | Relative hazard of breast cancer-specific mortality. The thick black line shows the logarithm hazard ratio, and the gray shades show the 95% confidence interval. The superimposed histogram demonstrates that the age distribution is approximately normal. **(A)** Overall, **(B)** HoR(+)/HER2(–) subtype, **(C)** HoR(+)/HER2(+) subtype, **(D)** HoR(–)/HER2(+) subtype, and **(E)** triple-negative subtype.

which have shown young patients with luminal subtype breast cancer may benefit from a more intensive anti-hormonal (35). Unfortunately, due to the limitations of the SEER database, we were unable to conduct an in-depth analysis of the impact of endocrine therapy upon patient survival. However, considering the fact that patients included in this study were diagnosed from 2010 to 2015, mostly before the results of the SOFT and TEXT trials (36, 37) and before the subsequent renewal of clinical guidelines, it can be expected that most of the premenopausal patients were treated with tamoxifen alone as adjuvant endocrine therapy (38). Therefore, we can estimate that many of these patients were undertreated according to the current standard that recommend the use of ovarian function suppression in many cases (39). This may be a potential explanation as to why HoR(+)/HER2(–) patients aged <40 years presented with poorer outcomes in this study. However, further analysis using a more detailed database containing the specifics of a patient's adjuvant treatment will be needed to support this conclusion.

It has been previously demonstrated that chemotherapy can reduce mortality for many female breast cancer patients, but not for those aged ≥80 years (40). Our results are in concurrence with the previous finding and show that elderly patients had

worse disease-specific survival in the overall cohort. Many studies have demonstrated an association between undertreatment and poor survival outcomes (41–43), and it is understandable that with the increase of age, the probability of undertreatment may increase as well. It has been reported in previous studies that older patients are less likely to receive the standard-of-care treatment, including surgical therapy, chemotherapy, adjuvant radiotherapy, and endocrine therapy (44–47). Older patients often have other underlying health issues and may suffer from more serious side effects when receiving standard therapy, which could increase disease-specific mortality in elderly patients (48). In our analysis, the difference in survival among age groups was still significant even after adjustment for radiotherapy and chemotherapy. While the presence of comorbidities may preclude the use of chemotherapy, it is unclear why the application of adjuvant endocrine therapy was suboptimal among eligible elderly women. Unfortunately, we could not control potential confounders such as patient frailty and undertreatment due to the lack of information regarding comorbidities and the incomplete treatment information in the SEER database.

This study was based on the SEER database, which includes cancer incidence and survival information from 18 registries, covering ∼27.8% of the U.S. population. However, our study has several limitations. First, as mentioned previously, detailed information regarding patient treatment (for example: whether patient received neoadjuvant or adjuvant chemotherapy, the type of chemotherapy used, the type of endocrine therapy received, whether anti-HER2-targeted therapy was used and whether patient completed radiotherapy) is not recorded in the SEER database, which limits further investigation regarding the impact of therapeutic regimens on clinical outcomes. Second, breast cancer subtypes are roughly defined by ER, PgR, and HER2 status in the SEER database. The lack of data regarding Ki67 expression and other detailed molecular indicators (without which the luminal A and luminal B subtypes could not be properly distinguished according to current standards) only allows us to categorize the patients into four IHC-defined breast cancer subtypes. Third, the median follow-up of this study is only 43 months, and it is possible that our study may have missed late recurrences, which are not uncommon in the luminal and HoR(+) subtype. Finally, because this study utilizes retrospective methodology, sampling bias may have been introduced. Therefore, our results should be confirmed and supplemented by further prospective studies with more information and precise molecular subtypes before clinical application. Despite the limitations mentioned above, our study contributes to the growing evidence that the relationship between age at diagnosis and BCSM varies by tumor subtype.

In conclusion, through analysis of the largest sample size available in the SEER database, the current study showed that the prognostic value of age in determining BCSM varies with IHC-defined breast cancer subtype. Younger age at diagnosis may be particularly prognostic in HoR(+)/HER2(–) breast cancer, but further evidence is needed to analyze the prognostic value of age in premenopausal patients receiving standard adjuvant endocrine therapy. The development of individualized treatment strategies for patients of different ages may be a viable direction for future research, with additional emphasis on intensified treatment for young patients with HoR(+)/HER2(–) breast cancer.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: www.seer.cancer.gov.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Lishui Hospital, Zhejiang University School of Medicine. 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 participated in this research. SCa and SCh: concepts and design. SCa and ZG: data acquisition, analysis, and interpretation. YZ, PL, and YP: material support. SCa and SCh: study supervision. SCa, XL, and WZ: writing, review, and revision of manuscripts. The final manuscript read and approved by all authors.

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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.2020.01729/full#supplementary-material

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SYSTEMATIC REVIEW
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Efficacy and Safety of Controlled Ovarian Stimulation With or Without Letrozole Co-administration for Fertility Preservation: A Systematic Review and Meta-Analysis

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Background: The co-administration of letrozole during controlled ovarian stimulation (COS) with gonadotropins is used to limit the potentially harmful effects of a supra-physiological rise in estrogen levels on hormone-sensitive cancers. However, the efficacy and safety of adding letrozole to COS remain debated.

Methods: This is a systematic review and meta-analysis of published studies that compared the efficacy and safety of COS with co-administration of letrozole vs. COS without letrozole in all patient populations. A secondary analysis was done including only the studies in breast cancer patients. The primary efficacy endpoint was the number of retrieved mature Metaphase II (MII) oocytes. Secondary efficacy and safety endpoints were total number of oocytes, maturation rate, fertilization rate, number of cryopreserved embryos, peak estradiol levels, progesterone levels, and total gonadotropin dose. Data for each endpoint were reported and analyzed thorough mean ratio (MR) with 95% confidence interval (CI).

Results: A total of 11 records were selected including 2,121 patients (990 patients underwent COS with letrozole and 1,131 COS without letrozole). The addition of letrozole to COS did not have any negative effect on the number of mature oocytes collected (MR = 1.00, 95% CI = 0.87–1.16; $P = 0.967$) and the other efficacy endpoints. COS with letrozole was associated with significantly decreased peak estradiol levels (MR = 0.28, 95% CI = 0.24–0.32; $P < 0.001$). Similar results were observed in the secondary analysis including only breast cancer patients.

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Conclusions: These findings are reassuring on the efficacy and safety of COS with gonadotropins and letrozole and are particularly important for fertility preservation in women with hormone-sensitive cancers.

Keywords: fertility, controlled ovarian stimulation, letrozole, gonadotropins, breast cancer

INTRODUCTION

Over the last years, cancer death rate has been continuously dropping thanks to improvement in screening techniques and therapies ensuring early diagnosis and increased survival (1). Life-saving treatments such as chemotherapy or radiotherapy have several potential long-term adverse effects including gonadotoxicity (2–8). The subsequent risk of treatment-related infertility and the loss of ovarian endocrine function represent important causes of distress for patients who are diagnosed during their reproductive years (9–11). Therefore, scientific societies strongly recommend fertility consultation before initiation of anticancer treatments in all patients of childbearing age (12–15).

In the last decades, oocyte and embryo cryopreservation have become standard procedures for fertility preservation (12–15). In order to increase the chance for success, controlled ovarian stimulation (COS) with high doses of gonadotropins is needed to maximize the number of oocytes retrieved and stored (16). COS exposes women to supra-physiological estrogen levels, raising concerns about the safety of the procedure in patients with hormone-sensitive cancers (17, 18). The use of both letrozole and tamoxifen was proposed, alongside classic COS protocols, to avoid unnecessary and potentially harmful effects of the rise in estrogen levels on the cancer (19, 20). Letrozole is an aromatase inhibitor that blocks androgen conversion into estrogen and it is used "off label" in infertility treatment in many countries, especially as ovulation inductor for women with either anovulatory cycles (21), including those with polycystic ovary syndrome (PCOS) (22), or unexplained infertility before planned intercourses or intra-uterine insemination (IUI) (23). Co-treatment with letrozole was proposed also alongside the COS for *in vitro* fertilization (IVF) in infertile women (24). However, the warning letter published by the original manufacturer still limits its general acceptance. Indeed, the safety concerns related to an increased number of reported malformations in pregnancies resulting from protocols that included letrozole were based only on a single abstract (including 150 babies from 130 pregnancies, compared to a large group of spontaneous low-risk pregnancies) and never confirmed by larger and methodologically sounder studies (25–28). Therefore, the concerns related to potential risks of congenital malformations have been dispelled by the scientific community, but the warning remains.

In terms of efficacy, some studies showed that letrozole co-administration was associated with comparable or even better oocyte yield than traditional protocols, without increasing serum estradiol levels (19, 29, 30), while others have demonstrated a reduction in the number of growing follicles, oocytes retrieved, and pregnancies as well as an increased incidence of cycle cancellations (31, 32). Moreover, data are not homogeneous, with most studies comparing COS with letrozole in oncologic patients to infertile women or donors as controls (29, 33, 34).

Because of the aforementioned controversial data about this important issue, we performed a systematic review and meta-analysis to clarify the efficacy and safety of adding letrozole to COS for IVF.

MATERIALS AND METHODS

This was a quantitative synthesis of studies that compared the efficacy and safety of COS with co-administration of letrozole (letrozole cohort) vs. COS without letrozole (no-letrozole cohort).

Study Endpoints

The primary efficacy endpoint was the number of retrieved mature Metaphase II (MII) oocytes. Secondary efficacy endpoints were total number of retrieved oocytes, maturation rate, and fertilization rate. Other secondary safety endpoints were peak estradiol levels, total gonadotropin dose, and length of the stimulation.

Pregnancy rate, live birth rate, relapse rate, and disease-free survival in cancer patients, adverse events, and progesterone levels were other pre-planned endpoints of interest. However, they could not be analyzed due to lack of data among the included studies.

As secondary analysis, the role of COS with or without letrozole was investigated specifically in the breast cancer patient population. All the analyses were repeated by including only the three studies that included breast cancer patients in both the letrozole and no-letrozole cohorts (35–37).

Data Sources and Search Strategy

A systematic literature search of PubMed was conducted to identify studies investigating protocols of COS with letrozole compared to those without letrozole. The search was not limited to studies about cancer patients who needed to cryopreserve their oocytes or embryos, but included also infertile patients and COS for elective fertility preservation. The search was restricted to full papers written in English and reporting original data; no restriction in terms of year of publication was applied. The final date of search was March 31, 2020. The terms used for the search strategy were "letrozole," "aromatase inhibitor," "controlled ovarian stimulation," "fertility preservation," "cancer," "breast cancer," "oocyte vitrification," and "oocyte freezing." Boolean operators were used to connect specific search keywords.

The effective combination of search terms was designed and organized by one reviewer (BB) and discussed with two other reviewers (ID and ML). The titles and abstracts obtained from the search were analyzed independently by two reviewers (BB and ML), and a third author (ID) evaluated the search results in order to apply the eligibility criteria.

Article Selection

Records eligible for this analysis had the following features: (a) studies comparing COS with or without letrozole; (b) in the experimental group, letrozole had to be included for the whole COS. Records with the following characteristics were excluded: (a) studies in which letrozole was given only for a few days and not for the whole duration of COS; (b) studies that used letrozole only for ovulation induction; (c) studies written in languages other than English; (d) studies without control group; and (e) studies that compared COS with letrozole vs. COS plus other drugs.

Two investigators (BB and ML) independently extracted data from all the eligible studies. From each eligible record, the following variables were collected: first author, year of publication, sample size and type of COS (letrozole and no letrozole), patients' characteristics (indications, age), characteristics of COS cycle (trigger method, estradiol level at triggering, total gonadotropin dose, and number of days of stimulation), efficacy outcomes (number of mature MII oocytes, total number of oocytes retrieved, number of cryopreserved mature oocytes, maturation rate, fertilization rate, number of cryopreserved embryos, and pregnancy rate/live birth rate), relapse rate and disease-free survival (in cancer patients), adverse events, and progesterone levels when available.

Statistical Analysis

Mean values with standard deviation or odds ratios (ORs) with 95% confidence intervals (CIs) were collected for all endpoints of interest (number of MII oocytes, total number of collected oocytes, maturation rate, fertilization rate, peak estradiol levels, total gonadotropin dose, and length of the stimulation). Statistical analysis was conducted with a random-effects model.

In order to analyze each endpoint and to compare the performances of COS with or without letrozole, data were studied via mean ratios (MRs), 95% CI, and *P*-values. A MR value >1 indicates that for a specific endpoint, the letrozole cohort has higher values while a MR < 1 means that the study favors standard COS without letrozole.

$P < 0.05$ were considered statistically significant. To evaluate heterogeneity among studies, I^2 values and relative *P*-values were also reported. A sensitivity analysis for each endpoint was performed to assess if the results were mostly driven by one or more studies.

RESULTS

The search strategy returned 625 records: after applying the inclusion and exclusion criteria, 15 records were potentially eligible for this meta-analysis (**Figure 1**). Among them, three records were excluded because they referred to the same study: in two cases [Goldrat et al. (38) vs. Goldrat et al. (34) and Cakman et al. (39) vs. Quinn et al. (36)], the article with the most updated data was selected (34, 36); for the other case [Haas et al. (24) vs. Haas et al. (40)], the least recent paper was included because of a larger sample size and the reporting of endpoints considered in the present meta-analysis (24). One article was excluded because it did not provide the required data for statistical analysis (41).

Therefore, a total of 11 records were selected for the current meta-analysis, including 2,121 patients, of whom 990 underwent COS with letrozole and 1,131 underwent COS without letrozole (24, 29, 33–37, 42–45). Six studies were conducted in cancer patients only (35–37, 42, 43, 45); one study in infertile patients only (24). COS with letrozole in cancer patients was compared to COS without letrozole in infertile controls in two studies (29, 34), to COS without letrozole in healthy elective fertility preservation patients in another study (33), and to both cancer patients and healthy elective fertility preservation patients in another study (44).

The main characteristics of the included studies are summarized in **Table 1**.

The total number of MII oocytes, as quantitative marker of efficacy, was reported in nine studies (24, 29, 34–37, 42–44). No difference between the letrozole and no-letrozole cohorts was found with a MR value of 1.00 (95% CI = 0.87–1.16; $P = 0.967$; **Figure 2**). Heterogeneity was high ($I^2 = 68.6\%$; $P = 0.001$). Sensitivity analysis is reported in **Supplementary Table 1**.

The total number of retrieved oocytes was reported in all studies (24, 29, 33–37, 42–45). No difference between the letrozole and no-letrozole cohorts was found (MR = 1.04; 95% CI = 0.93–1.17; $P = 0.493$; **Figure 3A**). Heterogeneity was high ($I^2 = 73.8\%$; $P < 0.001$). Sensitivity analysis is reported in **Supplementary Table 2**.

Seven studies reported on maturation rate (29, 34, 36, 37, 43–45). Higher maturation rate was observed in the no-letrozole cohort; however, the difference was not statistically significant (MR = 0.94, 95% CI = 0.88–1.01, $P = 0.118$, **Figure 3B**). Heterogeneity was high ($I^2 = 82.5\%$; $P < 0.001$). Sensitivity analysis is reported in **Supplementary Table 3**.

Fertilization rate was reported in three studies (29, 36, 43). A higher fertilization rate was observed in the no-letrozole cohort; however, the difference was not statistically significant (MR = 0.95, 95% CI = 0.89–1.00, $P = 0.064$; **Figure 3C**). No heterogeneity was observed ($I^2 = 0.0\%$; $P = 0.396$). Sensitivity analysis is reported in **Supplementary Table 4**.

Peak estradiol levels were reported in 10 studies (24, 29, 34–37, 42–45). Estradiol levels were significantly lower in the letrozole cohort (MR = 0.27, 95% CI = 0.23–0.32; $P < 0.001$; **Figure 3D**). Heterogeneity was high ($I^2 = 76.8\%$; $P < 0.001$). Sensitivity analysis is reported in **Supplementary Table 5**.

Total gonadotropin dose was reported in 10 studies (24, 29, 33–35, 37, 42–45). No statistically significant difference was observed between the letrozole and no-letrozole cohorts (MR = 0.97, 95% CI = 0.86–1.10, $P = 0.676$; **Figure 3E**).

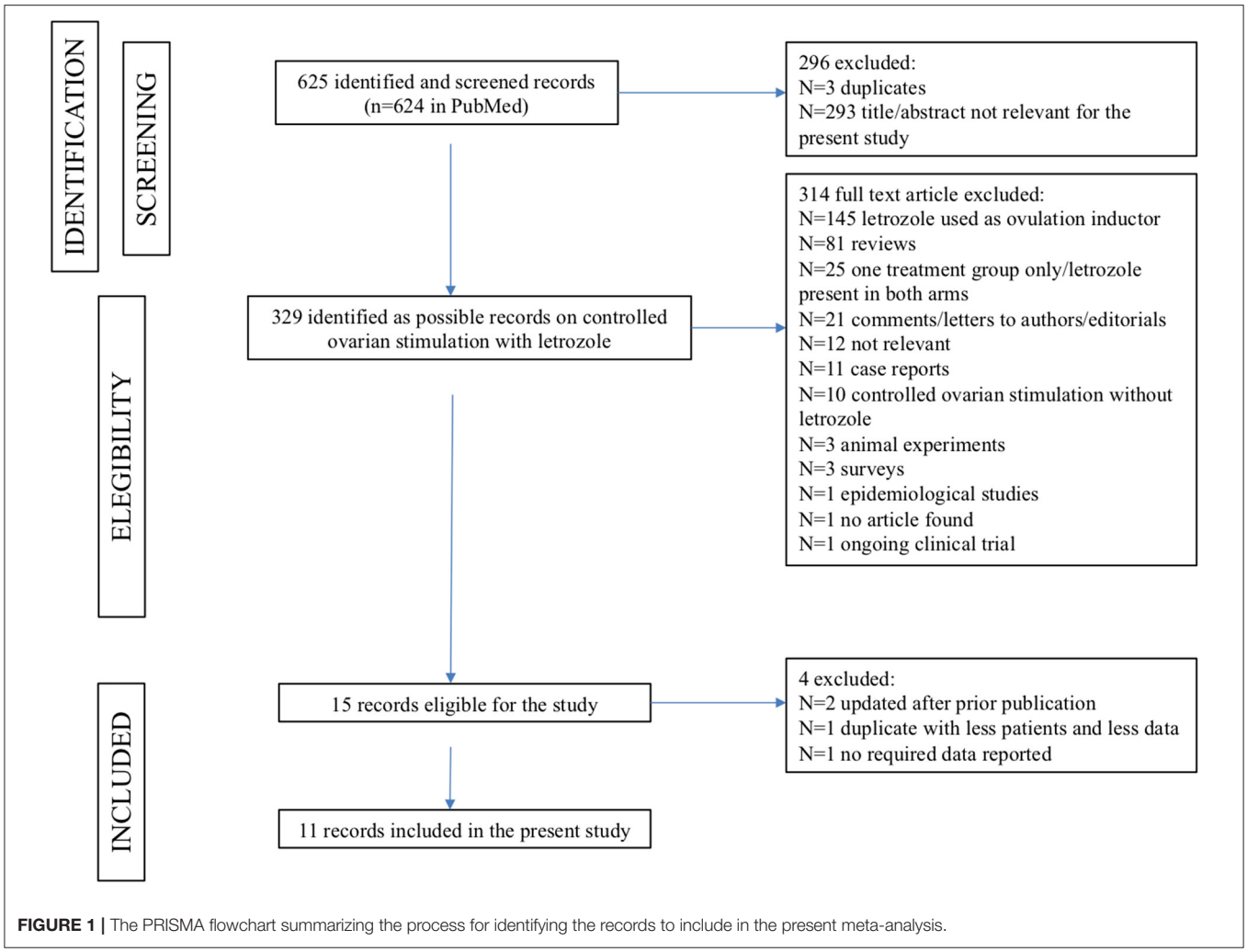

FIGURE 1 | The PRISMA flowchart summarizing the process for identifying the records to include in the present meta-analysis.

Heterogeneity was high (I^2 = 86%, P < 0.001). Sensitivity analysis is reported in **Supplementary Table 6**.

Length of the stimulation was reported in all included studies (24, 29, 33–37, 42–45). There was no difference between the letrozole and no-letrozole cohorts (MR =1.00, 95% CI = 0.96–1.04, P = 0.939; **Figure 3F**). Heterogeneity was high (I^2 = 65.9%; P = 0.001). Sensitivity analysis is reported in **Supplementary Table 7**.

All the analyses were repeated by including only the three articles comparing breast cancer patients in both the letrozole and no-letrozole cohorts (35–37). Based on data availability, four endpoints (number of MII oocytes, total number of oocytes, length of the stimulation, and peak estradiol levels) could be analyzed. The observed results were consistent with those of the primary analysis. No difference between the letrozole and no-letrozole cohorts was observed in terms of total number of MII oocytes (MR = 0.90; 95% CI = 0.68–1.20; P = 0.482; **Figure 4A**; I^2 = 76.9% and P = 0.013), total oocytes retrieved (MR = 0.96; 95% CI = 0.73–1.26; P = 0.771; **Figure 4B**; I^2 = 76.2%; P = 0.015), and length of the stimulation (MR = 1.00, 95% CI = 0.96–1.04, P = 0.985; **Figure 4C**; I^2 = 0.0%; P = 0.666). Peak estradiol levels were significantly lower in the letrozole group as compared to the no-letrozole group (MR = 0.28, 95% CI = 0.24–0.32; P < 0.001; **Figure 4D**; I^2 = 0.0% and P = 0.778).

DISCUSSION

While letrozole as an ovulation inductor is well-known and widely used (21, 22, 46), its role alongside a COS protocol is less studied and therefore less used in infertile patients, due to conflicting results and the safety warning of the producer. However, in the last years, it has become the standard of care for COS in patients with hormone-sensitive cancers to avoid potentially harmful supra-physiological estradiol levels (47), which are the main reason for oncologists to oppose oocyte or embryo cryopreservation (18). However, the evidence on the use of COS protocol that include letrozole is based on few observational studies, most of them with a small sample size and heterogeneous in nature.

The present meta-analysis aimed to assess the efficacy and safety of letrozole co-administration during COS. It showed that the addition of letrozole to COS does not have a negative effect

TABLE 1 | Main characteristics of the included studies and type of protocol of controlled ovarian stimulation.

		Number of patients		Type of patients		Age		COS protocol		
Author	**Year**	**Letrozole**	**No letrozole**	**Letrozole**	**No letrozole**	**Letrozole**	**No letrozole**	**Follicular development**	**Ovulation suppression**	**Trigger**
Sonigo et al.	2019	94	83	BC	BC	33.5 ±4.5 mean ± SD	33.6 ± 3.3 mean ± SD	rFSH	GnRH antagonist	GnRH agonist
Goldrat et al.	2019	23	24	BC	IN	30.4 ± 3.8 mean ±SD	30.8 ± 3.9 mean ±SD	rFSH	GnRH antagonist	GnRH agonist (for BC and at risk of OHSS) for the others hCG
Ben Harush et al.	2019	145	273	BC	K+EL	33.7 ± 5.1 mean ± SD	30.0±7.5 mean ± SD	rFSH	GnRH antagonist	GnRH agonist
Haas et al.	2017	87	87	IN	IN	36.5 ± 4.1 mean ± SD	37.0 ± 3.8 mean ± SD	rFSH	GnRH antagonist + rLH or hMG	hCG+GnRH agonist (GnRH agonist only for patients at risk of OHSS)
Quinn et al.	2017	151	40	BC (ER+)	BC (ER-)	NR	NR	rFSH	GnRH antagonist	hCG or GnRH agonist (decision taken singularly depending upon size of the follicular cohort and perceived risk of OHSS)
Pereira et al.	2016	220	439	BC	EL	36 (33-38) median (IQ range)	37 (34–39) median (IQ range)	rFSH	GnRH antagonist	hCG
Johnson et al.	2013	22	28	BC + endometrial k	BC+K	31.2 (19–43) mean (95% CI)	31.2 (21–41) mean (95% CI)	rFSH ± LH support	GnRH antagonist	hCG ± GnRH agonist
Revelli et al.	2013	50	25	BC (ER+)	BC (ER-)	34.4 ± 5.2 mean ± SD	35.1± 4.9 mean ± SD	rFSH or hMG	GnRH antagonist / long GnRH agonist	hCG
Checa Vizcaíno et al.	2012	9	10	BC	K	32 ± 2.87 mean ± SD	28 ± 4.13 mean ± SD	rFSH	GnRH antagonist	GnRH agonist
Domingo et al.	2012	142	66	BC	K	33.2 ± 4.3 mean ± SD	30.6 ± 5.7 mean±SD	rFSH	GnRH antagonist	GnRH agonist
Oktay et al.	2006	47	56	BC	IN	36.4 ± 3.6 mean ± SD	36.9 ± 3.9 mean ± SD	rFSH	GnRH agonist	hCG

COS, controlled ovarian stimulation; BC, Breast cancer; K, cancer; IN, infertile; EL, Elective; ER -, Estrogen receptor negative; ER +, Estrogen receptor positive; rFSH, recombinant follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; rLH, recombinant lutenizing hormone; hMG, human menopausal gonadotropin; hCG, human chorionic gonadotropin; BC, breast cancer; OHSS, ovarian hyperstimulation syndrome.

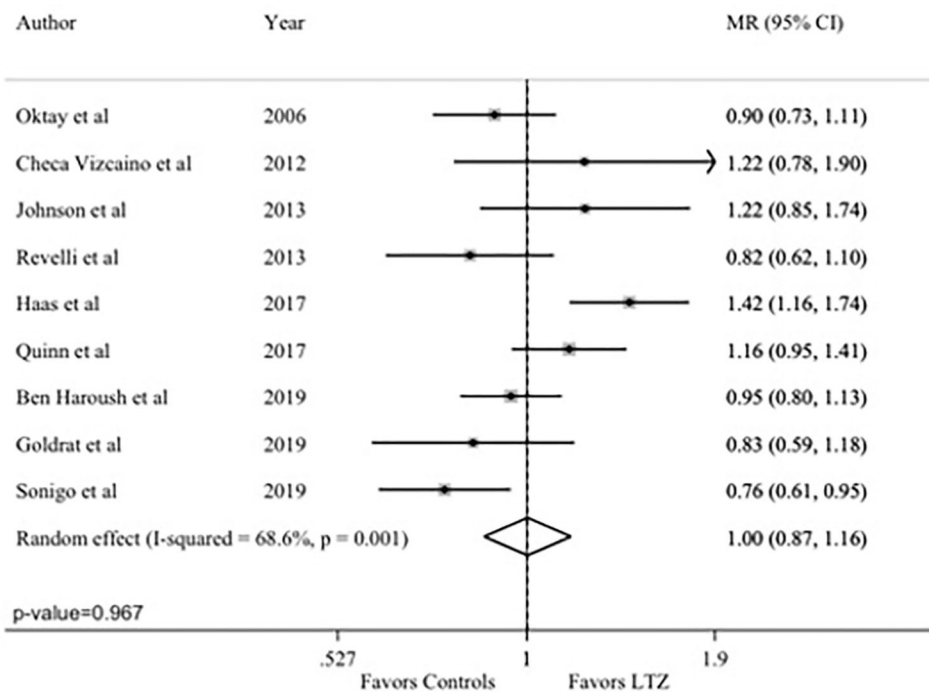

FIGURE 2 | Primary endpoint: number of Metaphase II (MII) oocytes. MR, mean ratios; CI, confidence intervals; LTZ, letrozole.

on the number of mature oocytes collected and on other efficacy endpoints, while it is associated with significantly decreased peak estradiol levels which may be of great importance particularly in patients with hormone-sensitive cancers.

A high heterogeneity among studies was observed in the majority of the analysis. This may be due to study design (none was a randomized trial), their low sample size, the different cohorts of patients included also in terms of age, as well as the non-homogeneous COS protocols. For example, Ben Haroush et al. included in the no-letrozole cohort healthy women who elected to have their oocyte cryopreserved for social reasons and very young patients (26.5 ± 7.1 years) with non-hormone-sensitive cancers (44). Both these groups of women are expected to be high-responders, but surprisingly, the authors reported similar number of retrieved oocytes between groups with a slightly higher maturation rate in favor of the breast cancer cohort. The sensitivity analysis reported in **Supplementary Table 3** showed that, after excluding the study by Ben Haroush et al., maturation rate results become statistically significant in favor of the no-letrozole cohort, supporting that this study strongly weights on the final statistical results for this parameter.

The study design, and specifically the choice of the controls, is the feature associated with the highest risk of bias for the included studies. By comparing results between cancer patients and healthy infertile women with the latter probably having a worse prognosis at start, a selection bias becomes impossible to avoid, especially in an observational study. Using healthy patients who elected to have their fertility preserved for social reasons is probably a more accurate choice; however, literature is not univocal on ovarian response to COS in cancer patients before gonadotoxic therapies, not excluding a worse ovarian reserve even before starting anticancer therapies (48, 49). Study comparing cancer patients in both study groups usually had smaller sample size and did not exclude potential bias due to the impact of the cancer type. Only three studies included exclusively breast cancer patients in both study cohorts. To specifically investigate the performance of COS with or without letrozole in breast cancer patients, we performed a secondary analysis that showed no influence of letrozole on all the evaluated efficacy endpoints. However, some issues remain to be clarified also in this setting. For example, *BRCA*-mutated women, which are described by some reports as less fertile (50, 51), have more frequently hormone receptor-negative cancers; therefore, they are more likely to be included in the no-letrozole cohorts. Recent data, demonstrating the safety of pregnancy in breast cancer survivors with germline *BRCA* pathogenic variants (52), further highlight the need to pursue with additional research efforts to define the optimal fertility preservation approaches in these patients. Only one study in this meta-analysis compared infertile patients both in the letrozole and no-letrozole cohorts (24). The authors hypothesized a beneficial effect of letrozole on ovarian response because of an androgen-mediated increase of FSH receptors on granulosa cells, as it was seen in primates (53). Their results in terms of number of oocytes and blastocysts obtained are promising, but the study design is retrospective and it analyzed only 174 IVF cycles. A well-designed randomized trial would be better suited to confirm or deny their findings.

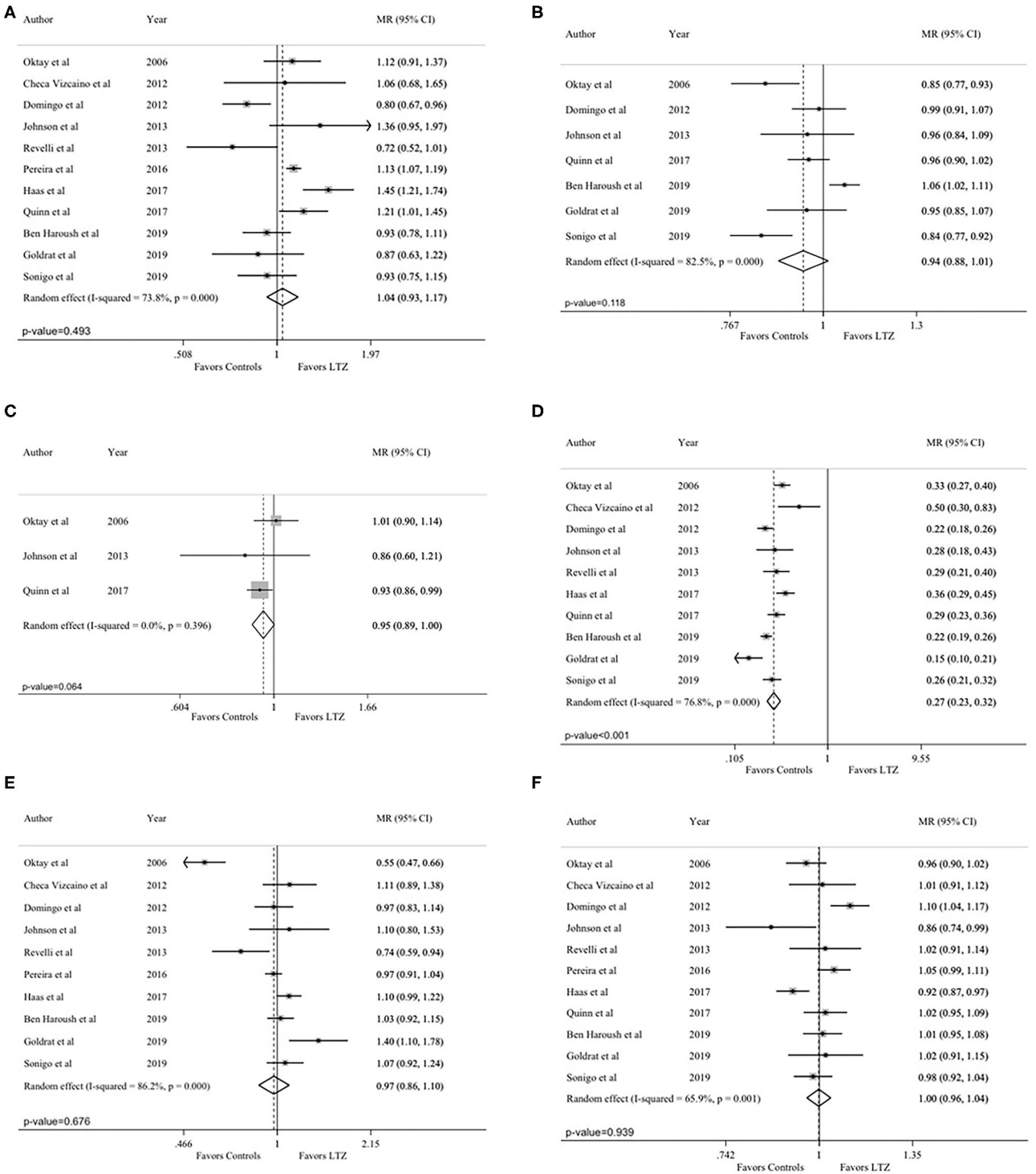

FIGURE 3 | Secondary endpoints: **(A)** total number of retrieved oocytes; **(B)** maturation rate; **(C)** fertilization rate; **(D)** peak estradiol levels; **(E)** total gonadotropin dose; **(F)** length of the stimulation. MR, mean ratios; CI, confidence intervals; LTZ, letrozole.

Another important potential explanation for the heterogeneity among studies is the ovulation trigger criteria that were used. In an earlier study, Oktay et al. showed lower oocyte maturation rates when trigger was achieved at a leading follicle size of 17 mm (29). Once trigger was done at a follicular size of 19–21 mm, maturation rates improved. In several studies included in our meta-analysis, ovulation trigger was performed in both groups either at 17 mm or "when appropriate"

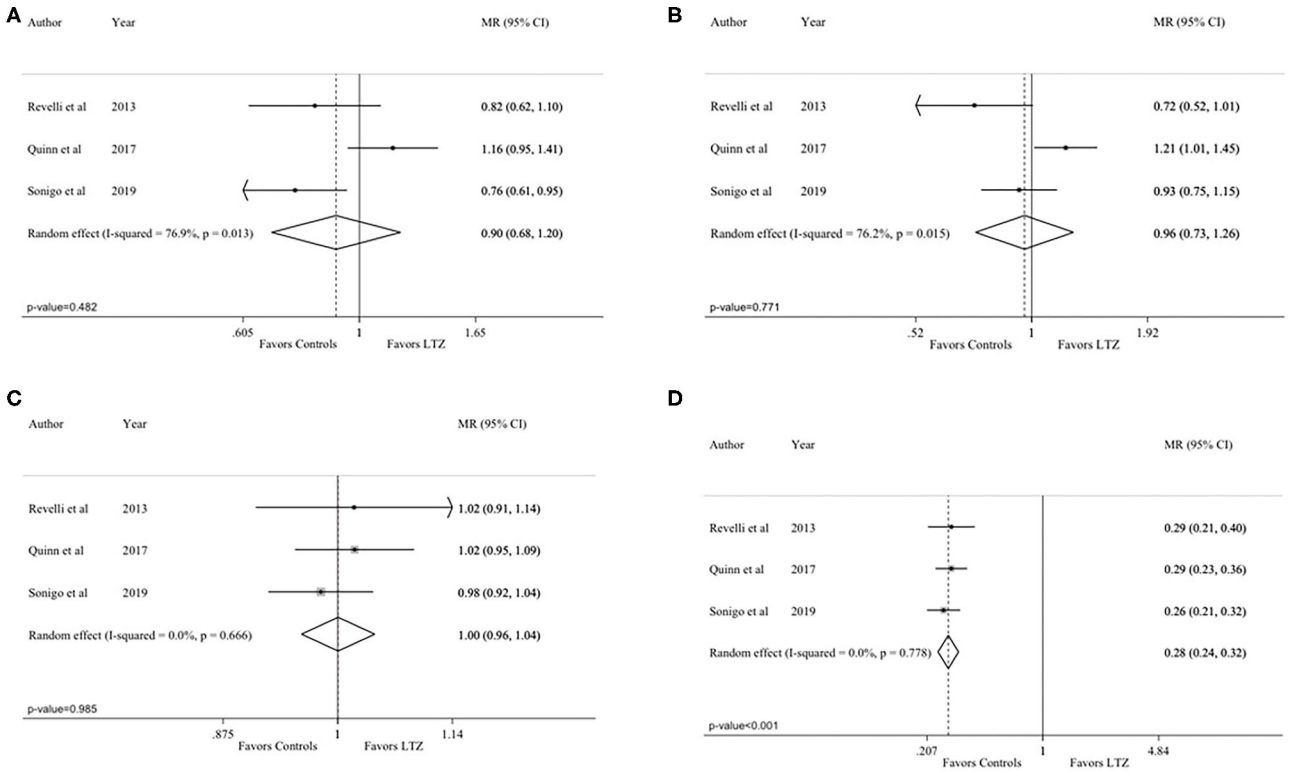

FIGURE 4 | Analysis including only the studies comparing breast cancer patients in both the letrozole and no-letrozole groups: **(A)** number of Metaphase II (MII) oocytes; **(B)** total number of retrieved oocytes; **(C)** length of the stimulation; **(D)** peak estradiol levels. MR, mean ratios; CI, confidence intervals; LTZ, letrozole.

(24, 35, 37, 44). This issue may account for the lower oocyte maturation rate in the letrozole cohort as compared to the non-letrozole cohort.

Importantly, the core outcome of fertility research should be the live birth rate being the ultimate chance that a specific treatment gives a patient the possibility to have a baby. Unfortunately, a meta-analysis on this outcome is not yet possible. Only one study included in our metanalysis reported this outcome in both the letrozole and no-letrozole cohorts (43). In this study, out of 50 patients, only six returned to thaw the embryos, one in the letrozole cohort and five in the no-letrozole cohort. For the patients who received COS with letrozole, one twin pregnancy via gestational carrier was obtained; it was complicated by pre-eclampsia, and two babies were born pre-term with a cesarean section. Among the five patients who received COS without letrozole only, three had their embryos transferred (the embryos did not survive the thawing for the other two). One patient used a gestational carrier, the pregnancy had no complications, and the baby was delivered vaginally at term. The other two patients had singleton pregnancies: one was complicated by pre-term labor but managed to deliver at term vaginally; the other was complicated by a baby large for gestational age and was delivered at term via cesarean section (43). Notably, utilization rate of cryopreserved material in cancer patients is reported to be quite low [around 10–23% for frozen embryos (54–56) and 5% for frozen oocytes (57–59)], considering that these women, also those who need to use their cryopreserved oocytes or embryos to have a pregnancy, have to complete their oncological therapies before. With time, more data on the utilization of such material will become available.

In terms of safety concerns, peak estradiol level was lower in the letrozole cohort. These data indirectly confirm the possible protective mechanism of letrozole for patients who are affected by hormone-sensitive cancers including breast tumors. Breast cancer patients who undergo COS with letrozole before starting chemotherapy does not appear to have higher risk of recurrence than those who do not undergo any fertility preservation procedure (60). The safety of this approach has also been shown for patients undergoing neoadjuvant chemotherapy, although the evidence is more limited in this setting (60, 61). The length of the stimulation is another safety parameter; indeed, this is of particular importance for cancer patients who need to start life-saving oncological treatments as soon as possible. Our study shows that standard protocols for COS with or without letrozole have the same stimulation length. Due to the paucity of information reported in the included articles, safety data remain largely incomplete. More evidence is needed on oncological outcomes (i.e., relapse rate, disease-free survival, adverse events, and delay in chemotherapy start) (62) as well as progesterone levels during COS (38, 63).

In conclusion, letrozole co-administration during COS resulted to be as effective as standard COS but with significantly decreased peak estradiol levels, suggesting its increased safety for patients with hormone-sensitive cancers. Although current data are reassuring, more studies, including randomized controlled trials, are needed to finally prove the efficacy and safety of letrozole co-administration during COS, particularly among cancer patients. Moreover, long-term outcomes in terms of both efficacy and safety should be strongly encouraged to be collected.

DATA AVAILABILITY STATEMENT

The data extracted from the original publications and supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ID and ML contributed to the conception and design of the study. BB, ID, and ML performed the literature search, study selection, and data extraction that were then reviewed by all authors. MB and MC performed the statistical analysis. The results were interpreted by BB, CM, ID, and ML The initial manuscript was drafted by BB, CM, ID, and ML. All authors revised the manuscript critically for important intellectual content and approved it.

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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.2020.574669/full#supplementary-material

Supplementary Table 1 | Sensitivity analysis for the endpoint "mature Metaphase II (MII) oocytes."

Supplementary Table 2 | Sensitivity analysis for the endpoint "total number of retrieved oocytes."

Supplementary Table 3 | Sensitivity analysis for the endpoint "maturation rate."

Supplementary Table 4 | Sensitivity analysis for the endpoint "fertilization rate."

Supplementary Table 5 | Sensitivity analysis for the endpoint "peak estradiol levels."

Supplementary Table 6 | Sensitivity analysis for the endpoint "total gonadotropin dose."

Supplementary Table 7 | Sensitivity analysis for the endpoint "length of the stimulation."

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Breast Cancer During Pregnancy: A Marked Propensity to Triple-Negative Phenotype

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‡This work is dedicated to the memory of my beloved ex-wife "Naima Bachnou" who passed away because of breast cancer during pregnancy

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Breast and cervical cancers comprise 50% of all cancers during pregnancy. In particular, gestational breast cancer is considered one of the most aggressive types of cancers, which is a rare but fatal disease. However, the incidence of this type of cancer is increasing over the years and its prevalence is expected to rise further as more women delay childbearing. Breast cancer occurring after pregnancy is generally triple negative with specific characterizations of a poorer prognosis and outcome. On the other hand, it has been pointed out that this cancer is associated with a specific group of genes which can be used as precise targets to manage this deadly disease. Indeed, combination therapies consisting of gene-based agents with other cancer therapeutics is presently under consideration. We herein review recent progress in understanding the development of breast cancer during pregnancy and their unique subtype of triple negative which is the hallmark of this type of breast cancer.

Keywords: breast cancer, pregnancy, gene deregulation, delayed childbearing, triple-negative

Abbreviations: AMBER, African American breast cancer epidemiology and Risk Consortium; ASCO, American Society of Clinical Oncology; AURKA, Aurora Kinase A; BIRC5, Baculoviral IAP Repeat Containing 5; BRCA, Breast cancer susceptibility gene; Ca2+, Calcium ions; cdc, Cell division control protein; CNB, Core needle biopsy; CTLA4, Cytotoxic T-Lymphocyte Associated Protein 4; CXCL, C-X-C Motif Chemokine Ligand; CXCR, C-X-C Motif Chemokine Receptor 4; ELN, Elastin; ER/ESR, Estrogen receptor; ERBB2, Erb-B2 Receptor Tyrosine Kinase 2; FAK, Focal adhesion kinase; FBN, Fibrillin; FNAC, Fine needle aspiration; GPCR, G protein-coupled receptor; GSK3-β, glycogen synthase kinase 3-beta; HER2, human epidermal growth factor receptor 2; IGF1, Insulin Like Growth Factor 1; IL, Interleukin; ILC, Invasive lobular carcinoma; irAEs, Immune-related adverse events; JAK, Janus Kinase; LAR, Luminal androgen receptor; mdm2, Mouse double minute 2 homolog; MMP, Matrix metallopeptidase; MMTV, Mouse mammary tumor virus; MRI, Magnetic resonance imaging; NCCN, National Comprehensive Cancer Network; NST, No-special-type; p53, Tumor protein 53; p63, Tumor protein 63; PABC, Pregnancy-associated breast cancer; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death-ligand 1; PGR/PR, Progesterone receptor; PI-MECs, Parity-induced mammary epithelial cells; PTEN, Phosphatase And Tensin Homolog; Rac1, Rac Family Small GTPase 1; RANKL, Receptor activator of the nuclear factor κB ligand; RhoA, Ras Homolog Family Member A; ROCK, Rho Associated Coiled-Coil Containing Protein Kinase; SLNB, Sentinel lymph node biopsy; src, SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase; STAT, Signal Transducer And Activator Of Transcription; TDO2, Tryptophan-2,3-dioxygenase; TGF, Transforming growth factor; TILs, Tumor-infiltrating lymphocytes; TNBC, Triple-negative breast cancer.

INTRODUCTION

Breast cancer is the most common type of cancer in females affecting more than 2.1 million women and causing more than half a million deaths annually (1). The etiology of breast cancer is complex and heterogeneous with numerous pathological characteristics; these directly correlate with available treatment options and disease prognosis (2). Based on microarray and unsupervised cluster analysis studies, breast cancer is classified into four molecular subtypes with distinct gene expression patterns and clinical outcomes (3). These subtypes include luminal (A and B), human epidermal growth factor receptor 2 (HER2)-type, and triple-negative breast cancers (TNBC) (4–6).

TNBC possesses molecular characteristics and clinical aggressiveness that is analogous to that of basal-like cancer (7). TNBC lacks estrogen receptor (ER), progesterone receptor (PR), and HER2 expression and accounts for ~15% of all breast cancer cases (7). More intriguingly, the described subtype of breast cancer is reportedly associated with high-grade invasive ductal carcinomas and, when compared with other subtypes, TNBC was found to be larger with higher metastatic propensity to lungs, brain and other visceral organs.

Since the majority of basal-like cancers are also TNBC and more than 80% of TNBC are basal-like breast cancers, it has been postulated that TNBC and basal-like phenotypes are essentially analogous (8). Using gene expression profiling, the molecular heterogeneity of TNBC was well defined. One study subclassified TNBC into six molecular subtypes including basal-like 1, basal-like 2, immunomodulatory, mesenchymal-like, mesenchymal stem-like, and luminal androgen receptor (LAR) subtype (9). Furthermore, TNBC molecular subtyping revealed three subtypes, LAR, basal-like with low immune response and high M2-like macrophages and basal-enriched with high immune response and low M2-like macrophages (10). Despite histological differences, the metastatic characteristics of the highlighted TNBC subtypes remain comparable (11). In addition to the metastatic potential of TNBC, it is vital to note that once TNBC metastasizes the window between relapse and death becomes very narrow (12). Dent et al. reports that patients with TNBC were more likely to experience significant relapses and higher rates of death when compared with women suffering from other types of breast cancers (12). The same group also reports a four folds increase in the likelihood of visceral metastasis in TNBC patients when compared with other types of breast cancer (13).

Breast cancer risk factors are various; nonetheless, a strong association between pregnancy and breast cancer has been well established (14, 15). Although early age pregnancy is considered generally protective against breast cancer, this protection is deferred. Nevertheless, the period immediately subsequent to pregnancy is characterized by a risk of breast cancer development (16). During the last 30 years, diagnosis of cancer during pregnancy has become more common due to the present trend of delaying pregnancy or childbearing to an older age (17). Pregnancy-associated breast cancer (PABC) is an upcoming issue; in this review, we aim at illustrating recent advances in understanding the development and progression of PABC and their associated genes with emphasis on TNBC to review current and potential management options.

Gestational cancer is defined as cancer diagnosed during pregnancy or the first postpartum year (18). Pregnancy-associated melanoma, breast and cervical cancers are the most common malignancies during pregnancy; both cervical and breast cancers account for 50% of all gestational cancers (19). Hematological cancers including leukemia and lymphoma comprise 25% of gestational cancer cases, while ovarian, thyroid and colon cancers are less common (19).

Pregnancy-associated breast cancer (PABC), also known as "gestational breast cancer" is defined as breast cancer diagnosed either during pregnancy or up to one year postnatal (20) and affects around 1 in 3,000 pregnant women (21). In comparison with nulliparous women, breast cancer in pregnant women is histologically similar; approximately 75%–90% of the tumors are invasive ductal carcinomas with no-special-type (NST) (21–26). While, invasive lobular carcinoma (ILC) and other histological types are uncommon in patients with PABC (23, 27–29). Previous studies have showed that postpartum period is linked to a higher risk of developing more aggressive, high-grade breast cancer (14, 16, 23, 26, 30–32) with high tumor nuclear grade (29, 33, 34) and poorly differentiated tumors (24). PABC is also associated with lymphovascular invasion (22, 23, 33), more frequent lymph node involvement and larger tumor size (21–23, 27, 35–40). Similar to nulliparous women, PABC tends to commonly metastasize to lung, liver, brain, and skeletal system (41). Women with PABC have a poorer clinical outcome and disease-free survival with a higher mortality rate compared with nulliparous women (42–45).

With regards to steroid receptors, the previous data showed that estrogen and progesterone play major roles in breast tumorigenesis (46–48), and their effects on breast cells are mediated by their respective receptors, the ER and the PR (49, 50). Earlier studies evaluated tumor histology as well as the prognostic and predictive markers (ER, PR, HER-2/*neu*, p53, and Ki-67) in PABC; in comparison to age-matched non-pregnant women, their findings show that PABC exhibit lower expression of ER/PR and higher expression Ki-67, p53 HER2 (23, 25, 26, 43, 51–53). However, a study by Shousha showed that during pregnancy or early lactation, the expression of HER-2/*neu* was negative; however HER2 expression was noticed after delivery or at the end of lactation indicating suppression of HER-2/*neu* expression during pregnancy and lactation (32). Low ER positivity was observed in women with PABC, plausibly due to decreased ER levels during pregnancy (22, 51, 54, 55). It has been indicated that increased estrogen levels can aid in preventing ER-positive tumors (56). Furthermore, multiparous women (≥3 live births) who never breastfed were at a higher risk of ER–/PR– breast cancers compared with multiparous women with a history of breastfeeding (57). A study by Harvell et al. analyzed the presence of breast cancer subtypes in PABC and found that the presence of Luminal A, Luminal B, Her2-positive, TNBC, and basal-like subtypes in PABC (58). Other studies also confirmed TNBC, Luminal B and HER2-positive as the most common subtypes among PABC while luminal A subtype was rare (25, 52, 59–61) (**Table 1**).

TABLE 1 | Prevalence of molecular subtypes of breast cancer in pregnancy-associated breast cancer (PABC).

Population (Year)	Prevalence of Molecular Subtypes (%)				Reference
	Luminal A	Luminal B	HER2-Positive	TNBC	
Chinese (2020)	10.8%	30.4%	15.8%	17.4%	(61)
Chinese (2019)	7.1%	47.1%	22.9%	22.9%	(25)
Korean (2018)	7.7%–21%	21.1%	17.3%	35.9%–40.4%	(59, 60)
Hungarian (2014)	0%	32.8%	18%	48.4%	(52)

Several risk factors have been associated with PABC including hormonal changes, immune suppression during pregnancy as well as diagnostic challenges related to increased postpartum breast density and subsequent breast cancer diagnosis (14, 30). Breast involution is considered an important risk factor due to its shared features with pro-inflammatory microenvironment (26, 62–64), thus, providing suitable grounds for tumor growth and spread (65). Possible mechanisms of PABC include breast differentiation and involution (14, 66, 67). Following lactation, breast remodeling is a regulated program that involves the stimulation of fibroblasts, endothelial cells and immune cells. These cells then activate breast cells enhancing wound closure and remodeling of damaged tissue leading to the growth and development of transformed cells (14, 66). Moreover, an *in-vitro* and *in-vivo* study showed that involuting breast can assist the growth of existing tumor cells (66). *In-vivo* data showed that weaning-induced involution maintained ductal development of normal cells, however, in tumor cells they promoted invasion. Intriguingly, Yang et al. reported that early age at menarche, nulliparity, and late age at first birth increased the risk of luminal A breast cancer without any association with TNBC (68). On the contrary, all highlighted factors were identified as risk factor for TNBC in several other studies (69–74). Women's race was also identified as a risk factor for TNBC. For instance, in comparison with white women, African-American women were found to be at a higher risk for TNBC, especially at a young age (<45 years) (71, 75, 76). A study by Ma et al. showed a protective effect of breastfeeding against development of TNBC (69). While data from the African American breast cancer epidemiology and Risk (AMBER) Consortium, showed that breastfeeding decreased the risk of TNBC associated with multiparity (77). Several other studies have revealed a significant correlation between PABC and high-grade breast cancers (16, 78–80); high grade morphology can be linked with PABC up to 10 years following pregnancy.

PABCs frequently display a higher incidence of the TNBC phenotype in comparison with cancers affecting nulliparous women. TNBCs comprise around 30%–40% of all PABC cases (52, 81) and are more likely to occur in recent pregnancy associated (within 1-2 years) breast cancers (52, 81). However, another study showed that TNBC risk can be present beyond 2 years postpartum; being one of the reasons for overall poor prognosis that characterizes tumors detected after pregnancy (16).

Molecular Features of Pregnancy-Associated Breast Cancer

To further understand the carcinogenic molecular pathways effected during pregnancy, leading to breast cancer development, it is crucial to investigate associated gene deregulation patterns as well as mutations and their role in breast cancer development. In comparison with normal epithelium, in PABC several hormone target genes regulating the mitotic phase were overexpressed (58); four of these genes *MKI6, AURKA, BIRC5*, and *MMP11* are included in the Oncotype DX (82). Furthermore, the expression of tumor suppressor, p63 was downregulated in PABC; its expression correlates with enhanced invasion and aggressive feature of PABC (58, 83).

Azim et al. aimed at identifying the effects of pregnancy and involution on certain gene expression patterns in breast cancer cells compared with normal breast tissue (84). The authors found that the expression of *PD-1*, *PD-L1*, and gene sets related to *SRC*, *IGF1*, and *β-catenin* were higher compared with non-parous breast cancer females. However, this difference in the expression did not reach statistical significance. Therefore, in order to confirm this important finding with high statistical significance, more studies and larger patient sample sizes are necessary, which may lead to important therapeutic avenues based on these gene targets. During pregnancy, in response to growth hormones, expression of ER, PR, and IGF-1 is elevated and is linked with increase in breast cancer cell proliferation (14). In this context, *in-vivo* studies showed loss of *PD-L1* to correlate with fetal resorption and increase in fetal lethality (85, 86), hence the expression pattern of PD-1 and its ligand PD-L1 in PABC was assessed. Another study analyzed PD-1 and PD-L1 expression in both tumor-infiltrating lymphocytes (TILs) in PABC and nulliparous women; PD-L1 was strongly elevated in PABC TILs in comparison with controls, independent of tumor characteristics (87). On the other hand, in TILs PD-1 was expressed in both PABC and nulliparous women (87). Research has shown that high stromal TILs and PD-L1 expression to be frequently present in TNBC (88). A similar study by Acs et al. (2017) assessed PD-1, PD-L1, and CTLA-4 expression in PABC and non-PABC women; the expression of PD-1, PD-L1 was seen in peritumoral lymphocytes, however there was no expression of CTLA-4 and elevated PD-L1 expression was associated with early-onset of breast cancer and poor prognosis (89).

Furthermore, certain pathways were also found to be highly activated in parous breast cancer females including the G protein-coupled receptor (GPCR) and the serotonin receptor signaling pathways (84). GPCR signaling pathway plays a vital role in multiple cellular processes and mediates the activation of around 3% of the genes (90). Consequently, any aberration within this pathway may contribute to various diseases including cardiac, inflammatory and neoplastic (91). However, GPCRs are large family of receptors and only two of these receptors (CXCR4 and GPR30) were found to be highly expressed in breast cancers (84). The upregulations of these genes may induce breast cancer growth and metastasis (92). Moreover, activation of those GPCRs happens upon their binding to their ligands, which triggers the subsequent

Ca2+ mobilization and kinase cascade activation leading to the induction of the expression of genes that are crucial for cellular growth (93).

The interplay between pregnancy and breast cancer has been an intriguing research topic over the years (94–96). Schedin et al. showed that many alterations- both inflammatory and non-inflammatory- occur in postpartum breasts causing a tumor inducing microenvironment (14). To further illustrate the mechanisms through which this takes place, Asztalos et al. reported that the involution process works to restore the status of breast tissue -prior to pregnancy- by inducing apoptosis, detachment of cells from the basement membrane (97), and other inflammation related events (62). The created inflammatory environment may initiate and contribute to the progression of breast cancer, especially through promoting tumor cell proliferation (14, 98). More importantly, a multitude of studies report different genetic patterns of PABC as compared to those detected in nulliparous women (16, 52, 81). Notably, estrogens can also bind to a known subtype of GPCRS, G protein estrogen receptor (GPER), thus contributing to breast cancer initiation and progression (99). The upregulation of serotonin induces tumorigenesis through induction of cellular proliferation (100). Furthermore, serotonin receptor pathway helps in the regulation of the expression of cathepsin S (CTSS) and is highly expressed in several cancer subtypes in which it correlates with their progression (101). The aggressiveness of pregnancy associated-TNBC, its poor prognosis and lack of treatment modalities makes it pivotal to study its genetic patterns and identify novel treatment options.

Molecular Features of Pregnancy Associated Triple-Negative Breast Cancers

Several variations are mainly ascribed to TNBC subgroup that was reportedly more predominant in PABCs (16, 52, 81); however, the exact mechanism by which pregnancy induces TNBC is yet to be fully elucidated. Among the different subtypes of breast cancer, CTSS is involved in invasion and is highly expressed in TNBC (102). Another factor that might contribute to the poor prognosis of TNBC is that TNBC increases the levels tryptophan-2,3-dioxygenase (TDO2) enzyme through inflammatory signals (103). TDO2 is a critical enzyme in catabolism of tryptophan, and its upregulation increases the production of tryptophan metabolites that exhibit antiapoptotic effects in TNBC cells (102).

Nevertheless, studies that specifically addressed the impact of pregnancy on development of TNBC are scarce. Asztalos et al. found a unique gene expression pattern for a specific set of genes in parous females who developed breast cancer in comparison to nulliparous breast cancer females. Differently expressed genes included 14 genes such as *CXCL1, CXCL12, ELN, ERBB2, ESR1, FBN1, IL1A, IL8, MMP12, MMP2, PGR, TGFB3, THBS1*, and *TIMP2*. Four of these genes (*CXCL1, IL1A, IL8, MMP12*) were upregulated, while the remaining 10 genes were down regulated in TNBC. Notably, downregulation of three of these genes (*ESR1, PGR, ERBB2*) are features of TNBC (104). Three of the upregulated genes (*CXCL1, IL1A, IL8*) are involved in inflammatory responses. Furthermore, inflammation and wound-healing involve macrophage cell influx, increased levels TGF-β1 and β3, MMPs-2, -3, and -9, and presence of fibronectin and laminin; these are linked with tumor progression and result in metastasis (14, 105–107). The interaction between cells and fibronectin *via* β_1 integrins results in the onset of human breast cancer (108). Upregulated expression of TGF-β triggers matrix deposition and growth of fibroblasts in the healing wound, thus, accelerating tumor growth (107, 109). Indeed, MMPs are essential for the process of angiogenesis and lymphangiogenesis; both processes are essential in wound healing and tumor initiation and progression (110, 111). These findings further support the hypothesis that inflammation could contribute to the development of TNBC after pregnancy.

Interestingly, Azim et al. reported that the receptor activator of the nuclear factor κB ligand (RANKL) is found to be repressed in TNBC compared with other types of breast cancer, while the receptor activator for nuclear factor κB (RANK) was found to be highly expressed in TNBC (112). However, the link between these TNBC patients and pregnancy was not been found (113). **Table 2** summarizes function of the identified genes in normal cells and PABC.

Tumor suppressors, *BRCA1/2* are involved in DNA damage repair, cell cycle control, transcription and ER type alpha activity (130). Mutations in *BRCA1/2* are considered as risk factors for the onset of breast cancer (131); Atchley et al. reported a significant association between mutations in breast cancer susceptibility gene 1 (*BRCA1*) and TNBC, with more than 2/3 of *BRCA1* mutations cases being of TNBC phenotype (132). Earlier studies have indicated that *BRCA1/2* mutation carriers can be at a higher risk for PABC (133, 134). A study by Johannsson et al. analyzed the incidence of PABC in carriers of *BRCA1* and *BRCA2* mutations in comparison with premenopausal Swedish women aged ≤ 40 with sporadic PABC (133). The study showed that *BRCA1/2* carriers are at an increased risk for PABC and hence should be monitored carefully during pregnancy and in the postpartum period (133). Another study revealed a significantly higher (25%) PABC frequency among *BRCA1/2* mutation carriers compared with non-PABC cases (135). Although deleterious *BRCA1* mutations are frequently encountered on both sporadic and hereditary TNBC (136), no link between pregnancy and these mutations has been found. Similarly, no association between TNBC and mutations in *BRCA2* gene were reported (137). Based on the previous discussion, we underline here that the exact link between pregnancy and TNBC remains to be elucidated.

Signaling Networks in Pregnancy-Associated Breast Cancer

Earlier investigations suggested various underlying molecular mechanisms underpinning the onset of PABC. In an *in-vivo* study by Wagner et al. (138), using WAP-Cre/Rosa-LacZ transgenic mice, the authors identified a mixed population of alveolar cells called parity-induced mammary epithelial cells (PI-MECs) in the mammary gland of parous, non-pregnant female mice; these cells were not present in nulliparous females. PI-MECs rely on the transcription factor p63 for survival (138–142); one-time pregnant mice (MMTV–Her2/Neu mouse model) lacking p63 have lower tumors, thus indicating a tumor-promoter role for PI-MECs (138). Furthermore, another study showed that increased expression of p63 inhibits the p53 and

TABLE 2 | Genes reported to having unique pattern of expression in triple-negative breast cancers (TNBC) patients and their associated functions.

Gene	Function in Normal Cells	Function in PABC	Reference
CTSS	Promotes antigen processing.	Angiogenesis, tumor progression, and invasion.	(102)
CXCL1	Inflammation.	Decreased relapse-free survival and metastasis.	(114)
CXCL12	Embryogenesis, Inflammation, and Immunity.	Promotes tumor growth and metastasis.	(115)
ELN	Provides elasticity to organs and tissues.	Enhances tumor migration and progression.	(116)
ERBB2/ HER2	Potentiates intracellular signaling.	Promotes metastasis and lower overall survival rates.	(117)
ESR1	Encodes estrogen receptor.	Downregulation is associated with worse outcome and poorly differentiated carcinomas.	(118)
FBN1	Structural support in elastic and non-elastic connective tissues.	Promotes tumor migration and invasion.	(119)
IL1A	Inflammation and hematopoiesis.	Promotes angiogenesis.	(120, 121)
IL8	Inflammation.	Promotes metastasis.	(122)
MMP12	Tissue remodeling.	Promotes angiogenesis and tumor progression.	(123)
RANKL	Regulation of T cell-dependent immune response.	Triggers endocrine therapy resistance and tumor progression.	(124)
PD-1	Negative regulator of immune response.	Promotes cancer immune evasion.	(125)
Src	Involved in embryonic development and cell growth.	Promotes malignancy and is associated with poor prognosis.	(126)
TGF-β3	Aids in embryogenesis, cellular differentiation and wound healing.	Promotes tumor progression and is associated with poor prognosis.	(127)
THBS1	Mediates cell-to-cell and cell-to-matrix interactions.	Involved in platelet aggregation, angiogenesis, and tumorigenesis.	(128)
TIMP2	Suppress proliferation of endothelial cells and maintain tissue homeostasis.	Promotes progression of cancer.	(129)

All information regarding the functions of those genes was collected form GeneBank (https://www.ncbi.nlm.nih.gov/genbank/).

STAT3 pathways; however, p63 enhances the expression of the pro-survival signaling STAT5 pathway, thus, initiating PI-MEC-induced tumorigenesis (142) (**Figure 1**). On the other hand, another study found loss of p63 during pregnancy reduced cyclin D1 levels, thus, suggesting a role of p63 in inducing PI-MEC survival post-partum (143). As shown in **Figure 1**, p63 levels increases cyclin D1which in turn inhibits estrogen receptor-alpha (ER-a) transactivation, further inhibiting the expression of BRCA1 (144). Following BRCA1 inhibition, PTEN is inactivated thereby activating mdm2 and blocking p53 (145) (**Figure 1**), which leads to genomic instability. Alternatively, pregnancy aids premalignant MECs evasion of apoptotic signaling through the activation of the JAK-STAT5 axis (146–148). In addition, receptor tyrosine kinases initiate downstream oncogenic signaling pathways such as PI3K/Akt which further activate either glycogen synthase kinase 3-beta (GSK3-β) or mdm2 (**Figure 1**).

On the other hand, in pregnant women, GPCRs are activated (84), these growth factors enhance focal adhesion kinase (FAK)

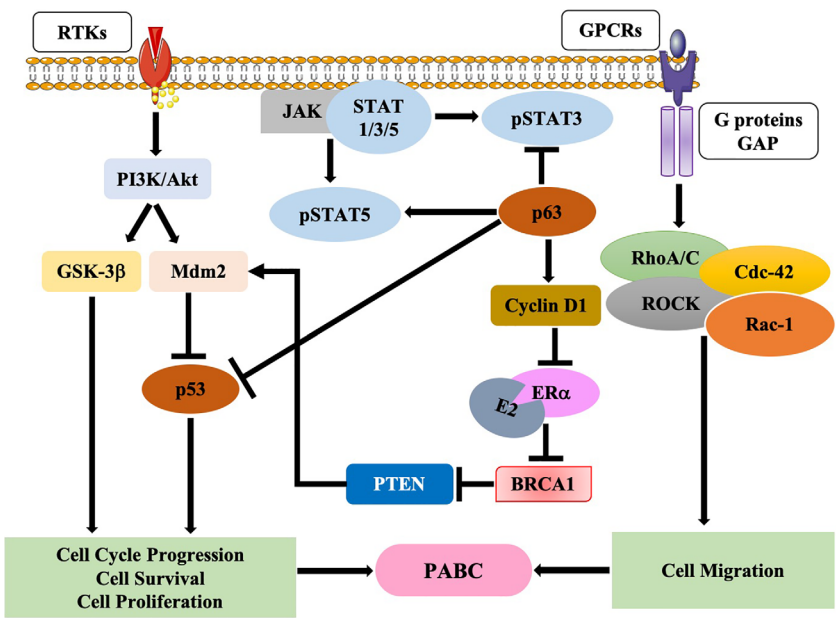

FIGURE 1 | Molecular pathways in pregnancy-associated breast cancer (PABC). Schematic diagram showing various pathways that are involved in the onset and progression of PABC.

which in turn activates RhoA/Rac1/cdc42/ROCK complex, thereby initiating cell migration (149) (**Figure 1**).

However, further understanding of the underlying molecular mechanisms of PABC is needed to help pave the way for the development of possible therapeutic strategies.

Diagnosis of Pregnancy-Associated Breast Cancer

Since pregnancy associated TNBCs have a poor prognosis and diagnostic delays may occur in pregnancy due to effects of pregnancy related hormones, increased awareness can help in paving the way for appropriate treatment.

Vis a vis PABC diagnosis, assessing breast symptoms during pregnancy and postpartum period can be perplexing due to hormonally induced changes in breast tissue that can result in augmented firmness and nodularity (150). Furthermore, postpartum lactational mastitis symptoms may mimic locally advanced or inflammatory breast cancer. The majority of PABCs are diagnosed after presenting with a palpable mass (151). To determine the scope of disease is critical in treatment decision-making.

Breast imaging includes mammography, ultrasound, and magnetic resonance imaging (MRI). While ultrasound is proven to be safe and commonly used in pregnancy (152), mammography confers minimal dose to the fetus with abdominal shielding (153). Contrast-enhanced breast MRI can be a useful diagnostic tool in non-PABC, however, in pregnancy, the safety of gadolinium still remains controversial. A free gadolinium is considered toxic as it can cross the placenta and stay in the amniotic fluid, which can be taken in by the fetus re-entering the fetal circulation (154). In addition, prone positioning required during breast MRI can apply a sustained pressure on the gravid uterus, disrupting uterine blood flow (154). In case of metastatic PABC, diagnostic workup prior to the delivery is required to enable therapeutic interventions. Generally, the pregnant patient is subjected to either chest X-ray with abdominal shielding, liver ultrasound or non-contrast supine MRI to check for lung, liver or bone metastasis, respectively (153).

Based on the imaging results, biopsy [fine needle aspiration (FNAC) or core needle biopsy (CNB)] is done for definite diagnosis of a breast mass (155). Although, FNAC is less traumatic with a low complication rate than CNB and generally does not require local anesthesia, FNAC provides inadequate information about the histopathological type, grade, steroid receptors, HER2 expression, and intrinsic behavior of the tumor (155, 156). Hence, CNB is considered as a more reliable method of pathological diagnosis of breast cancer (156). The tissue obtained from a biopsy is tested to determine the status of the hormone receptors (ER, PR) as well as Her2 and proliferation index (Ki-67) (157). Biopsies are performed either under ultrasound or stereotactic guidance (158). In addition, during the first and second trimesters of pregnancy, incisional or excisional biopsy can be safely done (159).

Once diagnosis of breast cancer has been completed by imaging methods and histopathology, it is essential not to postpone the treatment. It can be given post-delivery if the patient is in near term. If the patient is close to term, the treatment must commence (160).

Treatment of Pregnancy-Associated Breast Cancer

Treatment options for PABC remain challenging and may require special considerations. Surgery (e.g., modified radical mastectomy) is usually considered as the primary line of treatment in breast cancer during pregnancy but neoadjuvant chemotherapy has been widely used as a primary treatment option for advanced HER2-positive and TNBC (161). There are several concerns regarding the use of neoadjuvant chemotherapy that pertain to the potential peripartum complications and the impact on the fetal outcome (162). However, studies have shown that during the first trimester, chemotherapeutic agents are not advised as they may be potentially teratogenic (161). On the other hand, after completion of the first trimester, chemotherapeutic agents may be safely administered without the risk of fetal malformations (161). Multiple studies that explored the use of chemotherapeutic drugs for the treatment of breast cancer during pregnancy showed that the majority of drugs (taxanes and vinorelbine) are non-toxic during the second and third trimesters of pregnancy. However, these drugs may increase the risk of intrauterine growth restriction and preterm labor. Cytotoxic drugs may also induce both maternal and infant leukopenia, hence, chemotherapy after 35 weeks of gestation is contraindicated to avoid delivery of a leukopenic infant (161). Other drugs including methotrexate, trastuzumab and tamoxifen should also be avoided during pregnancy due to their effect on the central nervous system, cardiac, gastrointestinal and skeletal malformations, oligohydramnios (low levels of amniotic fluid), preterm labor, and spontaneous abortions (163–165). All these considerations should be taken into account when optimizing treatment options of breast cancer during pregnancy. A recent 4th ESO-ESMO guideline also emphasizes the need of an individual basis approach following the international guidelines and an expanded multidisciplinary team that will involve gynecologists/obstetricians as well as perinatologists, in addition to patients' own preferences (166).

Adjuvant chemotherapy is helpful and encouraged in patients with high-risk breast cancer including those with PABC. High-risk prognostic factors include estrogen and progesterone receptor negative status, HER2 status, high tumor grade, high TNM, and younger age of the patient (167). Patients that are treated with neoadjuvant chemotherapy and extensive residual disease (burden) are also strong candidates for adjuvant chemotherapy.

Radiation therapy is not advised during pregnancy as it can pose a high risk for fetal toxicity and malformations, childhood cancers and delays in neurocognitive development (168, 169). However, adjuvant radiotherapy (postpartum) can be safely used as in other breast cancer cases and following strict indications for adjuvant radiotherapy.

Breast cancer in young women (age < 40 years) tend to recur and therefore younger age of diagnosis, and hence longer lifespan places these patients at a statistically increased risk of recurrence and distant metastasis over time (170). Van Nes and van de Velde recommended mastectomy in younger patients over breast-conserving treatment (170). Any delay in treatment due to fallacies regarding risk of local and systemic therapy may worsens oncologic outcomes. Ambiguities regarding the safety of diagnostic modalities and treatment of PABC may lead to worse

outcomes in the group of younger pregnant women with breast cancer. However, recent studies provide robust data on the safety of diagnostic procedures that can enable a successful treatment of patients with this challenging malignancy.

Sentinel lymph node biopsy (SLNB) is a part of routine management of breast cancer and has been widely used in clinical practice. SLNB recommendation is proposed for patients with clinically node negative breast cancers, those with or without 1–2 suspicious lymph nodes on imaging, and for patients that were not treated with neoadjuvant systemic therapy (171). In contrast to the American Society of Clinical Oncology (ASCO) guidelines, the National Comprehensive Cancer Network (NCCN) guideline indicates lack of scientific evidence regarding the use of SLNB in pregnant women. While, the NCCN also advises that SLNB use should be an individualized decision, but not directly offered to pregnant women < 30 weeks' gestation. Of note, NCCN does not recommend the use of isosulfan or methylene blue dyes for SLNB in pregnancy while use of radioactive tracer (e.g., technetium 99m sulfur colloid) is also supported by limited scientific data regarding the fetal radiation dose (171).

CONCLUSIONS AND FUTURE DIRECTIONS

PABC incidence increases as women choose delayed childbirth, and while it is a rare form of breast cancer with a significant propensity for triple-negative phenotype; Nevertheless, PABC is a diagnostically and therapeutically challenging disease bearing various risks for affected woman and fetus. Although immunotherapy with immune checkpoint inhibitors may be a promising therapeutic approach for patients with PABC, it can trigger various autoimmune side effects or immune-related adverse events (irAEs); there are several endocrine-related irAEs (172). The most common endocrinopathies reported from clinical trials include hypothyroidism and hypophysitis in patients treated with anti-PD-1/PD-L1 antibodies and anti-CTLA4, respectively (173–178). In addition, hypopituitarism, type 1 diabetes mellitus and primary adrenal insufficiency have also been reported (172). On the other hand, treatment with Dasatinib alone or combined with immune checkpoint inhibitors could be another therapeutic rationale given that PABC frequently overexpress the corresponding receptors Src and PD-L1. Nevertheless, despite the overall poor outcome, we believe that the complete gene and miRNA profiles of PABC can aid in identifying novel therapeutic targets and biomarkers to manage this rare, but fatal disease. In conclusion, the etiology of PABC remains largely unknown, thus, further cellular and animal models in addition to preclinical and clinical studies in the field are necessary.

AUTHOR CONTRIBUTIONS

AA conceptualized the study. SA, IS, SM, HFA, SV and AA wrote, reviewed, and edited the manuscript. AA, HFA and SV acquired the funding. All authors have read and agreed to the published version of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Cancer During Pregnancy: How to Handle the Bioethical Dilemmas?—A Scoping Review With Paradigmatic Cases-Based Analysis

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Ethical issues that arise during the care of a pregnant woman with cancer are challenging to physicians, policymakers, lawyers, and the bioethics community. The main purpose of this scoping review is to summarize existing literature regarding the bioethical dilemmas when a conflict arises in the maternal-fetus dyad, like the one related to cancer and pregnancy outcomes. Moreover, we illustrate the decision-making process of real-life case reports. Published data were searched through the PubMed and Google Scholar databases, as well as in grey literature, using appropriate controlled keywords in English and Portuguese. After identification, screening, eligibility and data extraction from the articles, a total of 50 was selected. There are several established ethical frameworks for conflict resolution and decision-making. Pragmatic theoretical approaches include case-based analysis, the ethics of care, feminist theory, and traditional ethical principlism that scrutinizes the framework of autonomy, justice, beneficence, and non-maleficence. In addition, society and practitioner values could mediate this complex ethical interplay. The physician must balance autonomy and beneficence-based obligations to the pregnant woman with cancer, along with beneficence-based obligations to the fetus. Ethical challenges have received less attention in the literature, particularly before the third trimester of pregnancy. Best, unbiased and balanced information must be granted both to the patient and to the family, regarding the benefits and harms for the woman herself as well as for the fetal outcome. Based on a previously validated method for analyzing and working up clinical ethical problems, we suggest an adaptation of an algorithm for biomedical decision-making in cancer during pregnancy, including recommendations that can facilitate counseling and help reduce the suffering of the patient and her family.

Keywords: ethics, ethical, carcinoma, cancer, neoplasm, pregnancy, pregnant, gestation

BACKGROUND

Cancer is the second and first most common cause of death in women aged 25–34 and 35–65 years, respectively (1). However, cancer that occurs during pregnancy is a relatively rare event, with an estimate between 0.03 and 0.1% of all pregnancies. The incidence is expected to upsurge with later childbearing age and unplanned pregnancies. In Europe, 3,000–5,000 patients are diagnosed yearly with cancer during pregnancy, whereas 3,500 cases are reported in the USA (2–5). The most common neoplasms that occur during pregnancy are breast cancer, thyroid, cervical, ovarian and melanoma (2–5), but the currently available data is mainly limited to those areas of Western/Central Europe and North America (6, 7). Some recent data indicate that other cancers may be more prevalent in pregnant women in particular regions, as more cases of melanoma in Scandinavia (8) and gastrointestinal cancer in Asia (9).

The problematics of how to handle cancer during pregnancy has been a long-term matter of debate in the medical community. The many ethical issues that arise in the care of pregnant women involve many stakeholders—such as family, physicians, legislators, jurisdiction and the bioethics community - and its boundaries are imperfect since many contexts intersect. In the care of pregnant cancer women, it is important to consider the status of two biologically-related patients, but individually viable. However, cancer during pregnancy represents a dilemma given that treatment should be directed to keep two lives: maternal and fetal. Despite this complex ethical interplay, it should be emphasized, with the exception of special circumstances, that the patient has the final word in the decision-making process and that the remaining stakeholders contribute with a variable role and weight depending on each specific case and scenario.

This article focuses on the discussion to the clinical/pharmacological background and ethical issues that emerge from the medical management, especially before the third trimester, of a pregnant woman with cancer, which occurs whenever the therapy toxicity creates a conflict of interest that unbalances cancer and pregnancy outcomes. Furthermore, in order to permit a better framing of this problematic, we describe real-life paradigmatic cases that allow us to highlight the idiosyncrasies related to the ethical approach to cancer during pregnancy. Finally, we suggest an adaptation of an algorithm for biomedical decision-making in cancer during pregnancy, including some recommendations that can facilitate counseling and help reduce the suffering of the patient and her family.

Abbreviations: AFP, Alpha-fetoprotein; AMH, Anti-Müllerian hormone; ATRA, All-trans-retinoic acid; Bcr-Abl, Breakpoint cluster region protein-Abelson murine leukaemia viral oncogene homolog 1; CA, Carbohydrate antigen; G-CSF, Granulocyte colony-stimulating factor; GM-CSF, Granulocyte colony-stimulation factor; HE-4, Human epididymis protein-4; I-131, Radioactive iodine; LDH, Lactate dehydrogenase; mGy, mili Gray units; PD-1, Programmed cell death protein-1; PD-L1, Programmed cell death protein-ligand 1; SCC, Squamous cell carcinoma; US, Ultrasonography; USA, United States of America.

METHODS

Assessing reasons or arguments presented in the normative bioethics' literature can be a tricky task, as identifying any relevant data on a given topic in bioethics can be time-consuming and not always possible due to the high burden of grey literature, including books, edited book volumes and even predatory magazines, some of them with dubious content.

Therefore, some authors argue that, even if the systematic search should be maintained in bioethics research, the type of methodology will depend on the research question. In some cases, identifying all the literature on a given question may not be feasible and, even if it is, the time spent will not add significant value to the research (10, 11).

That is why some advocate a turn to critical interpretative reviews which might better serve bioethics research purposes (11). Based on that, we chose the scoping review as the best methodology for our research objectives, which were to rapidly map the existing literature (including the one not indexed in major databases, such as the grey literature), chart data from the studies, and clarify concepts. We were not interested in asking a single or precise question, but more focused on the identification of certain concepts in papers or studies, and in the mapping, reporting or discussion of the data collected. Our aim with this review is to summarize and clarify the existing published literature on the ethical dilemmas interwoven into the dimension of pregnant women with cancer, particularly before the third trimester. Moreover, we put in perspective the potential ethical problems and frameworks regarding the more appropriate approach for specific and representative case reports.

We developed an *a priori* protocol to define our research objective, and methods, which informed our selection for data extraction.

On October 8-9th, published literature was searched between 2010 and 2020 through the PubMed, using appropriate controlled keywords: ["ethics" (MeSH) OR "ethical" (MeSH)] AND ["carcinoma" (MeSH) OR "cancer" (MeSH) OR "neoplasm" (MeSH)] AND ["pregnancy" (MeSH) OR "pregnant" (MeSH) OR "gestation" (MeSH)]. References from the selected articles were scanned in order to identify other papers.

By the author's decision, other relevant articles beyond this scope, including grey literature, have been included. For that purpose, we used Google Scholar search engine with the controlled words in English and Portuguese ("ethics" AND "cancer" AND "pregnancy") OR ("ética" AND "cancro" AND "gravidez"), respectively.

Using Covidence (Covidence.org), we inputted our inclusion/exclusion criteria and selected the articles independently by two reviewers (DAC, JGN).

The inclusion criteria defined included: 1) patients who are pregnant; 2) patients who have active cancer; 3) articles addressing the problem of the triad: ethics, cancer and pregnancy; 4) articles including an ethical perspective during pregnancy; 5) articles and expert meeting reporting clinical practice guidelines or recommendations for cancer management during pregnancy; 6) study based on the toxicity of antineoplastic treatment during

pregnancy that includes one of the following: chemo-, hormone-, targeted-, immuno-, and radio-therapy; 7) study based on the toxicity of supportive medication during pregnancy; 8) article language in English or Portuguese; 9) articles must be available with full-text.

The exclusion criteria were: 1) premenopausal women and fertility issues; 2) cancer risk exclusively after pregnancy; 3) cancer risk and hormone replacement therapy; 4) active cancer and breastfeeding; 5) articles discussing cancer therapeutics exclusively; 6) articles not mentioning pregnancy, cancer, and ethics at all.

Data extraction was conducted in Microsoft Excel version 16.41 (20091302) using a data charting form developed for our protocol.

RESULTS

Results were mostly restricted to review articles, ethical perspectives, clinical practice guidelines and case-based teaching guides (only available English and Portuguese text). Afterwards, the screening and selection of articles, quality assessment, and data extraction were performed independently by two reviewers (DAC, JGN), according to the pre-planned inclusion and exclusion criteria. Conflicts were resolved by a third party (IG) when a consensus was not reached.

For the period between 2010 and 2020, the initial search in English yielded 633 publications after the possible combinations of keywords in PubMed. Excluding the 292 duplicates, the number was reduced to 341. Title screen reduced the selection to 75 papers for reviewing abstracts and 61 fulfilled the criteria for reading the full-text. In the final selection, 12 articles were chosen to be included in this review (**Figure 1**).

The initial search in Google Scholar with the English keywords ("ethics" AND "cancer" AND "pregnancy") resulted in 330.000 publications. By limiting the search between 2010 and 2020, 81.200 articles were retrieved. Furthermore, when searching for the Portuguese keywords ("ética" AND "cancro" AND "gravidez"), 3,150 papers were found. By limiting the search between 2010 and 2020, 2.680 publications were retrieved.

Twenty-nine additional articles in English and Portuguese, also indexed in PubMed, were included in this review and served as a reference to some of the previously searched articles. This second subgroup of articles included pioneering and relevant articles published in reference journals, as well as clinical trials and international guidelines/consensus.

Finally, a third subgroup of 10 publications was considered, which, despite not being indexed in databases, added value to the literature review. This group of articles was heterogeneous, with texts corresponding to international guidelines or to a health

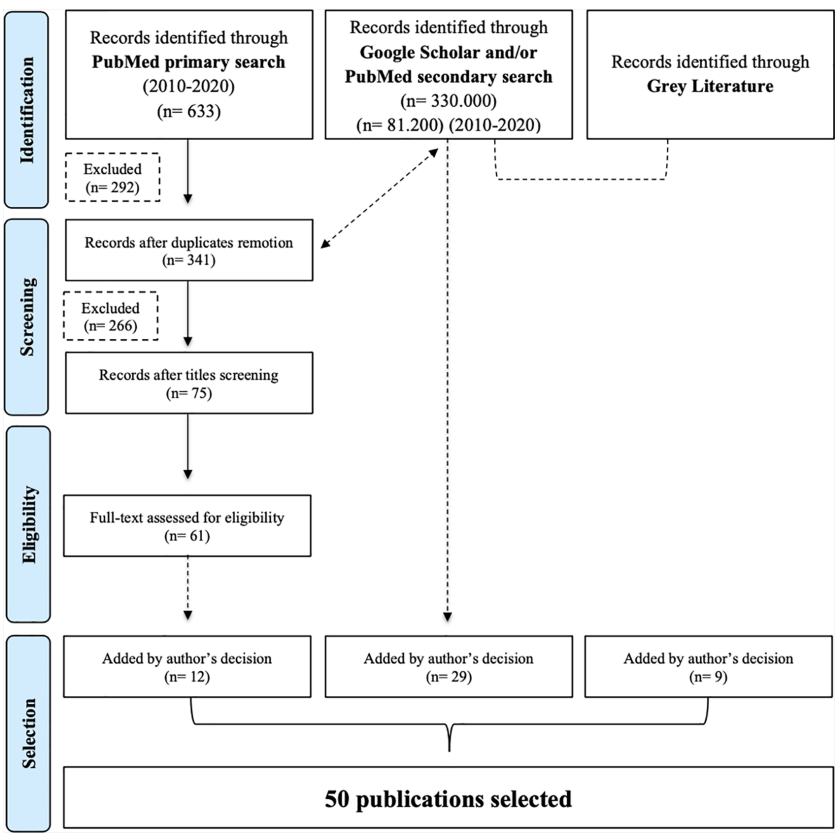

FIGURE 1 | Flowchart explaining the article selection strategy (adapted from PRISMA, 2009).

protection agency, book chapters or magazine sections (English and Portuguese), and case-based teaching guides.

DISCUSSION

About Cancer During Pregnancy

The knowledge about the effect of cancer during pregnancy and the effect of cancer progression in pregnancy is of crucial importance for the success of the mother's treatment and favorable outcome for the fetus. There are contradictory reports that have been published regarding the outcomes of these patients.

In 1880, Samuel Gross stated that breast cancer during pregnancy would behave like a rapidly growing disease, including with an "excessively malignant" clinical course (12). In 1943, after treating 20 patients with breast cancer, a group at Columbia University Presbyterian Hospital concluded that pregnancy "made the disease inoperable" (13). Ten years later, it was consensual that abortion was linked to improved patient survival (12). A population-based cohort study of 15,721 women diagnosed with breast cancer, of whom 1,110 (7%) had cancer during or within 2 years after pregnancy, revealed that this subset of patients had a worse prognosis, validating previous data (14). Conversely, a multicentric registry containing 447 women with breast cancer during pregnancy and 865 non-pregnant counterparts, showed similar overall-survival in both groups, after adjusting for known prognostic factors (15). Nonetheless, a careful interpretation of these studies should be taken, given the heterogeneity of the patient populations and treatments prescribed (16).

So far, there is no consolidated expert opinion on whether pregnancy can induce the occurrence or relapse of cancer and if it correlates only with maternal or also with other external or endogenous risk factors.

Complementary Diagnostic Exams and Trimester Considerations

During the last decades, imaging of the pregnant patient has been performed with radiography, computed tomography, magnetic resonance imaging, scintigraphy, positron emission tomography scan, and ultrasonography (US). US imaging has emerged as the primary imaging modality because it provides real-time images without the use of ionising radiation (16).

A clear link between the severity of fetus impairment, gestational stage, and cumulative radiation dose received has already been established (16). For instance, during the organogenesis, there is a higher likelihood of major induction malformations and the threshold dose is above 100 mGy. There are also other issues besides ionising radiation. The radioactive iodine (I-131) crosses the placenta and has the ability to affect fetal thyroid and gadolinium teratogenic in animal studies. More invasive imaging tests should only be performed if the diagnosis and/or staging is expected to contribute decisively to the prognosis of the mother or fetus and that the risks and benefits are perfectly clarified and understood by the mother (16, 17).

Although the fetus is unscathed by laboratory tests, our main concern will be the influence that pregnancy will have on diagnosis, staging and follow-up, due to the fact that the serum biomarkers lack sensitivity and specificity during this period. There are tumor biomarkers that may be increased, such as CA 15-3, SCC, CA 125, and AFP, and others that are not so much, such as the example of CEA, CA 19-9, LDH, AMH, and HE-4. Inhibin B and LDH increased in the last trimester may be a laboratory sign of hypertensive abnormalities linked to pregnancy (4).

Treatment Options and Trimester Considerations

The main challenge while managing cancer in pregnancy is balancing therapeutic regimen and fetus welfare. In addition, as an estimated 50% of pregnancies are unplanned, many women are exposed to teratogens before realising they are pregnant (13). This condition demands attention and careful protocols.

Approximately 0.5% of all births occur before the third trimester of pregnancy and the majority of these very early deliveries result in neonatal deaths and more than 40% in infant deaths. The delivery before 23 weeks of gestation, usually leads to neonatal death (5%–6% survival), and among rare survivors remains significant morbidity (98%–100%). When delivery is anticipated near the limit of viability, the patient, families and healthcare teams are faced with complex and ethically challenging decisions (18, 19). For most cytotoxic and targeted therapies, there is a lack of data regarding the risk of teratogenesis, based on case reports and retrospective series. The potential mutagenic, teratogenic and carcinogenic effects of ionising radiation and cytotoxic agents in the embryo are well known and depend on the dose, nature of the compound, treatment field and gestational stage. Some authors advocate that, if pregnancy occurs while the patient is under endocrine treatment (e.g., tamoxifen) or chemotherapy, a pregnancy termination should be recommended if it is done in the first trimester.

Surgery

In general, surgery can be performed during any stage of pregnancy with robust evidence demonstrating the safety of surgical procedures and most anaesthetic agents seem to be safe for the fetus. However, the risk of miscarriage is slightly incremented (1%–2%), especially in the first trimester. In addition, there is a higher risk of low birth weight and premature delivery (1.5–2 times relative risk), an increased rate of complications and higher morbidity in major abdominal and pelvic procedures. Relatively to anaesthetic drugs, there is a record of good safety and none of them stands in the drug list of proven teratogens. Given the fact that there is a minimal risk to the fetus and potential benefits of the treatment, there should not be any delay on the surgery, if indicated (2–4, 16).

Radiotherapy

The embryo-fetal risk can also be influenced by radiotherapy co-treatment and doses higher than 50–100 mGy should be avoided. Below these doses, there is a low risk of stochastic biological

effects (mutations), and non-stochastic effects (malformations, developmental disorders) are as frequent as in general population (3%–5%) (2, 4, 20). In certain cases, it is necessary to use radiotherapy in the tumor, so the clinician must use it, in the period that it is least harmful to the fetus. From 2 to 12 weeks, the use of radiation has the risk of teratogenesis and growth retardation. Until 20 weeks, the fetus can present mental and growth retardation, microcephaly, eye, palate and genital deformities and beyond that, there is an increased risk of sterility, malignancies, and genetic defects (2–4, 16).

Chemotherapy

The most sensitive and critical period of drug exposure is organogenesis, which occurs roughly 2–8 weeks post-conception (17), especially during the gastrulation period when tissues are differentiating rapidly, and damage becomes vast and irreparable (21). Therefore, during the first trimester, the risk of spontaneous abortions, fetal death and major congenital malformations are increased, reaching 10%–20% and decline to about 6% when folate antagonists like methotrexate are excluded. The effects of antineoplastic agents during the second trimester are related to intrauterine growth restriction, low birth weight, miscarriage, and premature birth (20%–40%) (2–4). During the perinatal period, the effects are related to maternal/fetal myelosuppression, infections, and haemorrhage. Long-term outcomes of children exposed to chemotherapeutic agents in utero are not well examined. It is known that it is safe to give some drugs during the third trimester without causing long-term damage to the baby, for example, for Hodgkin´s disease or breast cancer (22).

Endocrine Treatment

In contrast to non-pregnant counterparts, pregnancy-associated breast cancer is more likely to develop higher stage tumors, more poorly differentiated, and less common oestrogen or progesterone-receptor positivity. These results were corroborated by previous studies. Nevertheless, there is still a significant fraction of hormone-receptors positive breast cancer (23).

However, many of the physiological changes during pregnancy are hormone-driven. Furthermore, the blockade of oestrogen (e.g., with tamoxifen), which is frequently used in hormone-positive breast cancer, might interfere with these physiological modifications and can be teratogenic and associated with fetal death and birth defects, mainly craniofacial anomalies (preauricular skin tags, microtia, hemifacial microsomia), ambiguous genitalia (clitoromegaly, labial fusion), and acetabular and sacral dysplasia. Tamoxifen is also associated with vaginal bleeding and miscarriage. Moreover, tamoxifen is not recommended during the lactation period, as it delays milk production, and there are limited safety data regarding its excretion in human milk. Importantly, the decision to postpone the tamoxifen to allow lactation should be based on individual risk and include a balanced discussion between risk and benefit (3, 4, 16, 20, 24). However, the tamoxifen effects on the fetus and the course of pregnancy are not yet fully understood (24).

Targeted Agents

Most of these targeted agents commonly used in breast cancer, such as trastuzumab, pertuzumab, bevacizumab, among others, should not be used because they present some undesirable adverse effects, but also due to the fact that there is missing much information yet. In general, human epidermal growth factor receptor 2 agents are safe during the first trimester, although during the second and third trimesters oligohydramnios, pre-term delivery and neonatal deaths may be present. Rituximab, an anti-CD20, imatinib, an anti-Bcr-Abl tyrosine kinase, and ATRA, a trans-retinoic acid, can be used with caution, even though they cross the placenta. Rituximab is safe in the first trimester, but in the coming trimesters, it causes cytopenia and B cell depletion, reversible at birth, while imatinib is safe in the second and third trimesters, with the risk of causing major malformations in the first trimester. ATRA is mainly dangerous in the first trimester due to the risk of abortion. The only targeted agent safe throughout pregnancy is interferon-α (3, 4, 16, 20, 25, 26).

Immunotherapy

A plethora of immunotherapy options is being used in the investigation and active treatment of several malignancies. As it is a more recent treatment, there is not much information regarding the security of these drugs during human pregnancy. However, as we all know, mother and fetus are not genetically identical. Therefore, an immunological tolerance from the mother towards the fetus is necessary in order for the pregnancy to develop successfully (26).

Immune checkpoints, such as programmed cell death protein-1 (PD-1), PD-1 ligand (PD-L1), and cytotoxic T-lymphocyte–associated protein 4, play a crucial role in the process aforenamed. Consequently, the fetus can be harmed by an aggressive immune response after the inhibition of these immune checkpoints. Furthermore, the drugs that can inhibit the checkpoints are immunoglobulins G4 antibodies that have the ability to cross the placenta and cause toxicity directly to the fetus. In animal models, these drugs demonstrated that their use could increase abortion rates, stillbirths, premature delivery and higher incidence of infant mortality, namely in the third trimester. However, there was not an increase in fetus malformations. In summary, since these drugs are so recent and have so little information regarding their security among pregnant women, immune checkpoint inhibitors are not recommended (26).

Supportive Medication

Our concern about pregnancy in women with cancer should not only focus on antineoplastic agents, but even on non-antineoplastic agents used in clinical cancer practice, such as bisphosphonates, granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), antiemetics, analgesics, and anti-inflammatories (2–4, 16, 20, 26).

Because bisphosphonates inhibit bone resorption, they are used in the treatment of hypercalcemia, osteoporosis, metastatic bone disease, and Paget disease. The bisphosphonates inhibit

osteoclastic bone resorption *via* a mechanism that differs from that of other antiresorptive agents. In addition to their inhibitory effect on osteoclasts, bisphosphonates appear to have a beneficial effect on osteoblasts. These biological effects can lead to a reduction in serum calcium in the maternal blood and its availability to the fetus, which might induce skeletal malformations, reduced bone growth and low birth weight. It can, inclusively, affect parturition adversely by reducing uterine contractions. Therefore, it is contraindicated during pregnancy (3, 20).

G-CSFs/GM-CSFs are recommended in cases of severe neutropoenia or as primary/secondary prophylaxis during treatment with some chemotherapy regimens. Its safety during the pregnancy period is still unknown. In animal studies, it seems to cross the placenta and increase the rate of spontaneous abortion and low birth weight (2, 3). However, G-CSFs have already been used during pregnancy, without complications. Thus, these agents may also be considered during pregnancy if a high risk of neutropoenia is forwared (2, 3, 16, 26).

Antiemetics (metoclopramide, cyclizine, meclozine, alizapride, ondansetron, and aprepitant) can be safely used during the first trimester of pregnancy. The safety of corticosteroids is variable, with the use of hydrocortisone and prednisolone being preferred to dexamethasone or betamethasone, as they are extensively metabolized in the placenta and relatively little detected in the fetal compartment. Repeated administrations of betamethasone are associated with attention problems and cerebral palsy. Analgesics (paracetamol, opioids, anti-inflammatory agents), with the exception of the first trimester, did not generate side effects, but there is some risk of respiratory depression and fetal ductus arteriosus closure (3, 4, 16).

The Ethical Issue: Balancing Interests

Pregnancy appears as an exceptional circumstance in medical ethics as the primary medical principle *Primum non nocere* can be questioned, as the access to the fetus occurs exclusively through intervention on the pregnant mother and treating the mother may imply harming the fetus. This is a unique situation since the welfare of both mother and fetus must be considered on any treatment planning.

When a conflict arises in the maternal-fetus dyad, caregivers must understand the pregnant woman´s mindset, broad social network, values, cultural, and religious beliefs, as this may impact their decisions (27). Consequently, it is imperative to promote the autonomy and physical integrity of the pregnant woman, ensuring that all available information on pregnancy and cancer outcomes is provided in order to allow for a fully informed consent consistent with her values (28) since the woman's decision is absolute and unlimited. Therefore, in cases when the woman's decision may harm her fetus (e.g., treatment of cancer during the first trimester) coercion to force treatment is never justified.

To date, there is no data systematically collected reporting the decision-making process of women who had cancer and pregnancy at the same time. Although, there are two studies conducted in the United Kingdom reporting patient experiences with participation in ORACLE (29) (evaluate the possibility that treatment with antibiotics prolongs labor and improves neonatal outcomes in women who are less than 37 weeks pregnant and experiencing either pre-term labor or premature rupture of membranes) and in the Magpie Trial (30) (prophylactic use of anticonvulsants for women with severe preeclampsia). In these studies, the major motivating factors were identified as self-benefit (it can help treat mother condition), benefit for your child (it can minimize the associated risks for the fetus) and altruism (participation can help future women or is it for the sake of medical science). It was also shown that, although some women seek the opinion of family and friends, they have little involvement or influence in women's decisions. The partners played a role in providing a second opinion for many women, but study participants rejected the idea that their relatives or friends were in a position to influence their decision. In parallel, it may be reasonable to consider, in the future, the option of offering pregnant women with cancer the possibility of participating in clinical trials and/or enrolling in registries, increasing the motivation and the expectation of benefits for them and the fetus.

In the child-to-be perspective, there are extra layers of ethical complexity to address, because the antineoplastic treatment typically affects not only the pregnant woman but also the fetus. The developing fetus clearly has no capacity for autonomous choice, and there is no formula for balancing the interests and moral claims of the fetus with those of the mother. Furthermore, the welfare of the fetus is typically not independent of the interests of its mother (31).

When a conflict arises in the maternal-fetus dyad, such as cancer treatment and the risk of fetal demise, a range of ethical frameworks may be useful in the decision-making process. It is clear that the physician has beneficence-based and autonomy-based obligation to the pregnant cancer patient (32). Because of an immature central nervous system, the fetus cannot meaningfully be said to possess values and beliefs, although this is tremendously arguable.

Hence, scientifically there cannot be autonomy-based obligations to any fetus, although women's beliefs may hasten her to judge differently (32). However, the physician can have beneficence-based obligations to the fetus, if the fetus is considered as a patient (33). The pregnant woman is free to withhold patient status, confer patient status, or, after conferring it, withdraw it from her pre-viable fetus (32). The fetus has no claim to patient status independently of the pregnant woman's autonomy. When the woman is uncertain about or is not able to confer the status, the fetus can be provisionally regarded as a patient (32, 33). However, these approaches have been criticized for their tendency to emphasize the divergent rather than shared interests of the pregnant woman and the fetus. In fact, in most cases, the interests of the pregnant woman and fetus actually converge (28).

Whenever a pregnant woman is presented with a cancer diagnosis, several ethical concerns addressing technicalities must be approached, while keeping in mind the surrounding emotional issues.

There is no established *modus operandi* for the physician, which raises pertinent questions: i) should the patient be included in the decision-making to the best of her abilities in a limited way, or should paternalistic decision-making take over? ii) Should a proxy decision-maker decide based on the perceived patient's best interest? (20) These are queries that can be carefully grasped by solving real-life cases, in parallel those that will be presented later.

To allow for an informed decision, the patient must be well aware of multiple medical facts, such as cancer prognosis, the possibilities of antineoplastic therapy, its main toxicities and its aim, namely: whether curative or palliative, if it will improve quality of life, or overall and progression-free-survival, if there is a risk of pre-term delivery or if peripartum complications are expected.

The timing of treatment must also be considered—is the mother symptomatic and needs to initiate treatment quickly or is it possible to delay it until the third trimester, when there is no significant risk for fetal defects in a short and long-term? Besides technical issues, before starting the treatment, the physician must consider emotional issues, such as the possibility of the child-to-be meeting its mother.

There are several established ethical frameworks for conflict resolution and decision-making. Pragmatic theoretical approaches include case-based analysis, the ethics of care, feminist theory, and traditional ethical principlism that scrutinizes the framework of autonomy, justice, beneficence, and non-maleficence. In addition, society and practitioner values could benefit this complex process.

Illustrative Cases of Bioethical Dilemmas and a Proposed Algorithm for Ethical Decision-Making

For instance, the 1987 paradigmatic case of a pregnant woman in her late 20s, who had a late lung relapse 15 years after Ewing´s sarcoma diagnosis brought these issues to ahead. Although fully committed to saving her life, at the end of the second trimester, it became clear that the patient was dying. The Medical Centre tried to insist upon an early Cesarean section delivery in order to save her fetus. She refused the intervention with the support of her family, knowing it would almost certainly kill her, but the hospital forced the delivery through a court order. Both the patient and her extremely premature baby survived for only a short while after the surgery. In 1990, the Court of Appeals posthumously vacated the court-ordered Cesarean section, holding that the patient is totally autonomous to make healthcare decisions for herself and her fetus and that only in the most exceptional circumstances should a pregnant woman's right to refuse interventions be called into question (34). Despite the media exposure of this case, others with similar ethical issues were far from being elucidated (35–37).

This first case is an example of what should not be done in ethical terms when approaching such a complex and sensitive context. It was noticeable that there was no multidisciplinary management and strategy for a balanced approach to outline cancer and pregnancy facts of the case and the non-medical issues to achieve the best ethical possible scenario (**Figure 2**).

It is possible to infer that the woman would be in mental conditions to take a position. Hence, all the best possible information, at that moment, namely the cancer and pregnancy outcomes for the woman, evidence-based treatment options and available supportive measures for the situation, should have been presented to the patient. Furthermore, the comparison with previous similar ethical scenarios and the consultation of guidelines should have been assessed and discussed, in a multidisciplinary gathering, comprising the whole medical specialities related to this subject (e.g., Medical Oncology, Obstetrics, among others), a representative of the ethics committee, all the stakeholders (such as the patient's family and the fetus' father) and the patient itself. Finally, the patient's final decision, in collusion with the autonomy ethic's principle, should have been respected.

In 2016, on February 20, a 17-week-pregnant woman in her late 30s, collapsed after an intracerebral haemorrhage, probably related to a late recurrence of kidney cancer diagnosed 10 years earlier (40). Soon after physicians declared her brain dead (41). The hospital ethics committee and the family were enquired. It was explained to both parties - mother's family and the fetus' father—that, to allow for the fetus survival, the woman should be kept on life-sustaining treatment to reach at least its 32 weeks, the earliest date doctors felt that a successful Cesarean delivery would be possible (40). This emphasizes the role of the "mother's body as a cadaveric incubator", "mother as the organ donor and fetus as the recipient", and the concern for "possible damages to the fetus" (42–44). Some professionals believe that it is not ethically acceptable to maintain the mother's body after brain dead to use it as a "fetal container." Such a decision should not be assumed, but it must be debated. If the mother is to be considered a "cadaveric incubator" with no autonomous rights, the rights of the fetus should legally prevail. Another argument claims that the continued somatic support itself is actually organ donation with the fetus as the recipient (42–44). The family strongly expressed that the mother would have wanted her life preserved in order to give the fetus a chance for survival. The ethics committee equated the fetus life to a child at risk and allowed the support to the brain-dead mother. The decision was taken in a meeting of the neurosurgical, critical care, obstetrics, neonatal, transplant and ethical staff, along with the patient's family. One hundred seven days later, the baby was born healthy, and the life-sustaining machines were turned off (40, 41).

About 10 years earlier, a woman of the same age and gestational stage as in the previous case suffered a stroke secondary to the brain progression of melanoma. Soon after, the doctors declared brain dead. Her family also decided to keep her alive to give the fetus a chance. It became a race between the fetus' development and cancer that was ravaging the patient's body. The baby was born about 2 months prematurely, and tragically did not survive. At that time, the case was also considered to be notable because there was no controversy (43).

Put into perspective, the two aforementioned cases have analogous ethical issues, however, with different endings. Nevertheless, both these situations follow the best ethical possible scenario approach (**Figure 2**). Since both patients were declared

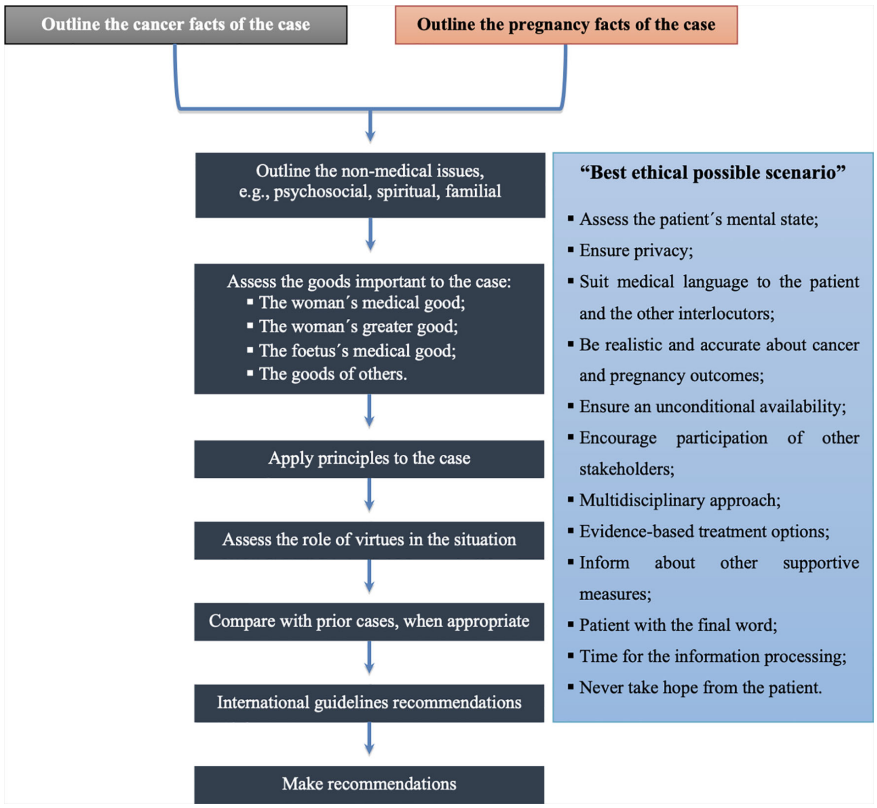

FIGURE 2 | Algorithm for biomedical ethical decision-making (adapted from Schenck (38) and Botha et al. (39)).

brain dead, none of them would be mentally suitable to take a position. Thus, the patient's family and the fetus' father should be responsible for the decisions regarding equally the patient and the fetus welfare. As observed, in these circumstances, all of the stakeholders had received the best information possible *vis-à-vis* the cancer and pregnancy outcomes, the evidence-based treatment options to follow pregnancy and supportive measures for maintenance of "cadaveric incubator", with the aim for the survival of the fetus. All the decisions were engaged with a multidisciplinary team, taking into consideration previous comparable clinical cases, and respecting the patient's family and fetus' father autonomy. Since the number of cases describing the management of extended maternal somatic support after brain death is limited, every case should be continuously reassessed and adapted along with the increasing experience and knowledge (42–44).

In these difficult cases, mainly before the third trimester, the sovereign decision should be taken after thorough discussion between mother (or legal substitute) and the treating physician (42–46). While respecting the principle of autonomy, another final ethical issue is the right of the physicians to conscientiously object to certain treatment options.

As stated before, physicians should not bias with their recommendations and should present to consider three scenarios: i) treatment during pregnancy, with close monitoring for side effects and reconsideration of termination before viability; ii) treatment with termination of the pregnancy; and iii) treatment delay until fetal lung maturity, when it's reasonably safe to deliver the baby (34, 47).

All over Europe, there are differences between countries regarding availability, conditions and gestational limit. In Portugal, since 2007, and after a National Referendum, the voluntary termination of pregnancy until 10 weeks of gestation was legalized (law n°16/2007). In that same legal document, it is stated that in case of danger of death or physical and/or psychic injury, the possibility of interrupting the pregnancy until 12 weeks of gestation is allowed (48).

The available international guidelines recommend that maternal fetal medicine consultation should include counseling on maintaining or terminating a pregnancy, including a review of the treatment options. These guidelines support a framework of shared decision-making in the context of maternal-fetal conflict to provide guidance for compassionate conflict resolution. An ethics consultation may be helpful to mediate conflict resolution. Intervention by the courts is rarely appropriate or indicated and should be avoided (27, 47, 49).

Based on a validated method for analysing and working up clinical ethical problems, we suggest an adaptation of an algorithm for biomedical decision-making in pregnancy-associated cancer (**Figure 2**).

The first task in this ethical decision-making process is to establish the medical and pregnancy facts of the case. The second

step is to determine pertinent non-medical issues, which is more challenging. These steps are followed by an assessment of the goods relevant in the case. The immediate concern is clearly what is suitable for the woman medically, but that is followed closely by an attempt to understand the patient's overall good—e.g., psychological good, good in terms of family and relations, spiritual good, and good in terms of the patient's preceding life history and values. While ensuring the good of the woman is the primary aim, this is insufficient in itself, as the goods of fetus and others must also be considered (38).

The principles that apply in the case at hand are then evaluated, specifying what a given principle means in this case and balancing it against the moral claims of each of the others. In themselves, principles can become mere abstractions, perhaps even sterile nostrums for dealing with these complex ethical dilemmas. Therefore, virtue ethics, another bioethical approach that has received increased attention in recent years, addresses the nature of the relationship between patient and healer, with particular attention to the character of the physician (38). Pellegrino and Thomasma have presented a detailed analysis of how they interpret the virtues that are essential to medical practice. These virtues include phronesis, compassion, fidelity, trust, integrity, self-effacement, justice, fortitude, and temperance (50).

In any case, a consideration of the virtues and principles on the one hand, and guidelines recommendations and prior similar cases analysis on the other, provide more guidance for a right answer to bioethical dilemmas.

Finally, the guidelines recommendations should be accompanied by several steps that must take place in the "best ethical possible scenario", in order to enhance the quality of the counseling and emotional support that are an essential part of management (**Figure 2**):

- Assess the mental state of the patient;
- Ensure privacy;
- Suit medical language to the patient and the other interlocutors;
- Be realistic and accurate about cancer and pregnancy outcomes;
- Ensure an unconditional availability for the discussion and re-discussion of each dough or clarification.
- Encourage participation in the decision-making process of the partner and the closest family members;
- Inform that medical management is not the responsibility of a single professional, but of a multidisciplinary team with a holistic approach;
- Provide evidence-based treatment options;
- Inform about other supportive measures;
- Inform that the patient has the final word in the decision-making process;
- Provide the necessary time for the information processing phases according to Kübler-Ross model (denial, anger, bargaining, depression, acceptance);
- Never take hope from the patient.

CONCLUSIONS

Scientific and clinical data addressing the risks and the efficacy of treating a pregnant woman with cancer have already been explored in the literature. However, the imbricated ethical challenges have received less attention, particularly before the third trimester of pregnancy.

A pregnant woman with cancer faces the choice between best antineoplastic treatment versus maximal fetal welfare. Best, unbiased and balanced information about the benefits and harms for the woman herself as well as for fetal outcome must be granted both to the patient and to the family.

Halting, in this scoping review, the authors identified certain concepts and discussed the heterogeneous data collected regarding bioethical decisions on cancer during pregnancy. Nevertheless, there are still some unsolved queries, that must be discussed in multidisciplinary groups, and personalized to each unique scenario. Each new decision should be included in an updated shared and anonymous database to put in perspective what should be done in a particular cancer situation that affects two unique lives (maternal and fetal), allowing to gather attitudes and experiences that fill a knowledge gap needed to develop ethical care guidelines.

AUTHOR CONTRIBUTIONS

All authors have read the submission and agreed to submit this manuscript. The present manuscript is the result of original work by the co-authors. Conception and design: DAC, JN. Development of methodology: DAC, JN. Acquisition, analysis, and interpretation of data: DAC, JN, IG. Writing, review, and/or revision of the manuscript: DAC, JN, SD, MF, IG. Manuscript supervision: SB, DP.

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Breast Cancer Mortality in Young Women in Brazil

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Objective: Malignant breast cancer is the leading cause of death by cancer in young women. The study aimed to determine if breast cancer mortality among young women has increased between the period from 1996 to 2017 in Brazil.

Methods: A time-series analysis of breast cancer mortality rate in young women (20–39 years old) was carried out. Mortality data, from 1996 to 2017, were collected from the Mortality Information System of the Health Ministry, and demographic data, from the Brazilian Institute of Geography and Statistics. Trends in mortality were performed by Joinpoint Regression, the spatial distribution of the mortality rate was done with the QGIZ Software version 2.18, and Spearman's correlation coefficient was used to correlate the mortality rates with the Human Development Index.

Results: There was an increase in breast cancer mortality rates in young women in the majority of Brazilian states, with an upward trend in all regions. The correlation with the Municipal Human Development Index, income, and education had a significant impact on the mortality rate for women from 30–39 years old in both time frames evaluated and for women from 20–29 years old, only from 1996 to 2000.

Conclusion: The data obtained in the study, showed that even though the breast cancer mortality rate of young women is lower than women over 40 years old, it has been increasing in all regions of Brazil, mostly for women from 30–39 years old, suggesting that this group should be included in screening programs.

Keywords: young women, breast neoplasm, trend analysis, epidemiology, mortality

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INTRODUCTION

Malignant breast cancer is the most common type of cancer in women, except for non-melanoma skin cancer. In Brazil, it is estimated in 2020, 66,280 new cases of women with breast cancer, representing 29.7% of all types of cancer with an incidence of 43.7 per 100 thousand women. As for mortality, in 2017, there were 16,724 deaths of women with breast cancer, corresponding to a death risk of 16.1 per 100 thousand (1).

In young women (20–39 years old), breast cancer is also the most prevalent type of cancer, as well as the leading cause of death by cancer in most countries, and it is considered a problem that is

still little discussed and studied, because when compared to the incidence and mortality in the age group over 40 years, the numbers are significantly lower (2).

The incidence of breast cancer in young women has shown a significant increase. In the Brazilian capitals of Porto Alegre (1993 and 2005) and Goiânia (1998 to 2008), there was a considerable increase of it in the 20 to 39 age group (3). Likewise, in the United States, it was observed a growth from 24.6 to 31.7 per 100 thousand women in the same age group, in 1975 and 2015, respectively (4).

In young women, breast cancer occurs heterogeneously, with worse prognosis and high mortality, and in more advanced stages, presenting with larger and more aggressive tumors (5). A study carried out in the United Kingdom showed poor survival in women with breast cancer under the age of 40, even with the modernization of the treatments performed, in addition to the risk of tumor recurrence (6). Compared to older women, the prognosis is worse and the chance of death is greater when diagnosed in stages I and II (7).

In the United States, the estimated breast cancer deaths in women under 40 years old, were 3% of the total deaths from this condition (8). Given this scenario, it is crucial to better understand breast cancer mortality in this age group to submit more effective measures for screening, early identification, and case management.

It was observed that the mortality rate of young women (under 40 years old) has been escalating in Brazil; hence, this study aimed to determine if breast cancer mortality among young women has increased between the period from 1996 to 2017 in Brazil and correlate it with socioeconomic variables.

METHODOLOGY

A time-series evaluation of breast cancer mortality rate in young women in Brazil was conducted with data from 1996 to 2017. The number of deaths related to breast neoplasm (C50) was used according to the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). The mortality data from the Mortality Information System of the Ministry of Health and demographic data of the IBGE (Brazilian Institute of Geography and Statistics) were obtained at the Department of Informatics of the Ministry of Health (https://datasus.saude.gov.br/).

For the analysis, the data were obtained by geographic regions: North, Northwest, Midwest, Southeast, South, and 26 states. For the mortality rate, a specific mortality rate was calculated for each year and location, using the formula: Number of deaths from breast cancer in the geographic region, divided by the reference population, multiplied by 100,000.

The SDR from breast neoplasms were analyzed as dependent variables (Y-axis) and the years 1996 to 2017 as independent variables (X-axis). Analysis were carried out using the Joinpoint Regression Program, Version 4.7.0.0. (February 2019). There was an increase in the mortality rate when the tendency was of growth and the minimum value of the 95% CI > 0. However, the reduction occurred when there was a decline in the tendency and the maximum value of the 95% CI < 0. Stability was defined when, regardless of the trend, 95% CI included the value of 0. The average annual percentage changes for particular mortality rates were described with the 95% confidence interval. Statistical hypotheses were verified at the significance level $\alpha = 0.05$.

A correlation analysis was used to verify the existence of a relationship between breast cancer mortality rates. For this analysis, the 5-year periods from 1996 to 2000 and 2013 to 2017 and the human development index (HDI) of 2000 and 2010, respectively, were chosen (9). The year 1996 was chosen because it was the year of the beginning of the registration of cases of breast cancer deaths in the Mortality Information System and 2017 because it was the last year of registration updated at the time of data collection.

The HDI follows the three dimensions assessed worldwide: income, longevity and education. In this work we use the HDI, HDI income and HDI education. It is through the HDI that countries are classified as developed, developing or underdeveloped.

Spearman's correlation coefficient was used for the analysis, which were interpreted according to the following parameters: if correlation coefficient is <0.4, the correlation is considered to be of low magnitude, if correlation coefficient is ≥0.4 to <0.5 the correlation is considered to be moderate magnitude and, finally, correlation coefficient of ≥0.5 represents a strong correlation. The significance level of 5% was considered. For the analysis, the software Statistical Package for the Social Sciences (SPSS), version 20.0 was used.

The cartographic base of Brazil that contains the borders of the States is publicly available online in shapefile (SHP) on the website of the Brazilian Institute of Geography and Statistics (IBGE). Color maps were created to demonstrate the distribution of overall breast cancer mortality rates in the age groups 20–29 and 30–39 years in the Brazilian states. All figures were designed using QGIS version 2.8. The spatial distribution of breast cancer rates was presented in the years 1996 and 2017, displayed in pink scales, in which the darkest shades illustrate the highest rates and the lightest shades the lowest rates.

The data collected are available in the public domain, with no need for authorization from the Committee of Ethics and Research with Human Beings.

RESULTS

From 1996 to 2017, there were 19,105 deaths of young women with breast cancer in Brazil. According to the spatial distribution of the mortality rates, in all Brazilian states, there was a raise for women under 40 years old (**Figure 1**). This increase was observed in the states of Pará, Mato Grosso, Mato Grosso do Sul and Rio de Janeiro, for women from 20–29 years old in 1996 (**Figure 1A**) to 2017 (**Figure 1B**). For the age group of 30–39 years, there was an increase in most states, and rates remained constant only in 10 states (**Figures 1C, D**).

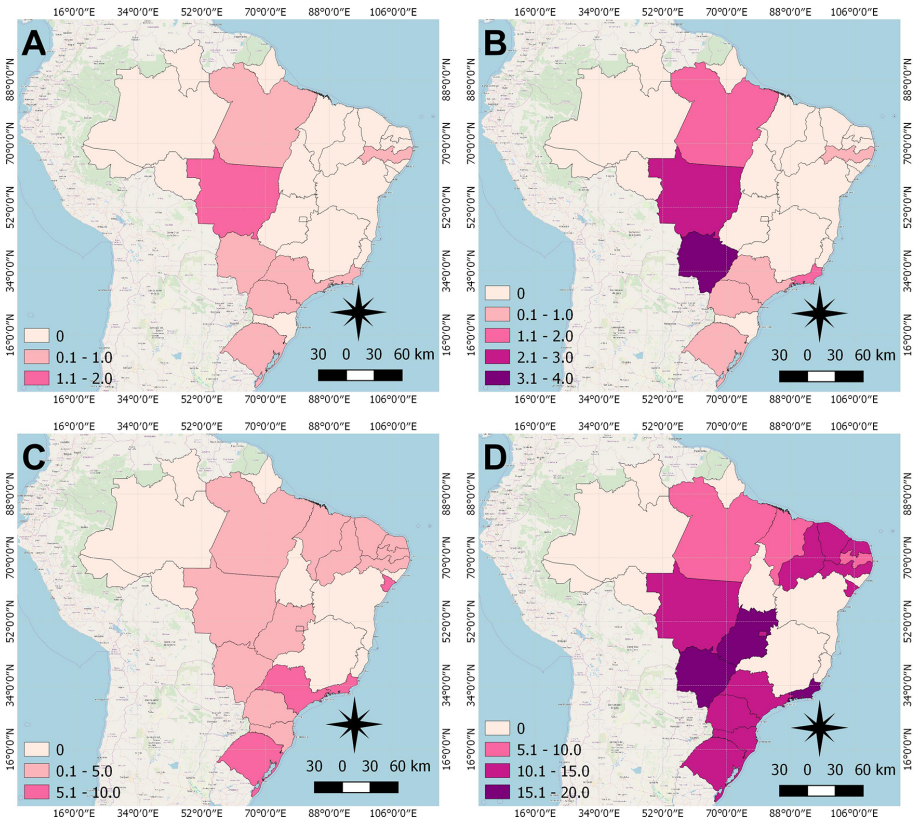

FIGURE 1 | Distribution of Breast cancer mortality rate of women under 40 years of age in Brazilian states, in the years 1996 and 2017. **(A)** 20–29 years, 1996; **(B)** 20–29 years, 2017; **(C)** 30–39 years, 1996; **(D)** 30–39 years; 2017.

According to the Joinpoint Regression (**Tables 1** and **2**), the mortality rate of young women with breast cancer in Brazil has been escalating. For women from 20–29 years old, there was an increase of 2.2% a year from 1996 to 2017 (**Figure 2A**). When we analyze the five regions of Brazil, for this age range, the state of Pará, in the North region, had an increase in the mortality ratev of 5.7% a year (**Figure 2B**). The whole Northeast region had an increase of 4.3% (**Figure 2C**), in the Midwest region, Mato Grosso and Mato Grosso do Sul state, increased in 3.4 and 3.1% a year, respectively (**Figure 2D**). The whole Southeast region had an increase of 1.9% a year (**Figure 2E**), with São Paulo and Rio de Janeiro state, being 1.7 and 2.6%, respectively. There were no significant values for the South region (**Figure 2F**), even though there was an increase in the mortality rate.

When analyzing the mortality rate trend for the age group of 30 to 39 years (**Tables 1** and **2**), there was an increase of 4.6% a year in Brazil (**Figure 3A**). For the North region, there was an increase of 3.7% a year (**Figure 3B**), and Pará was the only state in this region with a record of death in this age group, with 6.5 deaths/100 thousand women. In the Northeast, the increase was 2.9% (**Figure 3C**), with the state of Paraíba standing out with 16.9%, followed by Piauí (11.6%) and Maranhão (10.5%). In the Midwest, in general, the growth was 1.3% (**Figure 3D**), being higher in the state of Goiás with 7.1%. In the Southeast and South regions, the lowest growth rates were observed (**Figures 3E, F**, respectively), with 0.7% in each region, whereas in the Southeast, the state of Rio de Janeiro had the highest growth of 5.5% and in the South, the state of Santa Catarina obtained 4.5% between 1996 and 2017.

Still concerning the time trend of young women mortality due to breast cancer, some points of statistically significant increase or decrease were identified, as can be observed for the age group of 30 to 39 years where, in Brazil, from 2011 to 2014 there was a 28% variation in the mortality rate (**Figure 3A**). In the Southeast, between 1996 and 2005, there was a downward trend of -0.9%, followed by an increase of 1.9% between the years 2005 and 2017 (**Figure 3E**). Similarly, from 1996 to 2005, there was a downward trend of mortality in the South region of -1.3% and followed by an increase of 2.2% in the years 2005 to 2017 (**Figure 3F**).

When correlating breast cancer mortality in young women with sociodemographic indexes (**Table 3**), the municipal human development index (MHDI), the Income HDI and Education HDI had a significant impact in the mortality rate for women from 30–39 years old in both periods evaluated and for women from 20–29 years old, only in 1996 to 2000.

TABLE 1 | Trends of Breast cancer mortality in women under 39 years of age in Brazilian states from 1996 to 2017.

	Age 20-29					Age 30-39				
	MR 1996	**MR 2017**	**APC**	**IC**	**P**	**MR 1996**	**MR 2017**	**APC**	**IC**	**P**
Brazil	0.45	0.75	2.2	1.3 – 3.1	<0.05	4.79	12.42	4.6*	2.7 – 6.5	<0.05
North	0.40	0.50	2.1	-0.5 – 4.7	0.1	2.25	4.93	3.7	2.6 – 4.8	<0.05
Acre	–		–	–		–	–	–	–	
Amapá	–		–	–		–	–	–	–	
Amazonas	–		–	–		–	–	–	–	
Pará	0.60	1.79	5.7	4.1 – 7.3	<0.05	2.06	9.30	6.5	4.4 – 8.6	<0.05
Rondônia	–	–	–	–		–	–	–	–	
Roraima	–	–	–	–		–	–	–	–	
Tocantins	–	–	–	–		–	–	–	–	
Northeast	0.28	0.64	4.3	2.3 – 6.3	<0.05	3.18	5.29	2.9	2.0 – 3.6	<0.05
Alagoas	–	–	–	–		–	–	–	–	
Bahia	–	–	–	–		–	–	–	–	
Ceará	–	–	–	–		3.03	10.11	5.7	4.0 – 7.5	<0.05
Maranhão	–	–	–	–		0.64	7.02	10.5	8.0 – 13.2	<0.05
Paraíba	–	–	–	–		0.46	8.33	16.9*	10.6 – 23.5	<0.05
Pernambuco	0.45	0.26	-2.5	-5.6 – 0.6	0.1	4.40	13.38	4.0	1.9 – 6.2	<0.05
Piauí	–	–	–	–		0.58	11.51	11.6	8.1 – 15.3	<0.05
Rio Grande do Norte	–	–	–	–		4.94	12.91	8.4	5.6 – 11.3	<0.05
Sergipe	–	–	–	–		8.06	13.69	6.9	3.5 – 10.5	<0.05
Midwest	0.10	0.51	7.4*	-3.7 – 19.9	0.2	3.88	7.18	1.3	0.1 – 2.4	<0.05
Goiás	–	–	–	–		4.50	16.29	7.1*	2.7 – 11.6	<0.05
Mato Grosso	1.12	2.30	3.4	0.3 – 6.5	<0.05	3.65	11.46	3.4*	-2.0 – 9.0	0.2
Mato Grosso do Sul	0.56	3.99	3.1	0.2 – 6.0	<0.05	2.00	16.86	4.4	0.9 – 8.0	<0.05
Distrito Federal	–	–	–	–		4.53	10.14	2.5	-1.2 – 6.2	0.2
Southeast	0.59	0.83	1.9	0.8 – 3.0	<0.05	5.88	7.25	0.7*	0.1 – 1.3	<0.05
São Paulo	0.45	0.86	1.7	0.1 – 3.2	<0.05	6.21	13.17	4.4*	3.0 – 5.8	<0.05
Rio de Janeiro	0.77	1.18	2.6	1.2 – 4.1	<0.05	7.20	17.87	5.5*	2.9 – 8.2	<0.05
Espírito Santo	–	–	–	–		–	–	–	–	
Minas Gerais	–	–	–	–		–	–	–	–	
South	0.59	1.03	1.9	-0.1 – 3.9	0.1	5.478	6.33	0.7*	-0.4 – 1.8	0.2
Paraná	0.61	0.66	1.1	-1.7 – 3.9	0.4	4.74	11.78	4.3*	-2.3 – 11.2	0.2
Santa Catarina	–					3.98	13.29	4.5	2.7 – 6.5	<0.05
Rio Grande do Sul	0.77	1.42	1.7	-1.4 – 4.8	0.3	6.88	12.29	3.1*	0.1 – 6.1	<0.05

MR, Mortality rate per 100.000; APC, Annual percent changes calculated by Joinpoint Regression Analysis; [*] AAPC, Average Annual Percent Change calculated by Joinpoint Regression Analysis; CI, confidence interval.

DISCUSSION

In oncology, there is no common ground about the age group that specifies "young women", however, in the literature; most publications refer to young women, those under 40 years of age. Thus, for this study, the age group of 20 to 39 years was selected to be evaluated (10).

Breast cancer in young women is still poorly studied and there are a lot of uncertainties about the specific characteristics of the pathology, such as prognosis, recurrences, and mortality (11). The prognosis for young women is worse than for women aged 50 to 69 years, being related to late presentation and more aggressive tumor biology. The late diagnosis stands out the importance of community and health professionals that pays attention to complaints related to the breasts. Regarding that the tumor biology is aggressive, there are still several questions, lacking the intensification of research in the same degree of importance of women with triple-negative cancer (12).

With the escalating incidence and consequently, an increase in the mortality rate that rises with age is a concern because of the few existing studies.

In this study, we chose to include only cases of death from breast cancer coded as ICD10–C50, registered in the Ministry of Health's Information System, since the correction of mortality rates from ill-defined causes can overestimate mortality rates, especially in places where there are more deaths from ill-defined causes.

Breast cancer mortality in young women has increased in the last two decades in Brazil, its regions and states, presented rates higher in 2017 when compared to 1996 for the two age groups studied. The regions with the highest APC was the Midwest, with 7.4% for women between 20–29 years old and North, with APC of 3.7% for women between 30–39 years old.

Rocha-Brischiliari et al. (13) also described increasing mortality rates in young women (20–49), in all regions of Brazil but they described that the Northeast region was the one with the largest increase and Southeast and South regions with the highest average rates from 1996 to 2013. This gap could be due to the 5 years difference among data (2013 to 2017) and that women from 41 to 49 years old were included.

Balmant et al (14). described that breast cancer mortality rates, in Brazil and its regions, in the 2009–2013 period, for

TABLE 2 | Descriptive values for the Annual Percentage Change of the regions and states that presented Average Annual Percent Change calculated by Joinpoint regression.

	Age 20-29				Age 30-39			
	MR	**APC**	**CI**	**P**	**MR**	**APC**	**CI**	**P**
Brazil								
Brazil (1996–2011)	–	–	–	–	4.79–5.62	0.8	0.3–1.4	<0.05
Brazil (2011–2014)	–	–	–	–	5.62–10.66-	28	13.0–44.9	<0.05
Brazil (2014–2017)	–	–	–	–	10.66–12.42	2.7	-3.5–9.2	0.4
Northeast								
Paraíba (1996–1998)	–	–	–	–	0.46–2.26	119.2	20.2–299.9	<0.05
Paraíba (1998–2017)	–	–	–	–	2.26–8.33	9.4	7.5–11.4	<0.05
Midwest								
Midwest (1996–1998)	0.10–0.56	110.7	-36.2–595.2	0.2	–	–	–	
Midwest (1998–2017)	0.56–0.51	0,1	-3.4–3.7	1.0	–	–	–	
Goiás (1996–2011)	–	–	–		4.50–4.64	0.7	-2.6–4.1	0.7
Goiás (2011–2017)	–	–	–		4.64–16.29	9.4	9.4–42.3	<0.05
Mato Grosso (1996–2003)	–	–	–		3.65–0.98	-11.8	-23.3–1.5	0.1
Mato Grosso (2003–2017)	–	–	–		0.98–11.36	11.9	6.5–17.5	<0.05
Southeast								
Southeast (1996–2005)	–	–	–		5.88–5.30	-0.9	-2.1–0.3	0.1
Southeast (2005–2017)	–	–	–		5.30–7.25	1.9	1.2–2.7	<0.05
São Paulo (1996–2010)	–	–	–		6.21–5.68	-0.6	-1.8–0.6	0.3
São Paulo (2010–2017)	–	–	–		5.68–13.17	15.2	11.3–18.3	<0.05
Rio de Janeiro (1996–2007)	–	–	–		7.20–7.71	1.0	-1.4–3.3	0.4
Rio de Janeiro (2007–2017)	–	–	–		7.71–17.87	15.2	7.8–23.1	<0.05
South								
South (1996–2005)	–	–	–		5.47–4.56	-1.3	-3.3–0.7	0.2
South (2005–2017)	–	–	–		4.56–6.33	2.2	0.9–3.5	<0.05
Paraná (1996–2011)	–	–	–		4.74–5.39	1.3	-0.6–3.2	0.2
Paraná (2011–2014)	–	–	–		5.39–14.93	39.0	-10.0–114.8	0.1
Paraná (2014–2017)	–	–	–		14.93–11.78	-9.6	-27.3–12.3	0.3
Rio Grande do Sul (1996–2007)	–	–	–		6.88–4.62	-4.2	-8.0–0.1	<0.05
Rio Grande do Sul (2007–2017)	–	–	–		4.62–12.89	11.7	6.5–17.1	<0.05

MR, Mortality rate per 100.000; APC, Annual percent changes calculated by Joinpoint Regression Analysis calculated by Joinpoint Regression Analysis; CI, confidence interval.

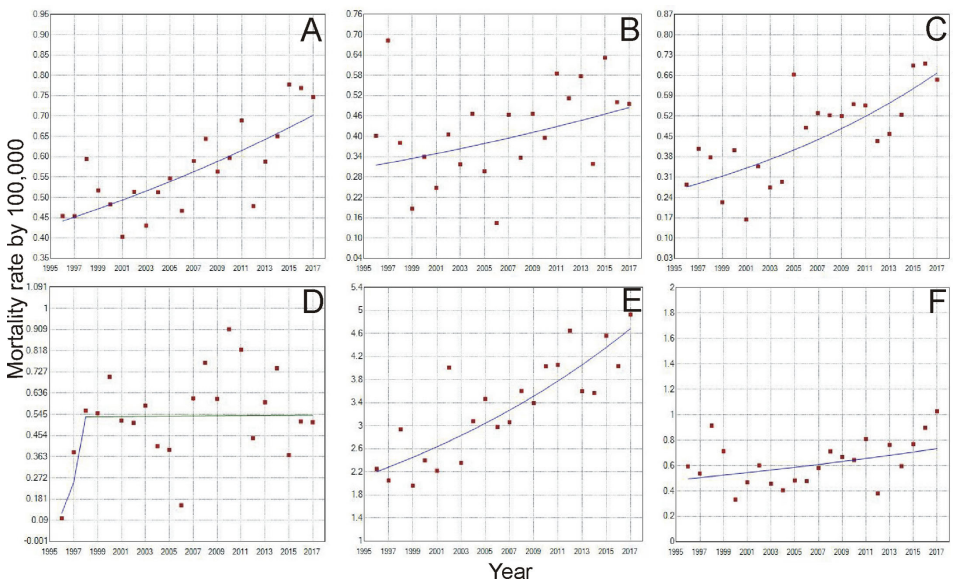

FIGURE 2 | Trends of Breast Cancer Mortality rate from 1996 to 2017, of women aged 20-29 years in Brazil **(A)**. North region **(B)**, Northeast region **(C)**, Midwest region **(D)**, Southeast region **(E)** and South Region **(F)**. [D] Period: 1996-1998; APC: 110.7; CI: -36.2 – 595.2; p=0.2; Period: 1998-2017; APC: 0.1; CI: -3.4 – 3.7; p=1.0.

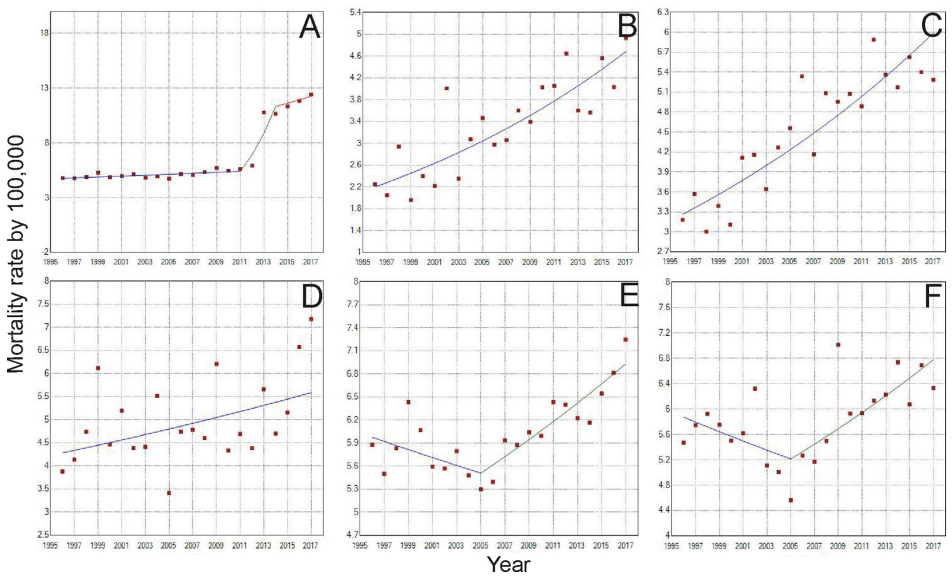

FIGURE 3 | Trends of Breast Cancer Mortality rate from 1996 to 2017, of women aged 30-39 years in Brazil **(A)**, North region **(B)**, Northeast region **(C)**, Midwest region **(D)**, Southeast region **(E)**, and South Region **(F)**. **(A)** Period: 1996 - 2011; APC: 0.8; CI: 0.3–1.4; p =<0.05; Period: 2011-2014; APC: 28.0; CI: 13.0–44.9; p =<0.050; 2011-2017; APC: 2.7; CI: -3.5-9.2; p =0.4; =0.3; **(E)** Period: 1996-2005; APC: -0.9; CI: -2.1–0.3; p =0.1; Period: 2005-2017; APC: 1.9; CI: 1.2–2.7; p =<0.05 **(F)** Period: 1996-2005; APC: -1.3; CI: -3.3–0.7; p =0.2; Period: 2005–2017; APC: 2.2; CI: 0.9–3.5; p = <0.05.

adolescents and young adults, were higher for the 25–29 years old range in all regions (above 4.4 per million). Nevertheless, these data reinforces that there has been an increase in mortality of young women, from breast cancer in Brazil, and that it has been happening differently in all regions.

A survey carried out on countries in Oceania and the Americas among women aged 20 to 49, from 2002 to 2012, pointed out that there was an increase in the mortality rate, due to breast cancer, in Brazil, Colombia, Venezuela, and the Philippines, while for the other countries, the trend was to have decreased rates (15).

In the United States, the American Cancer Society reveals that breast cancer is the second leading cause of death by cancer. The chance of an American woman dying from breast cancer is 2.6%, and since 2007, rates have remained stable in women under 50 years of age. However, between the years 2013 and 2017, there was a reduction in the annual rate of 1.3% per year and a significant improvement in the survival of young women with breast cancer, attributing this reduction to early diagnosis, improved awareness, and adequate treatment (4, 16).

Similarly in Chile, from 1995 to 2013, there was a downward trend in breast cancer mortality rates of 0.8% a year for women from 30 to 39 years old. This decrease is the result of implemented strategies such as prevention, early diagnosis, timely treatment, efficient measures that seek to reduce breast cancer mortality, and improve life quality (17).

While the mortality rates in low and middle-income countries are increasing, in high-income countries, the opposite has been observed. In several high-income countries, breast cancer mortality rates have decreased, mainly due to advances in treatment. However, there are still divergences in middle and low-income countries (15).

In France, even though the incidence of breast cancer for young women increased by 1.1% per year from 1990 to 2018, mortality decreased by 1.3% in the same period, corresponding to 5% of deaths inyoung women (10). Similar to Shanghai in

TABLE 3 | Correlation between breast cancer mortality rates and Municipal Human Development Index, according to age group.

1996-2000	MHDI		HDI Income		HDI Education	
	Correlation coefficient*	*p*	Correlation coefficient*	*p*	Correlation coefficient*	*p*
20–29 years	0.54	0.004	0.60	0.001	0.50	0.008
30–39 years	0.68	<0.001	0.66	<0.001	0.67	<0.001
2013–2017						
20–29 years	0.25	0.210	0.21	0.297	0.20	0.313
30–39 years	0.53	0.004	0.509	0.007	0.508	0.007

*MHDI, Municipal Human Development Index; HDI, Human Development Index; *Spearman's correlation coefficient.*

China where the trend was for decreasing mortality rates due to this condition (18). Also, a trend analysis of breast cancer mortality in women aged 30 to 39 years from 1996 to 2009 was carried out in Switzerland, showing a downward trend from three to 1.6 deaths per 100 thousand women (19).

In this sense, there is interference from socioeconomic level in the actions and adherence of women to breast cancer prevention measures. As for education, the higher, the better the search for help, when women understand and make themselves understood in health services (20).

In this study, it was possible to verify a positive correlation between the municipal human development index (MHDI), the HDI Income and Education in the HDI, which has a significant impact on the mortality rate of women aged 30–39 years.

The HDI follows three pillars: income, longevity and education. In this work we use the general HDI, the income HDI and the education HDI. It is through the HDI that countries are classified as developed, developing or underdeveloped. The measure is from zero to one, the closer to one, the better the HDI.

Although Brazil is economically considered a high-middle income country, the HDI is considered high and reflects breast cancer mortality rates, as shown in **Table 3**. The positive correlation of mortality in Brazil with the HDI means that the higher the HDI, the greater the chance of dying from breast cancer.

For the 20–29 age group in the 2013–2017 5-year period, there was no correlation with the MHDI, we believe that a more detailed study could reveal this phenomenon with greater precision. However, it must necessarily be related to an improvement in data recording, raising mortality rates and distancing the MHDI variable as a factor related to the increase in deaths.

Delays of diagnosis and initiation of treatment are the main aspects that determine the poor prognosis in this age group. In Brazil, the average time between diagnosis and the start of treatment was 59 days, with variation between regions, the longest recorded in the South and Southeast, with 61 and 65 days respectively, remembering that the recommendation is not to exceed 60 days (21). Another study carried out in Singapore showed that the delay of more than 90 days for the beginning of the treatment can interfere in the survival of women with invasive cancer (22).

In addition to the delay in starting treprognosisatment after diagnosis, we can add the delay in diagnosis after the identification of the first symptoms. A study carried out in Brazil identified that the delay between the signs and symptoms and the diagnosis was on average 102 days. Therefore, the delay of diagnosis and the delay in starting treatment can negatively reflect on survival and mortality. The delay in diagnosis can occur in two ways, the first related to the user's delay in seeking care after identifying the signs and symptoms of breast cancer, and the delay related to the health system, including problems with scheduling appointments and diagnostic tests, interfering with the initiation of therapies (23).

One of the most common ways of identifying breast cancer is screening, which is carried out through the mothers' clinicians and imaging tests, such as mammography and ultrasound. The most effective and that really shows an effect in reducing mortality is screening by mammography, however, there is no recommendation to perform it in women under 50 years old (24).

Although there are no studies that prove the effectiveness of clinical breast examination in reducing mortality from breast cancer in young women, it is still a strategy that can be easily performed by a medical professional or nurse, in addition to having a low cost. Clinical breast examination is usually performed opportunistically, a factor that justifies the low coverage of this form of screening, according to a study carried out in Brazil in which half of the women do not undergo a clinical breast examination (23).

If carried out in an organized manner, with awareness campaigns and through programs, it can reach a larger number of women, benefiting the age group of young women who are not part of the mammographic screening. A study carried out in Indonesia showed that clinical breast examination is a viable alternative for screening, since underdeveloped countries have difficulty maintaining a mammographic screening program (25).

Still, there is evidence that preventive measures should be used, including health education with incentives to change lifestyle, reducing alcohol and tobacco consumption, maintaining adequate weight and physical activity. The diagnosis in the shortest possible time and initiation of appropriate treatment improves prognosis, increasing chances of cure and reduced mortality from breast cancer in young women (26).

One of the limitations of the study was to use secondary data, in which gaps were identified in the database, where there was no record of cases of death in some states, especially in the age group of 20 to 29 years. In view of this limitation, it appears that there are possible flaws in the information about the deaths that have occurred, incurring a limitation in the planning of preventive actions, diagnosis and treatment. The fact that mortality from breast cancer in young women is lower in relation to other age groups, does not diminish the concern and the impact on public health.

Considering the gap verified in the reported data, mainly in the age group of 20 to 29 years old, the need for the commitment of Brazilian municipalities and states in the systematization of health information is highlighted, since the lack of data brings losses to the planning of actions and health management in some Brazilian states.

CONCLUSION

In 2017, the World Health Assembly presented a cancer prevention and control resolution through an integrated approach, urging governments and WHO to accelerate actions to achieve objectives specified in the Global Action Plan in order to reduce premature cancer mortality (27). The data obtained in the study, supported these actions, showing that even though the mortality rate of young women is lower than for women over 40 years old, it has been increasing in all regions of Brazil, mainly for women from 30–39 years old, suggesting that controlled actions

of prevention and screening by clinical breast examination should be carried out by public managers.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JS, RP, SP created the project. JS, RP also contributed to the data analysis and writing of the manuscript. JS, RP, RO acquired the original data and developed the methodology. RP, JS, RO, MS, contributed to conceptualize the study and edited the manuscript. RP, SP, MC also supervised the general work. All authors contributed to the article and approved the submitted version.

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Disease Spectrum of Breast Cancer Susceptibility Genes

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Background: Pathogenic variants in cancer susceptibility genes can increase the risk of a spectrum of diseases, which clinicians must manage for their patients. We evaluated the disease spectrum of breast cancer susceptibility genes (BCSGs) with the aim of developing a comprehensive resource of gene-disease associations for clinicians.

Methods: Twelve genes (*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RECQL, STK11*, and *TP53*), all of which have been conclusively established as BCSGs by the Clinical Genome Resource (ClinGen) and/or the NCCN guidelines, were investigated. The potential gene-disease associations for these 12 genes were verified and evaluated based on six genetic resources (ClinGen, NCCN, OMIM, Genetics Home Reference, GeneCards, and Gene-NCBI) and an additional literature review using a semiautomated natural language processing (NLP) abstract classification procedure.

Results: Forty-two diseases were found to be associated with one or more of the 12 BCSGs for a total of 86 gene-disease associations, of which 90% (78/86) were verified by ClinGen and/or NCCN. Four gene-disease associations could not be verified by either ClinGen or NCCN but were verified by at least three of the other four genetic resources. Four gene-disease associations were verified by the NLP procedure alone.

Conclusion: This study is unique in that it systematically investigates the reported disease spectrum of BCSGs by surveying multiple genetic resources and the literature with the aim of developing a single consolidated, comprehensive resource for clinicians. This innovative approach provides a general guide for evaluating gene-disease associations for BCSGs, potentially improving the clinical management of at-risk individuals.

Keywords: breast cancer, cancer susceptibility genes, disease spectrum, germline mutation, cancer genetic

INTRODUCTION

Hereditary predisposition is found in approximately 10% of all breast cancer cases (1). Most are related to germline mutations in high-penetrance genes such as *BRCA1* and *BRCA2* (2–5). Since the identification of *BRCA1* and *BRCA2* (6, 7), genetic testing has become a routine part of clinical care for individuals with possible hereditary breast cancer predisposition (1). With the substantial increase in knowledge of cancer genetics (8, 9), more than 30 potential breast cancer susceptibility genes (BCSGs) have been suggested, including genes with high (e.g., *BRCA1/2, TP53, CDH1, PTEN*, and *STK11*), moderate (e.g., *PALB2, CHEK2, ATM*, and *RECQL*), and low-to-disputed penetrance (e.g., *MLH1, MSH2, MSH6, PMS2, MEN1*, and *PPM1D*) (9–12). Among them, 12 genes with high or moderate penetrance for breast cancer have been definitively established by either the Clinical Genome Resource (ClinGen) (11) or the National Comprehensive Cancer Network (NCCN) (12), the top two authoritative resources.

Pathogenic variants in a BCSG can also increase the risk of other diseases. For instance, *CDH1* is not only associated with increased breast cancer risk, but also a predisposition to gastric cancer (13, 14). Furthermore, several BCSGs are responsible for rare hereditary cancer syndromes, such as *TP53*, which is responsible for Li-Fraumeni syndrome. Individuals with this syndrome have a very high risk of developing multiple malignancies, including but not limited to, breast cancer, sarcoma, brain cancer, leukemia, lung cancer, and adrenocortical cancer (15–18). As comprehensive panel genetic testing becomes the norm (19), clinicians are increasingly faced with the challenge of advising mutation carriers about genes they may be less familiar with or involving cancer susceptibility in organs outside their specialty.

A variety of existing resources, in addition to NCCN and ClinGen, describe the diseases associated with each gene (20), including but not limited to, Genetics Home Reference (https://ghr.nlm.nih.gov/), Online Mendelian Inheritance in Man (OMIM) (https://www.ncbi.nlm.nih.gov/omim), GeneCards (https://www.genecards.org/), and Gene-NCBI (https://www.ncbi.nlm.nih.gov/gene/). However, gene-disease associations described among these six resources are often ambiguous, incomplete, or confusing. For example, the association of *BRCA2* with melanoma is identified in NCCN and Genetics Home Reference but not in other genetic resources such as ClinGen, OMIM, GeneCards, or Gene-NCBI. Furthermore, some gene-disease associations are not found in any genetic resource, such as the association of *CHEK2* with gastric cancer, which has been established with high likelihood in the literature (21, 22). This poses a considerable dilemma for clinicians who are obligated to identify and assess gene-disease associations that require management in clinical practice.

In addition, the rapidly growing medical literature makes it not possible for clinicians to extract useful information precisely and quickly. To address this challenge, Natural language processing (NLP), a technology that trains a computational algorithm with many annotated examples to allow the computer to "learn" and "predict" the meaning of human language, may present a promising solution. Our previous studies illustrate how to train and evaluate an NLP algorithm and incorporate it into a semi-automated procedure to accurately identify the penetrance studies based on abstracts (23–25).

Relying on a patchwork of resources is cumbersome, time-consuming, and can lead to errors of omission. A single comprehensive resource is critically needed to streamline this process. In light of these issues, we have developed a novel approach to identify, evaluate, and curate the diseases or complex syndromes associated with cancer susceptibility genes based on six genetic resources and the NLP literature review.

METHODS

Established Breast Cancer Susceptibility Genes

Germline genetic testing is performed on non-cancer cells and mostly blood-based or saliva-based, and a germline pathogenic variant in a cancer susceptibility gene indicates the possibility that other family members have a hereditary susceptibility to developing cancer. In contrast, somatic testing is performed on cancer cells (e.g., tumor tissue), and a somatic variant may guide targeted therapy and other treatment decisions. The present study focused on germline BCSGs, and only monoallelic BCSGs were included. The BCSGs were initially identified using ClinGen (11) and NCCN (12). In 2019, Lee and other experts on the ClinGen Hereditary Cancer Clinical Domain Executive Committee published a list of 31 high-priority genes for curation using the ClinGen Gene Curation framework (11). Among these 31 genes, 11 classified as having a 'Definitive' or 'Moderate' association with breast cancer were included in our study. The NCCN Guidelines for 'Genetic/Familial High-Risk Assessment: Breast and Ovarian' identified 21 genes offered in multi-gene panels where breast cancer risk was classified as 'Very strong', 'Strong', or 'Limited' (12). Of these 21, the 12 genes that were classified as 'Very strong' or 'Strong' were also included in our study. Accounting for overlap between the two resources, 12 BCSGs were selected for breast cancer, namely, *ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RECQL, STK11*, and *TP53* (**Figure 1**).

Identification of Gene-Disease Association

Diseases associated with BCSGs were initially identified in the six genetic resources (ClinGen, NCCN, OMIM, Genetics Home Reference, GeneCards, and Gene-NCBI) and by reviewing the literature. For each of these sources, each potential association was coded in our database as '1' if the association was definitive, '9' if the association was possible, and '0' if there was no association, as shown in **Supplementary Table 1**. The date of last access to all resources was November 20, 2020. In the following sections we describe in detail each of these resources.

ClinGen

ClinGen is a database curated by the Clinical Genome Resource. It uses a standardized clinical validity framework to assess

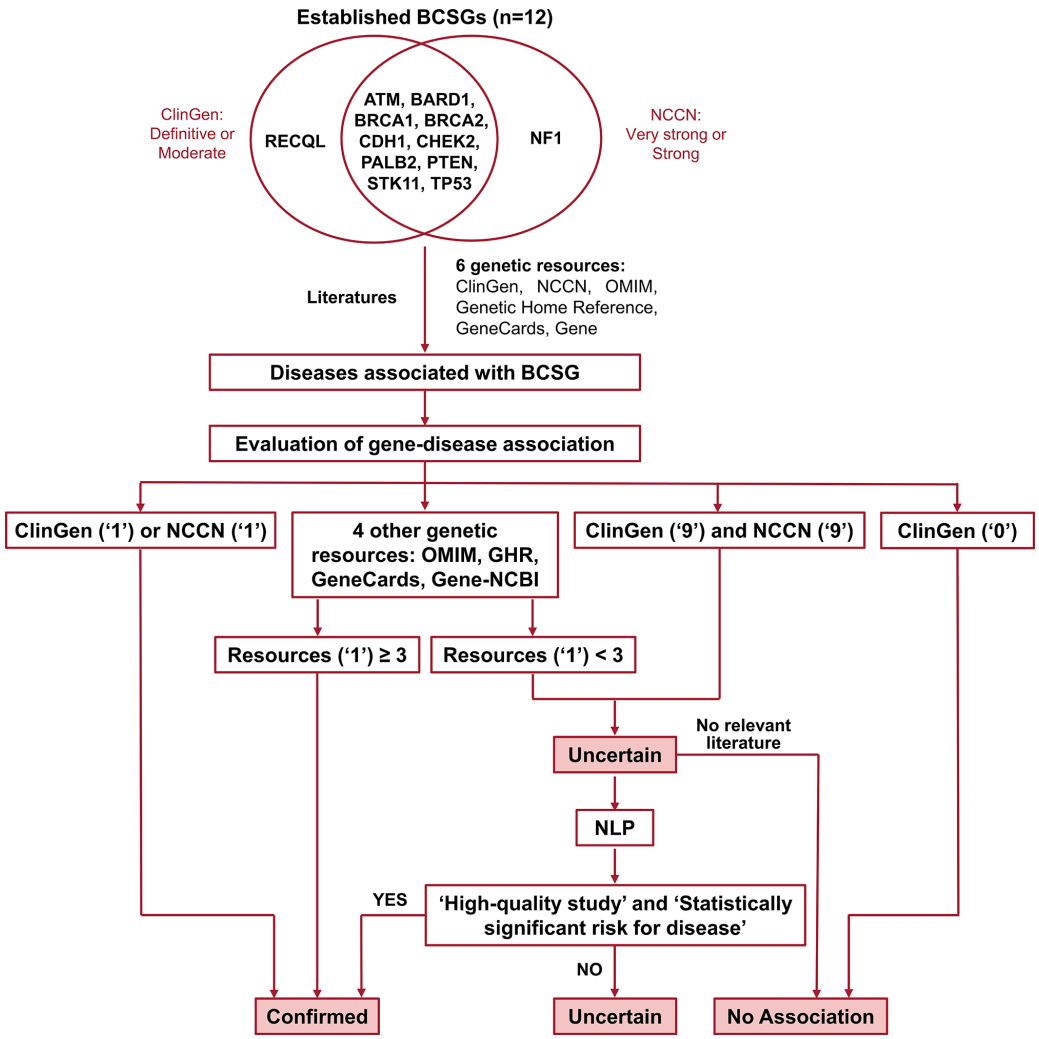

FIGURE 1 | Flow chart for identifying and evaluating gene-disease association. The number '1' indicates that the gene was associated with BCSG in the resource. The number '0' indicates that the gene's association with BCSG was refuted in the resource. The number '9' indicates that the gene's association with BCSG was unclear in the resource. Uncertain association indicates that the gene's association with BCSG is unclear, and further studies are required to refute or accept the association. BCSGs, breast cancer susceptibility genes; NLP, natural language processing.

evidence to validate a gene-disease association and to define disease management. We extracted data regarding gene-disease associations directly from the 'Gene-Disease Validity' reports in ClinGen (https://search.clinicalgenome.org/kb/gene-validity).

The strength of 'Gene-Disease Validity' was classified by ClinGen as 'Definitive', 'Strong', 'Moderate', 'Limited', 'Refuted', 'Disputed', or 'No Reported Evidence' based on the level of evidence. If an association was classified as 'Definitive', 'Strong', or 'Moderate', it was coded in our database as '1' in the field ClinGen Validity. If an association was classified as 'Limited', it was coded in our database as '9'. If an association was classified as 'Refuted', 'Disputed' or 'No Reported Evidence', it was coded in our database as '0'.

We also reviewed the 'Actionability' reports in ClinGen, where the gene-disease associations were identified indirectly (https://clinicalgenome.org/working-groups/actionability/). The 'Actionability' report in ClinGen summarizes secondary findings in patients and identifies diseases caused by susceptibility genes that can be prevented or palliated. A gene-disease association was coded as '1' in our database in the field ClinGen Actionability, if the disease was a manifestation of the genetic disorder, if management of that disease was recommended by screening or preventive intervention, or if the disease was verified in the 'Penetrance' section of the 'Actionability' report. The gene-disease association was coded in our database as '9', if the report suggested a possible relationship.

NCCN Guidelines

Data was extracted from the NCCN Guidelines on Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic (Version 2.2021) (12) and Colorectal (Version 2.2019) (26). A gene-disease association was coded as '1' in our database if a

disease or a feature was used to identify patients for genetic testing or if the management of a disease was recommended for mutation carriers. If NCCN identified a possible relationship, the gene-disease association was coded as '9'.

Other Genetic Resources

Other reputable databases such as 'OMIM', 'Genetics Home Reference', 'GeneCards', and 'Gene-NCBI' (described in detail below) were also used to identify gene-disease associations. If a gene-disease association was present in one of these resources, this association was coded as '1' in our database.

'OMIM' is an online compendium of human genes and genetic phenotypes that is written and regularly updated by the McKusick-Nathans Institute of Genetic Medicine. The "Clinical Synopses" table for each gene was used to identify gene-disease associations.

'Genetics Home Reference' is a free online resource that was created after the announcement of the human genome map in 2003 and is maintained by the National Library of Medicine. It is designed to make the connection between genetics and disease more transparent for the general public. The "health conditions related to the Genetic Changes" section for each gene was used to identify gene-disease associations. Of note, as of October 1, 2020, Genetics Home Reference was ended as a stand-alone website, and most of its content has been transferred to MedlinePlus Genetics (https://medlineplus.gov/genetics).

'GeneCards' is a comprehensive database of human genes. The content of this database is reviewed and updated by the GeneCards Suite Project Team. The "disorders" table for each gene was used to identify gene-disease associations.

'Gene-NCBI' is a resource of the National Center for Biotechnology Information (NCBI), which centralizes gene-related information into individual records. Many different types of gene-specific data are connected to the record including gene products and their attributes, expression, interactions, pathways, variation, and phenotypic consequences. The "Phenotypes" section for each gene was used to identify gene-disease associations.

Evaluation of Gene-Disease Association

The process of validating the gene-disease association is outlined in **Figure 1**. Of the six genetic resources, we considered ClinGen and NCCN the most authoritative and curated these as major resources. As shown in **Figure 1**, we designated the gene-disease association 'verified' if it was coded as '1' in either ClinGen or NCCN. Additionally, if the gene-disease association was coded as '1' in more than three other genetic resources (OMIM, Genetic Home Reference, GeneCard, and Gene-NCBI), it was also designated 'verified'. On the other hand, we designated the gene-disease association 'uncertain', if it was not coded as '1' in either ClinGen or NCCN and was found in fewer than three of the other genetic resources (OMIM, Genetic Home Reference, GeneCard, and Gene-NCBI). We designated the gene-disease association as 'no association' directly if it was coded as '0' in ClinGen.

All 'uncertain' gene-disease associations were further evaluated by literature review using an abstract classifier NLP procedure, which classifies abstracts as being relevant to cancer penetrance or not (23, 24). Our NLP abstract classifier was developed to cull germline penetrance papers from PubMed. In brief, it uses a Support Vector Machine algorithm to classify abstracts as relevant to penetrance, prevalence, both, or neither (24). This NLP abstract classifier has been incorporated into a semiautomated procedure. The sensitivity and specificity of this approach in identifying cancer penetrance studies have been validated (23).

In this study, we used standard gene and disease PubMed search terms (**Supplementary Table 2**) to run the procedure. The NLP abstract classifier was applied to identify the abstracts that were classified as relevant to prevalence or penetrance, and the abstracts were subsequently reviewed by two researchers independently. We then retrieved the full text of these penetrance studies and determined the gene-disease associations based on the quality of the penetrance study (including type of study, sample size, carrier numbers, and ascertainment criteria) as well as the statistical significance of the results.

If no relevant penetrance abstract was identified, the association was designated 'no association'. If relevant penetrance studies were identified, they were presented in a group consensus meeting with our principal investigator (KSH), one surgery resident, and four clinical researchers participating (two attending surgical oncologists and two research fellows in surgical oncology). The attendees selected high-quality penetrance studies based on study design, patient population, number of pathogenic variant carriers, and ascertainment mechanism, and reached a final consensus based on evaluating these high-quality studies. As a rule of thumb, we considered a gene-cancer association to be real if at least one high-quality penetrance study reported at least a two-fold increased risk that was statistically significant. If the attendees could not reach a consensus, the gene-disease association remained 'uncertain'. Of note, to ensure accuracy, the group meeting not only discussed the potential controversial gene-cancer associations but also examined all the evidence regarding every gene-cancer association reported in the study.

RESULTS

Breast Cancer Susceptibility Genes in Six Genetic Resources

As shown in **Table 1**, among the twelve established BCSGs, the association of breast cancer risk with *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, and *CHEK2* was identified in all six genetic sources; *PALB2*, *PTEN*, *STK11* and *TP53* were identified in at least two genetic sources. However, the association of breast cancer risk with *NF1* was only identified in NCCN, and *RECQL* was only identified in ClinGen.

Diseases Associated With BCSGs

There were 66 unique diseases initially identified, of which 42 diseases were determined to be associated with BCSGs by our evaluation (**Supplementary Table 3**). Besides breast cancer, malignant diseases including prostate cancer, pancreatic cancer, colorectal cancer, brain tumor, gastric cancer, ovarian

TABLE 1 | Associations between the 12 susceptibility genes and breast cancer in six genetic resources.

Gene	Genetic Resources						
	No. of resources	ClinGen	NCCN	OMIM	GHR	GeneCards	Gene-NCBI
ATM	6	Definitive	Strong	1	1	1	1
BARD1	6	Definitive	Strong for triple-negative disease	1	1	1	1
BRCA1	6	Definitive	Very strong	1	1	1	1
BRCA2	6	Definitive	Very strong	1	1	1	1
CDH1	6	Definitive	Strong	1	1	1	1
CHEK2	6	Definitive	Strong	1	1	1	1
STK11	4	Definitive	Strong	1	1		
PALB2	4	Definitive	Strong	1		1	
TP53	4	Definitive	Strong		1		1
PTEN	3	Definitive	Strong		1		
NF1	1		Strong				
RECQL	1	Moderate					

The number '1' indicates that the gene was associated with breast cancer in the resource.
GHR, Genetics Home Reference; NCBI, National Center for Biotechnology Information.

cancer, and sarcoma were associated with at least three BCSGs (range: 3 to 6). However, *BARD1* and *RECQL* were only associated with breast cancer, without increased risk for any other diseases.

The disease spectrum of each BCSG is shown in **Table 2**. Furthermore, several BCSGs are associated with specific syndromes, such as *NF1* with Neurofibromatosis Type 1, *PTEN* with Cowden Syndrome, *STK11* with Peutz-Jeghers Syndrome, and *TP53* with Li-Fraumeni Syndrome. The most common cancers associated with these syndromes were determined to be associated with the corresponding susceptibility genes by our procedure.

Disease Spectrum of BCSGs and the Corresponding Resources

A total of 160 gene-disease associations were initially identified in the six genetic resources and literature (**Supplementary Table 1**). As shown in **Figure 2**, a total of 86 gene-disease associations were identified by our evaluation. Among them, 90% (78/86) of

TABLE 2 | Diseases associated with the 12 breast cancer susceptibility genes.

BCSGs	Disease Spectrum		
	Malignant	Benign	Borderline
ATM	Breast Cancer, Colorectal Cancer, Gastric Cancer, Pancreatic Cancer, Prostate Cancer		
BARD1	Breast Cancer		
BRCA1	Breast Cancer, Ovarian Cancer, Pancreatic Cancer, Prostate Cancer		
BRCA2	Breast Cancer, Melanoma, Ovarian Cancer, Pancreatic Cancer, Prostate Cancer		
CDH1	Breast Cancer, Gastric Cancer	BCD Syndrome*	
CHEK2	Breast Cancer, Colorectal Cancer, Gastric Cancer, Kidney Cancer, Prostate Cancer, Osteosarcoma, Thyroid Cancer		
NF1	Brain Tumor, Breast Cancer, Leukemia, Sarcoma	Bone Dysplasia, Cafe-Au-Lait Spots, Intellectual Disability, Iris Hamartoma, Neurofibroma, Pulmonary Stenosis, Skin	GIST, Paraganglioma, Pheochromocytoma
PALB2	Breast Cancer, Ovarian Cancer, Pancreatic Cancer, Prostate Cancer		
PTEN	Brain Tumor, Breast Cancer, Colorectal Cancer, Endometrial Cancer, Kidney Cancer, Melanoma, Thyroid Cancer	Acral Keratoses, Autism, Cerebrovascular Malformation, Facial Papules, GI Hamartomatous Polyps, Lipoma, Macrocephaly, Macular Pigmentation, Oral Mucosal Papillomatosis, Palmoplantar Keratoses, Thyroid, Trichilemmoma, Uterine Fibroid	
RECQL	Breast Cancer		
STK11	Breast Cancer, Cervical Cancer, Colorectal Cancer, Endometrial Cancer, Gastric Cancer, Hepatobiliary Cancer, Lung Cancer, Pancreatic Cancer, Small Intestine Cancer	GI Hamartomatous Polyps, Skin	Non-Epithelial Ovarian Tumor, Ovarian SCST, Testicular SCST
TP53	Adrenocortical Carcinoma, Brain Tumor, Breast Cancer, Colorectal Cancer, Hepatobiliary Cancer, Pancreatic Cancer, Osteosarcoma, Soft Tissue Sarcoma		

GI, gastrointestinal; BCD, blepharocheilodontic; SCST, sex cord-stromal tumor; GIST, gastrointestinal stromal tumor.
**BCD syndrome consists of facial dysmorphism, hypertelorism, imperforate anus, distichiasis, clinodactyly, hypoplastic nails, choanal atresia, cleft palate, and benign teeth disorder.*

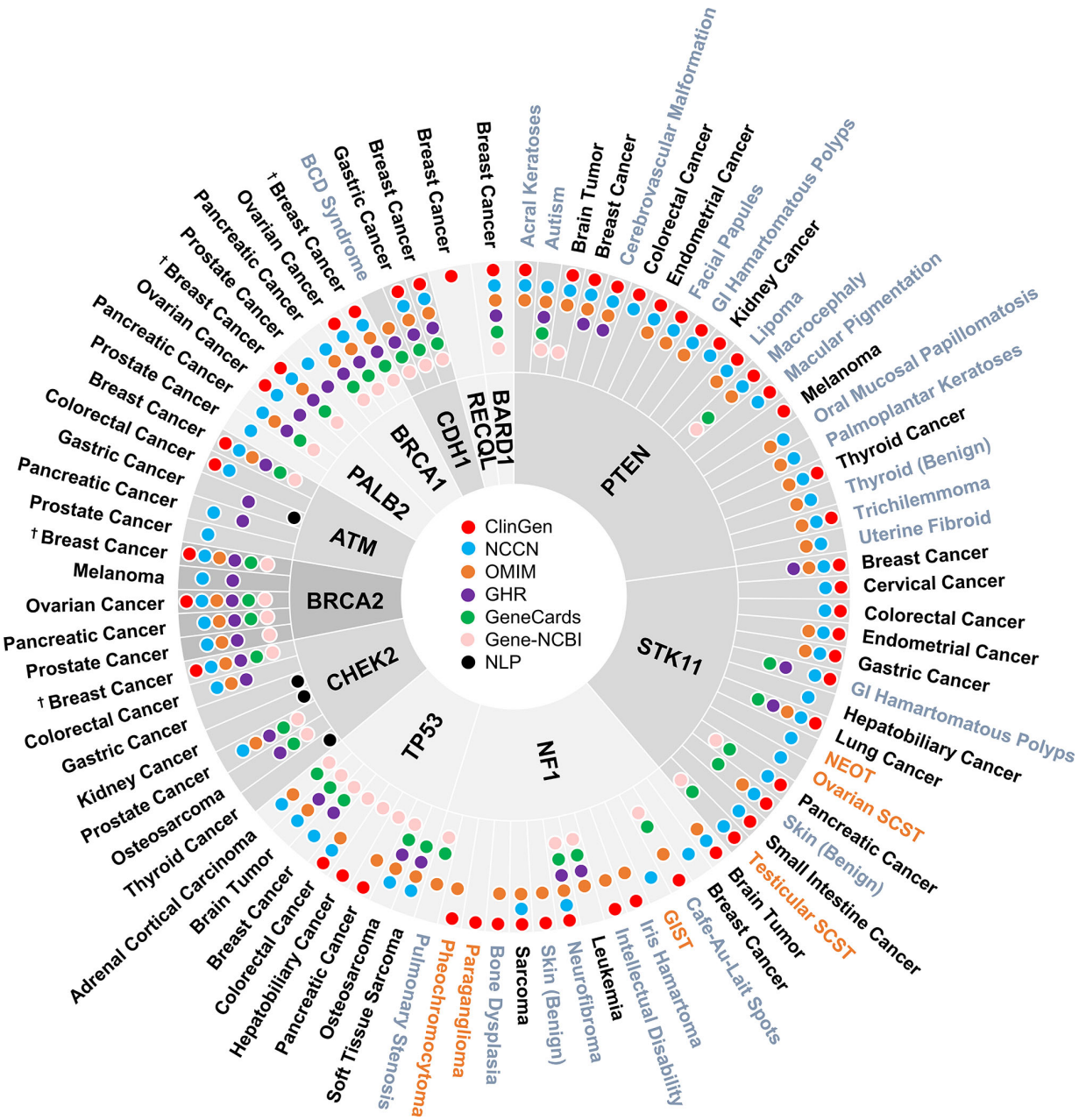

FIGURE 2 | Disease spectrum of breast cancer susceptibility genes. "†" refers to both female and male breast cancer. The three colors represent malignant disease (black), benign disease (grey), and borderline disease (orange), respectively. NLP, natural language processing; GI, gastrointestinal; BCD, blepharocheilodontic syndrome; SCST, sex cord-stromal tumor; GIST, gastrointestinal stromal tumor; NEOT, non-epithelial ovarian tumor.

gene-disease associations were verified by ClinGen and/or NCCN. Conversely, four gene-disease associations were absent from both ClinGen and NCCN but verified in three or more of the other four genetic resources. These included *CDH1*-Blepharocheilodontic (BCD) Syndrome, *CHEK2*-osteosarcoma, *NF1*-leukemia, and *NF1*-pulmonary stenosis. Notably, four gene-disease associations, namely, *ATM*-gastric cancer, *CHEK2*-gastric cancer, *CHEK2*-kidney cancer, and *CHEK2*-thyroid cancer, were verified by NLP literature review alone.

DISCUSSION

Although hereditary breast cancer is mainly associated with *BRCA1/2* pathogenic variants, it may also be associated with germline mutations in other genes. Thus, multi-gene panels usually include both high- and moderate-penetrance genes associated with breast cancer (8, 27, 28). The twelve BCSGs included in our study are those previously established by ClinGen and/or NCCN. To outline the disease spectrum for

the twelve BCSGs, we examined six reliable genetic resources combined with a literature review using NLP. Finally, 49 unique diseases were verified as being associated with the twelve BCSGs.

One of the authoritative resources used for this study is the NIH-funded ClinGen. In contrast to "expert panel" consensus assessments used by NCCN, ClinGen creates a framework that provides evidence for the strength of the association between a gene and a disease risk through semi-quantitative classification (29). The ClinGen classification is based on genetic evidence including case-level data and case-control data, as well as experimental evidence. The other authoritative resource employed for this study is the NCCN Guidelines - the recognized standard for clinical practice in cancer care - using its frequently updated set of clinical practice guidelines. More than 1,300 physicians and oncology researchers from the NCCN Member Institutions comprise the expert panels. Hence, the gene-disease association was designated 'verified' in our study if it was established by either ClinGen or NCCN. Although the standardized literature review method used by ClinGen is outstanding (11), this approach is time-consuming and leads to delay in reflecting the most recent findings. In addition, the gene-cancer associations listed on the NCCN guidelines may not be comprehensive. Therefore, it is necessary to include other genetic resources and find associations missed or not yet addressed by ClinGen and/or NCCN.

Four other genetic resources (OMIM, Genetics Home Reference, GeneCards, and Gene-NCBI) are also considered reputable and contain a comprehensive compendium of relationships between phenotypes and genotypes. However, these resources lack the strict curation processes for evaluating strength of evidence utilized by ClinGen or the expert panels employed by NCCN. Therefore, we rated the level of evidence from these four resources lower than ClinGen and NCCN, and the gene-disease association was designated 'verified' only if it was established by at least three of these sources when the relationship was not found in ClinGen or NCCN. Meanwhile, we understand that the likely valid gene-disease associations we identified that were not present in ClinGen or NCCN may be explained in part by the observation that the latter entities work in a slow and deliberate manner that might not yet have allowed a full review of all associations.

Forty-nine unique diseases were verified as being associated with BCSGs by our procedure. Each BCSG was associated with at least three diseases except *BARD1* and *RECQL*, which were only associated with breast cancer. *BARD1* shares strong structural homology with *BRCA1* and has been demonstrated to be involved in the cellular DNA repair process (30). The association between breast cancer and mutations in the *BARD1* gene was first found in a large case-control study of 65,057 women with breast cancer (8), where the prevalence of *BARD1* mutations was 0.18%, significantly greater than the controls (OR = 2.16, 95% CI: 1.31-3.63, $p < 0.05$). On the other hand, *RECQL* was first identified as a novel breast cancer susceptibility gene in 2015, by two independent research groups (31, 32). Bogdanova et al. compared 2596 breast cancer patients and 2132 healthy females from central Europe and indicated that *RECQL** c.1667_1667 +3delAGTA could represent a moderate-risk breast cancer susceptibility allele (33). A recent study found a moderate risk of breast cancer in African American women with *RECQL* mutation (34). In addition, *RECQL* is considered associated with hereditary breast carcinoma in ClinGen (gene-disease validity: moderate) (https://search.clinicalgenome.org/kb/genes/HGNC:9948). However, there is no high-quality penetrance study that showed statistical significance for additional diseases beyond breast cancer.

Generally speaking the BCSGs are thought to affect female breast cancer risk, but some are also associated with male breast cancer (MBC). Tai et al. evaluated 97 men with breast cancer from 1939 families. The cumulative risk of breast cancer was higher in both *BRCA1* and *BRCA2* male heterozygotes compared to those without a *BRCA1/2* pathogenic variant at all ages. The relative risk of developing breast cancer peaks in the 30s and 40s (35). Another study analyzed 321 families with *BRCA2* mutations both retrospectively and prospectively, suggesting a cumulative risk for male breast cancer of 8.9% up to age 80 (36). Based on these data, NCCN guidelines recommend that men with a *BRCA1/2* pathogenic variant should receive a clinical breast exam at a young age (12).

Notably, we found that *CHEK2* and *PALB2* were also associated with male breast cancer in GeneCards. We verified these associations by literature review based on the NLP procedure, with the literature showing strong evidence in penetrance studies. The *CHEK2/1100delC*, a truncating variant, is present in 13.5% of individuals from families with male breast cancer ($p = 0.00015$) and results in an approximately ten-fold increase of breast cancer risk in men (37). A population-based study found the *CHEK2/1100delC* was present in 4.2% of unselected male breast cancer cases, more prevalent than the frequency of 1.1% in 1,692 controls (OR = 4.1, 95% CI: 1.2-14.3, $p = 0.05$) (38). Recently, Yang et al. analyzed data from 524 families with *PALB2* pathogenic variants from 21 countries and found an association between *PALB2* and risk of male breast cancer (RR = 7.34, 95% CI: 1.28-42.18, $p = 0.026$) (39). Additionally, Pritzlaff et al. reviewed 715 male breast cancer patients who underwent germline multi-gene panel testing and found that pathogenic variants in *CHEK2* (OR = 3.7, $p = 6.24 \times 10^{-24}$) and *PALB2* (OR = 6.6, $p = 0.01$) were both significantly associated with breast cancer risk in men (40).

In the present study, 82% of gene-disease associations were verified by ClinGen and/or NCCN, underscoring the credibility of these two major resources. Nevertheless, six gene-disease associations were not found in ClinGen or NCCN but were instead identified in at least three of the other four genetic resources. Furthermore, these associations were similarly supported by published studies with strong evidence of the association, underscoring the reliability our review criteria.

Of note, four gene-disease associations, i.e., *ATM*-gastric cancer, *CHEK2*-gastric cancer, *CHEK2*-kidney cancer, and *CHEK2*-thyroid cancer, were not identified in any of the six resources but were verified by the NLP-aided literature review. In 2015, Helgason et al. reported a GWAS of gastric cancer in a European population, using information on 2,500 population-based gastric cancer cases and 205,652 controls. They found a new gastric cancer association with loss-of-function mutations in *ATM* (OR = 4.74, $p = 8.0 \times 10^{-12}$) (41). A recent study reported that *ATM* carriers were significantly associated with lower protein expression in five cancer types, including gastric cancer (42). A *CHEK2* mutation was also identified to predispose to

gastric cancer (OR = 1.6, $p = 0.004$), particularly in young-onset cases (OR = 2.1, $p = 0.01$) (21). Additionally, Näslund-Koch et al. examined 86,975 individuals from the Copenhagen General Population Study. The age- and sex-adjusted hazard ratio for *CHEK2/1100delC* heterozygotes compared with noncarriers was 5.76 (95% CI: 2.12-15.6) for gastric cancer and 3.61 (95% CI: 1.33-9.79) for kidney cancer (22). Furthermore, a case-control study reported a *CHEK2* mutation in 15.6% of unselected patients with papillary thyroid cancer, compared to 6.0% in age- and sex-matched controls (OR = 3.3, $p < 0.0001$) (43). Another *CHEK2* variant, c.470C allele, was shown to increase the risk of papillary thyroid carcinoma in female patients by almost 13-fold (OR = 12.81, $p = 0.019$) (44).

The NCCN guidelines for considering risk-reducing mastectomy and breast MRI are well established for carriers of high-risk genes (e.g., *BRCA1, BRCA2*, and *PALB2*), and guidelines on annual mammogram with consideration of breast MRI are also established regarding carriers with moderate-risk genes (e.g., *ATM* and *CHEK2*) (12). Women with genes such as *TP53, CDH1, PTEN, STK11*, and *NF1* may be managed according to established guidelines for the associated cancer predisposition syndrome. For instance, in Li-Fraumeni syndrome, annual whole-body MRI is advised in *TP53* pathogenic variant carriers (45, 46). More aggressive interventions may be recommended, such as consideration of prophylactic gastrectomy if a *CDH1* mutation is found, even in the absence of gastric cancer in the family (47). This necessitates that clinicians stay current with management guidelines and access reliable information resources to implement these updates effectively for their patients (e.g., resources such as ASK2ME could aid with this). Risks of other cancers for those BCSG carriers appear to be modestly elevated, but whether this should alter screening recommendations is unknown. For example, the risk of leukemia with "*TP53*" is 1.6 times as high as the general population, but since the general population risk of leukemia is 0.9%, this amounts to an absolute risk of only 1.4% by age 85 (48). Although a pathogenic mutation in *TP53* is statistically associated with leukemia, it would be hard to justify intensive screening or prevention measures based on this information. It is beyond the scope of this paper to identify the penetrance for each gene-disease association, but this will be the target of future work. Our proposed expansion of disease-gene association reporting will require clinicians to counsel patients appropriately about their risk of additional diseases and to refer them to genetic counselors or other specialists (e.g., neurologist, urologist).

Evaluation based on six genetic resources could result in omissions of some phenotypes associated with BCSGs. We attempted to lessen this effect by including a literature review as an additional step. Another limitation is that the strict criteria we set for gene-disease associations (e.g., verified by ClinGen/NCCN, or at least three genetic resources) could mean that some diseases are overlooked. By reviewing the literature using NLP, we reevaluated those uncertain gene-disease associations to lessen this effect as much as possible. Although the comprehensiveness of our data seems to be conducive to more individualized care, this raises the problem of absence of management guidelines for patients who carry such variants. Additionally, the clinical utility of identifying potential diseases in BCSG carriers may conflict with current cost-efficacy constraints (i.e., interpreting variants, genetic counseling, overdiagnoses, and resulting anxiety in patients). Of note, we are making assumptions based on the available evidence, and we recognize that authoritative sources, such as ClinGen and NCCN guidelines, are updated periodically. Thus, this study represents a snapshot of current knowledge and understanding, rather than a definitive conclusion.

In 2016, we built a clinical decision support tool for cancer susceptibility genes, called Ask2Me.Org (49). This tool provides labs, researchers, and clinical experts with the estimated cancer risk of germline pathogenic variants, including the disease spectrum for each susceptibility gene. Ask2Me.Org has been recommended as a resource in recent clinical practice guidelines (50). These disease spectrums we verified in the current study will be soon available in our website Ask2Me.Org, which is constantly updated. Ongoing research based on accurate estimates of cancer risk needs to be conducted in terms of appropriate management strategies.

CONCLUSIONS

To the best of our knowledge, this is the first study to collate the disease spectrum of BCSGs from multiple sources and make it available in a single resource. Notably, we developed an innovative assessment process based on six genetic resources and literature review using an NLP procedure. Throughout our evaluation process, we have kept in mind that frequent updates of the disease spectrum will be necessary to adjust for new data in these genetic resources. Our study provides a reference point for future studies, showing that BCSG mutation carriers should also be cautious of other diseases beyond breast cancer and highlights the necessity of broadening the criteria of management and improving outcomes for at-risk individuals.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

We used public database with no patient data, and individual informed consent was waived.

AUTHOR CONTRIBUTIONS

JW, KY, DB, and KSH were involved in the conceptualization and design of this study. JW, PS, KY, JZ, KP, and SKM collected the data. YB and MW were responsible for maintaining the natural language processing abstract classifier. JW and PS analyzed the data and interpreted the results. JW, PS, and KY drafted the initial manuscript with critical feedback from DB and KSH. All authors contributed to the article and approved the submitted version.

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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.2021.663419/full#supplementary-material

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The remaining 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.

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Cost-Effectiveness of Adding Ribociclib to Endocrine Therapy for Patients With HR-Positive, HER2-Negative Advanced Breast Cancer Among Premenopausal or Perimenopausal Women

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Purpose: To evaluate the cost-effectiveness of adding ribociclib to endocrine therapy for pre/perimenopausal women with hormone receptor-positive (HR+), human epidermal receptor 2-negative (HER2-) advanced breast cancer from the US payer perspective.

Methods: A partitioned survival analysis model with three health states (progression-free, progressed disease, and death) was developed to compare the cost and effectiveness of ribociclib in combination with endocrine therapy versus endocrine therapy alone based on clinical data from the MONALEESA-7 phase 3 randomized clinical trials. Life years (LYs), quality-adjusted life-years (QALYs), and total costs were estimated and used to calculate incremental cost-effectiveness ratio (ICER) over a lifetime. Deterministic and probabilistic sensitivity analyses were conducted to test the uncertainties of model inputs. Additional scenario analyses were performed.

Results: In the base-case, ribociclib plus endocrine therapy was more effective than endocrine therapy with an additional 1.39 QALYs but also more costly with an ICER of $282,996/QALY. One-way deterministic sensitivity analysis showed that overall survival associated with the treatments and the cost of ribociclib had the greatest impact on the ICER. The probabilistic sensitivity analysis showed that only beyond a willingness-to-pay (WTP) threshold of $272,867, ribociclib plus endocrine therapy would surpass endocrine therapy alone as a cost-effective option.

Conclusions: From the US payer perspective, ribociclib plus endocrine therapy for pre/perimenopausal patients with HR+/HER2- advanced breast cancer is not cost-effective at a WTP threshold of $100,000 or $150,000 per QALY in comparison of endocrine therapy alone.

Keywords: breast cancer, CDK4/6 inhibitor, cost-effectiveness, partitioned survival analysis, ribociclib, pre/perimenopausal

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and the second most common cancer among women in the United States (1, 2). The American Cancer Society projects that 276,000 new cases will be diagnosed, and about 42,000 women will die from breast cancer in 2020 (3). Approximately 30% of women diagnosed with early-stage breast cancer subsequently develop advanced or metastatic cancer (4). Currently, it is estimated that 155,000 women with metastatic breast cancer are living in the United States (5).

Cyclin-dependent kinase (CDK) 4/6 inhibitors in combination with endocrine therapy have been approved by the US Food and Drug Administration (FDA) for both first-line and second-line treatment of HR+/HER2- advanced or metastatic breast cancer (6–17).

Ribociclib (trade name: Kisqali®) is a CDK 4/6 inhibitor, approved by the FDA for postmenopausal women with HR+/HER2- advanced or metastatic breast cancer when used in combination with an aromatase inhibitor or with fulvestrant (18, 19). Ribociclib has also recently been approved for pre/peri menopausal women with HR+/HER2- advanced or metastatic breast cancer when used in combination with an aromatase inhibitor based on MONALEESA-7 (NCT02278120), a randomized, double-blind, placebo-controlled phase III trial (18, 19). In this pivotal trial, pre/perimenopausal women with advanced or metastatic breast cancer received endocrine therapy (tamoxifen or a non-steroidal aromatase inhibitor (NSAI)) either alone or in combination with ribociclib (9, 13). Ribociclib plus endocrine therapy significantly improved progression-free survival (PFS) (median PFS: 23.8 vs 13.0 months; hazard ratio (HR) 0.55; 95% confidence interval (CI) 0.44-0.69; p<0.0001) in comparison with endocrine therapy alone (placebo) (13). Additionally, the MONALEESA-7 clinical trial showed significantly prolonged overall survival (OS) for ribociclib plus endocrine therapy versus endocrine therapy alone (estimated OS rate at 42 months 70.2% (95% CI 63.5%-76.0%) vs. 46.0% (95% CI 32.0%-58.9%); death HR 0.71 (95% CI 0.54-0.95); p<0.01). MONALEESA-7 was the first trial to demonstrate a statistically significant overall survival benefit for a CDK 4/6 inhibitor plus endocrine therapy as the first-line treatment of advanced breast cancer. The most frequent grade 3 and 4 adverse events (AEs) for ribociclib compared to placebo were neutropenia (61% vs. 4%) and leukopenia (14% vs. 1%) (20).

While ribociclib has shown promising clinical effectiveness, it is associated with high cost. The wholesale acquisition cost (WAC) for ribociclib is $12,553 for a package of 63 200 mg tablets, which is the estimated amount needed for a 28-day treatment cycle. This study aims to evaluate the cost-effectiveness of ribociclib plus endocrine therapy versus endocrine therapy alone for pre/perimenopausal women with HR+/HER2- advanced breast cancer from a US payer perspective.

METHODS

Patient Population

The model cohort characteristics were based on the patients enrolled in the MONALEESA-7 clinical trial (13, 20). The target population for the cost-effectiveness evaluation was pre/perimenopausal women aged between 18 and 59 who had histologically or cytologically confirmed HR+/HER2- advanced breast cancer. Patients who received endocrine therapy during advanced disease state or previous CDK4/6 inhibitor treatment were excluded.

Intervention and Comparator

Treatment regimens in the economic analysis followed the MONALEESA-7 clinical trial protocol (20). The experimental intervention therapy was 600 mg of ribociclib (3×200 mg tablets) plus endocrine therapy administered orally once daily on day 1 to 21 in a 28-day cycle. Endocrine therapy alone without ribociclib was used as the comparator. Tamoxifen (20 mg orally once daily) or NSAI (letrozole 2.5 mg orally once daily or anastrozole (1 mg orally once daily) was used for endocrine therapy. For ovarian suppression treatment, goserelin 3.6 mg was administered subcutaneously on day 1 of the 28-day cycle (20). For both treatment and comparator groups, treatment was discontinued once the disease progressed (20).

Partitioned Survival Analysis Model

Model Structure

A cohort-based partitioned survival analysis (PartSA) model was constructed from a US payer perspective with three health states, progression-free (PF), progressed disease (PD), and death (D), to reflect the natural history of the disease and be consistent with clinical trial endpoints (**Figure 1**).

In this model, state occupancy of the cumulative probability of progression is estimated based on survival functions fitted to the original survival data. The PFS curve and the OS curve were obtained from the MONALEESA-7 clinical trial (13, 20). The web-based program WebPlotDigitizer was used to extract the PFS and OS from published Kaplan-Meier curves (21). The use of a fitted parametric distribution is generally preferred over using raw survival data in cost-effectiveness studies when the study time horizons are much longer than the study period. The extracted PFS and OS data were fitted by Exponential, Weibull, Gamma, Gompertz, Log-normal, and Log-logistic parametric distributions. Weibull parametric distribution, as illustrated in **Figure 2**, was chosen based on Akaike information criterion (AIC), Bayesian information criterion (BIC), and clinical relevance (22). The shape and scale parameters that determined the fitted Weibull distributions for PFS and OS for both arms were then used in the PartSA model, which mirrored disease progression by using the estimated state membership based on survival functions fitted to the PFS and OS data. The parametric survival functions obtained from the Kaplan-Meier curves of PFS and OS were provided for three health states: PF, PD, and D. The area under the PFS curve represents the cohort in the PF state. The area between the OS curve and the PFS curve represents the cohort with PD state. The area between the OS curve and the horizontal line of 100% represents the cohort in the D state (23). The model was run over a 20-year time horizon for the base case, which allowed for a follow-up of ≥ 99% of patients until death in the endocrine therapy group.

Results were expressed as total and incremental costs, lifeyears (LYs), quality-adjusted lifeyears (QALYs), and incremental cost-effectiveness ratios (ICERs). ICER was the main outcome measure

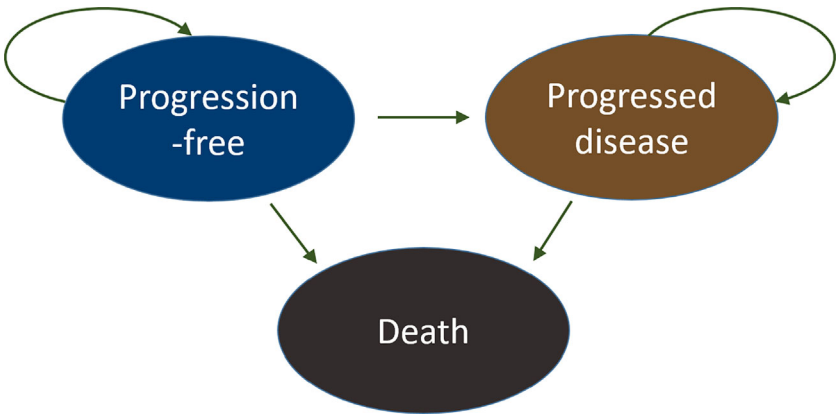

FIGURE 1 | Partitioned survival analysis and the state diagram. Three health states were considered in the model: progression-free (PF), progressed disease (PD), and death (D). All patients started in the progression-free state and transitioned over time to progressed disease or death. Eventually, all subjects moved to the absorbing state, the death state. The arrows illustrate the directions of movements between different health states.

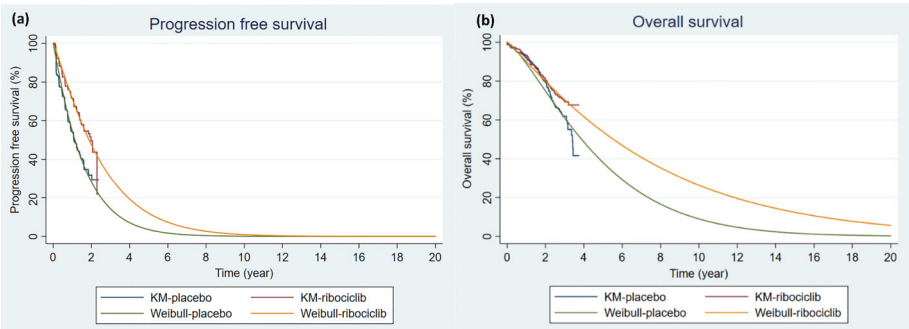

FIGURE 2 | Kaplan-Meier Survival Curves and Parametric Survival Curve Fitting. **(A)** Progression-free survival **(B)** Overall survival. The Kaplan-Meier curves were constructed based on MONALEESA-7 clinical trial (13, 20). Weibull parametric distributions were fitted to the Kaplan-Meier curves for progression-free survival and overall survival over a 20-year time horizon. KM, Kaplan-Meier survival curve; placebo: endocrine therapy only; ribociclib, ribociclib and endocrine therapy; Weibull, extrapolated Weibull parametric model.

and was expressed as the incremental cost per QALY gained. The PartSA was conducted using TreeAge Pro 2019.

Clinical Parameters

Efficacy

The PFS and OS data for ribociclib plus endocrine therapy and endocrine therapy alone were obtained from the phase III MONALEESA-7 study (13, 20). Fitted Weibull survival distributions as described above were used to populate the partitioned survival models.

Safety

The model included incidences and costs of two major grade 3/4 adverse events associated with the treatments as reported from the MONALEESA-7 clinical trial: neutropenia and leucopenia (**Table 1**) (13, 20).

Quality of life

To evaluate health outcomes, literature-based utilities and disutilities were applied to the model by downwardly adjusting life years to generate QALYs. QALYs were estimated by assigning health state-specific utility values for advanced metastatic breast cancer for time spent within each health state as simulated by the partition model (33, 34). Disutilities associated with adverse events were applied to the proportion of patients experiencing that AE for one month for each AE episode based on incidences reported in the clinical trials (35).

Economic Parameters

Cost

Medical costs from a US healthcare payer perspective were considered in the model, which included drug costs, costs of disease monitoring and management, costs of management of severe adverse events (AEs), cost of subsequent therapy, and end-of-life costs. Drug acquisition costs were obtained from the RED BOOK using wholesale acquisition cost (WAC) prices. Costs of clinical laboratory tests were obtained from the Medicare Clinical Laboratory Fee Schedule, and costs of other health care services including office visits and imaging were obtained from the Medicare

TABLE 1 | Model inputs.

Summary of Model Inputs				
Input	**Value**	**Distribution**	**Note**	**Source**
Economic Input				
Drug acquisition costs per 28-day of cycle, 2019 USD				
Tamoxifen	21.33	Gamma	WAC, pkg size: 30 of 20 mg	RED BOOK Online® (24)
NSAI (letrozole or anastrozole)	7	Gamma	WAC, pkg size: 30 of 2.5 mg(letrozole), 30 for 1 mg (anastrozole)	RED BOOK Online® (24)
Goserelin	635.25	Gamma	WAC, pkg size: 63 of 200 mg	RED BOOK Online® (24)
Ribociclib	12,552.97	Gamma	WAC, pkg size: 63 of 200 mg	RED BOOK Online® (24)
Disease management and monitoring costs per event, 2019 USD				
CT scan of chest	199	Gamma	1 for screening, every 8 weeks for 18 months and every 12 weeks after, and 1 for end of therapy	Physician Fee Schedule (CPT 71260) (25)
CT scan of abdomen and pelvis	324	Gamma	1 for screening, every 8 weeks for 18 months and every 12 weeks after, and 1 for end of therapy	Physician Fee Schedule (CPT 74177) (25)
Whole body bone scan	314	Gamma	1 for screening	Physician Fee Schedule (CPT 78306) (25)
Level 4 office visit (new)	131.18	Gamma	1 for screening	Physician Fee Schedule (CPT 99204) (25)
Level 4 office visit (established)	80.01	Gamma	2 for cycle 1, 1 for cycle 2, 2 for cycle3, 1 per cycle for subsequent cycles	Physician Fee Schedule (CPT 99214)
ECG (standard 12-lead)	8.65	Gamma	1 for screening, 2 for cycle 1 to cycle 3, 1 per cycle for subsequent cycles	Physician Fee Schedule (CPT 93010) (25)
Cardiac imaging (ECHO)	210.47	Gamma	1 for screening	Physician Fee Schedule (CPT 93306) (25)
Cardiac assessment (MUGA)	237.50	Gamma	1 for screening	Physician Fee Schedule (CPT 78472) (25)
CBC with auto diff WBC	8.63	Gamma	1 for screening, 2 for cycle 1 to cycle 3, 1 for each subsequent cycle after cycle3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 85025) (26)
CMP	11.74	Gamma	1 for screening, 2 for cycle 1 to cycle 3, 1 for each subsequent cycle after cycle3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 80053) (26)
Fasting lipid panel	14.88	Gamma	1 for cycle 1, 1 every 4th cycle for each subsequent cycle after cycle 3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 80061) (26)
Free Assay (ft-3)	18.82	Gamma	1 for cycle 1, 1 every 4th cycle for each subsequent cycle after cycle 3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 84481) (26)
TSH	18.67	Gamma	1 for cycle 1, 1 every 4th cycle for each subsequent cycle after cycle 3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 84443) (26)
FSH	20.65	Gamma	1 for cycle 1, 1 every 4th cycle for each subsequent cycle after cycle 3, 1 for end of therapy	Clinical Laboratory Fee Schedule (HCPCS 83001) (26)
Estradiol	31.04	Gamma	1 for cycle1, 1 for cycle 3	Clinical Laboratory Fee Schedule (HCPCS 82670) (26)
Prothrombin time/INR	4.37	Gamma	1 for screening, 1 for cycle 2, 1 for cycle 3, 1 for each subsequent cycle from cycle 3, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 85610) (26)
Urinalysis	4.02	Gamma	1 for screening, 1 as clinically indicated during cycle 1 through subsequent cycles, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 81000) (26)
Hepatic function panel	9.08	Gamma	1 for cycle 1,2,3, and 1 for cycle 4,5,6	Clinical Laboratory Fee Schedule (HCPCS 80076) (26)
Serum Pregnancy Test	16.73	Gamma	1 for screening, 1 for cycle 1, 1 for cycle 2, 1 for cycle 3, 1 for each subsequent cycle, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 84702) (26)
Urine Pregnancy Test	8.61	Gamma	1 for screening, 1 for cycle 1, 1 for cycle 2, 1 for cycle 3, 1 for each subsequent cycle, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 81025) (26)
Tumor tissue	273.00	Gamma	1 for screening, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 86152) (26)
Blood for circulating tumor DNA	22.28	Gamma	1 for cycle 1, 1 for each subsequent cycle from cycle 8 day 1 and day 1 of every 3rd cycle thereafter, 1 for EOT	Clinical Laboratory Fee Schedule (HCPCS 87149) (26)
Blood test for CYP2D6	450.91	Gamma	1 cycle for cycle 1	Clinical Laboratory Fee Schedule (HCPCS 81226) (26)
Quantitative assay for serum drug level	18.64	Gamma	Tamoxifen-treated group: 1 for cycle 1, 2 for cycle 3 NSAI-treated group: 1 for cycle1, 1 for cycle 3	Clinical Laboratory Fee Schedule (HCPCS 80299) (26)
Cost of managing grade ¾ adverse events per episode, 2019 USD				
Neutropenia	9649.00	Gamma	We assumed grade ¾ adverse events occur during the first cycle of the treatment	Reference (27)
Leukopenia	4934.00	Gamma		Reference (28)

(Continued)

TABLE 1 | Continued

Summary of Model Inputs				
Input	**Value**	**Distribution**	**Note**	**Source**
Cost of end-of-life-care, 2019 USD				
End-of-life care	20,409	Gamma	One time exit cost from progression-free and progressed disease state	Reference (29)
Subsequent therapy, 2019 USD				
Chemotherapy per month	5349.74	Gamma	Duration of therapy: 3.3 months	Reference (30, 31)
Hormone therapy per cycle	3687.86	Gamma	Drug acquisition cost and injection cost for the first 28 days	RED BOOK Online® (24)
	1843.93	Gamma	Duration of therapy: 2.9 months Drug acquisition cost and injection cost after the first cycle (28 days)	Medicare Part B Drug Average Sales Price (HCPCS J9395) (32) Reference (31)
Clinical inputs				
AEs, probability, %				
Placebo group				
Neutropenia	63.5%	Beta		Reference (20)
Leukopenia	1.61%	Beta		Reference (20)
Ribociclib group				
Neutropenia	4.50%	Beta		Reference (20)
Leukopenia	1.80%	beta		Reference (20)
Patient receiving subsequent therapy, %				
Placebo group				
Chemotherapy	36.5%	Beta		Reference (20)
Hormone therapy	42.9%	Beta		Reference (20)
Ribociclib group				
Chemotherapy	30.6%	Beta		Reference (20)
Hormone therapy	44.8%	Beta		Reference (20)
Weibull distribution parameters				
Input	Scale	Shape	Note	Sources of original KM curves
Placebo group				
Progression-free survival	1.608	1.068		Figure 2A in Reference (13)
Overall survival	5.127	1.317		Figure 1A in Reference (20)
Ribociclib group				
Progression-free survival	2.593	1.146		Figure 2A in Reference (13)
Overall survival	7.710	1.107		Figure 1A in Reference (20)
Humanistic outcome inputs				
Input	Utility	Distribution	Note	Source
Health-state utility values				
Progression-free	0.830	Beta		Reference (33)
Progressed disease	0.443	Beta		Reference (34)
AE disutility values				
Neutropenia	-0.007	Beta		Reference (35)
Leukopenia	-0.003	Beta		Reference (35)

Physician Fee Schedule. Costs of treating severe AEs and end-of-life costs were estimated using published costs from the literature and applied as one-time events (**Table 1**). The base-case model assumed that AEs occurred during the first four weeks of initiating the therapies. All costs were standardized to 2019 U.S. dollars using the Consumer Price Index's medical component and discounted by 3% annually (36).

Healthcare Resource Utilization

The drug utilization regimen, schedule for clinical lab tests, office visits, and imaging were modeled following the MONALEESA-7 clinical trial protocols per treatment cycle basis (20). Patients were assumed to be on treatment until disease progression. The types and probabilities of AEs were derived from the results of the MONALEESA-7 clinical trial and were modeled as a one-time event (20). Subsequent post-progression chemotherapy and hormone therapy were given to 36.5% and 42.9% of patients in the control arm and 30.6% and 44.8% of patients in the ribociclib, respectively (20).

Sensitivity Analyses

One-Way Sensitivity Analysis

Deterministic one-way sensitivity analyses were conducted by varying one variable (model input) at a time within its plausible range. The plausible range was set to be plus or minus 25% of the base case value or 95% confidence interval (CI) except costs, which were set to be 50% to 200% of the base case value.

Probabilistic Sensitivity Analysis

Probabilistic sensitivity analysis was conducted by varying all variables at the same time by running 10,000 Monte Carlo simulations with probabilities and health state utility values set to follow beta distributions and costs set to follow gamma

TABLE 2 | Final/base case cost effectiveness analysis.

Base case cost-effectiveness analysis		
Base-case-results	**Ribociclib**	**Placebo**
Costs (USD)		
Medication	$398,042	$12,615
Disease management and monitoring	$9,528	$10,410
Managing adverse events	$15,497	$11,330
Subsequent therapy after progression	$6,923	$523
End-of-life care	$16,140	$17,821
Total cost	$446,130	$52,699
Effectiveness		
Progression-free life years	2.47	1.57
Post-progression life-years	4.61	3.15
Total life years	7.08	4.72
Progression-free QALY	2.04	1.30
Post-progression QALY	2.04	1.40
Total QALY	4.09	2.70
Incremental Cost-effectiveness		
Incremental cost per life-year gained ($/LY)	166,690	
Incremental cost per QALY gained ($/QALY)	282,996	

Ribociclib cost itself was $378,561, which is 95.11% of total medication cost and 84.85% of the total cost ($446,130) in the ribociclib group.

TABLE 3 | Results of scenario analyses.

Parameter	Base case input	Alternative input(s)	ICER ($/QALY)
Base-case result			282,996
Time horizon	20 years	5 years	831,552
		10 years	434,562
		30 years	260,519
		40 years	256,529
Discount rate	3% for cost only	3% for cost and benefits	358,418
		No discount rate applied for cost and benefit	301,841
Adverse event cost/ disutilities	Included	Excluded	278,393
Subsequent therapies	Included	Excluded	283,631

distributions. Cost-effectiveness acceptability curves were plotted based on the probabilistic sensitivity analysis.

Scenario Analyses

Additional scenario analyses were conducted. The analyses examined the model uncertainties around model structural variations by varying time horizons, (5-, 10-, 30-, 40-year time horizons), discount rate (0% or 3% for costs and QALYs), and excluding adverse events and subsequent therapies from the analysis.

RESULTS

Base-Case Analysis Results

The base case cost-effectiveness results are summarized in **Table 2**. Within a 20-year time horizon, the partitioned survival analysis model predicted that ribociclib plus endocrine therapy was associated with 7.08 LYs and 4.09 QALYs as compared to 4.72 LYs and 2.70 QALYs in the endocrine therapy only arm. While the ribociclib group is associated with significantly longer LYs and QALYs, it is also associated with significantly higher costs. The expected annually discounted (3%) total costs for ribociclib plus endocrine therapy arm per patient were $446,130 as compared to $52,699 in the endocrine therapy only arm. The drug costs of $398,041 accounted for the most significant proportion of total costs followed by end-of-life care ($16,140), disease management and monitoring ($15,497), and subsequent therapy after progression ($9,528). Cost for ribociclib acquisition itself constituted 95% of total medication cost and 85% of total cost in the ribociclib and endocrine therapy group. As a result, the ICER for ribociclib plus endocrine therapy compared to the endocrine therapy alone is $166,689/LY and $282,996/QALY. At a willingness-to-pay (WTP) threshold of $150,000/QALY accepted in oncology treatments in the United States, this ribociclib treatment cannot be considered as a cost-effective option compared to endocrine therapy alone.

Scenario Analysis Results

Additional scenario analyses were conducted to explore the effect of varying time horizons, discount rate, and the exclusion of AEs, and the exclusion of subsequent therapies from the base case analysis (**Table 3**).

Varying Time Horizons

With the base case time horizon set to be 20 years, the scenario analysis varied the time horizon at 5, 10, 30 and 40 years. These changes resulted in the ICER varying from $831,551/QALY (with incremental cost: $358,692 & incremental QALY: 0.43), $434,562/QALY (with incremental cost: $389,793 & incremental QALY: 0.90), $260,519/QALY (with incremental cost: $393,871 & incremental QALY: 1.51), and $255,805/QALY (with incremental cost: $393,939 & incremental QALY: 1.54), respectively. ICERs are relatively stable beyond 20 years of follow-up in comparison with the base case ICER of $282,996/QALY with 20 years of time horizon, with none reaching cost-effectiveness.

Varying Discount Rates

In the base-case analysis, a 3% discount rate was applied only to cost, while the effectiveness outcome of QALY was not discounted. When a 3% discount rate was applied to both cost and QALY, the ICER increased to $358,417/QALY. When no discounting was applied, the ICER was similarly above the WTP threshold ($301,841/QALY).

Exclusion of Adverse Events

The base-case model included adverse events for neutropenia and leukopenia and assumed that an adverse event occurred during the first four weeks of initiating the therapies. When cost and disutility information related to these adverse events were excluded, the ICER changed from $282,996/QALY to $278,393/QALY. Despite the prevalence of these AEs, they appeared to have little impact on both the QALYs (<0.5%) and costs (<2%).

Exclusion of Subsequent Therapy Costs

In the base-case model, patients received subsequent chemotherapy or endocrine hormone therapies once they discontinued the main assigned medication treatments. When subsequent therapy costs were excluded from the model, the ICER result changed from $282,996/QALY to $283,630/QALY, which showed this had a negligible impact on the model.

One-Way Deterministic Sensitivity Analysis Results

The one-way sensitivity analysis results are shown in the tornado diagram in **Figure 3**, which lists the model inputs that have the biggest impact on the cost-effectiveness when individual model inputs were varied within their plausible ranges. The tornado diagram ranks the variability of each input parameter from the highest to the lowest across the top 18 model inputs based on their impact on the ICER. Key model drivers were scale parameters for OS Weibull distribution for both treatment arms as well as the drug cost of ribociclib. Overall, the model is robust with the ICER maintained above $150,000/QALY across all variations as validated by the one-way sensitivity analysis.

Probabilistic Sensitivity Analysis Results

A probabilistic sensitivity analysis using a Monte Carlo simulation of 10,000 iterations demonstrated that in most iterations (>99%), the ribociclib group yielded more QALYs and was more costly. The cost-effectiveness acceptability curves (CEAC) from the probabilistic sensitivity analysis (**Figure 4**) present the probabilities for each alternative that have the greatest net benefit (add up to 1 at any given WTP), expressed as a function of the WTP. The CEACs showed that ribociclib was less likely to be associated with more net benefit than placebo below a WTP of $272,867/QALY. Only above $272,867/QALY did ribociclib start to have an advantage over endocrine therapy. At a $100,000/QALY WTP threshold, the endocrine therapy is more cost-beneficial than ribociclib plus endocrine therapy in 99.12% of the iterations, and at a $150,000/QALY WTP threshold, in 92.91% of the iterations, showing the stability of the base-case results.

DISCUSSION

The approval of ribociclib for use in pre/perimenopausal women give patients access to a new treatment option that helps to improve progression-free survival and overall survival. Building on clinical trial data, this study provides a cost-effective evaluation of this new drug treatment. While significantly improving PFS and OS over endocrine therapy alone, ribociclib plus endocrine therapy for pre/perimenopausal women with HR+/HER2- advanced breast cancer is not cost-effective with an ICER of $282,996/QALY, despite assuming a generous WTP threshold of $150,000/QALY for the U.S. healthcare setting. As tested by different scenario, deterministic and probabilistic sensitivity analyses, the model is robust with the ICER remained above $150,000/QALY.

To the best of our knowledge, our study is the first study to use a partitioned survival analysis model to evaluate the cost-effectiveness of ribociclib for this indication in pre/perimenopausal women. Our findings showed that the drug price of ribociclib has one of the biggest impacts on the cost-effectiveness result. At an acquisition cost of $12,553 for 63 200 mg tablets for a 28-cycle treatment, ribociclib drug costs totaled $374,577, accounting for 84.9% of the total costs of the ribociclib treatment arm. In fact, the incremental cost of ribociclib plus endocrine therapy arm versus the endocrine therapy only arm was $393,431, most of which was contributed by ribociclib drug cost. The differentials in other cost categories, including disease monitoring, adverse event management, post-progression treatment and end-of-life costs are relatively low in comparison. Threshold analysis was conducted and shows that at a WTP of $150,000/QALY, the drug price needs reduction by about 48.84% (from $12,553 to $6,422 per 28-day treatment cycle for 63 200mg tablets) in order to make ribociclib a cost-effective treatment option in this population.

Our findings in the pre/perimenopausal patient population are in line with other studies evaluating cost-effectiveness of ribociclib plus endocrine therapy for HR+/HER2- advanced or metastatic breast cancer in the postmenopausal population, which also found the intervention to be not cost-effective with ICERs ranging from $210,369/QALY to $440,000 per QALY based on MONALEESA-2(NCT01958021) clinical trial (33, 37). It should be noted that MONALEESA-7 was different from the above trial in that it was designed for pre/perimenopausal women who also received ovarian function suppression (OFS) during treatment, yet in both patient populations, the high drug costs could not be justified by the received benefits at currently accepted WTP threshold from the US payer perspective.

The high drug costs also directly impact patients through high out-of-pocket costs which often place patients at risk of financial toxicity (38). The annual acquisition cost for ribociclib treatment is $163,189 in 2019 USD. Although patient out-of-pocket costs for ribociclib was not assessed in this study, it could be substantial even for insured patients given that ribociclib is typically listed as a specialty drug with formulary tier 4 or 5 with different insurance plans. The financial burden for patients and caregivers may lead to psychological distress and medication nonadherence, leading to diminished patient clinical and humanistic outcomes (38, 39).

Ribociclib is not alone in this regard. A recent study assessed the cost-effectiveness evaluations of cancer drugs conducted by the US's Institute for Clinical and Economic Review (ICER), and found that most new cancer drugs were not cost-effective (40). In the US, regulatory approvals are not tied to costs and the complicated network of multiple payers in the healthcare system is associated with less negotiating power to obtain substantial drug discounts with manufacturers as compared to other countries with single-payer health care systems. In general, the US pays the highest price for new drugs in the world, especially for specialty drugs, including oncology drugs. For example, the US pays about 46% of global expenditure on oncology medications (41). While high drug prices help incentivize innovation, there needs to be a fine balance

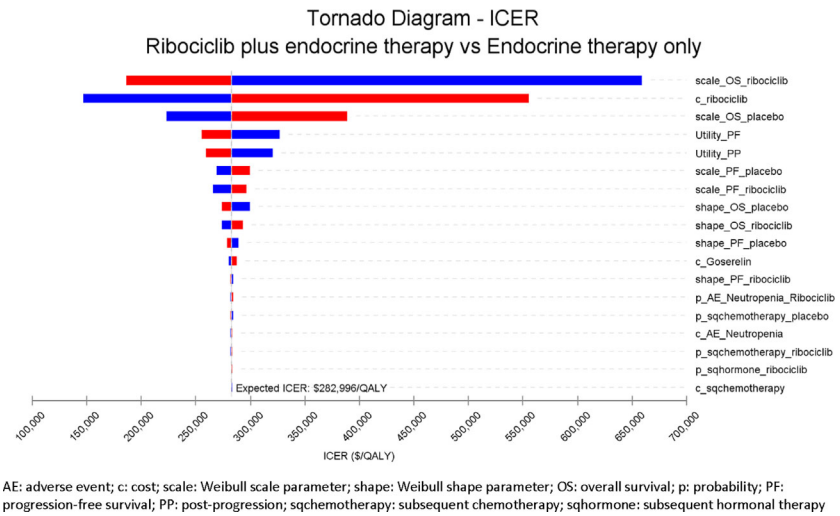

FIGURE 3 | Tornado Diagram for One-way Deterministic Sensitivity Analysis.

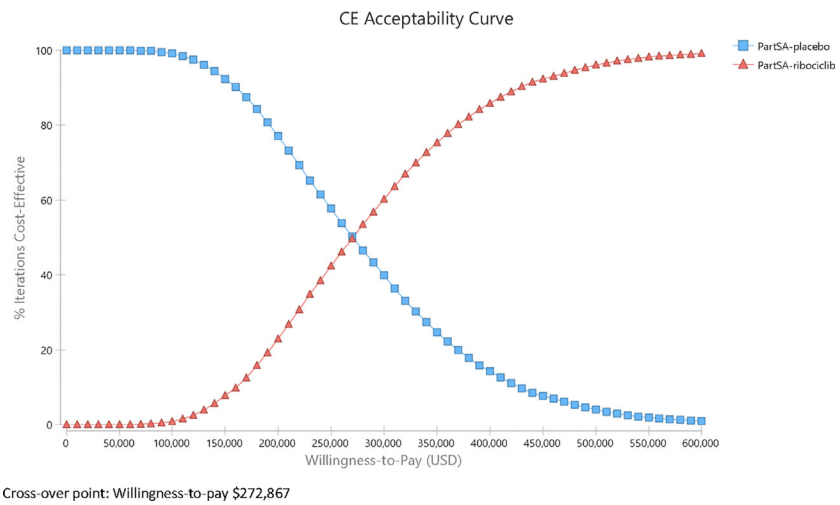

FIGURE 4 | Cost-effectiveness Acceptability Curve.

between profitability to the pharmaceutical industry and affordability to the health care systems (42). Cost-effectiveness evidence of new oncology drugs should be utilized to play a bigger role in guiding pricing and reimbursement decisions with US payers through novel payment models (43, 44).

This study has several strengths. First, the study applied a partitioned survival analysis (PSA) model to simulate the lifetime costs and QALYs among the patient population. Compared to traditional Markov models, PSA models directly estimate health state occupancy from the survival curves without needing to calculate transition probabilities needed for Markov models. Thus, PSA modeling is viewed as a relatively more straightforward approach to make estimates from clinical trial data and tends to provide a better estimate to the observed survival data. PSA models are the predominantly used model structure in oncology treatments in health technology assessment (HTAs) submitted to (conducted by) the UK National Institute for Health and Care Excellence (NICE), which represented some of the highest standards for cost-effectiveness analysis. Second, the study extrapolated both progression-free and overall survivals beyond the trial period using fitted parametric curves to allow for evaluation of costs and outcomes over life time. Third, the study extensively evaluated the model uncertainties by including not only one-way and probabilistic sensitivity analyses but also different scenario analyses to capture different possible clinical and modelling scenarios.

This study has limitations. One limitation is related to the model structure uncertainty, which is inherent to all pharmacoeconomic modeling. Currently, the use of PF, PD, and D as model health states are widely adopted in oncology cost-effectiveness studies because they follow the primary endpoints of oncology clinical trials. While it is possible to test the model input uncertainties using deterministic and probabilistic sensitivity analyses, the uncertainties around the model structure are more challenging to test.

Another limitation is related to uncertainties around model inputs. This model is based on large RCT efficacy and safety data and may not necessarily reflect real-world outcomes and cost-effectiveness. For new drugs or existing drugs for new indications, initial cost-effectiveness assessment typically is conducted based on clinical trial data as not much real-world evidence is available yet. It will be desirable to also evaluate the cost-effectiveness when real-world effectiveness data become available. Besides, the utilities used in the model were not directly from the study population but from the literature on similar populations. However, we used the best possible data estimates from the literature. Also, as the clinical trial publications did not report detailed data on dose reduction and discontinuation, their impacts on the cost of treatment were not included in this model.

In conclusion, based on the partitioned survival analysis, this study found that while ribociclib provides significant survival benefits, it does not appear to be cost-effective when compared to endocrine therapy alone in treating pre/perimenopausal women with HR+/HER2- advanced breast cancer with a WTP of $150,000/QALY. Healthcare decision-makers need to take this into consideration, along with clinical effectiveness and safety when selecting treatments across populations to ensure efficient allocation of limited health care resources. Reductions in drug cost, could bring this treatment more in-line with accepted cost-effectiveness WTP limits across cancer treatments.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

EJ and LZ conceived and designed the study. EJ and LZ performed data collection. EJ and LZ performed data analysis. EJ, CW, LW, and LZ wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Risk of Endometrial Cancer and Frequencies of Invasive Endometrial Procedures in Young Breast Cancer Survivors Treated With Tamoxifen: A Nationwide Study

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Background: Although the guidelines recommend gynecological assessment and close monitoring for symptoms of endometrial cancer in postmenopausal breast cancer survivors taking tamoxifen (TAM), the risk of endometrial cancer in young breast cancer survivors has not yet been fully assessed. This study aimed to investigate the risk of developing endometrial cancer and the frequencies of gynecological examinations in young breast cancer survivors taking TAM in South Korea.

Methods: A nationwide retrospective cohort study was conducted using the Health Insurance Review and Assessment Service claims data. Kaplan–Meier analyses and log-rank tests were used to assess the probability of endometrial cancer, benign endometrial conditions, and the probability of invasive endometrial procedure. To analyze the risk of endometrial cancer and benign endometrial conditions, we used a multivariable Cox proportional hazards regression model.

Results: Between 2010 and 2015, 60,545 newly diagnosed female breast cancer survivors were included. The total person–years were 256,099 and 140 (0.23%) patients developed endometrial cancer during the study period. In breast cancer survivors aged ≥60 years [hazard ratio (HR), 5.037; 95% confidence interval (CI), 2.185–11.613], 50–59 years (HR, 4.343; 95% CI, 2.122–8.891), and 40–49 years (HR, 2.121; 95% CI, 1.068–4.213), TAM was associated with an increased risk of endometrial cancer. In subjects aged below 40 years, TAM did not significantly increase the risk of endometrial cancer. However, among the TAM subgroups, breast cancer survivors aged below 40 years [1.61 per 1,000 person–years (PY); HR, 12.460; 95% CI, 2.698–57.522] and aged 40–49 years (2.22 per 1,000 PY; HR, 9.667; 95% CI, 4.966–18.819) with TAM-related endometrial diseases showed significantly increased risks of endometrial cancer.

Among the TAM subgroup with benign endometrial conditions, the ratios of the frequency of invasive diagnostic procedures to the incidence of endometrial cancer were higher in subjects under 40 than subjects aged 60 or more.

Conclusion: Young breast cancer survivors with TAM-related benign endometrial diseases are at a higher risk of developing endometrial cancer. Gynecological surveillance should be tailored to the risk of endometrial cancer in young breast cancer survivors to improve the early detection of endometrial cancer and avoid unnecessary invasive procedures.

Keywords: breast neoplasms, dilatation and curettage, gynecological examination, tamoxifen, endometrial neoplasms

INTRODUCTION

In hormone receptor-positive breast cancer, use of tamoxifen (TAM) as an adjuvant antihormonal treatment is important (1, 2). TAM is associated with various adverse effects, including hot flashes, vaginal discharge, menstrual irregularities, sexual dysfunction, thromboembolic complications, hyperplasia, and endometrial cancer (3, 4). These adverse effects can reduce patients' adherence to adjuvant endocrine treatment (5). Endometrial cancer is known as a serious adverse effect related to the use of TAM (6–8).

Most guidelines recommend gynecological assessment for endometrial cancer in postmenopausal breast cancer survivors receiving TAM (9–11). However, routine examinations are not warranted as such surveillance is not effective in enhancing the early detection of endometrial cancer and may lead to more invasive and costly diagnostic procedures in asymptomatic postmenopausal women. Gynecological assessments are recommended to be performed only in postmenopausal women with complaints of abnormal vaginal symptoms (9, 11).

No existing guidelines have recommended gynecological assessment for endometrial cancer in premenopausal women receiving TAM. This is because previous studies did not show a significantly increased risk of endometrial cancer in premenopausal women receiving TAM (1, 10). However, in these studies, the numbers of young breast cancer survivors were small, the incidence of endometrial cancer was low, and assessments for various TAM-related gynecological symptoms were not conducted. Therefore, the risk of endometrial cancer in premenopausal women were possibly underestimated.

Hence, this nationwide retrospective cohort study was conducted using claims data from the Health Insurance Review and Assessment Service (HIRA). This study aimed to investigate the incidence of endometrial cancer in young breast cancer survivors and to analyze the frequency of invasive endometrial procedures in breast cancer survivors who were treated with TAM.

MATERIALS AND METHODS

Data Source and Study Population

Healthcare in South Korea is delivered through a single-payer healthcare system supported by the government. The HIRA has a major role in collecting data of all healthcare services delivered to patients and assessing the healthcare services for reimbursement decisions (12). The HIRA data include patients' general information, diagnoses, and healthcare services provided such as medications and procedures.

The data registered between January 2008 and December 2018 were extracted from the HIRA database. Patients who were newly diagnosed with breast cancer from January 2010 to December 2015 were included. To exclude prevalent breast cancer patients, we selected a 2-year washout period (from January 2008 to December 2009). Male breast cancer patients, patients with a history of *in situ* carcinoma, patients with presumed metastatic or recurrent breast cancer, and patients who did not undergo breast cancer surgery were excluded. Patients with previous or recent history of other cancer types including endometrial cancer, who underwent hysterectomy, or who had an oophorectomy were also excluded. Moreover, patients without follow-up claims data 1 year after the initiation of endocrine treatment were excluded. Newly diagnosed breast cancer was defined using the C50 code (invasive breast cancer) based on the 10th revision of the International Classification of Diseases (ICD-10) plus the V193 code, which is a claim code for reimbursement of cancer patients (13).

From January 2010 to December 2015, 203,956 breast cancer patients who were assigned with the C50 and V193 codes were identified. We excluded 861 male patients, 6,535 patients with a previous history of *in situ* carcinoma, 13,493 patients with metastatic or recurrent breast cancer, 17,142 patients who did not undergo breast cancer surgery, 16,036 patients with preexisting or who were recently diagnosed with other cancer types, 1,642 patients with previous hysterectomy, 99 patients who previously underwent oophorectomy, and 366 patients who did not have follow-up data 1 year after the initiation of endocrine treatment (**Supplementary Figure 1**).

Variables and Operational Definitions

Patients' baseline characteristics and the Charlson Comorbidity Index (CCI) based on ICD-10 codes were analyzed (14). Hypertension (HT), diabetes mellitus (DM), and dyslipidemia were defined using ICD-10 codes (HT, I10–13, 15, and 16; DM, E10–14; and dyslipidemia, E78) and their related medications. Breast cancer treatments were evaluated based on claims data 1 year after the breast cancer diagnosis. Data on surgery, radiation, chemotherapy, endocrine therapy, and trastuzumab were reviewed.

Endometrial cancer was defined using the newly claimed endometrial cancer codes (C55, C54, and D07). Benign endometrial condition was defined using the newly claimed diagnoses such as vaginal bleeding (N93 and N90), endometrial hyperplasia (N851 and N850), and polyp of endometrium (N840). Procedures including endometrial evaluation (aspiration, biopsy, and polypectomy) and dilatation and curettage (D&C) were identified using electronic data interchange codes. In-hospital mortality was assessed.

For landmark analysis, the index date was defined as the date 1 year after the initiation of endocrine therapy or the date of surgery if antihormonal medications were not prescribed.

Statistical Analysis

The incidence rates of endometrial cancer were compared between the TAM group and no TAM group. In the TAM subgroup stratified according to age at diagnosis (<40, 40–49, 50–59, and ≥60 years), the incidence of endometrial cancer and benign endometrial condition, total frequency of procedures, frequency of procedures per 1,000 person–years, and the ratio of the frequency of invasive diagnostic procedures to the incidence of endometrial cancer were calculated. Kaplan–Meier analysis and the log-rank test were used to assess the disease-free probability of patients with endometrial cancer and benign endometrial condition, and the procedure-free probability of the endometrial evaluation and D&C.

To analyze the risk of endometrial cancer and benign endometrial conditions, we used a Cox proportional hazards regression model adjusted for age at diagnosis, insurance, CCI, previous dyslipidemia, previous diabetes mellitus, previous hypertension, previous polycystic ovarian syndrome, chemotherapy, radiation, and trastuzumab.

Statistical analyses were conducted using R software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria) and SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). This study was approved by the Institutional Review Board of Asan Medical Center (IRB no. S2019-1702-0001).

RESULTS

Baseline Characteristics

A total of 60,545 breast cancer survivors were included in this analysis. The total person–years were 256,099, and the mean duration after cohort entry was 66 months. Of these patients, 27,034 (44.65%) received TAM, while 33,511 (55.35%) were not treated with TAM (**Table 1**). Of the total patients, 29,635 (48.9%) breast cancer survivors were aged below 50 years, and 7,519 (12.4%) young breast cancer survivors were aged below 40 years (**Supplementary Table 1**). The proportion of breast cancer survivors who received any type of chemotherapy was 60.74% (n = 36,776). During the study period, 68.66% (41,569) of these patients received radiation therapy. Trastuzumab was prescribed in 8,619 (14.23%) patients. All patients underwent breast cancer surgery.

Endometrial Cancer in Breast Cancer Survivors

Of the total study population, 140 (0.23%) patients developed endometrial cancer during the study period (**Table 1**). There were 98 (0.36%) and 42 (0.12%) endometrial cancer cases in the TAM group and non-TAM group, respectively.

In breast cancer survivors aged 40 or over, TAM significantly increased the risk of endometrial cancer (**Table 2** and **Figure 1**). The incidence of endometrial cancer in subjects ≥60 years who

TABLE 1 | Baseline characteristics of the study population.

	Tamoxifen (n = 27,034, 44.65%)		No tamoxifen (n = 33,511, 55.35%)		Total (n = 60,545, 100%)	
Age at diagnosis (years, mean ± SD)	45.79 ± 8.44		55.39 ± 10.78		51.11 ± 10.90	
Insurance						
Health insurance	26,567	98.27	32,616	97.33	59,183	97.75
Medicare	467	1.73	895	2.67	1,362	2.25
CCI (mean ± SD)	1.59 ± 1.57		2.34 ± 2.04		2.01 ± 1.88	
Previous diabetes mellitus	1,158	4.28	4,018	11.99	5,176	8.55
Previous hypertension	3,804	14.07	11,679	34.85	15,483	25.58
Previous dyslipidemia	3,625	13.40	11,864	35.40	15,489	25.58
Previous PCOS	118	0.44	59	0.18	177	0.29
Chemotherapy	16,045	59.35	20,731	61.86	36,776	60.74
Radiation	19,620	72.58	21,949	65.5	41,569	68.66
Trastuzumab	2,961	10.95	5,658	16.88	8,619	14.23
(Neo)adjuvant endocrine therapy						
None	0		17,521	52.28	17,521	28.94
Tamoxifen	26,374	97.56	0		26,374	43.56
Tamoxifen +AI	660	2.44	0		660	1.09
AI	0		15,990	47.71	15,990	26.41
Endometrial cancer*	98	0.36	42	0.12	140	0.23
Benign endometrial conditions*	7,254	26.83	2,888	8.61	10,142	16.75
In-hospital mortality	465	1.72	1,303	3.89	1,768	2.92
Duration after cohort entry (mean ± SD, month)	66.47 ± 20.34		65.68 ± 20.84		66.03 ± 20.62	

*1 year after the initiation of treatment.

SD, standard deviation; CCI, Charlson Comorbidity Index; PCOS, polycystic ovary syndrome; AI, Aromatase inhibitor.

were treated with TAM was the highest in all age subgroups [1.38 per 1,000 person–years (PY); hazard ratio (HR), 5.037; 95% confidence interval (CI), 2.185–11.613; P<0.001]. However, in subjects aged below 40 years, TAM did not significantly increase the risk of endometrial cancer, and the incidence rate of endometrial cancer was low (0.62 per 1,000 PY; HR, 2.048; 95% CI, 0.658–6.377; P = 0.216).

Benign Endometrial Conditions in Breast Cancer Survivors

TAM significantly increased the risk of benign endometrial conditions in all age subgroups (**Supplementary Table 2** and **Supplementary Figure 2**). The incidence of benign endometrial conditions in subjects under 40 years who were treated with TAM (88.60 per 1,000 PY) was the highest in all age subgroups. The incidence of benign endometrial conditions in subjects 60 years or more who were not treated with TAM (10.80 per 1,000 PY) was the lowest in all age subgroups.

Endometrial Cancer in Young Breast Cancer Survivors With Benign Endometrial Conditions

Among the TAM subgroups, benign endometrial conditions were significantly related to an increased risk of endometrial cancer in all age subgroups (**Table 3**). The incidence of endometrial cancer in subjects ≥60 years with benign endometrial conditions was the

TABLE 2 | Univariate analysis and multivariable Cox regression analysis of endometrial cancer risk related to tamoxifen by age at diagnosis.

Age	Tamoxifen	N	No. of events	Person–years	Incidence rate, per 1,000 person–years	p[a]	Crude HR (95% CI), p				Adjusted HR (95% CI)[b], p			
<40	No	2,613	4	11,695	0.34		1	(Reference)			1	(Reference)		
	Yes	4,906	13	20,895	0.62	0.300	1.904	0.620	5.851	0.261	2.048	0.658	6.377	0.216
40–49	No	6,053	10	26,577	0.38		1	(Reference)			1	(Reference)		
	Yes	16,063	55	66,919	0.82	0.020	2.187	1.114	4.291	0.023	2.121	1.068	4.213	0.032
50–59	No	13,739	15	58,315	0.26		1	(Reference)			1	(Reference)		
	Yes	4,351	20	18,038	1.11	<0.001	4.308	2.205	8.415	<0.001	4.343	2.122	8.891	<0.001
60≤	No	11,106	13	46,405	0.28		1	(Reference)			1	(Reference)		
	Yes	1,714	10	7,255	1.38	<0.001	4.876	2.138	11.120	<0.001	5.037	2.185	11.613	<0.001

[a]*Log-rank test.*

[b]*Adjusted for age at diagnosis (continuous), insurance (health insurance, Medicare), Charlson comorbidity index (continuous), previous hypertension (yes or no), previous diabetes mellitus (yes or no), previous dyslipidemia (yes or no), previous polycystic ovarian syndrome (yes or no), chemotherapy (yes or no), radiation (yes or no), and trastuzumab (yes or no).*

CI, confidence interval; HR, hazard ratio.

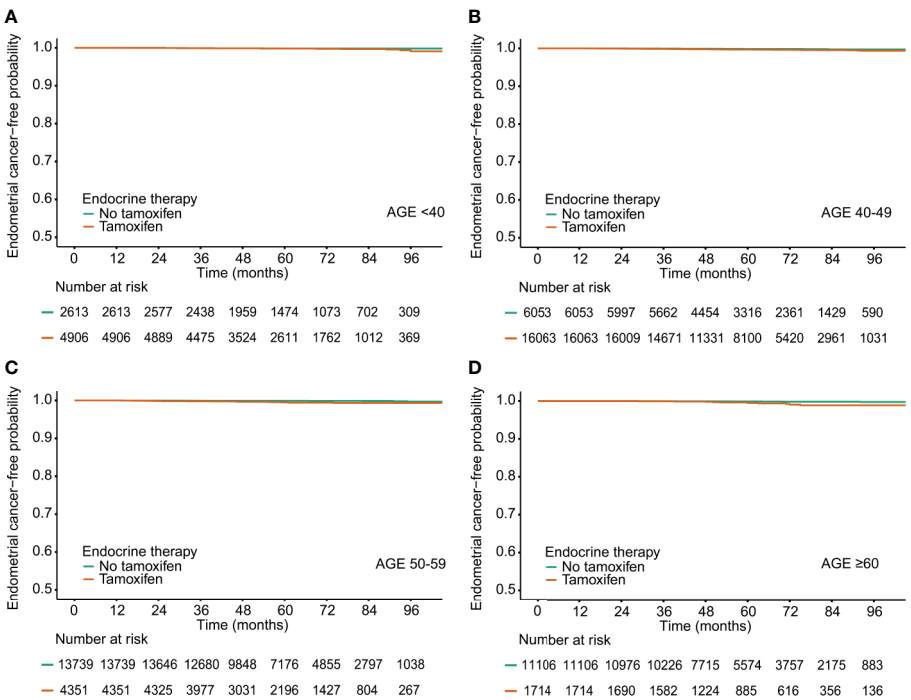

FIGURE 1 | Endometrial cancer-free probability in breast cancer survivors by tamoxifen and age at diagnosis.**(A)** Age<40, **(B)** Age 40-49, **(C)** Age 50-59, **(D)** Age <60.

TABLE 3 | Univariate analysis and multivariable Cox regression analysis of endometrial cancer risk related to benign endometrial condition in patients treated with tamoxifen.

Age	Benign endometrial condition	N	No. of events	Person–years	Incidence rate, per 1,000 person–years	p[a]	Crude HR (95% CI)			Adjusted HR (95% CI)[b]		
<40	No	3,396	2	14,049	0.14		1	(Reference)		1	(Reference)	
	Yes	1,510	11	6,846	1.61	<0.001	10.810	2.393 48.830	0.002	12.460	2.698 57.522	0.001
40–49	No	11,587	11	47,139	0.23		1	(Reference)		1	(Reference)	
	Yes	4,476	44	19,780	2.22	<0.001	9.543	4.927 18.450	<0.001	9.667	4.966 18.819	<0.001
50–59	No	3,306	5	13,449	0.37		1	(Reference)		1	(Reference)	
	Yes	1,045	15	4,589	3.27	<0.001	8.817	3.203 24.270	<0.001	8.815	3.179 24.444	<0.001
60≤	No	1,339	0	5,586	0.00		1	(Reference)		1	(Reference)	
	Yes	375	10	1,669	5.99	<0.001		NA			NA	

[a]*Log-rank test.*

[b]*Adjusted for age at diagnosis (continuous), insurance (health insurance, Medicare), Charlson comorbidity index (continuous), previous hypertension (yes or no), previous diabetes mellitus (yes or no), previous dyslipidemia (yes or no), previous polycystic ovarian syndrome (yes or no), chemotherapy (yes or no), radiation (yes or no), and trastuzumab (yes or no).*

CI, confidence interval; HR, hazard ratio.

highest in all age subgroups (5.99 per 1,000 PY). In subjects without benign endometrial conditions, the incidence of endometrial cancer was low.

In younger breast cancer survivors aged under 40 years (HR, 12.460; 95% CI, 2.698–57.522; $P = 0.001$) and 40–49 years (HR, 9.667; 95% CI, 4.966–18.819; $P < 0.001$), benign endometrial conditions significantly increased the risk of endometrial cancer, although the actual incidence rates of endometrial cancer were lower than those of their older counterparts (1.61 per 1,000 PY in subjects under 40 years; 2.22 per 1,000 PY in subjects aged 40–49 years).

Frequencies of Invasive Endometrial Procedures in Breast Cancer Survivors

The frequencies of invasive endometrial procedures were higher in the TAM groups than those in the non-TAM subgroups (**Table 4** and **Figure 2**). Among the TAM group aged 60 years or more, invasive endometrial evaluations and D&C were performed about 18 and 23 times to detect one endometrial cancer, respectively. However, in the TAM group aged below 40 years, invasive endometrial evaluations and D&C were conducted more than 46 and 54 times to find one endometrial cancer, respectively.

Among breast cancer survivors treated with TAM, the rates of invasive endometrial procedures were higher in subjects with benign endometrial conditions (**Supplementary Table 3**). Among the TAM-treated subjects with benign endometrial conditions, the ratios of the frequency of invasive diagnostic procedures to the incidence of endometrial cancer were 49.0 (endometrial evaluation) and 59.5 (D&C) in subjects under 40 years. However, in subjects aged 60 or more, the ratios were 14.6 (endometrial evaluation) and 21.2 (D&C), respectively.

DISCUSSION

This study demonstrated that the risk of endometrial cancer increased in young breast cancer survivors on TAM. Even young breast cancer survivors aged below 40 years who had TAM-related benign endometrial conditions such as vaginal bleeding, endometrial hyperplasia, and endometrial polyp showed a significantly increased risk of endometrial cancer compared with those without benign endometrial conditions. Although the incidence rates of endometrial cancer in younger breast cancer survivors were lower than those of their older counterparts; the frequencies of invasive endometrial procedures were higher in younger breast cancer survivors than in their older counterparts.

To our knowledge, this is the first nationwide study to demonstrate the increased risk of endometrial cancer in young breast cancer survivors treated with TAM. Previous studies were limited by the fact that the sample size was not enough to assess the endometrial cancer risk in premenopausal breast cancer survivors on TAM treatment. The National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 study, where the current guidelines are based, indicated that treatment with TAM did not increase the endometrial cancer risk in premenopausal women and that additional monitoring was not required (10). However, this study enrolled 5,077 patients aged below 50 years (2,596 in the placebo group and 2,581 in the TAM group), and the number of participants aged below 40 years was 244 (185 in the placebo group and 159 in the TAM group). Of the total sample aged below 50 years, 17 (eight in the placebo group and nine in the TAM group) patients developed endometrial cancer. Treatment with TAM did not significantly increase the risk of endometrial cancer in women aged below 50 years (risk ratio, 1.21; 95% CI, 0.41–3.60). From the NASBP B-14 study, the incidence of endometrial cancer in TAM-treated breast cancer survivors were investigated (1). This study enrolled 2,843 participations (1,424 in the placebo group and 1,419 in the TAM group), and only 62 patients were aged below 50 years. A total of 15 patients (zero in the placebo group and 15 in the TAM group) developed endometrial cancer; of them, only one patient in the TAM group was aged below 50 years. These results suggest that the previous studies may underestimate the risk of endometrial cancer in TAM-treated premenopausal breast cancer patients. In our study, 29,635 breast cancer survivors aged below 50 years were included, and 68 of them developed endometrial cancer. Meanwhile, 7,519 young breast cancer survivors aged below 40 years were investigated.

TABLE 4 | Frequencies of endometrial procedures in breast cancer survivors 1 year after initiation of treatment.

	N	Endometrial evaluation				Dilatation and curettage			
		Patients, no. (%)	Procedures, no.	Procedure rate (per 1,000 person–years)	Ratio[a]	Patients, no. (%)	Procedures, no.	Procedure rate (per 1,000 person–years)	Ratio[b]
Tamoxifen									
<40	4,906	492 10.0	606	29.0	46.6	557 11.4	707	33.8	54.4
40–49	16,063	1,592 9.91	2,021	30.2	36.7	1,799 11.2	2,269	33.9	41.3
50–59	4,351	399 9.17	505	28.0	25.2	465 10.7	581	32.2	29.0
60≤	1,714	144 8.40	179	24.7	17.9	184 10.7	234	32.0	23.4
No tamoxifen									
<40	2,613	128 4.90	154	13.20	38.50	111 4.25	141	12.10	35.20
40–49	6,053	230 3.80	290	10.90	29.00	232 3.83	279	10.50	27.90
50–59	13,739	187 1.36	215	3.69	14.30	190 1.38	218	3.74	14.50
60≤	11,106	124 1.12	153	3.30	11.80	157 1.41	178	3.84	13.70

[a]*Ratio = the rate of endometrial evaluation/the rate of endometrial cancer.*
[b]*Ratio = the rate of dilation and curettage/the rate of endometrial cancer.*

Because the current international guidelines are based on the previous studies, gynecological assessment is not recommended for premenopausal women taking TAM. According to the American Cancer Society/American Society of Clinical Oncology Survivorship Care Guidelines, clinicians should perform annual gynecological assessments in postmenopausal women taking TAM, and patients should inform their physicians if unexpected bleeding occurs (11). According to the 2014 American College of Obstetricians and Gynecologists guidelines, postmenopausal women taking TAM should monitor for symptoms of endometrial disease or cancer (9). In the European Society for Medical Oncology guidelines for postmenopausal breast cancer survivors, appropriate diagnostic tests are recommended to be carried out in those with symptoms of endometrial hyperplasia (15).

In our study, young breast cancer survivors aged below 50 years who had TAM-related endometrial diseases or symptoms showed a significantly increased risk of endometrial cancer. This suggests that gynecological assessments of endometrial cancer in these young breast cancer survivors on higher risk of developing endometrial cancer should be considered. However, the actual incidence rates of endometrial cancer in younger breast cancer survivors (1.61 per 1,000 PY in subjects under 40 years; 2.22 per 1,000 PY in subjects aged 40–49 years) were lower than those of their older counterparts (3.27 per 1,000 PY in subjects in their 50s; 5.99 per 1,000 PY in subjects ≥60 years), and therefore balanced decisions on screening for endometrial cancer should be made.

The guidelines indicated that more attention should be paid to the symptoms related to endometrial hyperplasia. In patients who did not develop any adverse effects after taking TAM, initial screening and regular Papanicolaou smear examinations are usually recommended because the benefits of routine endometrial surveillance in asymptomatic patients on TAM therapy remain unknown. There are no clear guidelines on which gynecological examination should be performed (16, 17). In previous studies, endometrial biopsy or transvaginal ultrasound was not used to screen asymptomatic women receiving TAM (18, 19). Although another previous study reported that there was no significant increase in the incidence of endometrial cancer in young breast cancer patients aged below 40 years, the study did not analyze the presence or absence of benign endometrial symptoms and disease (20). In our study, the risk of endometrial cancer increased only in young breast cancer survivors with TAM-related endometrial benign disease. Hence, it may be appropriate to assess for signs of endometrial hyperplasia like abnormal bleeding, including spotting and abnormal vaginal discharge in young breast cancer survivors on TAM.

In a large-scale randomized controlled trial of female breast cancer survivors, treatment with TAM for 10 years further reduced the recurrence and mortality rates, particularly after 10 years, compared with discontinuing this treatment after 5 years (21, 22). Extended adjuvant TAM therapy is associated with an increase in the risk of endometrial cancer (23). The longer the TAM treatment period is, the more important endometrial cancer screening through gynecological examination can become. Young breast cancer survivors undergoing extended endocrine therapy with TAM up to 10 years should be informed of the possible risk of developing endometrial cancer and to monitor for symptoms related to benign endometrial disease. TAM administered at dose of 5 mg/d for 3 years can reduce the recurrence of breast cancer by 50% with a limited toxicity and may be used as a new treatment option for these patients (24).

To the best of our knowledge, the frequencies of gynecological diagnostic procedures among young breast cancer survivors in the real-world setting have rarely been reported (25). Invasive procedure can cause harm to these young patients. In this study, young breast cancer survivors with TAM-related endometrial conditions showed a significantly increased risk of endometrial cancer. However, the ratios of endometrial evaluation and D&C to the incidence of endometrial cancer in breast cancer survivors aged below 50 years were higher than those in breast cancer survivors aged 50 years and older. Considering the actual incidence of endometrial cancer by age, these invasive procedures should be cautiously opted to avoid causing unnecessary harm.

This study has some limitations. First, the HIRA data lacked information on laboratory examinations, imaging studies, family

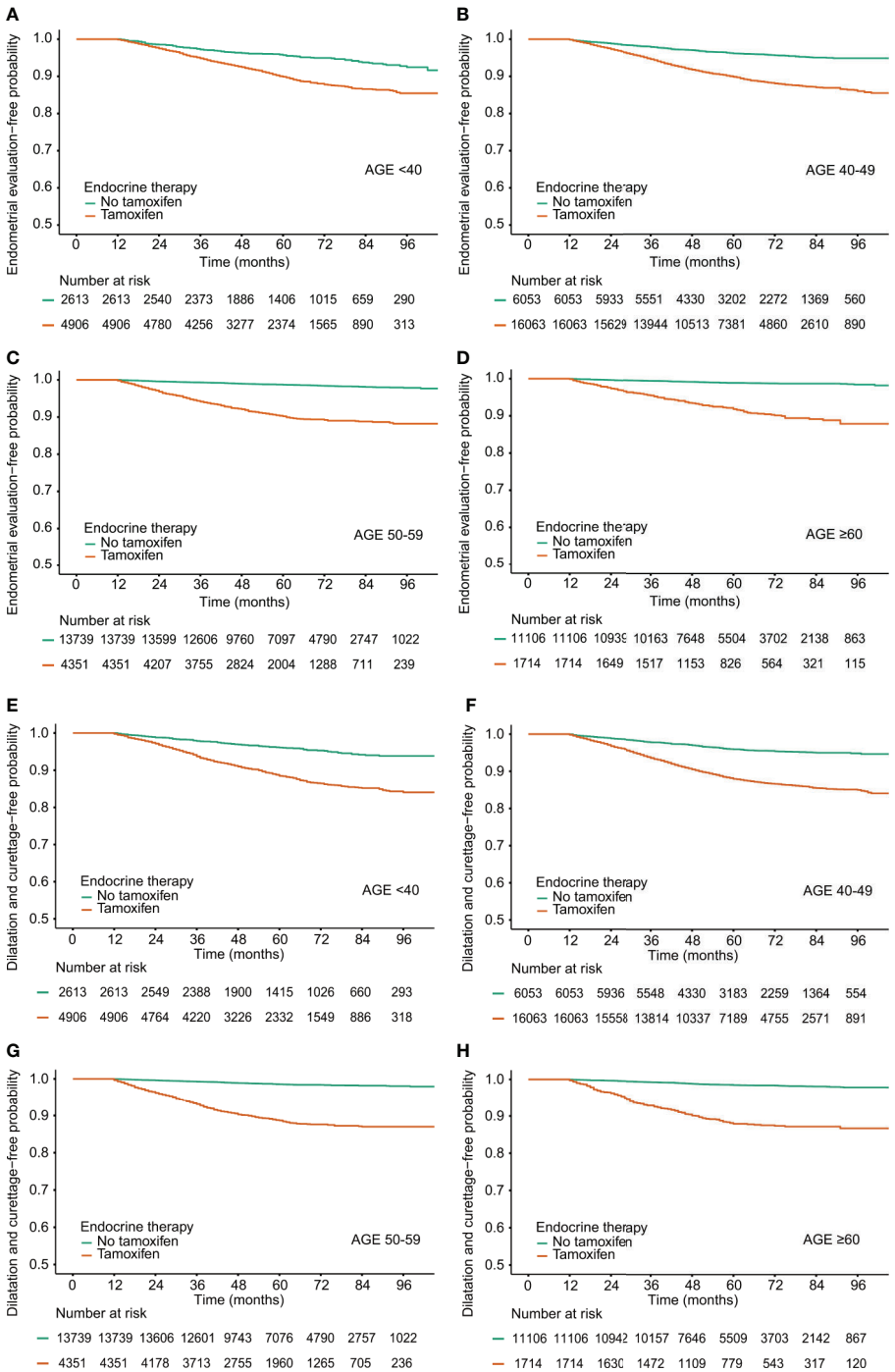

FIGURE 2 | Procedure-free probability in breast cancer survivors by tamoxifen and age at diagnosis. **(A–D)** Endometrial evaluation, **(E–H)** Dilatation and Curettage.

history, and pathological outcomes such as cancer stage, hormone receptor status, and HER2 overexpression. Second, patients' adherence to individual treatments could not be specified. Third, vaginal ultrasound exams were not able to be analyzed because information about ultrasound is not archived in the HIRA database. Fourth, clinical results such as recurrence, metastasis, or the cause of death were not available. Only in-hospital mortality was assessed. Lastly, although pregnancy is known to reduce the risk of endometrial cancer, the multivariate analyses in this study were not adjusted by this factor due to insufficient data.

In conclusion, young breast cancer survivors with TAM-related endometrial benign disease are at a higher risk of

developing endometrial cancer. Clinicians should assess for benign endometrial conditions in premenopausal breast cancer survivors who are taking TAM. Gynecological surveillance should be tailored to the risk of endometrial cancer in young breast cancer survivors to improve the early detection of endometrial cancer and avoid unnecessary invasive procedures.

DATA AVAILABILITY STATEMENT

The datasets generated for this study is not publicly available. These datasets are from the Korean national database, and are not allowed to be extracted from the server by laws. Requests to access the datasets should be directed to the Health Insurance Review and Assessment Service.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Asan Medical Center (IRB no. S2019-1702-0001). Written informed consent from the participants' legal guardian/next of kin was not required in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SC: planned, wrote, and revised the article. JHJ, YJL, JJ, JL, HK, BK, BS, and SA reviewed and edited the article. YL: planned, analyzed the data, and performed the statistics. IC: planned, wrote, revised, supervised, and designed the concept of the article. All authors contributed to the article and approved the submitted version.

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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.2021.636378/full#supplementary-material

Supplementary Figure 1 | Study population.

Supplementary Figure 2 | Benign endometrial condition-free probability in breast cancer survivors by tamoxifen and age at diagnosis.

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The PREgnancy and FERtility (PREFER) Study Investigating the Need for Ovarian Function and/or Fertility Preservation Strategies in Premenopausal Women With Early Breast Cancer

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Background: Offering ovarian function and/or fertility preservation strategies in premenopausal women with newly diagnosed breast cancer candidates to undergo chemotherapy is standard of care. However, few data are available on uptake and main reasons for refusing these options.

Methods: The PREFER study (NCT02895165) is an observational, prospective study enrolling premenopausal women with early breast cancer, aged between 18 and 45 years, candidates to receive (neo)adjuvant chemotherapy. Primary objective is to collect information on acceptance rates and reasons for refusal of the proposed strategies for ovarian function and/or fertility preservation available in Italy.

Results: At the study coordinating center, 223 patients were recruited between November 2012 and December 2020. Median age was 38 years (range 24 – 45 years) with 159 patients (71.3%) diagnosed at ≤40 years. Temporary ovarian suppression with gonadotropin-releasing hormone agonists (GnRHa) was accepted by 58 out of 64 (90.6%) patients aged 41-45 years and by 151 out of 159 (95.0%) of those aged ≤40 years. Among patients aged ≤40 years, 57 (35.8%) accepted to access the fertility unit to receive a complete oncofertility counseling and 29 (18.2%) accepted to undergo a cryopreservation technique. Main reasons for refusal were fear of delaying the initiation of antineoplastic treatments and contraindications to the procedure or lack of interest in future childbearing. Patients with hormone-receptor positive breast cancer had a

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tendency for a higher acceptance rates of ovarian function and/or fertility preservation strategies than those with hormone-receptor negative disease.

Conclusions: More than 90% of premenopausal women with early breast cancer, and particularly those with hormone receptor-positive disease, were concerned about the potential risk of chemotherapy-induced premature ovarian insufficiency and/or infertility and accepted GnRHa administration. Less than 1 out of 5 women aged ≤40 years accepted to undergo cryopreservation strategies.

Keywords: breast cancer, premenopausal patients, premature ovarian insufficiency, fertility preservation, gonadotoxicity

INTRODUCTION

Among women of reproductive age, breast cancer is the most frequent diagnosed malignancy (1). Chemotherapy still remains an important component of the care of many premenopausal breast cancer patients also taking into account their higher risk of developing more aggressive breast cancer subtypes (2, 3). The long-term side effects of chemotherapy including the potential damage to women's ovarian function and fertility potential are of high concern for a significant proportion of women diagnosed during their reproductive age (4, 5). Two main approaches are available for trying to counteract the long-term side effects of chemotherapy on breast cancer patients' reproductive health (6). Firstly, ovarian function preservation aims to reduce the potential long-term side effects of chemotherapy-induced premature ovarian insufficiency (POI) that include menopause-related symptoms, psychosocial issues and other health problems (4). This approach can be of importance also to patients not interested in future conception. Secondly, fertility preservation aims to increase the chances of achieving a post-treatment pregnancy in patients willing to complete their family plan after breast cancer treatment (7–10).

Current guidelines recommend to perform a complete oncofertility counseling to all premenopausal women at the time of cancer diagnosis (7–10). During this counseling, the potential risk of chemotherapy-induced POI and subsequent possible impaired ovarian function and fertility should be discussed, and patients interested in avoiding these side effects are offered the available strategies for preserving ovarian function and/or fertility (7–10). In premenopausal breast cancer patients, temporary ovarian suppression with gonadotropin-releasing hormone agonists (GnRHa) during chemotherapy is considered as standard strategy for ovarian function preservation (7–10). Available strategies for fertility preservation include cryopreservation of embryos, oocytes and/or ovarian tissue to be preferably proposed to women diagnosed at ≤40 years (≤36 years for ovarian tissue cryopreservation) considering the low success rate in older patients (7–10). Despite the widespread use of these strategies among breast cancer patients, few data are available on the uptake and on the main reasons for refusal of these options.

The prospective PREgnancy and FERtility (PREFER) study aims to investigate the actual needs and preferences of patients regarding the proposed options for ovarian function and/or fertility preservation available in Italy (11). Previous results of the PREFER study indicated that a significant proportion of young women with newly diagnosed breast cancer are concerned about the possible risk of chemotherapy-induced POI and/or infertility but only 12% decide to undergo the proposed cryopreservation procedures (12). Here, we present updated results from the PREFER study.

METHODS

Study Design and Participants

Details of the PREFER study design and methods were previously reported (11, 12). Briefly, this is an ongoing multicenter prospective observational study aiming to optimize care and improve knowledge on ovarian function and/or fertility preservation in premenopausal women with early breast cancer.

The study includes premenopausal women with early breast cancer aged between 18 and 45 years who are candidates to undergo (neo)adjuvant chemotherapy. Exclusion criteria are *de novo* metastatic disease, prior exposure to chemotherapy and/or radiation therapy and severe psychiatric disorders.

In the present analysis, we updated previously reported data of patients included at the coordinating center (12). Moreover, further analyses were conducted to explore the impact of breast cancer hormone-receptor status on patients' choices. In addition, preliminary efficacy results of cryopreservation strategies in terms of number of retrieved and cryopreserved oocytes and response rate to controlled ovarian stimulation are reported.

Due to the slow opening of the other Italian participating centers, we believe that the current updated analysis can provide additional important information before the possibility to analyze the data from all centers, expected to occur in 2 years from now.

All patients provided a written informed consent before study entry. The study was approved by the Ethics Committee of the coordinating center in November 2012.

The study is registered on ClinicalTrials.gov (NCT02895165).

The PREFER Algorithm for the Oncofertility Counseling

A specific algorithm was developed to implement a proper oncofertility counseling (11, 12) (**Figure 1**). Briefly, the risk of chemotherapy-induced POI and/or infertility and the available strategies for ovarian function and/or fertility preservation are

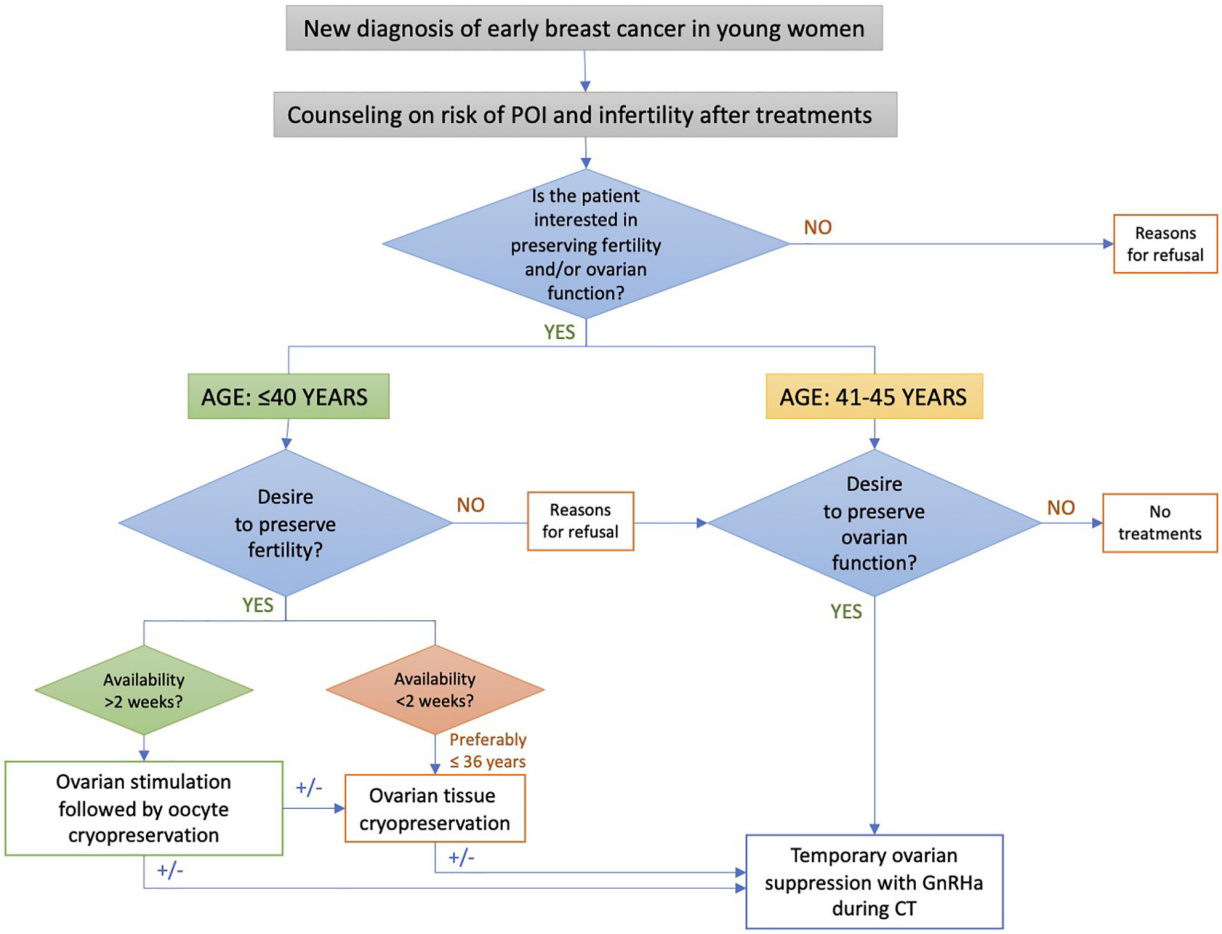

FIGURE 1 | Oncofertility counseling algorithm for patients enrolled in the PREFER study. POI, premature ovarian insufficiency; GnRHa, gonadotropin-releasing hormone agonist.

discussed by the oncologist with all premenopausal patients as soon as possible after diagnosis and before starting any systemic anti-cancer treatment. For patients diagnosed at ≤40 years and interested in fertility preservation, a complete oncofertility counseling with a fertility specialist is offered and both oocyte and/or ovarian tissue cryopreservation are proposed according to the time available before anticancer treatment initiation. Notably, in Italy, embryo cryopreservation is prohibited by law in these patients. After cryopreservation techniques are performed, temporary ovarian suppression with GnRHa during chemotherapy is offered. Whereas, for patient diagnosed between 41 and 45 years and interested in ovarian function preservation, only temporary ovarian suppression with GnRHa during chemotherapy is offered. Cryopreservation strategies are not proposed in this age group due to their low success rate in breast cancer patients older than 40 years (13).

Study Objectives and Statistical Analysis

Primary objective of the PREFER study is to assess patients' preferences and choices of the different available strategies for ovarian function and/or fertility preservation, in terms of acceptance rate, and to collect the reasons for refusal of the proposed strategies. To investigate the efficacy of cryopreservation strategies in patients aged ≤40 years at diagnosis is a secondary objective. Potential differences in acceptance rates of ovarian function and/or fertility preservation strategies according to hormone-receptor status were explored.

Patient, tumor and treatment characteristics, types of strategies for ovarian function and/or fertility preservation offered and accepted by patients, and reasons for refusal are prospectively collected in electronic case report forms. Statistical analyses are mainly descriptive. Means and standard deviations were used to summarize continuous variables, whereas counts and percentages were used for categorical variables. Statistical analyses were performed using SAS 9.2 (SAS Institute).

RESULTS

From November 2012 to December 2020, 223 consecutive newly diagnosed premenopausal breast cancer patients were included at the Breast Unit of the coordinating center.

Baseline characteristics are reported in **Table 1**. Median age at study entry was 38 years (range 24-45). A total of 64 (28.7%) women were diagnosed between the age of 41 and 45 years and 159 (71.3%) at ≤40 years. At the time of breast cancer diagnosis, 150 (67.3%) patients had at least one child.

Overall, the majority of patients (209, 93.7%) was concerned about the potential risk of developing chemotherapy-induced POI and/or infertility (**Figure 2**). Specifically, 58 (90.6%) patients aged between 41 and 45 years and 151 (95.0%) aged ≤40 years were sensitive to these issues (**Figure 2**). For the 14 (6.3%) patients not concerned about potential risk of developing chemotherapy-induced POI and/or infertility, main reasons were lack of interest in ovarian function preservation in 10 patients (71.4%), prior completion of family planning in 3 (21.4%), and lack of interest in future childbearing in 1 (7.1%).

TABLE 1 | Baseline characteristics of the patients included in the PREFER study.

Characteristics	Total cohort N = 223 No. (%)
Age, median (range), years	38 (24.0-45.0)
Age distribution, characteristics	
≤ 40	159 (71.3)
41 - 45	64 (28.7)
Previous pregnancy	150 (67.3)
Number of previous pregnancies, median (range)	2 (1-6)
Partner at breast cancer diagnosis	
Present with stable relationship	158 (70.9)
No partner present	58 (26.0)
Other	7 (3.1)
Tumor size	
≤ 2 cm	101 (45.3)
> 2 cm	121 (54.3)
Unknown	1 (0.5)
Nodal status	
Node negative	96 (43.1)
Node positive	122 (54.7)
Unknown	5 (2.2)
Hormone receptor status	
ER-positive and/or PgR-positive	173 (77.6)
ER-negative and PgR-negative	50 (22.4)
HER2 status	
Positive	76 (34.1)
Negative	147 (65.9)
Timing of chemotherapy	
Adjuvant	129 (57.9)
Neoadjuvant	92 (41.3)
Missing	2 (0.9)
Type of chemotherapy	
Anthracycline- and taxane-based	184 (82.5)
Others	37 (16.1)
Type of endocrine therapy*	
Tamoxifen ± GnRHa	59 (34.1)
Aromatase inhibitor + GnRHa	77 (44.5)
Tamoxifen ± GnRHa → Aromatase inhibitor + GnRHa	26 (15.0)
No endocrine therapy	1 (0.6)
Chemotherapy ongoing	7 (4.0)
Missing	1 (0.6)

Percentages calculated on the total number of patients with hormone receptor positive disease (n = 173).

ER, estrogen receptor; PgR, progesterone receptor; GnRHa, gonadotropin-releasing hormone agonist.

Among the 64 patients diagnosed between 41 and 45 years of age, 58 (90.6%) were concerned about the possible chemotherapy-induced POI and accepted the use of temporary ovarian suppression with GnRHa during chemotherapy as a strategy for ovarian function preservation (**Figure 3**). Two patients diagnosed between 41 and 45 years underwent a complete reproductive counseling with the fertility specialists because of a strong pregnancy desire; however, none of them underwent a cryopreservation strategy. When assessing acceptance rates by hormone receptor status, active steps towards the offered strategy for ovarian function preservation with GnRHa administration were perused by 45 out of 49 (91.8%) women with hormone receptor-positive breast cancer and by 13 out of 15 (86.7%) with hormone receptor-negative disease (**Figure 4**).

Among the 159 patients aged ≤40 years at diagnosis, a complete reproductive counseling conducted at the fertility unit was accepted by 55 (34.6%) women. Among the 94 patients that refused to undergo a complete reproductive counseling, main reasons were previous completion of family planning in 63 (67.0%), concerns about a possible delay in cancer treatment in 11 (11.7%), lack of interest in future childbearing in 10 (10.6%) and oncological ineligibility for cryopreservation procedures in 5 (5.3%), unknown reason in 4 (4.3%) and availability of cryopreserved oocytes before breast cancer diagnosis in 1 (1.1%). Among the 55 patients that underwent a complete reproductive counseling by the fertility specialists, 5 (9.1%) were deemed medically ineligible by the fertility specialist to a cryopreservation technique mainly due to low ovarian reserve or high risk of complications, 21 (38.2%) refused the proposed cryopreservation strategies and 29 (52.7%) accepted to receive at least one of cryopreservation option. Specifically, among the 29 patients that accepted fertility preservation procedures, 24 underwent oocyte cryopreservation, 4 ovarian tissue cryopreservation, and one both oocyte and ovarian tissue cryopreservation. Among the 25 patients that underwent oocyte cryopreservation, median number of retrieved oocytes was 12 (range 0-42) and median number of cryopreserved oocytes was 9 (range 0-24). Poor response rate (i.e. retrieval of ≤4 oocytes) was observed in 3 out of 25 (12%) patients.

Among the 21 patients that refused the proposed cryopreservation strategies, main reasons were fear of delaying the initiation of antineoplastic treatments for 6 (28.8%), refusal of further medicalization after complete counseling (23.8%), lack of interest in the procedure after complete counseling for 6 (28.8%), and lack of support from a partner for 2 (9.5%), prior completion of family planning for 2 (9.5%).

Overall, among women aged ≤40 years at diagnosis, 151 (95.0%) took active steps towards the offered strategy for ovarian function and/or fertility preservation. The use of temporary ovarian suppression with GnRHa during chemotherapy was accepted by 122 (76.7%) women, while the use of cryopreservation strategies (followed by temporary ovarian suppression with GnRHa during chemotherapy) was accepted by 29 (18.2%) patients (**Figure 3**).

When assessing acceptance rates by hormone receptor status, active steps towards the offered strategy for ovarian function and/

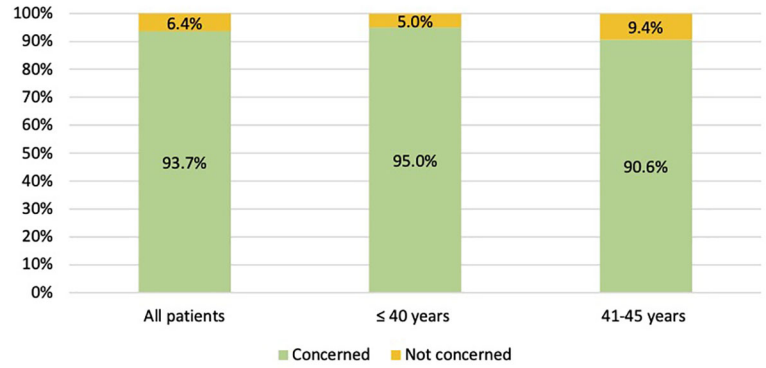

FIGURE 2 | Number of patients concerned about the potential risk of chemotherapy-induced premature ovarian insufficiency AND/OR subsequent impaired fertility.

or fertility preservation were pursued by 119 out of 124 (96%) women with hormone receptor-positive breast cancer and by 32 out of 35 (91.4%) with hormone receptor-negative breast cancer. In particular, the use of temporary ovarian suppression with GnRHa during chemotherapy was accepted by 119 (96%) women with hormone receptor-positive breast cancer and by 32 (91.4%) with hormone receptor-negative disease. Cryopreservation strategies were accepted by 24 (19.4%) women with hormone receptor-positive breast cancer and by 5 (14.3%) with hormone receptor-negative disease (**Figure 5**).

DISCUSSION

Although performing a complete oncofertility counseling to discuss the potential risk of chemotherapy-induced POI and infertility and to offer the available strategies for ovarian function and/or fertility preservation is mandatory in all premenopausal women with new cancer diagnosis (7–10), limited evidence exists on the actual use of these techniques (14). The PREFER study was designed to overcome this knowledge gap. This is important information to acquire for improving the oncofertility care and resource allocation in this area. Updated results of the PREFER study shows that the possibility of developing POI and/or subsequent impaired fertility worried most of premenopausal patients with breast cancer (93.7%). All patients concerned about the risk of developing POI accepted GnRHa use during chemotherapy as an option to preserve ovarian function. Among young women aged ≤40 years at diagnosis, approximately one out of 3 (34.6%) was interested in accessing the fertility unit but less than 1 out of 5 (18.2%) decided to undergo one or more of the offered cryopreservation options. The main reasons for refusal were prior completion of family planning, fear of delaying the initiation of antineoplastic treatments, refusal of further medicalization after complete counseling and lack of interest in the procedure after complete reproductive counseling.

An important issue in premenopausal patients facing breast cancer diagnosis and treatment is represented by the development of chemotherapy-induced POI with its subsequent infertility but also menopause-related consequences that include vasomotor symptoms, sexual dysfunction, body image chance, bone loss,

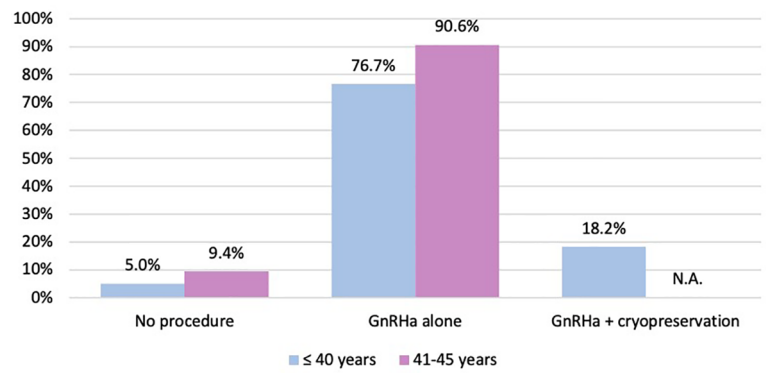

FIGURE 3 | Number of patients who took active steps towards the offered strategies for ovarian function and/or fertility preservation. GnRHa, gonadotropin-releasing hormone agonist; NA, not applicable.

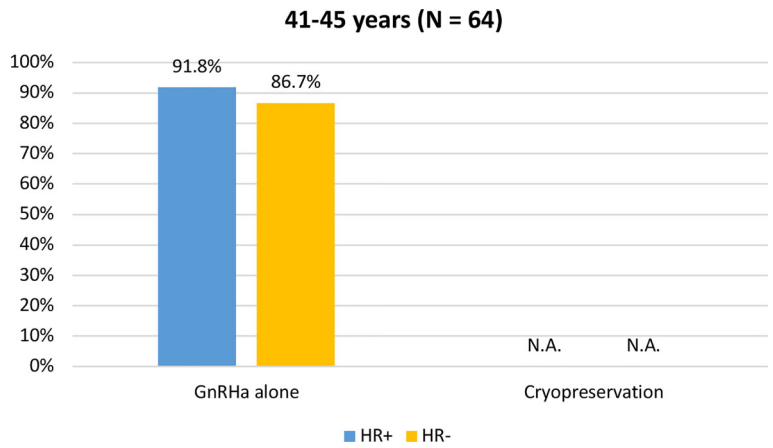

FIGURE 4 | Acceptance rate of the offered strategies for ovarian function preservation according to hormonal receptor status in patients diagnosed between 41 and 45 years of age. GnRHa, gonadotropin-releasing hormone agonist; NA, not applicable.

cardiovascular risk and psychosocial issues (4). Therefore, informing premenopausal breast cancer patients about the risk of chemotherapy-induced POI is independent of future pregnancy desire (15, 16). The PREFER study aims to give important information also on this regard. Hence, unlike other studies that recruited only patients diagnosed at ≤40 years, our study allowed the inclusion of patients diagnosed between the age of 41 and 45 years to whom ovarian suppression with GnRHa is currently recommended as a standard strategy for reducing the risk of developing POI (15, 16).

In the PREFER study almost all patients (91% and 95% for patients diagnosed between 41 and 45 years and at ≤40 years, respectively) accepted the use of GnRHa during chemotherapy as a strategy to preserve ovarian function. A lower percentage of acceptance was demonstrated among the women enrolled in the American HOHO study (3.1% of patients aged ≤40 years) (17), and a slightly higher percentage in those included in the European HOHO study (24% of patients aged ≤40 years) (16). This difference can be partially explained by the publication, and subsequent recommendation from Italian guidelines (15), of the results of the Italian PROMISE-GIM6 study (18, 19) available since 2011. PROMISE-GIM6 study is the largest multicenter randomized study evaluating the efficacy and safety of GnRHa use during chemotherapy in premenopausal patients (aged less than 45 years) (18, 19). Moreover, another possible explanation for this difference is that the treatment with GnRHa during chemotherapy is reimbursed by the Italian National Health System (15). Notably, ovarian suppression with GnRHa is standard strategy for ovarian function preservation but it does not represent an alternative to cryopreservation techniques in young women interested in fertility preservation (7, 8, 10, 20). Premenopausal women with hormone receptor-positive breast

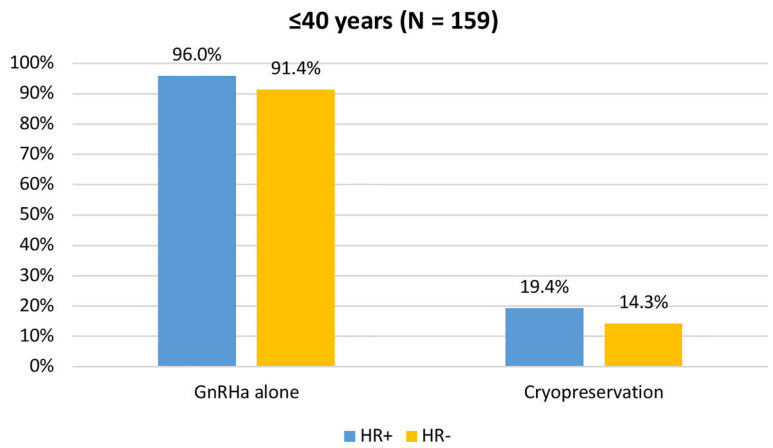

FIGURE 5 | Acceptance rate of the offered strategies for ovarian function and/or fertility preservation according to hormonal receptor status in patients diagnosed at ≤40 years of age. GnRHa, gonadotropin-releasing hormone agonist.

cancer are candidate to adjuvant hormonal therapy for at least 5 years (7, 21, 22). In women at increased risk of disease recurrence including those exposed to prior chemotherapy use, ovarian function suppression with an aromatase inhibitor showed to be superior to either tamoxifen alone or tamoxifen combined with ovarian function suppression (23, 24). In premenopausal women developing chemotherapy-induced amenorrhea, administering an aromatase inhibitor alone may increase the risk of ovarian function recovery (25). Thus, starting GnRHa before chemotherapy could avoid the issues of defining ovarian function assessment following chemotherapy and the choice of the best endocrine therapy partner in this setting (24).

Besides the need for ovarian function preservation, young women with breast cancer may not have completed their family building plans at the time of diagnosis and might be interested in fertility preservation. Cryopreservation strategies are standard fertility preservation strategies (7–10). Preferably, ovarian tissue cryopreservation is proposed to women younger than 36 years, while oocyte and embryo cryopreservation is indicated up to the age of 40 years (9). Several barriers exist in discussing these options, including patient-related factors, cost of the strategies, lack of collaboration with a fertility unit, physicians' inadequate knowledge of the different available strategies, or their concerns about the safety of pregnancy following breast cancer treatment (26). Although implementing a proper oncofertility program is crucial, our results highlight that only a minority of patients (18.2%) are finally motivated to undergo cryopreservation options. This is in line with other studies in both the US and Europe showing that, despite important concerns related to the development of this side effect, less than 10% of patients decide to undergo cryopreservation techniques (12, 16, 17). With a growing availability of efficacy and safety data, improved knowledge and financial coverage of these strategies, it is expected that the acceptance of such strategies will increase in the future (27–30). In fact, we found a higher percentage of patients accepting a cryopreservation technique (18.2%) compared to the previous analysis where only 12% of the patients accepted these surgical procedures (12). Nevertheless, the low number of patients undergoing cryopreservation strategies should be considered to improve the care in this setting. The creation of a solid oncofertility network with a hub and spoke regional distribution would be desirable (9, 31, 32). Thus, patients, from different oncology units that are interested in cryopreservation techniques can be referred to a smaller number of highly specialized fertility units in order to better optimize the access and success to these procedures. The collaborative network between oncology units and fertility centers might be useful also for counseling patients following anticancer treatment completion not only on pregnancy and conception but also about other reproductive issues including contraception and management of gynecological side effects of anticancer treatments (33). For achieving this goal, it is essential to establish a collaborative network between oncology units and fertility centers. Because of the many barriers existing in discussing ovarian and fertility preservation and building such network, we decided to extend the PREFER program to other Italian institutions. Multicenter data will become available in the near future and will help to further understand potential regional differences in oncofertility care and patients' attitudes towards these issues.

Another important unresolved factor is the optimal timing for attempting pregnancy, especially in patients with hormone receptor-positive disease. Previous study demonstrated no difference in the access of reproductive counseling according to hormone receptor status, but a lower pregnancy rate among women with hormone receptor-positive disease (34, 35). We found a tendency for lower rates of access to cryopreservation strategies in patients with hormone receptor-negative disease as compared to those with hormone receptor-positive disease (10% *vs.* 19.4%). The longer period of anticancer treatment for patients with hormone-receptor positive disease with subsequent ovarian aging and need to postpone family planning might explain this attitude.

In conclusion, we demonstrated that the possible onset of chemotherapy-induced POI chemotherapy-induced and/or infertility worries the majority of newly diagnosed premenopausal breast cancer patients, and this appears to be particularly relevant in those with hormone receptor-positive disease. Use of GnRHa is a widely used and accepted method for ovarian function preservation. In women diagnosed at ≤40 years of age, approximately one out of 3 breast cancer patients accepted to undergo a counseling accepted to with a fertility specialist and less than 1 out of 5 decided to undergo a cryopreservation strategy. Our findings are relevant to improve the oncofertility counseling, for which it is essential to have a strong collaboration between oncologist and fertility specialist.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable request to the corresponding author without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Regionale, IRCCS Ospedale Policlinico San Martino. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: LDM, ML. Collection and assembly of data: EB, CM, LDM, ML. Data analysis and interpretation: EB, VF, LB, LDM, ML. All authors contributed to the article and approved the submitted version.

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Validation of the Clinical Treatment Score Post–Five Years in Breast Cancer Patients for Predicting Late Distant Recurrence: A Single-Center Investigation in Korea

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Background: Endocrine therapy is administered to hormone-positive breast cancer patients to prevent distant metastasis. It is important to evaluate the risk of recurrence and to determine which patients are viable candidates for such treatment because hormone therapy has side effects that can include postmenopausal symptoms. The Clinical Treatment Score post–five years (CTS5), a simple tool for identifying candidates for endocrine therapy, was recently introduced; however, CTS5 only has been applied in validation studies with postmenopausal women. We aimed to validate CTS5 among premenopausal breast cancer patients.

Methods: We identified patients treated between 1994 and 2014 at Samsung Medical Center in Seoul, Korea, and followed their treatment outcomes for more than 60 months after surgery using clinicopathologic parameters. According to menopausal status, we divided the study population into two groups: pre- and postmenopausal women. After calculating CTS5 values based on some parameters, we stratified the rate of late distant recurrence (DR) and analyzed the correlation between CTS5 value and late DR by risk.

Results: Among 16,904 patients treated surgically for breast cancer, 2,605 with hormone receptor–positive breast cancer who received endocrine therapy were included. Of these, 1,749 (67.14%) patients were premenopausal women, and the median age was 44.00 years. When categorizing study participants according to CTS5-related risk for late DR, 86.79% were categorized as low risk, 5.95% were categorized as intermediate risk, and 7.26% were categorized as high risk. The annual rate of DR was 1.41% for those in the present study and was similar between pre- and postmenopausal participants (1.40 vs. 1.42). Distant metastasis-free survival was not different between the two groups (hazard ratio: 0.817, 95% confidence interval [CI]: 0.547–1.221). The area under the receiver operating characteristic curve at 10 years for premenopausal and postmenopausal patients was 61.75 (95% CI: 52.97–70.53) and 72.71 (95% CIs: 63.30–82.12), respectively.

Conclusions: Although CTS5 was able to predict late DR, it should be applied with caution in premenopausal women. A CTS5 calculator for premenopausal women might be needed to not underestimate the risk of recurrence in Korea.

Keywords: hormone receptor-positive breast cancer, premenopausal patients, hormone replacement therapy, CTS5, late distant recurrence

INTRODUCTION

Endocrine therapy is inevitable for patients with hormonal status–positive breast cancer to prevent local recurrence and distant metastasis (1–3). Generally, patients with estrogen receptor (ER)- or progesterone receptor (PR)-positive breast cancer are treated with adjuvant endocrine therapy for five years after surgical treatment (4, 5). It is important to evaluate the recurrence risk and determine whether to maintain or stop endocrine therapy after five years based on side effects, such as postmenopausal symptoms, and patient quality of life (6–8). Therefore, it is necessary to decide whether to stop or continue endocrine therapy after weighing the side effects of therapy and the risk for recurrence or metastasis of breast cancer.

Recently, *Dowsett* and colleagues introduced a tool called the Clinical Treatment Score post–five years (CTS5) as a scoring system to help decide whether to stop or continue treatment after five years of endocrine therapy using several clinicopathologic parameters including tumor size, nodal status, and histopathologic grade (9, 10). This scoring system was developed using data from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial, which included postmenopausal women with ER-positive or ER-unknown early breast cancer (11, 12). The ATAC trial categorized patients into three risk groups (low, intermediate, and high) for estimating the prognostic performance for late distant metastasis.

However, the CTS5 scoring system might not be as effective in Asian countries because there are many more young breast cancer patients than in Western society (13). In prior research, CTS5 was applied to postmenopausal women in the ATAC and BIG 1-98 study cohorts at diagnosis, and the algorithm was not applied to premenopausal patients (11, 14, 15). CTS5 provides a convenient way to predict distant recurrence (DR) but has limitations in extending its use to all ER- or PR-positive breast cancer patients.

In the present study, we aimed to validate the CTS5 score and develop a modified scoring system to predict distant metastasis not only in postmenopausal women, but also in premenopausal women. We used data from a single institution as the validation set and analyzed participants after subdividing them into pre- and postmenopausal groups to differentiate existing CTS5 scores and identify the prognostic value of CTS5 according to menopausal status.

PATIENTS AND METHODS

Study Populations

We retrospectively reviewed the medical records of patients who were treated surgically for breast cancer at Samsung Medical Center in Seoul, Korea, between January 1994 and December 2014. Among them, patients with hormone receptor–positive early breast cancer who received adjuvant endocrine therapy and were followed for more than 60 months after surgery were included. We excluded data from women with a final pathologic stage equal to or higher than T3 or N3, ductal carcinoma in situ, or a diagnosis of bilateral breast cancer. Patients who received neoadjuvant chemotherapy also were excluded. Patients with poor drug compliance—those with discontinuation of tamoxifen or aromatase inhibitors such as anastrozole or letrozole intake after starting— also were excluded. Additionally, patients who showed DR prior to five years after diagnosis were excluded from the study cohort. Finally, we excluded patients with extension of adjuvant endocrine therapy after five years (**Figure 1**). According to menstrual cycle period, date of last menstruation, and hormonal test results including follicle-stimulating hormone and estradiol levels, we divided the study population into two groups of pre- and postmenopausal women. Human epidermal growth factor receptor 2 (HER2)-positive patients were included.

Validation as a Prognostic Tool

Dowsett et al. (9) suggested the use of CTS5 for predicting late DR rates after five years of adjuvant endocrine therapy in patients with hormonal receptor–positive breast cancer. Using the formula CTS5 = 0.438 × nodes + 0.988 × (0.093 × size – 0.001 × size^2 + 0.375 × grade + 0.017 × age), we validated CTS5 as a prognostic tool for DR onset. We assigned three risk categories in each group of women according to cutoff values of 5% and 10% of DR risk as calculated by CTS5. The cutoff criteria for classifying risk were the same as those of CTS5 values for the combined dataset (ATAC training set and BIG 1-98 validation set).

For survival analysis, the five- to 10-year DR risk was analyzed for each group by Kaplan–Meier plots. The hazard ratio (HR) and 95% confidence interval (CI) of the premenopausal group were calculated and compared to those of the postmenopausal group through univariate analysis. Time-dependent areas under the receiver operating characteristic curve (AUC) at 10 years with 95% CIs were calculated to evaluate matching of the DR rate prediction in between pre- and postmenopausal groups.

Statistical Analyses

Patient characteristics were compared using the independent t-test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Univariable and multivariable analyses were conducted using Cox regression analysis models.

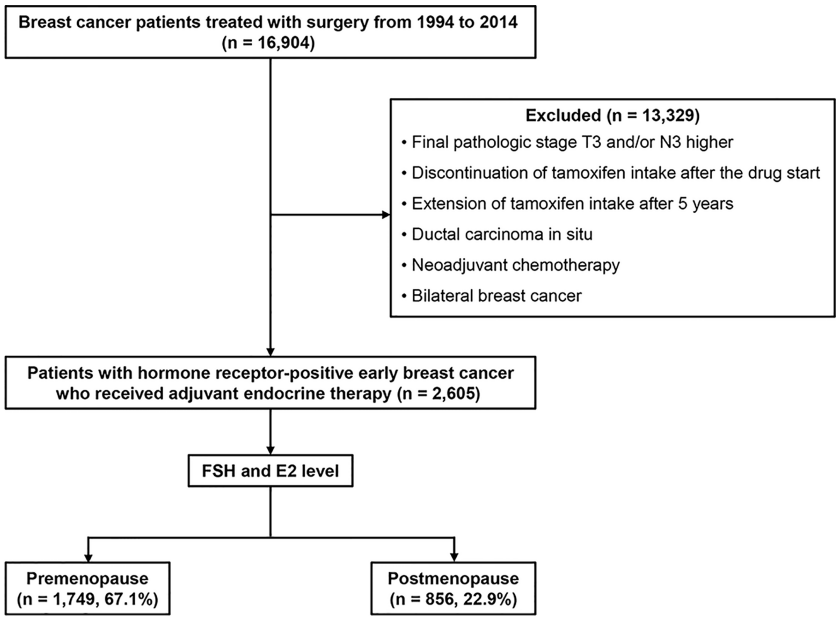


FIGURE 1 | Consort diagram of the study population.

The five- to 10-year DR risk was estimated using the Kaplan–Meier method. Kaplan–Meier curves, with corresponding log-rank tests, were constructed for DR. Values are reported as mean ± standard deviation (SD) or median with range.

Statistical significance was established at $p < 0.05$. All statistical analyses were performed using the Statistical Analysis System (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA) and R Statistical Programming Language Version 2.13.2 (The R Foundation, Vienna, Austria; available at http://www.R-project.org/). The predictability of each CTS5 model for 10-year distant metastasis-free survival was assessed with the time-dependent area under the curve (AUC) and its 95% CI by constructing the receiver operating characteristic (ROC) curve at 10 years post-surgery using R package (16). The present study was approved by the Review Committees (no. 2020-09-143), and work was conducted according to the principles outlined in the Declaration of Helsinki.

RESULTS

The demographics and baseline characteristics of this study are described in **Table 1**. Among 2,605 patients included in the present study, 1,749 (67.14%) were premenopausal, and 856 (32.86%) were postmenopausal. The median follow-up period was 94.69 months [59.97 – 233.85]. The median age of the premenopausal women was 44.00 years, and that of the postmenopausal women was 56.50 years; overall, the average median age of the study population was 46.00 years. Nodal status, tumor grade, and tumor size were not significantly different between the two groups. A total of 1,902 patients (73.04%) received adjuvant chemotherapy, and more premenopausal women than postmenopausal women received chemotherapy (77.02% *vs.* 64.91%; $p < 0.0001$). There was no difference between pre- and postmenopausal patients in terms of tumor size, tumor grade, or nodal status. Tamoxifen was administered in 1,481 patients (56.85%) total, 1,325 of whom were premenopausal. Goserelin treatment as a subcutaneous injection of a depot formulation was observed in 159 patients, all of whom were premenopausal. As for aromatase inhibitors, 336 postmenopausal patients (39.25%) received anastrozole, and 278 (32.48%) received letrozole. During the first five years of treatment, 126 patients were switched to aromatase inhibitors, and all 126 patients were classified as premenopausal. Moreover, 110 (4.22%) cases of late DR were recorded, with an annual hazard rate of 1.41% (95% CI: 1.16%–1.70%). There was no significant difference between the two groups in terms of DR cases or annual rate of DR after five years of adjuvant endocrine therapy. The rate for late DR of HER2-positive patients was significantly lower than that of HER2-negative patients (1.64% *vs.* 4.49%; $p = 0.0351$) (**Supplementary Table 1**).

Tumor size, tumor grade, and nodal status were arranged in a separate table according to CTS5 risk category status in pre- and postmenopausal women (**Table 2**). Overall, 86.79% (n = 2,261 patients) were categorized as low risk, 5.95% (n = 155 patients) were categorized as intermediate risk, and 7.26% (n = 189 patients) were categorized as high risk for late DR. Notably, more than 90% of patients in the moderate to poor tumor grade group were categorized high risk, as were all patients with more than two positive nodes. Combined ATAC and BIG 1-98 cohort data and data from participants of this study are compared in **Table 3**. Compared with the ATAC and BIG 1-98 cohorts, there

TABLE 1 | Demographic and clinical characteristics between pre- and postmenopausal women.

Characteristic	Total (n = 2,605)	Postmenopausal (n = 856)	Premenopausal (n = 1,749)	p
Age, years				<0.0001
Median	46.00	56.50	44.00	
Interquartile range	42-53	52-62	40-47	
Nodal status				
Negative	1,608 (61.73)	515 (60.16)	1,093 (62.49)	0.1403
1	455 (17.47)	148 (17.29)	307 (17.55)	
2-3	316 (12.13)	112 (13.08)	204 (11.66)	
4-9	226 (8.68)	81 (9.46)	145 (8.29)	
Tumor grade				0.2671
Well	789 (30.29)	242 (29.44)	537 (30.70)	
Moderate	1,279 (49.10)	416 (48.60)	863 (49.34)	
Poor	537 (20.61)	188 (21.96)	349 (19.95)	
Tumor size, mm				0.5903
<10	468 (17.97)	143 (16.71)	325 (18.58)	
10-20	1,229 (47.18)	415 (48.48)	814 (46.54)	
21-30	604 (23.19)	197 (23.01)	407 (23.27)	
31-50	304 (11.67)	101 (11.80)	203 (11.61)	
Chemotherapy				<0.0001
No	702 (26.96)	300 (35.09)	402 (22.98)	
Yes	1,902 (73.04)	555 (64.91)	1,347 (77.02)	
Hormonal therapy				<0.0001
Tamoxifen	1,481 (56.85)	156 (18.22)	1,325 (75.76)	
Toremifene	165 (6.33)	85 (9.93)	80 (4.57)	
Anastrozole	490 (18.81)	336 (39.25)	154 (8.81)[a]	
Letrozole	465 (17.85)	278 (32.48)	187 (10.69)[a]	
Unknown	4 (0.16)	1 (0.12)	3 (0.17)	
GnRH agonist				<0.0001
Goserelin	159 (6.10)	0 (0.00)	159 (9.09)	
Distant recurrence (>5 yrs)				0.8594
No	2,495 (95.78)	819 (95.68)	1,676 (95.83)	
Yes	110 (4.22)	37 (4.32)	73 (4.17)	
Distant recurrence (>5 yrs)				
Annual rate, %	1.41	1.42	1.40	
95% CI	1.16-1.70	1.00-1.96	1.10-1.77	

[a]*126 patients were switched from tamoxifen to aromatase inhibitors during the first 5 years of treatment.*

was no difference in tumor size or nodal status. The rate of chemotherapy in our study was more than 70% higher than that of the ATAC and BIG 1-98 cohorts, representing a significant difference, and low risk was identified in more than 80% of patients with the CTS5 score at the time of validation. The HR of five- to 10-year DR risk among premenopausal women was 0.817 (95% CI: 0.547–1.221; p = 0.3236), which was lower than that among postmenopausal women (**Figure 2**).

The time-dependent AUC at 10 years is presented with 95% CI value (**Figure 3**). The AUC for all patients was 64.71 (95% CI: 57.75–71.67). Among postmenopausal women, the AUC exceeded the total population AUC at 72.71 (95% CI: 57.75–71.67), whereas that in premenopausal women was 61.75 (95% CI: 52.97–70.53).

Histograms for CTS5 score are shown according to menopausal status with validated prognostic values of CTS5 for risk of DR between five and 10 years (**Figure 4**). Importantly, the premenopausal group included a greater proportion of patients at low risk than did the postmenopausal group. That is, premenopausal women unexpectedly had lower CTS5 scores than postmenopausal women, and this result correlated with the many patients at low risk.

DISCUSSION

There is a need for prognostic tools that can predict late recurrence rate after five years of endocrine therapy; CTS5 is a useful tool for satisfying this need and supporting clinicians in decision-making regarding extension of endocrine therapy (9, 17). This study is significant in that CTS5 was validated in premenopausal women, who account for the majority of breast cancer patients in Korea. After validation, we found that premenopausal women occupied a large portion of the low-risk recurrence group—in other words, the risk for late DR was underestimated by CTS5 in premenopausal women. Therefore, development of a predictive late DR model for premenopausal women is necessary.

Notably, compared with our study population, the ATAC cohort, used as the training set in CTS5, and the BIG 1-98 cohort, used as the validation set, both included postmenopausal women. Nodal status, tumor grade, and tumor size were similar between the population in our study and the ATAC plus BIG 1-98 cohort. In our study, there was no significant difference in tumor size, tumor grade, or nodal status between pre- and postmenopausal women. There was also no difference

TABLE 2 | Distribution of risk categories according to menopausal status.

Characteristic	Total (n = 2,605)	Low Risk (n = 2,261)	Intermediate Risk (n = 155)	High Risk (n = 189)
Postmenopausal (n = 856)				
Tumor size, mm				
<10	143 (16.71)	142 (19.8)	1 (1.61)	0 (0)
10-20	415 (48.48)	375 (52.3)	23 (37.1)	17 (22.08)
21-30	197 (23.01)	142 (19.8)	18 (29.03)	37 (48.05)
31-50	101 (11.8)	58 (8.09)	20 (32.26)	23 (29.87)
Tumor grade				
Well	252 (29.44)	239 (33.33)	8 (12.9)	5 (6.49)
Moderate	416 (48.6)	351 (48.95)	27 (43.55)	38 (49.35)
Poor	188 (21.96)	127 (17.71)	27 (43.55)	34 (44.16)
Nodal status				
Negative	515 (60.16)	515 (71.83)	0 (0)	0 (0)
1	148 (17.29)	135 (18.83)	13 (20.97)	0 (0)
2-3	112 (13.08)	66 (9.21)	33 (53.23)	13 (16.88)
4-9	81 (9.46)	1 (0.14)	16 (25.81)	64 (83.12)
Premenopausal (n = 1,749)				
Tumor size, mm				
<10	325 (18.58)	322 (20.85)	1 (1.08)	2 (1.79)
10-20	814 (46.54)	779 (50.45)	16 (17.2)	19 (16.96)
21-30	407 (23.27)	315 (20.4)	44 (47.31)	48 (42.86)
31-50	203 (11.61)	128 (8.29)	32 (34.41)	43 (38.39)
Tumor grade				
Well	537 (30.7)	523 (33.87)	5 (5.38)	9 (8.04)
Moderate	863 (49.34)	751 (48.64)	55 (59.14)	57 (50.89)
Poor	349 (19.95)	270 (17.49)	33 (35.48)	46 (41.07)
Nodal status				
Negative	1,093 (62.49)	1,093 (70.79)	0 (0)	0 (0)
1	307 (17.55)	302 (19.56)	5 (5.38)	0 (0)
2-3	204 (11.66)	140 (9.07)	58 (62.37)	6 (5.36)
4-9	145 (8.29)	9 (0.58)	30 (32.26)	106 (94.64)

TABLE 3 | Comparison of combined ATAC and BIG 1-98 cohorts and the present cohort.

Characteristic	ATAC + BIG 1-98 (n = 11,446)	Total (n = 2,605)	Premenopausal (n = 1,749)	p
Nodal status				0.1519
Negative	7,309 (63.86)	1,608 (61.73)	1,093 (62.49)	
1	1,807 (15.79)	455 (17.47)	307 (17.55)	
2-3	1,303 (11.38)	316 (12.13)	204 (11.66)	
4-9	1,027 (8.97)	226 (8.68)	145 (8.29)	
Tumor grade				<0.0001
Well	2,673 (23.35)	789 (30.29)	537 (30.70)	
Moderate	6,215 (54.30)	1,279 (49.10)	863 (49.34)	
Poor	2,558 (22.35)	537 (20.61)	349 (19.95)	
Tumor size, mm				0.6291
<10	2,036 (17.79)	468 (17.97)	325 (18.58)	
10-20	5,562 (48.59)	1,229 (47.18)	814 (46.54)	
21-30	2,599 (22.71)	604 (23.19)	407 (23.27)	
31-50	1,249 (10.91)	304 (11.67)	203 (11.61)	
Chemotherapy				<0.0001
No	8,896 (77.72)	702 (26.96)	402 (22.98)	
Yes	2,550 (22.28)	1,902 (73.04)	1,347 (77.02)	
Distant recurrence (>5yrs)				<0.0001
No	10,746 (93.88)	2,495 (95.78)	1,676 (95.83)	
Yes	700 (6.12)	110 (4.22)	73 (4.17)	
CTS5				<0.0001
Low	4,850 (42.37)	2,261 (86.79)	1,544 (88.28)	
Intermediate	3,620 (31.63)	155 (5.95)	93 (5.32)	
High	2,976 (26.00)	189 (7.26)	112 (6.40)	

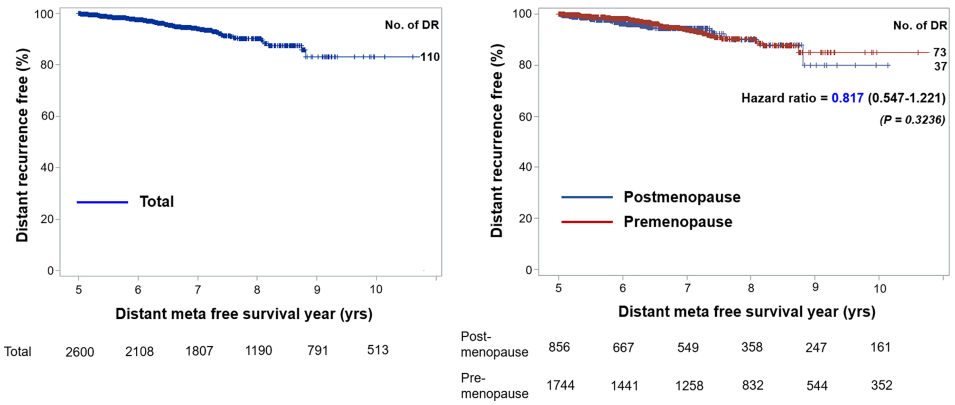


FIGURE 2 | Kaplan-Meier curves of distant recurrence-free survival.

in DR or annual DR between the two groups. However, when risk was divided according to the cutoff value of CTS5, patients at low risk were more than twice as numerous relative to the cohorts of the ATAC and BIG 1-98 trials. In addition, the premenopausal group contained a greater number of patients at intermediate and high risk than did the postmenopausal group. Interestingly, the rate of receiving chemotherapy was close to 80% in our study, while about 20% of the ATAC plus BIG 1-98 cohort received adjuvant chemotherapy. For this reason, it is thought that the frequency of adjuvant chemotherapy was high among premenopausal women. The AUC had a greater predictive rate for postmenopausal women than for total patients but was less predictive for premenopausal women than for the entire cohort. In other words, as CTS5 was created based on data of postmenopausal women, there is a high probability that it cannot efficiently be applied to premenopausal women in many Asian countries (18). Premenopausal women are likely to be classified in an underestimated risk group when CTS5 is used, and it is necessary to introduce a new scoring system to address this.

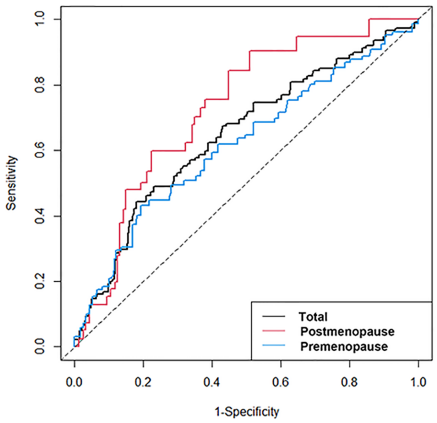
Recently, many studies have analyzed the prognostic value of CTS5. Villasco et al. (19) found that CTS5 has prognostic value in predicting late DR in both pre- and postmenopausal women by testing its clinical validity in a retrospective cohort, while Lee et al. (20) similarly concluded that CTS5 is a good prognostic tool for evaluating the risk of late distant recurrence in both pre- and postmenopausal women using the Ki-67 labeling index and confirmed its prognostic performance in premenopausal women. Although Lee et al. reported that their risk groups presented differences in tumor grade relative to the ATAC and BIG 1-90 cohorts, there was no significant difference in tumor grade between our group and the ATAC plus BIG 1-90 cohort. We think that the reason for the different risk groups might be related to whether or not adjuvant chemotherapy was available.

HER2 gene amplification is known to have an impact on breast cancer, and the intracellular signaling pathway of estrogen receptors and *HER2* has a complex connection (21). Wang et al. (22) recently suggested that *HER2* status has an effect on late DR in hormone receptor–positive breast cancer. In *HER2*-positive patients, such as those with luminal type B disease, a less effective prognostic value was found in their study. Cases of *HER2*-positive breast cancer are considered high-risk for DR but, due to the development of anti-*HER2*–targeted therapy, it is not effective for the *HER2*-positive model, and a further prediction model is required.

Recently, Noordhoek et al. (23) published a validity and accuracy study of the CTS5 for predicting the rate of late DR in the TEAM and IDEAL trials, arguing the CTS5 overestimates the risk of late DR in high-risk subgroups and suggesting that CTS5 should be used cautiously for interpreting the DR rate among patients at high risk despite its ease of use. Based on these findings, CTS5 must be applied carefully, and unique validation is justified in Korea, where there are many young breast cancer patients.

Various multigene assays have been developed to complement predictions using existing clinicopathologic parameters because of the lack of predictive values for late DR. Among them, GenesWell™ BCT has been validated as a tool to predict late DR (24). In some patients who underwent CTS5 validation, GenesWell™ BCT also was applied, and the CTS5-derived risk group correlated with the gene assay risk group (**Supplementary Figure 1**). However, the use of multigene assays is controversial in predicting late DR because some assays have not been validated, and the importance of clinical risk has been emphasized, especially in multigene panels of patients under 50 years of age (25). In conclusion, balanced application of a clinicopathologic prediction model and a multigene assay should be conducted to predict late DR, and further study is needed for proper selection of patients to receive extended adjuvant endocrine therapy.

The main limitation of this study is that it was a retrospective study conducted in a single center. It is necessary to conduct multicenter studies to further explore the limitations of CTS5, and a modified version of CTS5 should be developed and validated in large sets through multicenter research in South Korea. In addition, it is necessary to accurately stratify risk



	AUC	95% CI	
Total	64.71	57.75	71.67
Post	72.71	63.30	82.12
Pre	61.75	52.97	70.53

FIGURE 3 | Time-dependent AUC at 10 years post-surgery with 95% CI.

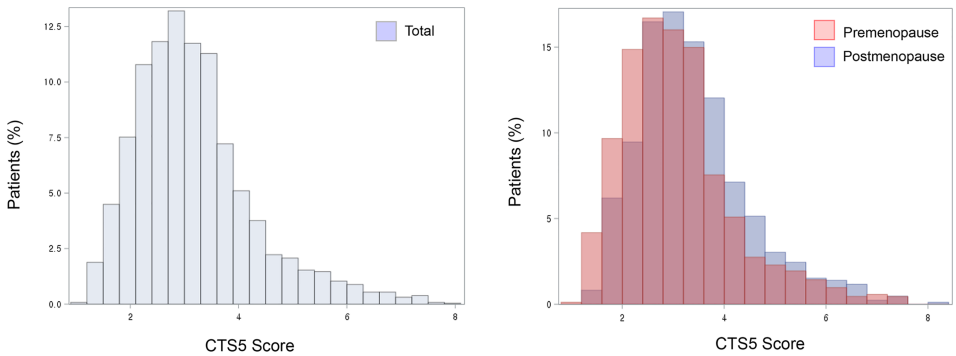


FIGURE 4 | Histogram of CTS5 scores.

groups by creating new cutoff values suitable for Korean patients. Accordingly, we plan to create a web-based search tool suitable for use in Korea.

When the CTS5 calculator was developed, HER2-positive patients were included despite the small population. Because the diagnostic technique has not been applied, such as silver-enhanced *in situ* hybridization in our data prior to 2003, it is not clear to describe the HER2 status. Due to development of HER2 gene amplification diagnosis, HER2 status can be accurately described, allowing not only chemotherapy, but targeted therapy to be administered. HER2-positive breast cancer is different from hormone receptor-positive breast cancer in terms of molecular biology, and further study is needed to predict late DR excluding the HER2-positive subtype. In addition, this study did not confirm the difference in CTS5 risk among premenopausal women according to use of GnRH agonists because of the small population (156 patients) of premenopausal patients received GnRH agonists. GnRH agonists have been used to suppress ovarian function in young patients with luminal-type breast cancer and premenopausal patients with high risk for DR due to poor prognosis (26, 27). In the SOFT-TEXT and ASTRRA trials, it was found that the prognosis of young breast cancer patients could be improved depending upon the use of GnRH agonists (28–31). In the future, additional CTS5 evaluation is required according to the use of GnRH agonists in premenopausal women, and it is necessary to evaluate the late prediction rate according to the combination of CTS5 and multigene assay by GnRH agonist use.

CTS5 can be prognostic, but risk evaluation is dependent upon traditional clinicopathologic parameters such as tumor size, tumor grade, and nodal status; it cannot provide customized guidance for each individual. Therefore, its combination with a multigene assay is important for deciding whether to extend endocrine therapy (17, 32). Furthermore, in addition to a clinical calculator, such as CTS5, and a multigene assay, further combination with an immunohistochemistry assay and radiographic imaging (computed tomography, positron-emission tomography, and magnetic resonance imaging) or tumor marker assessments is important for predicting patient prognosis (25, 33).

In conclusion, although CTS5 was created to support decision-making by clinicians about extending adjuvant endocrine therapy in postmenopausal women with positive hormonal receptors, there are limitations in predicting late DR in premenopausal women. For populations of premenopausal women with greater rates of breast cancer, a modified scoring system for late DR prediction is needed so as not to underestimate the recurrence risk.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The present study was approved by the Review Committees (no. 2020-09-143). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-HL performed literature search, the data analysis, and drafted the manuscript. SL and BC performed revised the manuscript. JY performed literature investigation and reviewing. JEL SK, and SN performed supervision and reviewing. JR designed the concept of article. All authors contributed to the article and approved the submitted version.

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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.2021.691277/full#supplementary-material

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Effects of Surgery on Prognosis of Young Women With Operable Breast Cancer in Different Marital Statuses: A Population-Based Cohort Study

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Background: The influence of surgical approaches [including mastectomy, breast-conserving therapy (BCT) and post-mastectomy breast reconstruction (PMBR) on prognosis of young women (<40 years old) with operable breast cancer has not been determined yet, and this might vary in patients with different marital statuses. Therefore, we aimed to investigate the effect of surgery on survival outcomes for young women with operable breast cancer in different marital statuses.

Methods: We used the Surveillance, Epidemiology, and End Results (SEER) database to identify young women with operable breast cancer between 2004 and 2016, who underwent mastectomy, BCT or PMBR. We assessed overall survival (OS) and breast cancer-specific survival (BCSS) using the Kaplan–Meier method and hazard ratios using multivariate Cox proportional hazard regression.

Results: Compared to mastectomy, both of BCT and PMBR conferred better OS (BCT: HR = 0.79, 95%CI: 0.69–0.90, p <0.001; PMBR: HR = 0.70, 95%CI: 0.63–0.78, p <0.001) and BCSS (BCT: HR = 0.79, 95%CI: 0.69–0.91, p = 0.001; PMBR: HR = 0.73, 95%CI: 0.65–0.81, p <0.001), but there was no significant difference of survival between BCT and PMBR group. The survival benefit of BCT compared to mastectomy remained significant in unmarried young women (OS: HR = 0.68, 95%CI: 0.55–0.83, p <0.001; BCSS: HR = 0.69, 95%CI: 0.56–0.86, p = 0.001) but not in the married (OS: HR = 0.89, 95%CI: 0.75–1.05, p = 0.177; BCSS: HR = 0.89, 95%CI: 0.75–1.05, p = 0.161), while no matter married or not, PMBR group had better OS and BCSS than mastectomy group but not BCT group.

Conclusion: Both of BCT and PMBR had improved survival compared to mastectomy for young women with operable breast cancer. The survival benefit of BCT compared to mastectomy remained significant in unmarried patients but not in married patients.

Keywords: breast cancer, breast-conserving surgery, mastectomy, breast reconstruction, survival

INTRODUCTION

Nowadays, treatment strategies for breast cancer have been improved largely, including surgery, radiation, chemotherapy, endocrine therapy, target therapy and immune therapy (1). For operable breast cancer, surgical treatment, such as mastectomy alone, breast-conserving therapy (BCT) and post-mastectomy breast reconstruction (PMBR), is still considered to be the most significant treatment. Previous randomized controlled trials and large retrospective studies have demonstrated that BCT have equal or better survival outcomes compared with mastectomy (2–5), and there are also researches reported that PMBR brought survival benefits compared with mastectomy alone (6).

For breast cancer in young women, which are defined as women under the age of 40 at breast cancer diagnosis, the survival benefit of BCT compared with mastectomy was uncertain, though some studies had been reported that BCT brought better body image and less anxiety for young breast cancer survivors (7, 8). There were also few evidences regarding the survival outcomes of PMBR compared with mastectomy alone for young breast cancer patients. Therefore, the survival outcomes after different surgical options for young breast cancer patients need to be further investigated.

Psychosocial factors have been reported to be associated with survival outcomes of cancer patients, and marital status is one of the most important psychosocial factors for breast cancer patients (9). Previous studies have demonstrated that married patients had prolonged overall survival (OS) and breast cancer-specific survival (BCSS) compared with unmarried patients (including patients who were single, divorced, separated and widowed) (9, 10). Married patients could acquire more financial and emotional support and have better adherence when undergoing treatments (9), while unmarried patients, with less psychosocial support, might have higher expectations on treatments, especially when choosing surgical approaches. The body image after breast cancer local surgery seems to have more effects in young unmarried patients' psychosocial life compared with those who are married, thus influencing their survival outcomes as well. Therefore, we hypothesized that the impact of surgical options on the prognosis of young breast cancer patients might be influenced by marital status.

The present study aimed to investigate the impact of surgical approaches (mastectomy, BCT or PMBR) on the overall survival (OS) and breast cancer-specific survival (BCSS) for young patients with operable breast cancer in different marital statuses using the Surveillance, Epidemiology, and End Results (SEER) database.

MATERIALS AND METHODS

Study Population

We extracted data from the SEER database that was released in April 2019; specifically, the dataset named "Incidence-SEER 18 Res Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2018 Sub (1975–2016 varying)" in the Case Listing and Frequency Sessions was obtained from the SEER*Stat software, version 8.3.6. The SEER database, including 18 tumor registries and representing approximately 28% of the population across the United States, contained information about patients' demographics, characteristics of tumor, surgery type, hormone receptor status (HRs), survival months and vital status (11). Since the year 2004 was selected as the first year of the study given that several employed covariates were introduced in SEER in 2004, we identified 35,128 young women (<40 years old) diagnosed with breast cancer (International Classification of Diseases for Oncology, third edition (ICD-O-3) morphology code 8500, 8501, 8502, 8510, 8512, 8513, 8514, 8520, 8521, 8522, 8523, 8524, 8525, 8530, 8541 and 8543) from January 2004 to November 2016[9]. Then, only patients with primary operable breast cancer were included according to the 6th edition AJCC system for cases between 2004 and 2009, and the 7th edition for cases between 2010 and 2016. Patients with unknown details including marital status, race, tumor grade, HRs, surgery and cause of death and those without radiation along with breast-conserving surgery were excluded. Finally, 20,885 cases were selected into our study, and the entire cohort was divided into three groups according to their surgery type: mastectomy, BCT and PMBR (**Figure 1**). Marital status was categorized as either married or unmarried, and the unmarried included patients who were single, divorced, separated and widowed.

Statistical Analysis

The patients' baseline characteristics among mastectomy, BCT and PMBR group were compared using Pearson's chi-square test. Survival outcomes, including OS and BCSS, were examined using the Kaplan–Meier method and compared among the three surgical groups using Log-rank tests. Meanwhile, the survival outcomes among the three surgical groups were further analyzed in subgroups stratified by marital status. Hazard ratios (HR) with 95% confidence intervals (CIs) to assess the survival difference among different surgical groups were calculated using multivariate Cox proportional hazard regression. A two-sided P value <0.05 was considered to indicate statistical significance. All analyses in our study were performed using Statistical Product and Service Solutions (SPSS) software (version 26.0).

RESULTS

Patients' Characteristics

Some 20,885 young women with primary operable breast cancer were included in our study, among which 7,418 (35.5%) underwent mastectomy, 5,966 (28.6%) underwent BCT and 7,501 (35.9%) underwent PMBR. The median follow-up time was 66 months. The patients' characteristics including demographics, age of diagnosis, characteristics of tumor, surgery approach, radiation, and chemotherapy are showed in **Table 1**. Most patients were 30–39 years old (89.6%), married (64.2%), White people (73.1%), in AJCC stage II (49.9%) and had poorly differentiated or undifferentiated tumor (57.8%). Among the three surgical groups, BCT group had highest percentage of

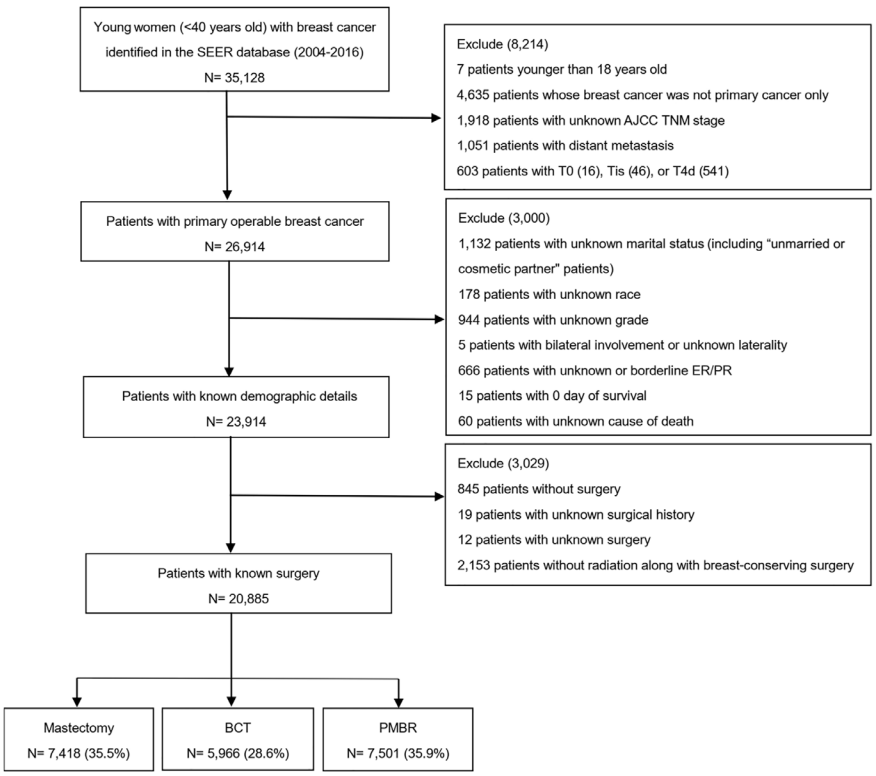

FIGURE 1 | Flow diagram for creation of the study cohort. SEER, Surveillance, Epidemiology, and End Results; AJCC, American Joint Committee on Cancer; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction.

unmarried patients (39.5%), while mastectomy group had 35.8% and PMBR group had 32.9%. Consistent with the entire cohort, approximately half of the patients in each group were AJCC stage II; besides, the mastectomy group had more stage III patients (31.6%) while BCT group and PMBR group had more stage I patients (38.6 and 32.2%, respectively). The mastectomy group had a higher percentage of larger tumor size (>2 cm) (67.2%) than BCT group (48.1%) and PMBR group (54.9%).

Effects of Surgery on Survival Outcomes in Overall and Stratified by Marital Status

Kaplan–Meier curves were generated by surgical approach to estimate OS and BCSS of patients with operable breast cancer. In log-rank tests, the BCT and PMBR group showed significantly (P <0.001) better OS and BCSS than mastectomy group, while less significant difference of OS and BCSS was observed between the BCT and PMBR group (**Figure 2**). After adjusting the possible confounding variables via multivariate Cox regression analysis, it turned out that compared to mastectomy, both of BCT and PMBR conferred better OS (BCT: HR = 0.79, 95%CI:0.69–0.90, p <0.001; PMBR: HR = 0.70, 95%CI: 0.63<0.78, p <0.001) and BCSS (BCT: HR = 0.79, 95%CI: 0.69<0.91, p = 0.001; PMBR: HR = 0.73, 95%CI: 0.65–0.81, p <0.001), but there was no significant difference of survival between BCT group and PMBR group (OS: HR = 1.04, 95%CI: 0.88<1.23, p = 0.644; BCSS: HR = 1.06, 95%CI: 0.90–1.26, p = 0.490) (**Figure 2**). The superiority of BCT in survival outcomes compared to mastectomy remained significant in unmarried young women (OS: HR = 0.68, 95%CI: 0.55–0.83, p <0.001; BCSS: HR = 0.69, 95%CI: 0.56–0.86, p = 0.001) but not in the married (OS: HR = 0.89, 95%CI: 0.75–1.05, p = 0.177; BCSS: HR = 0.89, 95%CI: 0.75–1.05, p = 0.161), while no matter married or not, PMBR group had better OS and BCSS than mastectomy group but not BCT group (**Figure 3**).

Effects of Surgery Stratified by Demographic and Pathological Subgroups

To further investigate the prognostic effect of surgery on survival by different demographic and pathological subgroups, we also stratified all cases according to age, race, AJCC stage, HRs, and whether receiving chemotherapy or not and conducted multivariate analyses (**Tables 2** and **3**). Compared with mastectomy, better OS of PMBR was observed in almost all subgroups except for HRs of ER+/PR− and non-chemotherapy group, while the superiority of BCT in OS was existed in all subgroups of age and race, and in stage I/II, HRs of ER+/PR+ and chemotherapy group. As for BCSS, the superiority of BCT was

TABLE 1 | Comparison of baseline characteristics of operable breast cancer among various surgical groups.

Characteristics	Total (%)	MAS (%)	BCT (%)	PMBR (%)	p value
Overall	20,885	7,418	5,966	7,501	
Age (years)					<0.001
18–29	2,174 (10.4)	785 (10.6)	516 (8.6)	873 (11.6)	
30–39	18,711 (89.6)	7,418 (89.4)	5,450 (91.4)	6,628 (88.4)	
Marital status					<0.001
Married	13,402 (64.2)	4,761 (64.2)	3,607 (60.5)	5,034 (67.1)	
Unmarried	7,483 (35.8)	2,657 (35.8)	2,359 (39.5)	2,467 (32.9)	
Race					<0.001
White	15,272 (73.1)	5,316 (71.7)	4,169 (69.9)	5,787 (77.1)	
Black	2,941 (14.1)	1,068 (14.4)	959 (16.1)	914 (12.2)	
Others	2,672 (12.8)	1,034 (13.9)	838 (14.0)	800 (10.7)	
Year of Diagnosis					<0.001
2004–2009	8,971 (43.0)	3,684 (49.7)	3,098 (51.9)	2,189 (29.2)	
2010–2016	11,914 (57.0)	3,734 (50.3)	2,868 (48.1)	5,312 (70.8)	
Grade					<0.001
Well differentiated	1,560 (7.5)	464 (6.3)	564 (9.5)	532 (7.1)	
Moderately differentiated	7,245 (34.7)	2,410 (32.5)	2,001 (33.5)	2,834 (37.8)	
Poorly differentiated	11,886 (56.9)	4,450 (60.0)	3,346 (56.1)	4,090 (54.5)	
Undifferentiated/Anaplastic	194 (0.9)	94 (1.3)	55 (0.9)	45 (0.6)	
AJCC stage					<0.001
I	6,115 (29.3)	1,398 (18.8)	2,302 (38.6)	2,415 (32.2)	
II	10,426 (49.9)	3,673 (49.5)	3,068 (51.4)	3,685 (49.1)	
III	4,344 (20.8)	2,347 (31.6)	596 (10.0)	1,401 (18.7)	
Tumor size					<0.001
≤2 cm	8,822 (42.2)	2,390 (32.2)	3,089 (51.8)	3,343 (44.6)	
>2 cm,≤5 cm	9,456 (45.3)	3,614 (48.7)	2,631 (44.1)	3,211 (42.8)	
>5 cm	2,519 (12.1)	1,374 (18.5)	239 (4.0)	906 (12.1)	
Unknown	88 (0.4)	40 (0.5)	7 (0.1)	41 (0.5)	
LN status					<0.001
Negative	10,799 (51.7)	2,968 (40.0)	3,779 (63.3)	4,052 (54.0)	
Positive	9,773 (46.8)	4,339 (58.5)	2,099 (35.2)	3,335 (44.5)	
No examined/Unknown	313 (1.5)	111 (1.5)	88 (1.5)	114 (1.5)	
HRs					<0.001
ER+/PR+	12,362 (59.2)	4,141 (55.8)	3,592 (60.2)	4,629 (61.7)	
ER+/PR–	2,162 (10.4)	811 (10.9)	500 (8.4)	851 (11.3)	
ER–/PR+	407 (1.9)	150 (2.0)	112 (1.9)	145 (1.9)	
ER–/PR–	5,954 (28.5)	2,316 (31.2)	1,762 (29.5)	1,876 (25.0)	
Radiation					<0.001
No	9,210 (44.1)	4,197 (56.6)	0 (0.0)	5,013 (66.8)	
Yes	11,675 (55.9)	3,221 (43.4)	5,966 (100.0)	2,488 (33.2)	
Chemotherapy					<0.001
No/Unknown	3,771 (18.1)	1,158 (15.6)	1,119 (18.8)	1,494 (19.9)	
Yes	17,114 (81.9)	6,260 (84.4)	4,847 (81.2)	6,007 (80.1)	

MAS, mastectomy; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction; AJCC, American Joint Committee on Cancer; LN, lymph node; HRs, hormone receptor status; ER, estrogen receptor; PR, progesterone receptor.

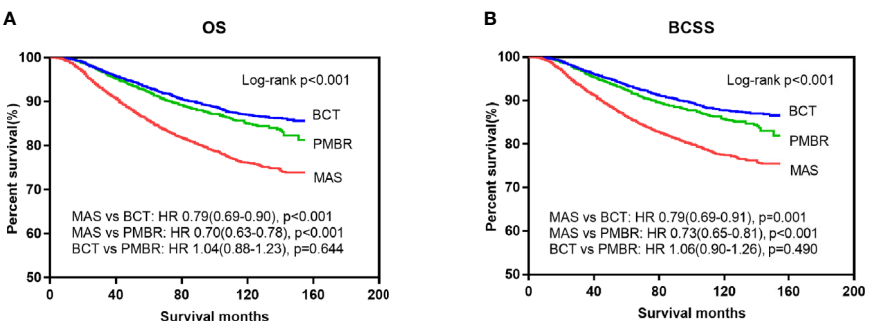

FIGURE 2 | Kaplan–Meier survival curves: OS **(A)** and BCSS **(B)** of young women with operable breast cancer according to surgical type. OS, overall survival; BCSS, breast cancer-specific survival; MAS, mastectomy; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction; HR, hazard ratios.

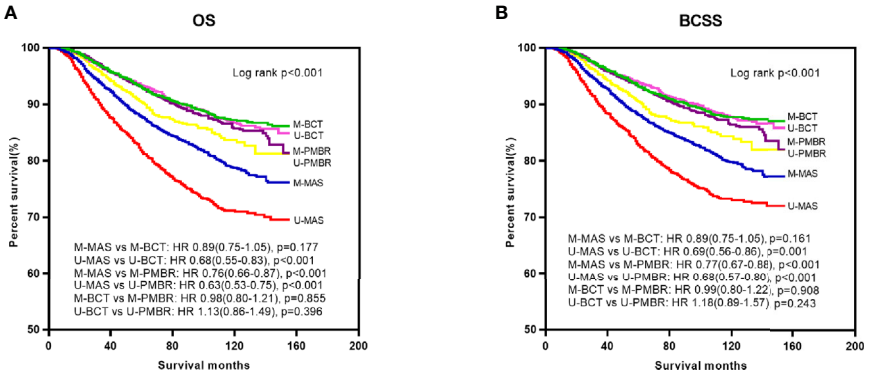

FIGURE 3 | Kaplan–Meier survival curves: OS **(A)** and BCSS **(B)** of young women with operable breast cancer according to surgical type and marital status. OS, overall survival; BCSS, breast cancer-specific survival; M-MAS, married patients who underwent mastectomy; U-MAS, unmarried patients who underwent mastectomy; M-BCT, married patients who underwent breast-conserving therapy; U-BCT, unmarried patients who underwent breast-conserving therapy; M-PMBR, married patients who underwent post-mastectomy breast reconstruction; U-PMBR, unmarried patients who underwent post-mastectomy breast reconstruction; HR, hazard ratios.

TABLE 2 | Effects of surgery in overall survival by demographic and pathological subgroups.

OS	BCT *vs* MAS		PMBR *vs* MAS		PMBR *vs* BCT	
Variable	**AHR* (95%CI)**	**p value**	**AHR* (95%CI)**	**p value**	**AHR* (95%CI)**	**p value**
Age (years)						
18–29	0.66 (0.46–0.95)	0.024	0.67 (0.50–0.90)	0.008	0.90 (0.56–1.43)	0.650
30–39	0.81 (0.70–0.93)	0.003	0.70 (0.63–0.79)	<0.001	1.07 (0.89–1.28)	0.468
Race						
White	0.85 (0.72–0.99)	0.041	0.74 (0.65–0.83)	<0.001	1.02 (0.84–1.24)	0.846
Black	0.72 (0.54–0.94)	0.017	0.62 (0.49–0.79)	<0.001	1.12 (0.76–1.64)	0.561
Others	0.64 (0.43–0.96)	0.029	0.62 (0.42–0.92)	0.017	1.04 (0.58–1.85)	0.906
AJCC stage						
I	0.37 (0.21–0.65)	0.001	0.59 (0.41–0.84)	0.003	2.24 (1.12–4.47)	0.022
II	0.75 (0.62–0.91)	0.004	0.82 (0.70–0.96)	0.013	1.22 (0.96–1.55)	0.110
III	0.84 (0.69–1.02)	0.070	0.65 (0.56–0.76)	<0.001	0.85 (0.67–1.08)	0.187
HRs						
ER+/PR+	0.73 (0.60–0.89)	0.002	0.73 (0.62–0.86)	<0.001	1.09 (0.85–1.41)	0.497
ER+/PR−	0.75 (0.48–1.16)	0.197	0.85 (0.63–1.14)	0.273	1.45 (0.87–2.40)	0.151
ER−/PR+	0.74 (0.34–1.61)	0.440	0.27 (0.13–0.56)	<0.001	0.46 (0.14–1.57)	0.216
ER−/PR−	0.85 (0.70–1.03)	0.098	0.68 (0.58–0.81)	<0.001	0.92 (0.71–1.19)	0.505
Chemotherapy						
No/Unknown	0.97 (0.48–1.96)	0.931	0.74 (0.51–1.07)	0.106	1.74 (0.66–4.55)	0.260
Yes	0.78 (0.69–0.89)	<0.001	0.70 (0.63–0.79)	<0.001	1.02 (0.86–1.21)	0.816

**With adjustment for race, age, marital status, T stage, N stage, histological grade, hormone receptor status, tumor size, surgery, radiation and chemotherapy.*
OS, overall survival; AHR, adjusted hazard ratios; CI, confidential interval; MAS, mastectomy; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction; HRs, hormone receptor status; ER, estrogen receptor; PR, progesterone receptor.

noticed in all age subgroups, in black people, HRs of ER+/PR+, and chemotherapy group compared with mastectomy; the benefit of PMBR in BCSS was significant in all age subgroups, in white and black people, stages I and III, all HRs except ER +/PR−, and chemotherapy group. In addition, there was no significant difference of OS and BCSS between BCT and PMBR in almost all subgroups except for stage I, in which PMBR had worse survival than BCT (OS: HR = 2.24, 95%CI: 1.12–4.47, p = 0.022; BCSS: HR = 2.51, 95%CI: 1.24–5.05, p = 0.010).

Effect of Various Factors on Survival Outcomes

Univariate analysis and adjusted multivariate analysis showed that unmarried status, black people, higher tumor grade (poorly differentiated or undifferentiated), lager tumor size (>2 cm), AJCC stage III and positive lymph node were independent risk factors for OS and BCSS, while receiving BCT or PMBR was protective factor for OS and BCSS (**Table 4**). In univariate

TABLE 3 | Effects of surgery in breast cancer-specific survival by demographic and pathological subgroups.

BCSS	BCT *vs* MAS		PMBR *vs* MAS		PMBR *vs* BCT	
Variable	**AHR* (95%CI)**	**p value**	**AHR* (95%CI)**	**p value**	**AHR* (95%CI)**	**p value**
Age (years)						
18–29	0.68 (0.47–0.98)	0.038	0.71 (0.53–0.96)	0.026	0.94 (0.58–1.52)	0.804
30–39	0.81 (0.70–0.94)	0.004	0.73 (0.65–0.82)	<0.001	1.09 (0.91–1.31)	0.356
Race						
White	0.86 (0.73–1.01)	0.074	0.76 (0.67–0.86)	<0.001	1.01 (0.83–1.24)	0.892
Black	0.69 (0.52–0.92)	0.011	0.64 (0.49–0.82)	<0.001	1.33 (0.91–1.96)	0.142
Others	0.65 (0.43–0.98)	0.040	0.68 (0.46–1.01)	0.053	1.15 (0.64–2.07)	0.640
AJCC stage						
I	0.34 (0.19–0.62)	<0.001	0.61 (0.42–0.88)	0.009	2.51 (1.24–5.05)	0.010
II	0.75 (0.62–0.92)	0.006	0.87 (0.74–1.03)	0.095	1.24 (0.97–1.59)	0.088
III	0.83 (0.68–1.01)	0.065	0.66 (0.56–0.77)	<0.001	0.87 (0.68–1.11)	0.257
HRs						
ER+/PR+	0.76 (0.62–0.93)	0.008	0.76 (0.64–0.89)	0.001	1.08 (0.83–1.40)	0.563
ER+/PR−	0.81 (0.51–1.27)	0.352	0.88 (0.65–1.19)	0.399	1.49 (0.89–2.49)	0.127
ER−/PR+	0.80 (0.35–1.79)	0.578	0.30 (0.15–0.62)	0.001	0.48 (0.14–1.69)	0.255
ER−/PR−	0.81 (0.67–0.99)	0.043	0.71 (0.60–0.84)	<0.001	0.96 (0.74–1.25)	0.784
Chemotherapy						
No/Unknown	0.82 (0.39–1.74)	0.604	0.73 (0.50–1.07)	0.108	2.04 (0.70–5.90)	0.189
Yes	0.79 (0.69–0.91)	0.001	0.73 (0.65–0.82)	<0.001	1.04 (0.88–1.24)	0.659

**With adjustment for race, age, marital status, T stage, N stage, histological grade, hormone receptor status, tumor size, surgery, radiation and chemotherapy.*
OS, overall survival; AHR, adjusted hazard ratios; CI, confidential interval; MAS, mastectomy; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction; HRs, hormone receptor status; ER, estrogen receptor; PR, progesterone receptor.

analysis, receiving radiation or chemotherapy were associated with lower OS and BCSS; however, after adjustment for confounding variables with multivariate analysis, receiving radiation was proved to have no significant effect in survival outcomes of young breast cancer patients while receiving chemotherapy had little effect in either OS or BCSS (HR = 1.18, 95%CI: 1.00–1.38, p = 0.049; HR = 1.18, 95%CI: 1.00–1.40, p = 0.047; respectively).

DISCUSSION

By investigating the survival outcomes of young women with operable breast cancer treated with mastectomy, BCT or PMBR in a population of 20,885 patients from the SEER database, our study found that BCT or PMBR had improved OS and BCSS compared with mastectomy for young women with operable breast cancer, which remained significant in subgroup of unmarried patients. In subgroup of married patients, PMBR still conferred better OS and BCSS than mastectomy, but BCT did not. In addition, BCT and PMBR had equal OS and BCSS for young breast cancer patients, which were not affected by marital status.

Previous randomized controlled trials and large retrospective studies have demonstrated that BCT had better or at least equivalent survival outcomes compared with mastectomy (2–5). However, only a low percentage of younger patients has been included and adequately evaluated in these researches. Young breast cancer patients who were considered to have more aggressive tumors and higher risk of local recurrence after breast surgery, the surgical management of breast cancer might be more aggressive even without clear demonstration of benefit (12). Both the European Society of Breast Cancer Specialists (EUSOMA) working group and the fourth international consensus conference for breast cancer in young women recommended breast-conserving surgery as the first option whenever suitable, as it provides same overall survival compared with mastectomy (7, 13). A systemic meta-analysis also declared that BCT provided equivalent survival compared with mastectomy in operable breast cancer patients younger than 40 years old (12). In 2020, Wang et al. (14) noticed that BCT did not have survival benefit compared with mastectomy for young patients with breast cancer; however, the number of young patients in the study was relatively small and the results were only adjusted for tumor size, hormone receptor, HER2 and lymph nodes statuses. As for breast cancer patients who are not suitable for breast conservation, PMBR has been proved to have better or at least equivalent impact on both overall survival and breast cancer recurrence rates compared with mastectomy alone (15). Furthermore, Bezuhly et al. (6) found that immediate breast reconstruction after mastectomy was associated with higher BCSS compared with mastectomy alone among younger women, consistent with the result in our study.

In our finding, there was no significant difference in survival between BCT and PMBR, though PMBR usually brought more injuries to local tissues and needed more time to recover. This result might be attributable to the fact that both BCT and PMBR maintained patients' body image to some extents and improved their psychosocial life, as the breast is a significant aspect of women's body image and has an effect in how women are perceived by others or the society as well as in women's self-perception (16–18). It has been reported that patients with greater psychological stress and less psychosocial support were more likely to have tumor progression and immune

TABLE 4 | Univariate and multivariate analysis of OS and BCSS for young women with operable breast cancer diagnosed between 2004 and 2016.

Characteristics	OS				BCSS			
	Univariate Analysis		Multivariate Analysis*		Univariate Analysis		Multivariate Analysis*	
	HR† (95%CI)	p value	HR† (95%CI)	p value	HR† (95%CI)	p value	HR† (95%CI)	p value
Age (years)								
18–29	Reference	–	Reference	–	Reference	–	Reference	–
30–39	0.78 (0.72–0.85)	<0.001	0.89 (0.78–1.01)	0.081	0.78 (0.71–0.85)	<0.001	0.89 (0.78–1.02)	0.081
Marital status								
Married	Reference	–	Reference	–	Reference	–	Reference	–
Unmarried	1.30 (1.19–1.41)	<0.001	1.22 (1.12–1.34)	<0.001	1.27 (1.17–1.39)	<0.001	1.20 (1.10–1.32)	<0.001
Race								
White	Reference	–	Reference	–	Reference	–	Reference	–
Black	1.67 (1.50–1.85)	<0.001	1.33 (1.19–1.49)	<0.001	1.63 (1.46–1.82)	<0.001	1.31 (1.17–1.47)	<0.001
Others	0.85 (0.73–0.98)	0.021	0.89 (0.77–1.02)	0.100	0.84 (0.73–0.98)	0.022	0.89 (0.76–1.03)	0.106
Year of Diagnosis								
2004–2009	Reference	–	Reference	–	Reference	–	Reference	–
2010–2016	0.97 (0.88–1.07)	0.549	1.06 (0.96–1.17)	0.256	0.96 (0.87–1.06)	0.426	1.05 (0.95–1.16)	0.379
Grade								
Well differentiated	Reference	–	Reference	–	Reference	–	Reference	–
Moderately differentiated	2.87 (2.09–3.95)	<0.001	1.93 (1.40–2.66)	<0.001	3.15 (2.24–4.44)	<0.001	2.09 (1.48–2.95)	<0.001
Poorly differentiated	5.34 (3.91–7.28)	<0.001	2.59 (1.88–3.55)	<0.001	5.92 (4.24–8.28)	<0.001	2.81 (1.99–3.95)	<0.001
Undifferentiated/Anaplastic	5.30 (3.40–8.26)	<0.001	2.55 (1.63–4.00)	<0.001	6.05 (3.80–9.63)	<0.001	2.85 (1.78–4.57)	<0.001
AJCC stage								
I	Reference	–	Reference	–	Reference	–	Reference	–
II	2.81 (2.42–3.26)	<0.001	1.15 (0.93–1.42)	0.193	2.96 (2.53–3.47)	<0.001	1.17 (0.94–1.46)	0.153
III	7.95 (6.85–9.23)	<0.001	2.23 (1.75–2.84)	<0.001	8.57 (7.33–10.03)	<0.001	2.33 (1.81–2.99)	<0.001
Tumor size								
≤2 cm	Reference	–	Reference	–	Reference	–	Reference	–
>2 cm,≤5 cm	2.31 (2.08–2.57)	<0.001	1.40 (1.23–1.60)	<0.001	2.40 (2.16–2.68)	<0.001	1.43 (1.25–1.64)	<0.001
>5 cm	4.81 (4.26–5.43)	<0.001	1.70 (1.45–2.00)	<0.001	4.95 (4.37–5.62)	<0.001	1.70 (1.44–2.00)	<0.001
LN status								
Negative	3.27 (2.97–3.61)	–	Reference	–	Reference	–	Reference	–
Positive	3.47 (2.59–4.65)	<0.001	2.08 (1.83–2.37)	<0.001	3.40 (3.07–3.76)	<0.001	2.12 (1.85–2.41)	<0.001
HRs‡								
ER+/PR+	Reference	–	Reference	–	Reference	–	Reference	–
ER+/PR–	1.58 (1.37–1.82)	<0.001	1.31 (1.14–1.51)	<0.001	1.62 (1.40–1.87)	<0.001	1.34 (1.16–1.55)	<0.001
ER–/PR+	1.93 (1.49–2.50)	<0.001	1.98 (1.53–2.58)	<0.001	1.97 (1.51–2.57)	<0.001	2.02 (1.54–2.64)	<0.001
ER–/PR–	2.06 (1.88–2.26)	<0.001	1.77 (1.60–1.95)	<0.001	2.10 (1.91–2.31)	<0.001	1.80 (1.62–1.99)	<0.001
Surgery								
MAS	Reference	–	Reference	–	Reference	–	Reference	–
BCT	0.48 (0.44–0.54)	<0.001	0.76 (0.67–0.86)	<0.001	0.48 (0.43–0.54)	<0.001	0.76 (0.67–0.86)	<0.001
PMBR	0.56 (0.50–0.62)	<0.001	0.71 (0.64–0.79)	<0.001	0.57 (0.51–0.63)	<0.001	0.73 (0.66–0.82)	<0.001
Radiation								
No	Reference	–	Reference	–	Reference	–	Reference	–
Yes	1.30 (1.19–1.42)	<0.001	0.95 (0.85–1.05)	0.287	1.33 (1.21–1.45)	<0.001	0.96 (0.86–1.07)	0.465
Chemotherapy								
No/Unknown	Reference	–	Reference	–	Reference	–	Reference	–
Yes	2.40 (2.06–2.79)	<0.001	1.18 (1.00–1.38)	0.049	2.48 (2.12–2.91)	<0.001	1.18 (1.00–1.40)	0.047

**With adjustment for age, race, marital status, year of diagnosis, AJCC stage, tumor size, lymph node status, histological grade, hormone receptor status, surgery, radiation and chemotherapy.*

OS, overall survival; BCSS, breast cancer-specific survival; HR†, hazard ratios; CI, confidential interval; LN, lymph node; HRs‡, hormone receptor status; ER, estrogen receptor; PR, progesterone receptor; MAS, mastectomy; BCT, breast-conserving therapy; PMBR, post-mastectomy breast reconstruction.

dysfunction (9), which might partly explain the result that young breast cancer patients who chose BCT or PMBR had better survival outcomes. After both univariate and multivariate analysis using Cox regression model, we found that marriage was a protective factor to prognosis for young breast cancer patients, which was consistent to the results of previous studies (9, 10). The possible underlying reasons why married patients with breast cancer had better prognosis included greater financial resources, more prompt treatments and more psychological support (10). It has also been documented that patients who are married display less depression and anxiety than those who are unmarried after diagnosis of breast cancer, since a partner can share the emotional burden and provide appropriate social support (19, 20). Therefore, clinical doctors are supposed to pay more attention to assessing and relieving the psychological stress of unmarried young patients with breast cancer as well as maximizing their treatment adherence.

To explore the differences of demographic and pathological factors between married and unmarried young patients, we found that unmarried patients had higher AJCC stage and larger tumor size, but had higher percentage of BCT (see **Supplementary Material 1**). The result that unmarried patients had higher tumor stage and larger tumor size was similar to the findings of other studies (9) and could easily explained, since unmarried patients generally had lesser financial resources or psychological support, impeding them to undergo timely physical examination, obtain better insurance coverage and receive more treatments (21). Although unmarried patients were found to have larger tumor size at diagnosis, the percentage of them who underwent BCT was higher than that of married patients, reflecting the importance of breast conservation for unmarried young patients. To further investigate the effect of marital status in survival outcomes of patients who underwent different surgical approaches, we compared the OS and BCSS of patients in the three surgical groups stratified by marital status. As shown in **Figure 3**, we have noticed that unmarried patients who underwent mastectomy had worst OS and BCSS while married patients who underwent BCT had best OS and BCSS, which can be well explained by the finding that both of being unmarried and undergoing mastectomy are adverse predictors for prognosis of breast cancer patients. Furthermore, BCT conferred survival benefit compared with mastectomy in unmarried young patients but not in the married after eliminating confounding bias *via* multivariate analysis. This result is consistent to our assumption, since unmarried young patients were considered to be more concerned about maintaining the shape of their breast after surgery compared with the married. For patients with breast cancer, young women usually have stronger willing to conserve their breast compared with the older, so as to keep their body image and improve confidence in their psychosocial life. Meanwhile, compared with the married, it is more difficult for unmarried young patients to take a hit when told to dissect their breast, as they often need to face with more psychosocial stress while obtain less psychological support than the married. Therefore, even though unmarried young patients are more likely to have higher tumor stage at diagnosis, breast-conserving surgery should be recommended as the first option whenever suitable.

Unlike studies from single institution which had referral bias unavoidably, our study used SEER database, a large population-based cancer registry containing information from all levels of healthcare institutions, to present a more generalizable environment of clinical practice. There are several limitations in our study. Firstly, as a retrospective study including a large population from SEER database, there might existed data-entry errors and selection bias. Secondly, some information about marital status and prognosis of breast cancer patients could not be accessible in SEER database, including levels of hormone, reproductive history and subsequent treatments. Therefore, we could not further investigate the mechanism of the relationship between marital status and the prognosis of breast cancer patients; however, it might have little influence on the results of our research, which mainly focused on the impact of surgical approaches in survival outcomes for young women with operable breast cancer in different marital statuses. Thirdly, the information of ER and PR status was gathered from various pathology laboratories, possibly increasing bias of the data. Finally, information related to local recurrence and regional recurrence were unavailable in the SEER database, thus we failed to recognize patients with breast cancer recurrence who might have more advanced therapies.

CONCLUSION

By investigating the impact of surgical approaches in survival outcomes for young women with operable breast cancer in different marital statuses using the SEER database, our study demonstrated that both BCT and PMBR had improved survival compared with mastectomy for young women with operable breast cancer. The superiority of BCT in survival benefit to mastectomy was seen in unmarried patients but not in married patients. Meanwhile, BCT and PMBR had equal survival benefit for young breast cancer patients, which was not affected by marital status. According to our study, BCT should be recommended as the first option for young women with operable breast cancer whenever suitable; otherwise, PMBR is suggested to maintain the patients' body image as much as possible.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. The data can be found at Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov/).

ETHICS STATEMENT

This study was exempted by the ethics committee of Guangdong Provincial People's Hospital because our data were from the SEER database, which is de-identified and open to the public. And the SEER program approved the use of these data without the need for individual subject consent.

AUTHOR CONTRIBUTIONS

JZ, CY and YZ were involved in the design and coordination of the study as well as in data analysis, interpretation of results, and drafting the manuscript. KW was in charge of all study procedures. The others participated in the study procedures and critically revised the content of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.666316/full#supplementary-material

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ORIGINAL RESEARCH
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Patient-Reported Outcomes From Phase III Neoadjuvant Systemic Trial Comparing Neoadjuvant Chemotherapy With Neoadjuvant Endocrine Therapy in Pre-Menopausal Patients With Estrogen Receptor-Positive and HER2-Negative, Lymph Node-Positive Breast Cancer

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We aimed to evaluate the patient-reported outcomes (PROs) in a prospective phase III clinical trial, comparing neoadjuvant endocrine therapy (NET) with conventional neoadjuvant chemotherapy (NCT) in patients with hormone status positive, lymph node-positive premenopausal breast cancer (NCT01622361). The patients were randomized prospectively to either 24 weeks of NCT with adriamycin plus cyclophosphamide followed by taxane or NET with gonadotropin-releasing hormone agonist and tamoxifen. The patients were examined at the surgery unit of a large tertiary care hospital with a comprehensive cancer center. PROs were assessed on the first day of the trial (day 1, baseline) and at the end of treatment, using the breast cancer module of the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 23 (EORTC QLQ BR23). One hundred and eighty-seven patients were randomly assigned to chemotherapy (n=95) or endocrine therapy (n=92), and 174 patients completed 24 weeks of the neoadjuvant treatment period (n=87, in each group). Baseline scores were similar between the groups. After treatment, there were no statistically significant differences in

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the function scales, including body image, sexual functioning, and sexual enjoyment between the groups, although the endocrine treatment group showed a significant improvement in the future perspective (hazard ratio, 8.3; 95% confidence interval, 1.72–18.38; P = 0.021). Similarly, there were no statistically significant differences in the symptom scales between the groups, including adverse effects of systemic therapy, breast symptoms, arm symptoms, and upset about hair loss. In conclusion, overall PROs were similar in both treatment groups, except for "future perspective," which was significantly better in the NET group than in the NCT group.

Clinical Trial Registration: ClinicalTrials.Gov, identifier NCT01622361.

Keywords: quality of life, neoadjuvant chemotherapy, neoadjuvant endocrine therapy, patient-reported outcomes, Neoadjuvant stusdy of chemotherapy versus Endocrine therapy in premenopausal patient with hormone responsive, HER2-negative, lymph node-positive breaST cancer (NEST)

INTRODUCTION

Neoadjuvant chemotherapy (NCT) is becoming a more common treatment of choice for locally advanced breast cancer patients. Down-staging could lead to a lower extent of surgery, e.g., an increase in the breast conservation rate and a better cosmetic outcome (1–3). However, the adverse effects caused by chemotherapy for breast cancer have both short- and long-term consequences, and the frequency, duration, and severity, as well as challenges in controlling the adverse effects, should be considered in decision making (4–11). Short-term adverse effects typically occur during the treatment and usually resolve within months of the completion of therapy; these adverse effects include emesis, nausea, stomatitis, myelosuppression, myalgia, and alopecia. Long-term adverse effects might have a delayed onset and sustained impact, often lasting for many years (7). In contrast, although the neoadjuvant endocrine therapy (NET) is not yet considered as a standard of care in premenopausal women and should be studied in the context of a clinical trial, the therapy could be an alternative treatment option because the adverse effects and their negative impact on quality of life (QoL) associated with endocrine therapy are relatively mild compared to those with chemotherapy for breast cancer (12–14). However, although the overall impact of ET-induced adverse effects is relatively milder than those of cytotoxic chemotherapy, these adverse effects, such as hot flashes and mood disorders, also affect the QoL of the patients and might lead to discontinuation of the therapy (15–19).

These adverse effects may cause physiological and emotional changes that could affect the patients' QoL. Some studies reported that young patients with breast cancer might have a poorer QoL than older patients because of the distinct impact on their physical and psychosocial well-being (20–22). Improving the ability to predict an individual woman's risk of both long- and short-term adverse effects with various treatments will help her make a better-informed decision regarding therapy. More importantly, the impact of therapy-related adverse effects on young women with breast cancer has not been adequately evaluated.

In our phase III study among premenopausal patients with hormone-responsive, human epidermal growth factor receptor-2 (HER2) negative, lymph node-positive breast cancer [NEST] (NCT01622361) (23), the efficacy, safety, and patient-reported outcomes (PROs) of NET were compared with that of NCT. This study aimed to evaluate short-term treatment-related outcomes using breast cancer-specific PROs from the NEST trial. We hypothesized that different treatment types, namely NCT or NET, would have different impacts on QoL.

MATERIALS AND METHODS

Study Design and Participants

The NEST study was a prospective, multicenter, randomized, parallel-group, comparative phase III clinical trial. Seven centers attached to the Korean Breast Cancer Society Group participated in this study (KBCSG-012). This study protocol was approved by the Korea Food and Drug Administration as well as the institutional review board of every trial center and was conducted in accordance with the Declaration of Helsinki, good clinical practice, and the applicable local regulatory requirements on bioethics.

Patients were randomly assigned (1:1) to receive either adriamycin and cyclophosphamide (60 mg/m^2 adriamycin plus 600 mg/m^2 cyclophosphamide intravenously) every 3 weeks for four cycles followed by taxol (75 mg/m^2 docetaxel intravenously) every 3 weeks for four cycles, or gonadotropin-releasing hormone agonist (3.6 mg) every 4 weeks with tamoxifen 20 mg daily. The treatment was continued for 24 weeks before surgery (**Figure 1** and **Supplement 1**).

PRO Assessments

PROs were assessed using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core Breast Cancer Module 23 (EORTC QLQ-BR23, version 3.0) on day 1 (baseline) and at the end of treatment. The EORTC QLQ-BR23 is a breast cancer-specific module comprising four functional scales and four symptom scales. Responses to all items were converted to a 0 to 100 scale using a standard scoring algorithm (24). For functional scales, the higher scores represent a better level of functioning and QoL.

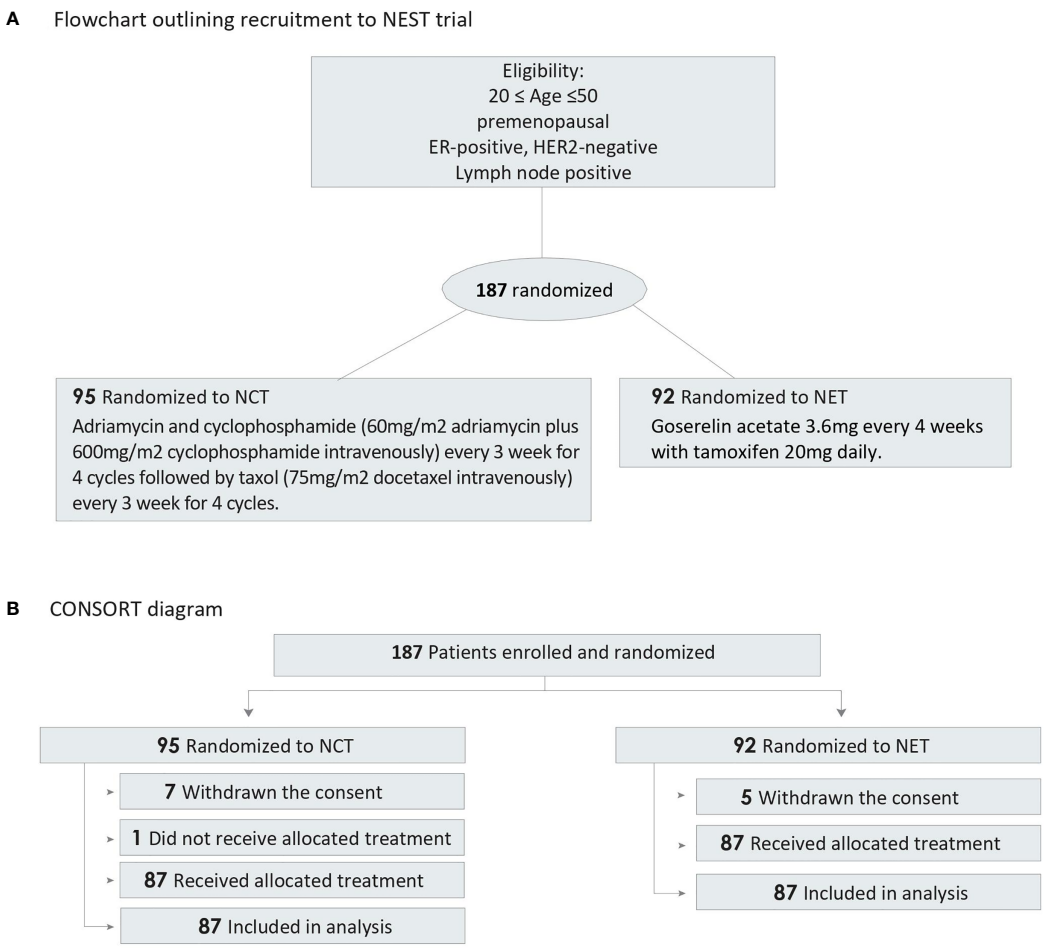

FIGURE 1 | Flowchart and CONSORT Diagram. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy. **(A)** Flowchart outlining recruitment to NEST trial, **(B)** CONSORT diagram of participant randomization.

For symptom scales, a higher score represents a greater severity in the symptoms. Hence, a negative change from baseline in the symptom scales reflects an improvement and a positive change reflects a deterioration. Conversely, a negative change from baseline in the functional scales reflects a deterioration, and a positive change reflects an improvement. Hair loss and alopecia were evaluated according to CTCAE Ver 5.0 (25).

Statistical Analyses

Descriptive statistics and graphical methods were used to describe the degree of change in the EORTC QLQ-BR23 scores at baseline and follow-up. Higher scores indicated better functioning or higher symptom severity. The main analysis was based on the changes from baseline for EORTC QLQ-BR23 scales. To compare between the two groups, Mann-Whitney test was used. The means of difference between two groups were presented as a forest plot. A two-sided $p<0.05$ was considered statistically significant. All analyses were conducted using SAS version 9.4 (SAS Institute; Cary, NC) and R version 3.6.1.

RESULTS

Between July 5, 2012, and May 30, 2017, a total of 187 patients from seven participating centers were included and randomly allocated to one of the two treatment arms. Seven patients in the NCT group and five patients in the NET group withdrew their consent. One patient who was randomly allocated to the NCT group did not receive the treatment. Therefore, a total of 174 patients completed the scheduled treatment and were finally analyzed (87 patients received NCT and 87 patients received NET). Patient characteristics and consort diagram are shown in **Table 1** and **Figure 1**, respectively.

The PROs analysis showed the results of the functional scales and symptom scales. The sample sizes for scores related to "sexual enjoyment" and the "upset by hair loss" scales were considerably smaller than those for the other symptom scales because these questions were only answered if the patients responded that they were sexually active (sexual enjoyment) and/or if the patient experienced hair loss (upset by hair loss), respectively.

TABLE 1 | Patient demographics and baseline characteristics.

	NCT group (n=87)	NET group (n=87)	*p* value
Age			0.255
Mean (SD)	42.5 ± 5.6	41.5 ± 5.8	
20-29	2 (2.3%)	2 (2.3%)	
30-39	20 (23.0%)	31 (35.6%)	
40-49	59 (69.0%)	50 (59.8%)	
50-55	6 (5.7%)	4 (2.3%)	
BMI (kg/m2)			0.921
<18.5	5 (5.7%)	4 (4.6%)	
18.5-24.9	54 (62.1%)	56 (64.4%)	
25-29.9	28 (27.6%)	27(23.0%)	
≥30	4 (4.6%)	7 (8.0%)	
Clinical T stage			0.746
T1	13 (14.9%)	9 (10.3%)	
T2	58 (66.7%)	62 (71.3%)	
T3	16 (18.4%)	16 (18.4%)	
Clinical N stage			0.808
N1	78 (89.7%)	76 (87.4%)	
N2	5 (5.7%)	5 (5.7%)	
N3	4 (4.6%)	6 (6.9%)	
Grade			0.616
G1/2	52 (59.8%)	61 (70.1%)	
G3	3 (3.4%)	4 (4.6%)	
N/A	32 (36.8%)	22 (25.3%)	
Ki 67 expression (%)			0.891
≤20%	49 (56.3%)	48 (55.2%)	
>20%	36 (41.4%)	37 (42.6%)	
Unknown	2 (2.3%)	2 (2.3%)	
Planned operation			0.141
Mastectomy	45 (51.7%)	53 (60.6%)	
Breast Conserving Surgery	42 (48.3%)	34 (39.1%)	

Data are n (%), unless otherwise stated.
NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; SD, standard deviation.

Patient-Reported Functional Scales (QLQ-BR23)

The mean baseline scores of the functional scales (NCT *vs.* NET) of body image (80.69 *vs.* 83.21), sexual function (21.23 *vs.* 20.24), sexual enjoyment (37.93 *vs.* 40.23), and future perspective (45.88 *vs.* 36.40) were generally similar, and the differences were not statistically significant between the treatment groups (**Table 2**).

However, a statistically significant and greater overall change from baseline favoring the NET group than in the NCT group was observed in the score for future perspective (**Figure 2**). The mean change from baseline for future perspective decreased by 8.75 in the NCT group and increased by 8.33 in the NET group, and the difference between the two groups was statistically significant (p=0.021) (**Figure 2**).

No statistically significant differences between the groups were observed in the functional scales for body image, sexual function, and sexual enjoyment (**Figure 3A**).

Patient-Reported Symptom Scales (QLQ-BR23)

The mean baseline scores of the symptom scales (NCT *vs.* NET) of systemic therapy-related adverse effects (19.66 *vs.* 19.38), breast symptoms (21.86 *vs.* 22.51), and arm symptoms (19.61 *vs.* 20.82) were similar between both the treatment arms except for "upset by hair loss," which was considerably lower in the NET arm (42.98 *vs.* 32.35). The question was to be answered only if the patient experienced hair loss; hence, the sample size for the "upset by hair loss" symptom was relatively small (NCT, 23; NET, 17) compared to that for other symptoms (**Table 2**).

No statistically significant differences between the groups were observed in the symptom scales for systemic therapy-related adverse effects, breast symptoms, and arm symptoms (**Figures 2**, **3B**). Grade 2 alopecia, which is defined as hair loss of ≥50% normal for that individual that is readily apparent to others, according to CTCAE Ver 5.0 (25), was not reported in the endocrine group; however, a greater overall change from baseline in the symptom scale for "upset by hair loss" was observed in the NET arm than in the NCT arm; nevertheless, this difference was not statistically significant (**Figures 2**, **3B**). The mean change from baseline score for "upset by hair loss" increased by 1.45 in the NCT group and 15.69 in the NET group; however, the difference between the two groups was not statistically significant. (p=0.557) (**Figures 2**, **3B**).

Among the 87 NET patients, six refused to undergo surgery after treatment. These six patients showed worse scores compared to the other patients in body image functional scales, systemic therapy-related adverse effects, arm symptoms, and "upset by hair loss" symptoms scales; however, none of them were statistically significant (**Figure 4**).

DISCUSSION

We have presented the first detailed cancer-related and breast cancer-specific PROs of a randomized clinical trial comparing NCT *vs.* NET in premenopausal patients with estrogen receptor-positive and HER2-negative, lymph node-positive breast cancer. In both treatment groups, no statistically significant differences were observed between the baseline and post-treatment scores in the overall PROs, including functional scales and symptom scales, except for "future perspective," which was better in the NET group than in the NCT group. However, in the study conducted by Ferreira et al, although it was an adjuvant setting, future perspective recovery was smaller among the groups treated with endocrine therapy (26). This means endocrine therapy seems to attenuate the recovery in domains that typically improve over time such as emotional function and future perspectives. In contrast, the impact of chemotherapy seemed to be transient. These findings suggest long-term follow-up for our study group which may lead to different findings compare to current results.

In general, 15–33% of patients with breast cancer experience concerns related to body image, according to a cross-sectional study by Falk Dahl et al. (27). In our study, the scores for "body image" dropped by more than 10 points from the baseline to post-treatment regardless of the treatment type. This difference is very important, as mean differences of 10 points or more have been considered clinically significant (28). Considering that body image is significantly correlated with adverse psychosocial consequences, such as depression (29) and poor QoL (30),

TABLE 2 | Baseline and follow-up EORTC QLQ-BR23 scores.

	NCT group (n=87)			NET group (n=87)			*p* value
	n	mean	95% CI	n	mean	95% CI	
Baseline							
Functional scales[a]							
Body image	85	80.69	(76.27, 85.1)	87	83.21	(79.38, 87.03)	0.457
Sexual functioning	84	21.23	(16.8, 25.66)	84	20.24	(15.81, 24.66)	0.775
Sexual enjoyment	29	37.93	(31.39, 44.47)	29	40.23	(31.67, 48.79)	0.905
Future perspective	85	45.88	(39.14, 52.63)	87	36.40	(29.75, 43.04)	0.055
Symptom scales/items[b]							
Systemic therapy side effects	85	19.66	(16.73, 22.59)	87	19.38	(17.03, 21.72)	0.639
Breast symptoms	85	21.86	(18.29, 25.44)	87	22.51	(18.92, 26.1)	0.870
Arm symptoms	85	19.61	(16.09, 23.13)	87	20.82	(16.83, 24.8)	0.890
Upset by hair loss	38	42.98	(32.82, 53.14)	34	32.35	(20.73, 43.98)	0.104
Follow up							
Functional scales[a]							
Body image	80	68.54	(62.02, 75.06)	80	70.21	(64.6, 75.81)	0.942
Sexual functioning	80	12.92	(9.28, 16.55)	78	11.54	(7.74, 15.34)	0.468
Sexual enjoyment	19	38.60	(30.54, 46.65)	16	37.50	(26.5, 48.5)	0.903
Future perspective	80	37.92	(30.76, 45.07)	80	42.92	(36.32, 49.51)	0.309
Symptom scales/items[b]							
Systemic therapy side effects	80	41.33	(36.09, 46.57)	80	36.80	(31.79, 41.8)	0.201
Breast symptoms	80	16.98	(13.62, 20.34)	80	14.48	(10.93, 18.03)	0.159
Arm symptoms	80	34.31	(29.26, 39.35)	80	28.75	(23.72, 33.78)	0.101
Upset by hair loss	51	45.10	(34.69, 55.51)	46	44.93	(35.32, 54.54)	0.891
Difference							
Functional scales*							
Body image	80	-13.44	(-21.21, -5.67)	80	-12.36	(-19.62, -5.1)	0.851
Sexual functioning	79	-9.07	(-14.98, -3.16)	75	-9.78	(-14.86, -4.69)	0.678
Sexual enjoyment	6	0.00	(-22.12, 22.12)	9	-11.11	(-33.3, 11.08)	0.462
Future perspective	80	-8.75	(-19.02, 1.52)	80	8.33	(-1.72, 18.38)	0.021
Symptom scales/items							
Systemic therapy side effects	80	21.51	(15.81, 27.2)	80	17.75	(12.3, 23.19)	0.294
Breast symptoms	80	-3.75	(-8.31, 0.81)	80	-7.71	(-12.49, -2.93)	0.438
Arm symptoms	80	15.00	(9.27, 20.73)	80	8.61	(1.75, 15.47)	0.352
Upset by hair loss	23	1.45	(-20.91,23.81)	17	15.69	(-8.64,40.01)	0.557

**Mann-Whitney test.*

[a]*Larger values indicate improvement.*

[b]*Larger values indicate deterioration.*

CI, confidence interval; EORTC, European Organisation for Research and Treatment of Cancer; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; QLQ-BR23, Quality of Life Questionnaire Breast Cancer Module.

physicians should monitor the distress related to altered appearance and help breast cancer patients cope with the related problems not only during the treatment period but also after completion of treatment.

In this study, the overall post-treatment scores in the symptom scales were similar in both treatment groups, including systemic therapy-related adverse effects, breast symptoms, arm symptoms, and "upset by hair loss" symptoms. Notably, only the scores of "breast symptoms" in the symptom scales were increased in both treatment arms, which indicated an improvement. This might be due to the relief derived from the treatment. Although there was no grade 2 alopecia in the endocrine group, post-treatment scores for "upset by hair loss" were similar in both groups. This result is unusual as it would usually be expected that patients in the chemotherapy arm would report higher rates of "upset by hair loss". In this section, we analyzed both baseline and follow-up answers and also did an analysis with a policy that for assumed a "not at all" category for women who did not answer, and it showed a similar result (**Supplementary Table 1**). This might be due to the patients' awareness and preparedness for chemotherapy-induced alopecia, which is a well-known adverse effect. Whereas in NET, hair loss is generally considered as an uncommon adverse effect, so if NET patients experience hair loss, it would be more disappointing. Among the adverse effects induced by therapies, hair loss or hair thinning has one of the highest negative effect on the QoL in patients (31, 32). Although hair thinning or hair loss induced by therapies is a temporary effect, it might cause considerable psychological and emotional distress in patients with breast cancer. While physicians often consider skin reactions such as hair loss as relatively minor compared to the other adverse effects, patients report a higher concern about the dermatological toxicity from anti-cancer therapy (31). These negative effects might lead to the discontinuation of treatment, and indeed some patients might refuse chemotherapy only because of the alopecia (33). Endocrine therapy might also

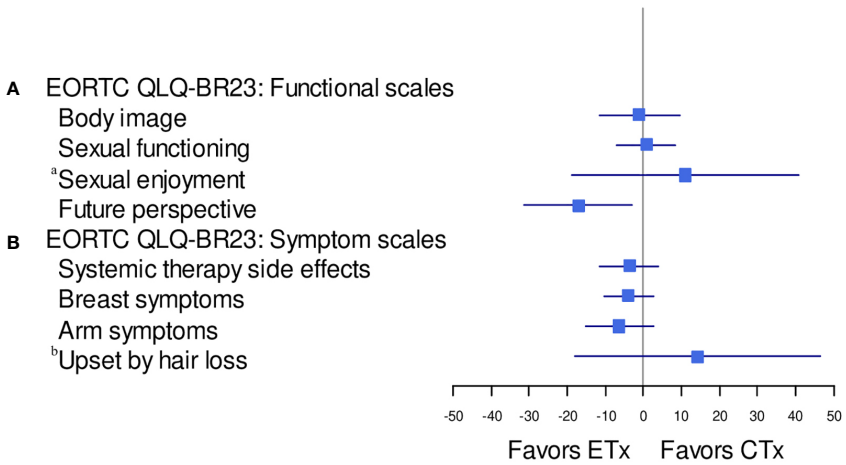

FIGURE 2 | Forest plot model of estimated difference (NET: ETx *vs.* NCT: CTx) in overall change from baseline (repeated-measures mixed-effect model) in PRO-evaluable population. CTx, chemotherapy; EORTC, European Organisation for Research and Treatment of Cancer; ETx, endocrine therapy; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; PRO, patient-reported outcome; QLQ-BR23, Quality of Life Questionnaire Breast Cancer Module; QoL, quality of life. **(A)** EORTC QLQ-BR23: functional scales, **(B)** EORTC QLQBR23 symptom scales. [a]The sample sizes for the "sexual enjoyment" functional scale were smaller than other functional scales because patients were asked to respond question that they were sexually active. [b]The sample sizes for the "upset by hair loss" symptom scale were smaller than other symptom scales because patients were asked to respond to this question only if they responded in a previous question that they were experiencing hair loss.

cause hair loss due to the anti-androgenic effects of the therapy, although the reported incidence of high-grade alopecia with endocrine therapy is relatively low compared with chemotherapy (34, 35). Endocrine therapy (Tamoxifen or gonadotropin-releasing hormone agonist) in hormone receptor-positive breast cancer patients reduces the estrogen levels and might cause hair loss or thinning (36). Freites-Martinez et al. (37) reported that patients receiving endocrine therapies might develop pattern alopecia similar to an androgen type, consistent with the mechanism of action of the causal agents. In a meta-analysis of 35 trials, Saggar et al. (34) reported that the overall incidence of ETs-induced alopecia was 4.4% and ranged from 0% to 25.4%, with the highest incidence in tamoxifen-treated patients. Gallicchio et al. (38) reported that approximately 25% of the patients receiving endocrine therapy experienced hair loss or thinning, and similar incidences of flushes and arthralgia related to endocrine therapy, which are known to affect the QoL (39, 40).

This relatively unexpected and disappointing outcome could have been mitigated by counseling and detailed education by the physicians about the adverse effects, especially hair loss or thinning, before the initiation of treatment (31, 41). However, it should be emphasized that emotional and psychological support to manage the impact of the adverse effect is also crucial, especially in patients receiving NET, in which hair loss or hair thinning is generally considered as unexpected or underrated compared to patients undergoing NCT. Studies have shown the effect of intervention or education in managing the adverse effects in patients receiving chemotherapy (42–46). Bourmaud et al. (47) showed promising efficacy for the educational program to improve treatment adherence and side effect management. Blanckenburg et al. (48) showed that the optimization of expectations might be a potential pathway in health care to improve patients' QoL. Recently, Jacobs et al, designed a randomized controlled trial that employs a patient-centered, evidence-based, virtual videoconference intervention to reduce the impact of adverse effects and to improve adherence to adjuvant endocrine therapy as well (49).

This educational and emotional support might have a positive influence on patients undergoing NET by emphasizing that NET-induced hair loss or hair thinning could be more distressing than expected and encourage the patients to be prepared for the impact.

Limitations of this study include that the QoL assessment was conducted only using the EORTC QLQ-BR23 tool. QoL of the studied patient population could have been further assessed by the World Health Organization Quality of Life-BREF questionnaire, and subsequent comparison with the EORTC QLQ-BR23 could have been valuable and would have strengthened the QoL information of the current study. Other limitations are the small sample size in the "upset by hair loss" section (38 and 34 patients) which results in a major comparison limitation. These factors could have caused the bias on "upset by hair loss" in symptom scale. In addition, in this study, we only measure one time of follow-up at six months, and the perception of "upset by hair loss" and all other domains of symptom scales may change over time. And adjuvant endocrine therapy persists for years, thus, it would be much better to analyze the time to deterioration (TTD) in symptom and functional scales based on the median time for treatment side effects to appear. Further research on QoL in a larger patient population might help

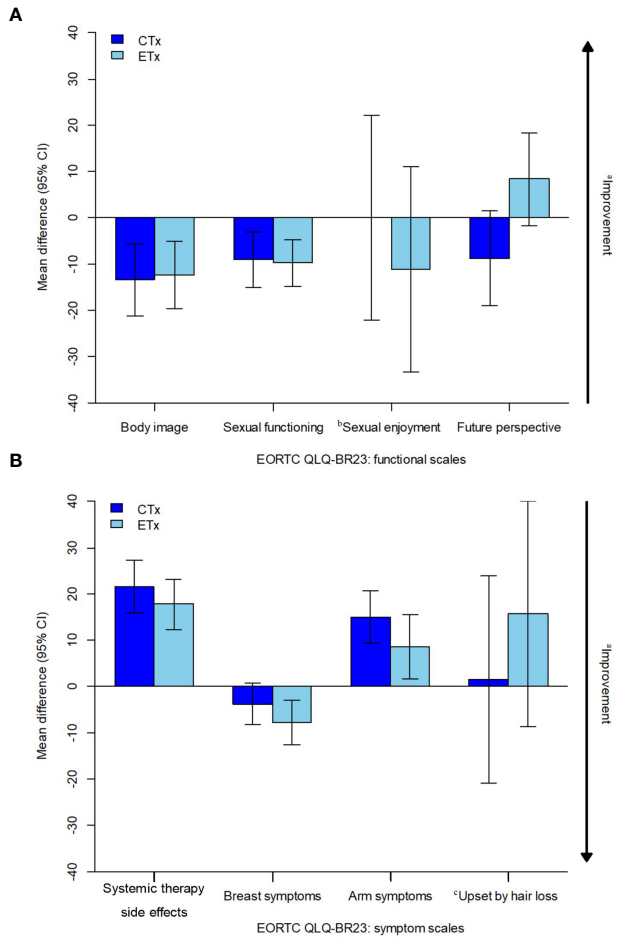

FIGURE 3 | Estimated overall change from baseline in PRO-evaluable population. CI, confidence interval; CTx, chemotherapy; EORTC, European Organisation for Research and Treatment of Cancer, ETx, endocrine therapy; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; PRO, patient-reported outcome; QLQ-BR23, Quality of Life Questionnaire breast cancer module; QoL, quality of life. **(A)** EORTC QLQ-BR23: functional scales, **(B)** EORTC QLQ-BR23: symptom scales. [a]Arrow denotes direction of improved outcome. [b]The sample sizes for the "sexual enjoyment" functional scale was smaller than other functional scales because patients were asked to respond to this question only if they responded in a previous question that they were sexually active. [c]The sample sizes for the 'upset by hair loss' symptom scale was smaller than other symptom scales because patients were asked to respond to this question only if they responded in a previous question that they were experiencing hair loss.

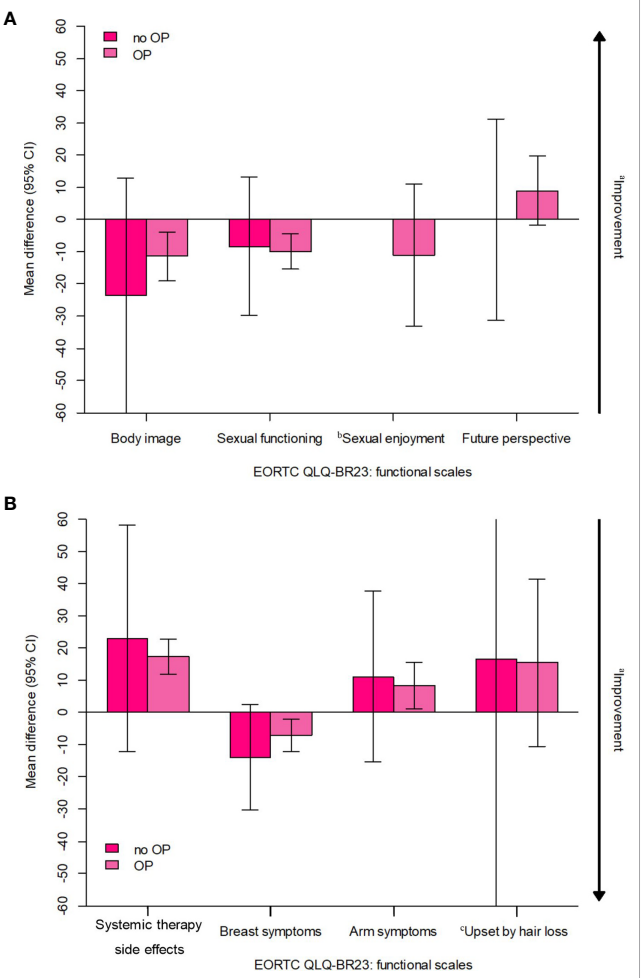

FIGURE 4 | Estimated overall change from baseline in 6 patients who refuse to undergo surgery after treatment (all received NET). CI, confidence interval; EORTC, European Organisation for Research and Treatment of Cancer; NET, neoadjuvant endocrine therapy; PRO, patient-reported outcome; QLQ-BR23, Quality of Life Questionnaire Breast Cancer Module; QoL, quality of life. **(A)** EORTC QLQ-BR23; functional scales, **(B)** EORTC QLQ-BR23: symptom scales. [a]Arrow denotes direction of improved outcome. [b]The sample sizes for the "sexual enjoyment" functional scale was smaller than other fuctional scales because patients were asked to respond to this question only if they responded in a previous question that they were sexually active. [c]The sample sizes for the "upset by hair loss" symptom scale was smaller than other symptom scales because patients were asked to respond to this question only if they responded in a previous question that they were experiencing hair loss.

clinicians to further understand the true impact on the QoL of the patients.

In conclusion, overall PROs were similar in both treatment groups, except for "future perspective" in the functional scales of EORTC QLQ-BR23 which was significantly better in the NET group than in the NCT group. The result provides a clinical rationale to emphasize pre-treatment education or emotional support, including that for expected effects on hair, to patients receiving NET as well as those undergoing NCT.

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 Asan Medical Center Institutional Review Board.

The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HK designed the study. SG and HK drafted the manuscript and HK wrote the original protocol for the study. All authors participated in the design of the study. HK filed for ethical approval from the Korea Food and Drug Administration and registered the trial on clinicaltrials.gov. GG was responsible for the pathology reports. SK-O performed the statistical analysis. SA conceived of the study and participated in its design. SA, WN, EL, YJ, LK, WH, and SN were involved in the study design and inclusion of patients in this trial. All authors contributed to the article and approved the submitted version.

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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.2021.608207/full#supplementary-material

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Circulating Tumor DNA to Interrogate the Safety of Letrozole-Associated Controlled Ovarian Stimulation for Fertility Preservation in Breast Cancer Patients

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Background: Current fertility preservation strategies for young breast cancer patients planning a future motherhood include the association of controlled ovarian stimulation with the aromatase inhibitor letrozole (let-COS) to harvest mature oocytes while maintaining low estradiol levels. Despite this is a widely adopted protocol, the safety of let-COS on breast cancer outcomes has been poorly investigated and its use remains off-label. We assessed the safety of let-COS in breast cancer patients using circulating tumor DNA (ctDNA) as a surrogate biomarker of disease recurrence.

Methods: BROVALE is an interventional non-randomized prospective study designed to evaluate the efficacy and safety of let-COS for fertility preservation in early breast cancer patients before starting (neo)adjuvant chemotherapy. Letrozole was administered throughout the COS cycle, until ovulation triggering. Safety was a secondary endpoint. Data on oncological outcomes were collected during the follow-up as well as plasma and whole blood for evaluation of ctDNA levels at the time of enrollment (i.e. before starting let-COS) and oocyte retrieval (i.e. 48 hours after the last administration of letrozole). Targeted gene sequencing on the primary tumor samples was performed to identify specific mutations used for ctDNA analysis by digital PCR. DNA extracted from whole blood samples was used to discriminate between somatic and germline mutations.

Results: From April 2014 to May 2017, 29 young early breast cancer patients enrolled in the BROVALE study who had available tissue samples participated to the ctDNA substudy. Among them, 15 had at least one validated somatic mutation. ctDNA was undetectable neither before nor after let-COS in 9 of them. Six patients had detectable

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ctDNA in the plasma samples collected before Let-COS. No change in ctDNA level after let-COS was observed in 3 patients and the level decreased (fold-change ≤ 0.5) in two women. One patient experienced an increased (fold-change ≥ 2) in ctDNA level but without disease relapse 34 months after diagnosis.

Conclusions: No increase in ctDNA level was observed in 93% (14/15) of the patients receiving let-COS supporting its use as a safe strategy for young women with early breast cancer interested in fertility preservation before chemotherapy.

Keywords: breast cancer, fertility preservation, letrozole, ovarian stimulation, circulating tumor DNA

INTRODUCTION

Recent advances in screening procedures and anticancer treatments have markedly improved survival in young early breast cancer patients (1). The majority of young women with newly diagnosed early breast cancer are candidates to receive neoadjuvant or adjuvant chemotherapy including gonadotoxic drugs that might severely impact their reproductive function and future fertility (2, 3). Therefore, oncofertility counseling is currently mandatory in all patients diagnosed during their reproductive years and, for women planning a future motherhood, fertility preservation before starting chemotherapy is standard of care (4–6).

Oocyte and/or embryo cryopreservation is currently the first strategy for fertility preservation to be offered to young early breast cancer patients (7). The standard approach to collect a maximum number of mature oocytes includes 10-15 days of controlled ovarian stimulation (COS) with gonadotropins using a gonadotropin-releasing hormone (GnRH) antagonist protocol to avoid premature spontaneous luteinizing-hormone (LH) peak (8). As this protocol is associated with a supraphysiological raise in estradiol levels, concerns have been raised on its potential detrimental prognostic effect in hormone-sensitive cancer such as breast cancer (9, 10). The co-administration of an aromatase inhibitor (letrozole) during COS allows to harvest several mature oocytes while maintaining low estradiol levels (11–13). A recent meta-analysis of 11 studies comparing standard COS with protocols including the administration of letrozole confirmed a similar efficacy in terms of oocyte yield, maturation and fertilization rates, but with significantly reduced estradiol levels when letrozole is included in the COS protocol (14). Despite this is a widely adopted protocol, the safety of letrozole-associated COS (let-COS) on breast cancer outcomes has been poorly investigated and its use is currently off-label in this indication.

Liquid biopsy evaluating the presence of circulating tumor DNA (ctDNA) is widely used as a minimally invasive tool offering a wide range of clinical applications (15). Among them, the detection of ctDNA during follow-up has been shown to be associated with a high risk of disease relapse in patients with early breast cancer (16–19).

In this study, we aimed to explore the safety of let-COS for oocyte and/or embryo cryopreservation in a prospective cohort of young women with early breast cancer who preserved their fertility before chemotherapy. For this purpose, in addition to oncological outcomes, we explored potential changes in ctDNA levels before and after let-COS as a possible surrogate measure of tumor development and predictor of disease relapse.

METHODS

Patient Population

BReast cancer OVAry LEtrozole (BROVALE) (NCT02661932) is an interventional non-randomized prospective study designed to evaluate the efficiency and safety of let-COS for fertility preservation in young women with early breast cancer. Details of the study have been previously reported (12). The present biomarker analysis addressed one of the planned secondary endpoints of the study focusing on the safety of let-COS. For this purpose, the changes in ctDNA levels before and after let-COS as well as oncological outcomes were assessed.

In BROVALE, standard or random start COS protocol using gonadotropins (150 to 300 IU/day) and GnRH antagonist (0.25mg/d from day 6, or when follicles reached 14 mm) was applied in all patients. GnRH agonist or human chorionic gonadotropin (hCG) were used for triggering when at least two follicles exceed 18mm and transvaginal ultrasound-guided oocyte retrieval occurred 36 hours later. Letrozole (5mg/day per os) was administered throughout the COS cycle, starting one day before or concomitantly with gonadotropins until ovulation triggering as previously described (12).

The Ethic Committee of Erasme Hospital approved the study. Informed consent was obtained from all participants before study inclusion.

Study Procedures

Whole blood samples for genomic DNA preparation were collected in EDTA tubes at the time of enrollment (i.e. before let-COS) and at oocyte retrieval (i.e. 36 hours after last administration of letrozole). Plasma and whole blood were immediately stored at -80°c until DNA extraction. Formalin fixed paraffin embedded (FFPE) tumor samples were collected from participating patients.

Plasma cell-free DNA (cfDNA) was extracted using the QIAamp circulating nucleic acid kit (Qiagen). Genomic DNA was extracted from whole blood samples using the Qiagen DNeasy Blood & Tissue Kit to discriminate somatic from germline mutations. DNA from primary tumor samples

(FFPE) was extracted using the Qiagen QIAamp DNA FFPE tissue kit.

Somatic mutations were identified from primary tumor samples by targeted gene sequencing using the Truseq Amplicon Cancer 48-gene Panel (Illumina, reference FC-130-1008). Sequence reads from the tumor and normal samples were aligned against the human genome reference version hg19/GRCh37 using the BWA (v.0.7.15) aligner with default parameter settings. In order to correct for mapping errors made by BWA around indels, a local realignment step was performed using IndelRealigner from the GATK (v.4.0.3.0) suite. When matched normal genomic DNA was available, somatic mutation calling was performed with two distinct variant callers, Manta (v.1.3.2)/Strelka (v.2.9.2) and Mutect 2 (v.4.0.3.0), using default parameters. When matched normal genomic DNA was not available, mutation calling was performed with two distinct variant callers, Pisces (v.5.1.6.54) & Mutect 2 (v.4.0.3.0), using default tumor mode only. Somatic mutations were annotated using ANNOVAR. Mutations were then filtered by selecting only exonic, non-synonymous single nucleotide variant (SNV) with a variant allele frequency (VAF) ≥ 8% and a coverage ≥ 1000 reads. Only known COSMIC (v.81) mutations with a frequency lower than 1% in the ExAC (v.0.3.1) database were used in further analysis.

The presence of plasma ctDNA was evaluated using the highly sensitive and precise digital PCR, a refined method of the conventional polymerase chain reaction (PCR). In particular, patient-specific droplet digital PCR (ddPCR) assays (Biorad PrimePCR ddPCR Mutation Assay or custom Assay) were used to detect the mutations identified in the tumor samples, with a single mutation being selected for each patient as previously reported (20).

RESULTS

Between April 2014 and May 2017, 31 early breast cancer patients with available tissue samples participated in the BROVALE ctDNA study. Two patients were excluded from further analysis due to low tumor DNA quantity (<50 ng; **Supplementary Figure 1**). All included patients underwent let-COS for fertility preservation before starting (neo)adjuvant chemotherapy. Out of 29 patients included in the present analysis, 12 (41.4%) had estrogen receptor (ER)-positive/HER2-negative tumors, 12 (41.4%) HER2-positive disease and 5 (17.2%) triple-negative breast cancer. Patients' and oncological characteristics are summarized in **Table 1**.

Targeted gene sequencing was performed on primary tumor samples of the 29 included patients in order to identify somatic mutations for subsequent plasma ctDNA detection. Sixteen (55%) tumor samples presented at least 1 somatic mutation either in *TP53* (44.8%) or *PIK3CA* (17.2%) genes. No mutations could be identified in the other interrogated genes. A single mutation was selected for each of the 16 patients, being *TP53* and *PIK3CA* mutations in 11 and 5 patients, respectively (**Figures 1A, B**). Fifteen of them (93.8%) were further

TABLE 1 | Patients and tumor characteristics (n=29).

	All patients (n = 29)	Patients without mutation (n = 14)	Patients with mutation (n = 15)	P value*
Age, IQR	31 (29-35)	31.6 (28.5-34.8)	32.4 (30-35)	0.57
Clinical setting				
Adjuvant	15 (51.7)	9 (64.3)	6 (40.0)	0.35
Neoadjuvant	14 (48.3)	5 (35.7)	9 (60.0)	
Tumor size				
0.1-5 cm	26 (89.7)	12 (85.7)	14 (93.3)	0.95
>5 cm	3 (10.3)	2 (14.3)	1 (6.7)	
Nodal status				
Negative	20 (69.0)	9 (64.3)	11 (73.3)	0.9
Positive	9 (31.0)	5 (35.7)	4 (26.7)	
Grade				
I/II	11 (37.9)	6 (42.9)	5 (33.3)	1
III	18 (62.1)	8 (57.1)	10 (66.7)	1
Ki67%, IQR	54.3 (20-80)	50.3 (20-84)	58 (35-78)	0.66
Estrogen receptor status				
Negative	8 (27.6)	3 (21.4)	5 (33.3)	0.88
Positive	21 (72.4)	11 (78.6)	10 (66.7)	
Progesterone receptor status				
Negative	12 (41.4)	5 (35.7)	7 (46.7)	0.76
Positive	17 (58.6)	9 (64.3)	8 (53.3)	
HER2 status				
Negative	17 (58.6)	8 (57.1)	9 (60.0)	0.83
Positive	12 (41.4)	6 (42.9)	6 (40.0)	
Parity				
Parous	5 (17.2)	2 (14.3	3 (20.0)	1
Nulliparous	24 (82.8)	12 (85.7)	12 (80.0)	

**Wilcoxon rank sum test for continuous variables and Fisher test for categorical variables, for the comparison between patients with and without mutation.*
IQR, interquartile range.

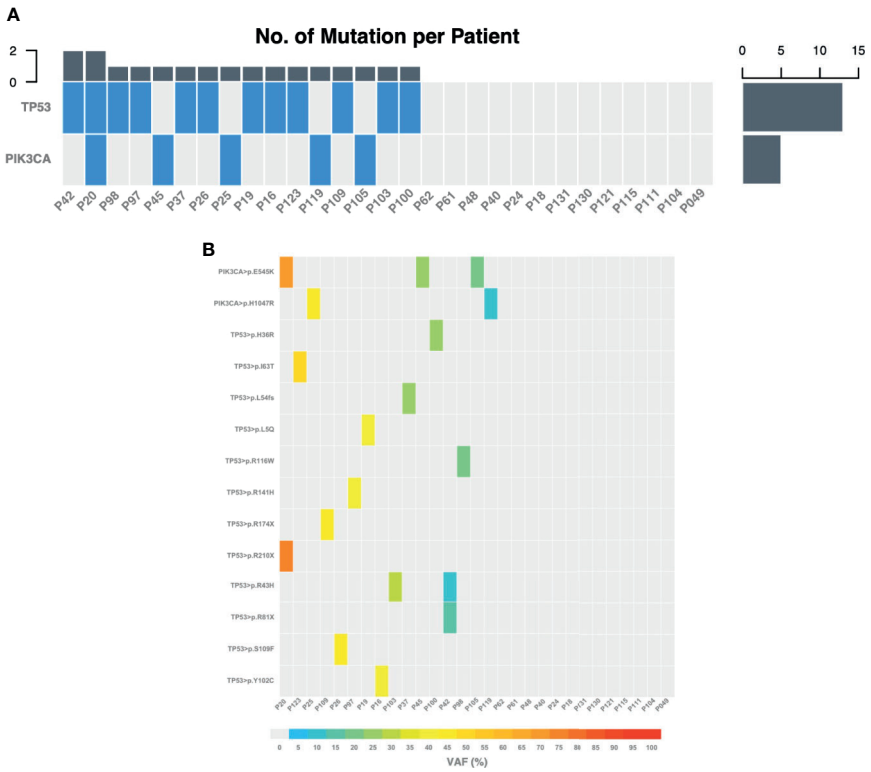

FIGURE 1 | Somatic mutations identified using targeted gene sequencing on the primary tumor samples. **(A)** Heatmap of genes for which at least one mutation was indexed across the 29 patients. **(B)** Heatmap of the variant allele frequency for each specific mutation indexed across the 29 patients. VAF, variant allele frequency.

validated using highly sensitive patient-specific mutation ddPCR assays with a high concordance being observed in the variant allelic frequency (VAF) between targeted gene sequencing and ddPCR (**Figure 2**).

For these 15 patients, the median duration of the stimulation was 9 days (range 5-14) and median estradiol peak reached 291pg/ml (range 55-928). A median of 6 mature oocytes were collected (range 1-21) (**Table 2**).

The presence of ctDNA was assessed in the plasma samples collected before and after Let-COS using ddPCR. In 9 out of 15 patients, ctDNA was not detectable before nor after let-COS. None of them had disease relapse during follow-up (**Table 3**). Six patients had detectable ctDNA in the plasma samples collected before Let-COS (**Figure 3**). An increase in ctDNA level after let-COS (fold-change ≥ 2) was observed in only one patient without disease relapse at the last follow-up visit 34 months after breast cancer diagnosis (P123). On the contrary, 3 patients had no change in ctDNA level after let-COS (P103-P16-P26), one of whom developed disease-relapse after 13 months of follow-up and died (P26). This patient was diagnosed with triple-negative breast cancer (T2N2) and had the highest average number of mutated copies in the plasma before and after the procedure (427.03 and 467.67 ctDNA copies/ml, respectively). Other 2 patients (P20-P37) had a decrease in ctDNA level after let-COS (fold-change ≤ 0.5), one of whom developed disease-relapse (P37) (**Table 3**).

DISCUSSION

In young women with early breast cancer interested in preserving fertility before starting neoadjuvant or adjuvant chemotherapy, oocyte and/or embryo cryopreservation following Let-COS protocol is widely adopted and recommended (21–23). However, the safety of this approach relies mainly on one single-center prospective non-randomized study showing no difference in risk of recurrence between 120 breast cancer patients who performed Let-COS for oocyte and/or embryo cryopreservation and a control group of 217 patients who did not preserve their fertility before starting chemotherapy (13). In a recent large prospective multicenter Swedish study including 380 women with breast cancer who underwent COS for fertility preservation between 1995 and 2017, the 5-year survival proportion was similar compared to breast cancer patients who did not perform COS (24). In this study, Let-COS was offered to only 59% of the patients. Moreover, oncological characteristics of the population were not reported, leading to important potential biases in the survival analysis (24).

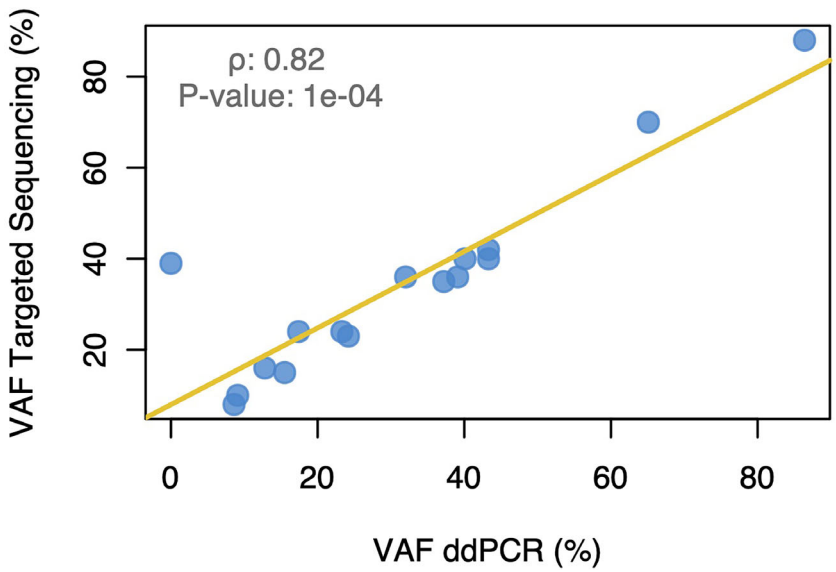

FIGURE 2 | Correlation between the variant allele frequency of the somatic mutations identified using targeted gene sequencing and droplet digital PCR. VAF, variant allele frequency; ddPCR, droplet digital PCR.

Therefore, defining the safety of performing COS for oocyte and/or embryo cryopreservation in breast cancer patients remains a clinical research priority (25). As shown in a recent survey involving breast cancer specialists, more than one third of them are concerned about the potential detrimental prognostic effect of COS in patients with breast cancer (10).

To our knowledge, this biomarker analysis is the first study addressing the safety of performing let-COS for fertility preservation in young breast cancer patients using ctDNA as a surrogate biomarker of disease recurrence. Indeed, among the wide range of clinical applications of this sensitive minimally invasive tool, molecular relapse detection is one of the most promising (16–19).

We first performed targeted gene sequencing in the primary tumors in order to identify the somatic mutations to be assessed for ctDNA detection in the plasma samples. Mutations were only present in *TP53* and *PIK3CA* genes that are known to be the two most frequently mutated genes in breast cancer (26). Notably, 15 out of the 16 mutations identified in the primary tumors were further validated using ddPCR. In our study, ctDNA was detected in 40% of the plasma samples before let-COS and the initiation of chemotherapy. This is similar to previous studies reporting a detection rate of approximately 50% in patients with newly diagnosed early breast cancer irrespective of molecular subtype and prior to any treatment (27).

Reassuringly, let-COS did not induce the emergence of ctDNA in these patients, although the majority of patients had hormone receptor-positive disease and supraphysiological estradiol levels (>500pg/ml) were reached in a third of them.

TABLE 2 | Characteristics of the COS cycles.

Patients ID	AMH (ng/ml)	Total doses of gonadotropins (IU)	COS duration (days)	E2 peak at triggering (pg/ml)	Triggering	Oocytes yield (N)
P20	0.42	3300	13	55	hCG	2
P103	1.9	3950	14	238	GnRHa	6
P37	6.1	1338	8	469	GnRHa	21
P16	0.1	2775	11	95	hCG	3
P26	0.54	450	5	65	hCG	1
P123	3.9	2250	9	472	GnRHa	15
P19	0.67	1700	10	487	hCG	7
P25	0.24	2250	9	291	hCG	4
P42	0.44	2700	9	92	GnRHa	3
P45	–	2400	8	133	GnRHa	2
P98	1.7	2250	9	468	GnRHa	10
P100	4.5	3500	13	747	GnRHa	10
P105	2.9	1800	8	615	GnRHa	5
P109	5.7	2200	11	928	GnRHa	16
P119	2.2	2025	9	291	GnRHa	11

AMH, Anti-Müllerian Hormone; COS, Controlled Ovarian Stimulation; hCG, human Chorionic Gonadotropin; GnRHa, Gonadotropin Releasing Hormone Agonist.

TABLE 3 | Characteristics of the patients with identified mutations, targeted gene sequencing and droplet digital PCR results and changes in circulating tumor DNA before and after controlled ovarian stimulation (total n = 15).

Patients characteristics					Tumor characteristics									ctDNA before COS		ctDNA after COS	
Patient ID	Age	Relapse	Alive	DFS follow-up (month)	Subtype	T	N	Ki67%	Gene	Mutation	ddPCR assay type	Primary VAF NGS %	Primary VAF ddPCR %	Plasma VAF %	Copies mutated average/ml plasma	Plasma VAF %	Copies mutated average/ml plasma
P20	27	No	Yes	68	ER+/PR+/HER2+	2	0	75	PIK3CA	pE545K	PrimePCR ddPCR Mutation Assay	70	65.1	0.75	3.68	0	0
P103	34	No	Yes	40	TNBC	2	–	70	TP53	p.R175H/ R43H	PrimePCR ddPCR Mutation Assay	35	37.2	0.27	1.99	0.14	1.61
P37	28	Yes	Yes	57	ER+/PR-/HER2-	2	0	75	TP53	p.L54fs	PrimePCR ddPCR Custom Assay	24	17.4	1.79	58.27	1.95	28.37
P16	35	No	Yes	65	TNBC	2	0	90	TP53	p.Y102C/ Y234C	PrimePCR ddPCR Custom Assay	36	39.1	0.22	2.91	0.26	5.10
P26	35	Yes	No	13	TNBC	2	2	95	TP53	p.S109F/ S241F	PrimePCR ddPCR Custom Assay	40	40.1	22.5	427.03	28.75	467.67
P123	34	No	Yes	34	ER-/PR-/HER2+	2	0	90	TP53	p.I63T	PrimePCR ddPCR Mutation Assay	88	86.4	9.05	65.93	14.35	188.60
P19	34	No	Yes	56	ER+/PR+/HER2+	1	1	80	TP53	p.L5Q	PrimePCR ddPCR Custom Assay	36	32	0	0	0	0
P25	35	No	Yes	27	ER+/PR+/HER2-	1	0	35	PIK3CA	p.H1047R	PrimePCR ddPCR Mutation Assay	42	43.3	0	0	0	0
P42	35	No	Yes	13	ER+/PR+/HER2-	1	0	64	TP53	p.R175H/ R43H	PrimePCR ddPCR Mutation Assay	10	9.1	0	0	0.045	0.61
P45	36	No	Yes	46	ER+/PR+/HER2-	2	0	60	PIK3CA	pE545K	PrimePCR ddPCR Mutation Assay	24	23.3	0.07	0.69	0	0
P98	24	No	Yes	50	ER+/PR-/HER2+	3	0	10	TP53	p.R248W/ R116W	PrimePCR ddPCR Mutation Assay	16	12.8	0	0	0.0095	0.54
P100	32	No	Yes	41	ER-/PR-/HER2+	1	1	60	TP53	p.H36R	PrimePCR ddPCR Custom Assay	23	24.2	0	0	0	0
P105	31	No	Yes	37	ER+/PR+/HER2-	2	0	16	PIK3CA	pE545K	PrimePCR ddPCR Mutation Assay	15	15.5	0	0	0.13	0.46
P109	29	No	Yes	30	ER+/PR+/HER2+	1	–	15	TP53	p.R174X	PrimePCR ddPCR Mutation Assay	40	43.3	0.014	2.76	0	0
P119	37	No	Yes	15	ER+/PR+/HER2-	2	0	35	PIK3CA	p.H1047R	PrimePCR ddPCR Mutation Assay	8	8.6	0	0	0	0

ctDNA, circulating tumor DNA; COS, controlled ovarian stimulation; NGS, targeted gene sequencing; ddPCR, droplet digital PCR; DFS, disease-free survival; ER, estrogen receptor; PR, progesterone receptor; TNBC, Triple-Negative Breast cancer; VAF, variant allele frequency.

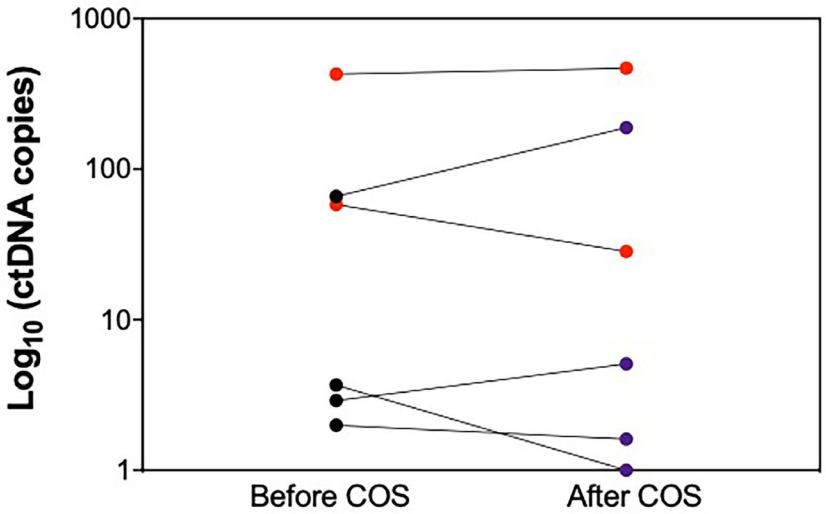

FIGURE 3 | Changes in circulating tumor DNA levels between the time of enrollment (i.e. before starting letrozole-associated controlled ovarian stimulation) and oocyte retrieval (i.e. 48 hours after the last administration of letrozole). Red dots = disease relapse during oncologic follow-up. Violet = no disease relapse during oncologic follow-up. ctDNA, circulating tumor DNA; COS, controlled ovarian stimulation.

In our study, ctDNA was detected in 6 patients at enrollment but increased in only one of them. Importantly, no negative effect on her oncological outcomes was observed. Notably, the patient exhibiting the highest ctDNA level at both time-points relapsed shortly after entering the study and died. She was affected by triple-negative breast cancer and had the shortest stimulation duration characterized by very low estradiol levels during COS. On the contrary, all patients with undetectable or very low ctDNA levels remained disease-free at the time of the last follow-up. The observation that there was no increase in ctDNA levels in the majority of the patients indirectly supports the lack of potential detrimental prognostic effect of a short-course of hormonal manipulation with let-COS in young women with early breast cancer before exposure to chemotherapy.

In terms of study limitations, this biomarker analysis has a relatively limited sample size. Formal statistical calculations could not be performed. Moreover, despite promising, to date the role of ctDNA as a tool for disease monitoring in patients with early breast cancer remains experimental without direct clinical application yet. However, importantly, this analysis was conducted within an interventional non-randomized prospective study and all biological samples were prospectively collected.

In conclusion, this biomarker analysis of the BROVALE study showed no increase in ctDNA levels in 93% of young women with early breast cancer who received let-COS for oocyte and/or embryo cryopreservation as a strategy to preserve fertility before starting neoadjuvant or adjuvant chemotherapy. These data indirectly support the use of this strategy as a safe approach in young early breast cancer patients interested in fertility preservation before chemotherapy initiation. Further validation of these findings in a large prospective clinical trial is warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study involving human participants was reviewed and approved by Erasme Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FR, ML, MI, and ID contributed to the conception and design of the study. OG and ID contributed to patients' enrollment in the BROVALE study and sample collection. FR, ML, MM, YB, JB, GR, DL, CS, and MI contributed to sample storage, processing, analysis and interpretation. The results were interpreted by FR, ML, MI, and ID. The initial manuscript was drafted by FR, ML, MI, and ID. All authors contributed to the article and approved the submitted version.

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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.2021.686625/full#supplementary-material

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The reviewer FS declared a past co-authorship with one of the authors ML to the handling editor.

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"High-Risk Breast Cancer Screening in BRCA1/2 Carriers Leads to Early Detection and Improved Survival After a Breast Cancer Diagnosis"

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Background: Germline *BRCA1/2* pathogenic variant (PV) carriers have high lifetime risk of developing breast cancer and therefore subjected to intense lifetime screening. However, solid data on the effectiveness of high-risk screening of the *BRCA1/2* carrier population is limited.

Patients and Methods: Retrospectively, we analyzed 346 women diagnosed with breast tumors. Patients were divided according to the timing of *BRCA1/2* PVrecognition, before (BRCA-preDx awareness, N = 62) or after (BRCA-postDx awareness group, N = 284) cancer diagnosis.

Results: Median follow-up times were 131.42 and 93.77 months in the BRCA-preDx awareness and BRCA-postDx awareness groups, respectively. In the BRCA-preDx awareness group, 78.7% of the patients had invasive tumors and 21.3% were diagnosed with pure ductal carcinoma *in situ*. In contrast, in the BRCA-postDx awareness group over 93% of women were diagnosed with invasive cancer and only 6.4% had *in situ* disease. The mode of tumor detection differed significantly between the groups: 71.9% in the BRCA-postDx awareness group and 26.2% in the BRCA-preDx awareness group were diagnosed after personally palpating a lump. Tumor size and nodal involvement were significantly more favorable in the BRCA-preDx awareness group. T stage was significantly lower in the BRCA-preDx awareness group: 54.84% at T1 and 20.96% at Tis. In the BRCA-postDx awareness group, only 37.54% were at T1 and 6.49% at Tis. The N stage was also significantly lower in the BRCA-preDx awareness group: 71% had no lymph node metastases, compared with 56.1% in the BRCA-postDx awareness group. Additionally, therapeutic procedures varied between the groups: BRCA-preDx awareness group patients underwent more breast conserving surgeries. Axillary lymph node dissection was done in 38% of women in the BRCA-postDx

awareness group and in only 8.7% of the BRCA-preDx awareness group patients. Interestingly, improved survival was found among patients who underwent high-risk screening (hazard ratio=0.34).

Conclusions: High-risk screening might facilitate downstaging of detected breast tumor among *BRCA1/2* carrier population.

Keywords: breast cancer, *BRCA1/2*, high-risk, survival, screening, downstaging

INTRODUCTION

Breast cancer is the most prevalent non-cutaneous cancer among women (1). In general, once diagnosed, early and accurate detection of the tumor size and degree of spreading is very important, since treatment in the early stages of the disease can improve the prognosis and save lives (2).

Women who carry a *BRCA1* or *BRCA2* pathogenic variant (PV) are at an increased risk of developing breast cancer. These women hold a lifetime risk as high as 60% to 90% (3), and also a risk of developing it at younger age than women in the general population (4). Finding *BRCA1/2* PV significantly alters medical management (5) and prompts earlier and more frequent screening and risk-reduction surgeries (6).

As part of the high-risk screening, the National Comprehensive Cancer Network's (NCCN) Guidelines state that for *BRCA1/2* carriers, annual magnetic resonance imaging (MRI) and clinical breast exams should start at age 25, and mammograms should start at the age of 30 (7).

There is evidence that for carriers of *BRCA2* PV, a combination of MRI screening and annual mammography can have a survival benefit (8, 9). On the other hand, for *BRCA1* PV carriers the high-risk screening appears to be less effective. This might be because of the high prevalence of triple-negative breast cancer (TNBC) in *BRCA1* carriers, an aggressive subtype with a poor prognosis (10).

Mammography can detect lesions at a minimal size of 1 mm and reveal breast cancer several years before it can be detected in a physical examination (11). MRI is even more sensitive screening modality than mammography alone (11), and the combination of the two is the most sensitive method for detecting breast cancer (12). However, information on the effectiveness of high-risk screening for the *BRCA* carrier population is limited. Frequent physical examination, mammography, and MRI, starting as early as possible in the high-risk population of *BRCA* carriers, are commonly used.

Here we aimed to determine whether high-risk screening has the potential to benefit *BRCA1/2* PV carrier population.

METHODS

Study Design & Patients

This retrospective study included 346 high-risk women who were diagnosed with breast cancer in 1996–2020 at the Oncology Department of the Hadassah-Hebrew University Medical Center in Jerusalem. The study focused on patients diagnosed during 1996-2020 due to the data accessibility.

The high-risk women are *BRCA1/2* PV carriers. The 346 patients were divided into two groups in order to determine the impact of high-risk screening. The BRCA-preDx awareness group comprised 62 women who knew that they were carriers of *BRCA* PVs. Therefore, they were offered intensified screening prior to breast cancer diagnosis. The BRCA-postDx awareness group consisted of 284 patients who first were diagnosed with breast cancer and only then they were found to carry *BRCA* PV. Therefore, the BRCA-postDx awareness group was not under high-risk screening. In 2009, the Israeli Ministry of Health added the reimbursement of annual MRI as a standard screening modality for *BRCA1/2* carriers. Therefore, we re-analyzed the data based on a cutoff at 2009 and separated the patients diagnosed before and after 2009 in each group.

Before 2009, the recommended screening included biannual clinical evaluation, breast ultrasound from the age of 25 years or 10 years prior to the age of diagnosis of family member, whatever comes first, and annual mammography from the age of 35 years. After 2009, annual MRI as a standard screening modality for *BRCA1/2* carriers.

The Hadassah Institutional Review Board approved the study and all patients gave written informed consent.

Clinical data were obtained from electronic medical records of Hadassah Medical Center. The data included demographics and information regarding the breast cancer: tumor size, lymph node status and distant metastasis (TNM), date of first diagnosis, pathology, receptor status, type of surgery, *BRCA* PV type, how the first diagnosis was made, family history, and follow-up.

Exclusion criteria were prior diagnosis of cancer and high-risk mutation other than *BRCA* PV.

Statistical Analysis

Association between two categorical variables was tested using the $\chi 2$ test and Fisher's exact test. Continuous variables were compared between two independent groups by use of the two-sample t-test or the non-parametric Mann-Whitney test. The non-parametric test was used for variables that were not normally distributed. The Kaplan-Meier survival model was used for assessing survival, with the log-rank test for the comparison of survival curves. The Cox regression model was applied as the multivariable model for survival. Lead time bias correction was done as described previously (13), it assumes an exponential distribution of the sojourn time, the period during which the tumor is asymptomatic but screen-detectable, with a rate of transition to symptomatic disease λ. Thus, $1/\lambda$ is the mean

sojourn time and is typically around 4. Duffy calculates an expected additional follow-up time to be subtracted from the calculated time-to-event of the study group. Where T is the last know follow-up time: follow-up correction time = (1-e^(-λT))/λ.

All statistical tests used were two-tailed, and a *P*-value of 0.05 or less was considered statistically significant. We used SSPS software for the statistical analysis.

RESULTS

Study Population

The total study population included 346 female patients (**Table 1**). The median age at diagnosis was 45.9 years (range 25–81). The BRCA-preDx awareness group included 62 patients and the BRCA-postDx awareness group 284 with similar mean age at diagnosis. In the BRCA-preDx awareness group, the majority of the patients (55/62, 88.7%) had at least one immediate family member who had a history of cancer, and all had a family history of cancer. In the BRCA-postDx awareness group, only 64.2% (177/276) of the patients had at least one immediate family member who had history of cancer, and 12% (33/276) had no family history of cancer ($P < 0.001$).

The patients' breast tumors characteristics are described in detail in **Table 2**. Interestingly, *BRCA1* PV was more frequent in the BRCA-preDx awareness group (48/62, 77.4%) compared with the BRCA-postDx awareness group (170/284, 59.9%; $P = 0.009$). One patient was positive for both *BRCA1/2* PVs. DCIS (ductal carcinoma *in situ*) was a more common pathology result in the BRCA-preDx awareness group, with 21.3% (13/61) of the patients having a pure DCIS at the time of diagnosis, compared with the BRCA-postDx awareness group's 6.4% (18/280; $P = 0.001$). IDC (invasive ductal carcinoma) was the pathologic diagnosis in 78.7% (48/61) of the patients in the BRCA-preDx awareness group, and in 90.4% (253/280) of the patients in the BRCA-postDx awareness group ($P = 0.001$). There were no statistically significant differences in receptor status or tumor grade between the groups.

Mode of Tumor Detection

The mode of detection differed significantly between the groups; 71.9% of the patients in the BRCA-postDx awareness group were diagnosed with breast cancer after they personally palpated a lump in their breast (**Figure 1**). That appears in contrast to the BRCA-preDx awareness group, where 26.2% of the patients personally palpated a lump ($P < 0.001$). In the BRCA-preDx awareness group, MRI was the diagnostic tool in 37.7% (23/61) of the cases, versus the BRCA-postDx awareness group where it accounted for only one case (0.4%, 1/267). In patients of younger ages, tumors were detected by self-palpation more frequently than by mammography in both study groups (**Supplementary Figure 1**).

Tumor Stage at Diagnosis

Furthermore, TNM staging was significantly more favorable in the BRCA-preDx awareness group. T stage was significantly lower in the BRCA-preDx awareness group (**Figure 2**): the majority of the patients were diagnosed at T1 (34/62, 54.8%), and 21% (13/62) of the patients were diagnosed at Tis. In the BRCA-postDx awareness group, only 37.5% (104/277) of the patients were diagnosed at T1 and 6.9% (19/277) of patients at Tis ($P < 0.001$). The N stage was also significantly lower in the BRCA-preDx awareness group. Within the BRCA-preDx awareness group, 71% of the patients were diagnosed with no lymph nodal metastases (44/62), while in the BRCA-postDx awareness group only somewhat more than half of the patients were diagnosed with no lymph node metastases (157/280, 56.1%; $P = 0.007$). Distant metastases were found in 3.4% of the patients in the BRCA-preDx awareness group and in 7.1% of the patients in the BRCA-postDx awareness group (non-significant trend).

Therapeutic Procedures Among the Study Population

Significantly, less patients in the BRCA-postDx awareness group underwent breast conserving surgeries compared with the BRCA-preDx awareness group (**Table 3**). Thirty-eight percent of patients from the BRCA-postDx awareness group had axillary

TABLE 1 | Characteristics of the study population.

Characteristic	BRCA-pre Dx awareness (*N*= 62)	BRCA-post Dx awareness (*N*=284)	*P* value	All (*N* = 346)
Age mean (SD), yr	47.4 (12.3)	45.6 (11.65)	**0.27**	45.9 (11.8)
Age<50	35/61 (57.4)	179/277 (64.6)		214/338 (63.3)
Age >50	26/61 (42.6)	98/277 (35.4)		124/338 (36.7)
Female sex, no (%)	62 (100)	284 (100)		346 (100)
Months of follow-up, median (min, max)	131.42 (3.06,271.9)	93.77 (0.95,282.4)		99.8 (0.95,282.35)
Ancestry, no (%)				
Ashkenazi Jewish	53/62 (85.5)	228/281[a] (81.1)	**0.4**	281/343 (81.9)
Sephardi Jewish	8/62 (12.9)	36/281[a] (12.8)		44/343 (12.8)
Other/Unknown	1/62 (1.6)	17/281[a] (6)		18/343 (5.2)
Family history, no (%)			**<0.001**	
First degree	55/62 (88.7)	177/276[b] (64.2)		232/338 (68.6)
Second degree	5/62 (8.1)	66/276[b] (23.9)		71/338 (21)
Third degree	2/62 (3.2)	0/276[b] (0)		2/338 (0.6)
None	0/62	33/276[b] (12)		33/338 (9.8)

[a]*Missing data of ancestry was missing for three patients.*
[b]*Missing data of family history was missing for eight patients.*
(Right) All patients. (Left) Comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group.

TABLE 2 | Breast tumor characteristics.

Characteristic	BRCA-pre Dx awareness (*N*= 62)	BRCA-post Dx awareness (*N*=284)	*P* value
BRCA1 positive	48/62 (77.4)	170/284 (59.9)	0.009
BRCA2 positive	15/62 (24.2)	114/284 (40.1)	0.019
Receptor status			0.248
Invasive			
ER/PR positive, HER2 negative	13/60[a] (21.67)	103/277[b] (37.2)	
ER/PR negative, HER2 positive	6/60[a] (10)	15/277[b] (5.4)	
Triple negative	25/60[a] (41.67)	100/277[b] (36.1)	
ER/PR positive, HER2 positive	4/60[a] (6.67)	27/277[b] (9.74)	
ER/PR positive, HER2 NA	0 (0)	6/277[b] (2.2)	
ER/PR negative, HER2 NA	0 (0)	9/277[b] (3.24)	
In situ			
ER/PR positive	9/60[a] (15)	15/277[b] (5.42)	
ER/PR negative	3/60[a] (5)	2/277[b] (0.72)	
Grade			0.119
Invasive			
Grade 1	3/46[c] (6.5)	9/232[d] (3.9)	
Grade 2	8/46[c] (17.4)	59/232[d] (25.4)	
Grade 3	28/46[c] (60.9)	150/232[d] (64.7)	
In situ			
Low	2/46[c] (4.3)	3/232[d] (1.3)	
Intermediate	2/46[c] (4.3)	6/232[d] (2.6)	
High	3/46[c] (6.5)	5/232[d] (2.2)	
Invasive vs. not invasive pathology			0.001
DCIS			
Positive	13/61[e] (21.3)	18/280[f] (6.4)	
Negative	48/61[e] (78.7)	262/280[f] (93.6)	
IDC			
Positive	48/61[e] (78.7)	253/280[f] (90.4)	
Negative	13/61[e] (21.3)	27/280[f] (9.64)	
ILC			
Positive	0/61[e]	9/280[f] (3.2)	
Negative	61/61[e] (100)	271/280[f] (96.8)	

[a]*Missing data of Receptor status was missing for two patients.*
[b]*Missing data of Receptor status was missing for seven patients.*
[c]*Missing data of grade was missing for sixteen patients.*
[d]*Missing data of grade was missing for fifty two patients.*
[e]*Missing data of Pathology was missing for one patient.*
[f]*Missing data of Pathology was missing for four patients.*
(Right) All patients. (Left) Comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; NA, not applicable; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; LCIS, lobular carcinoma in situ; ILC, invasive lobular carcinoma.

lymph node dissection (ALND) compared with only 8.8% (5/57, $P < 0.001$) in the BRCA-preDx awareness group. In addition, mastectomy was more frequently performed within the BRCA-postDx awareness group compared with the BRCA-preDx awareness group. Prophylactic bilateral mastectomy after diagnosis was less common in the BRCA-postDx awareness group (55/280, 19.6%) than in the BRCA-preDx awareness group (29/57, 50.9%; $P < 0.001$).

Outcomes Following Inclusion of MRI in the National Health Services

The BRCA-preDx awareness group included 29 patients diagnosed before and 33 patients after 2009, and the BRCA-postDx awareness group included 183 patients diagnosed before 2009 and 101 after 2009 (**Table 4**). Not surprisingly, the wider use of MRI had a clear effect. Until 2009, pathology results were similar between the groups, however, after 2009 the differences became significant: 31.3% (10/32) of patients had pure DCIS in the BRCA-preDx awareness group, while only 8.1% (8/99) did within the BRCA-postDx awareness group($P = 0.005$). Additionally, before 2009, the tumor was diagnosed through MRI in 13.8% (4/29) of the patients in the BRCA-preDx awareness group ($P < 0.001$), and this rose to 59.4% after 2009 ($P < 0.001$). Furthermore, the staging of invasive tumors at diagnosis was also affected, and a major downstaging in the BRCA-preDx awareness group was noted (T2–T4: 34.5% before 2009, 15.2% after 2009; $P = 0.012$). The difference in N stage between BRCA-preDx awareness and BRCA-postDx awareness is apparent only after 2009.

Overall Survival

During our study period (1996–2020), 72 patients died. Sixty-six (23.2%) women died in the 238 BRCA-postDx awareness group, compared with six patients (9.7%) from the BRCA-preDx awareness group. In the overall study period analysis, we have found significantly improved survival in the BRCA-preDx awareness group ($P = 0.008$, **Figure 3A**). Correction for lead time bias was done and the results remained statistically

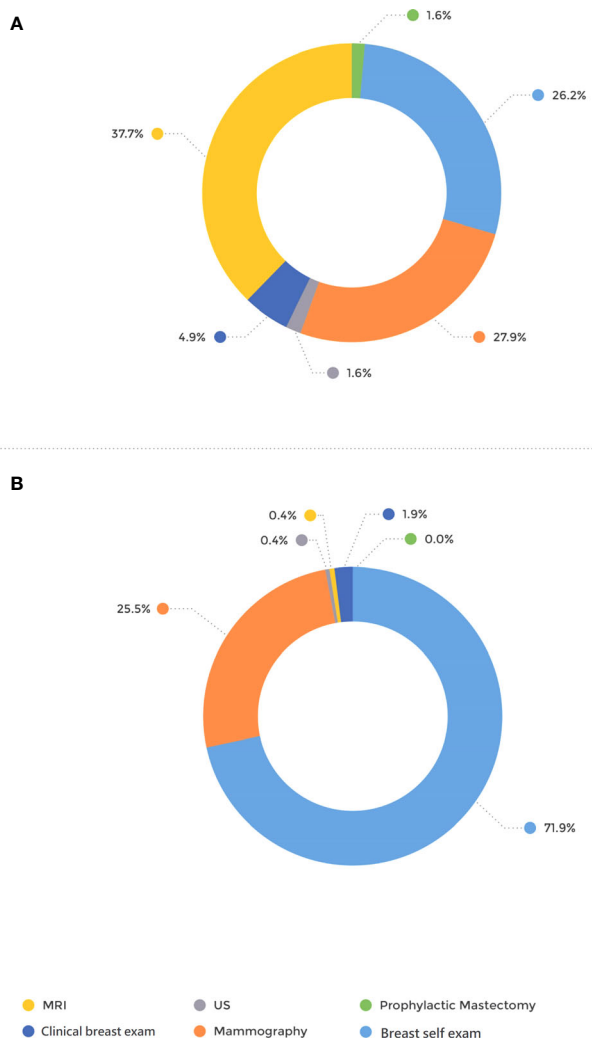

FIGURE 1 | Mode of tumor detection. **(A)** BRCA-preDx awareness group. **(B)** BRCA-postDx awareness group. MRI, magnetic resonance imaging; US, ultrasound.

significant (**Supplementary Figure 2**, p=0.0135). Further univariate analysis of our data found that there is no statistical difference in survival when patients are divided according to BRCA status (**Supplementary Table 1**, **Supplementary Figure 3**). Additional factors which are also significantly associated with improved survival are PR status and TNM staging. In a multivariate analysis, only PR status and M stage were significant (**Supplementary Table 1**).

In the sub-group analysis, there was no significant different in survival for between the *BRCA*-preDX group and the *BRCA*-postDx group prior to 2009 when MRI was not covered (p=0.237, **Figure 3B**): but there was statistically significant difference in survival after 2009 when MRI introduced (p=0.011, **Figure 3C**). Young women (age<50) with a diagnosis of breast cancer and *BRCA*-preDx awareness had improved survival (p=0.01) compared to women who were identified to harbor a *BRCA* PV after their breast cancer diagnosis (**Figure 3D**). Among older women (age>50), awareness of their *BRCA* status before their diagnosis of breast cancer did not improve their survival compared to women identified to have *BRCA* after their initial diagnosis (p=0.305, **Figure 3E**).

DISCUSSION

In this retrospective study, we found a potential downstaging effect of high-risk screening with several key differences between patients who were offered high-risk screening and those who were not.

First, breast cancer in the BRCA-preDx awareness group was more often detected by imaging, mostly MRI, while in the BRCA-postDx awareness group self-palpation was most prevalent. We attribute this difference to the use of MRI in the BRCA-preDx awareness group, as suggested by international

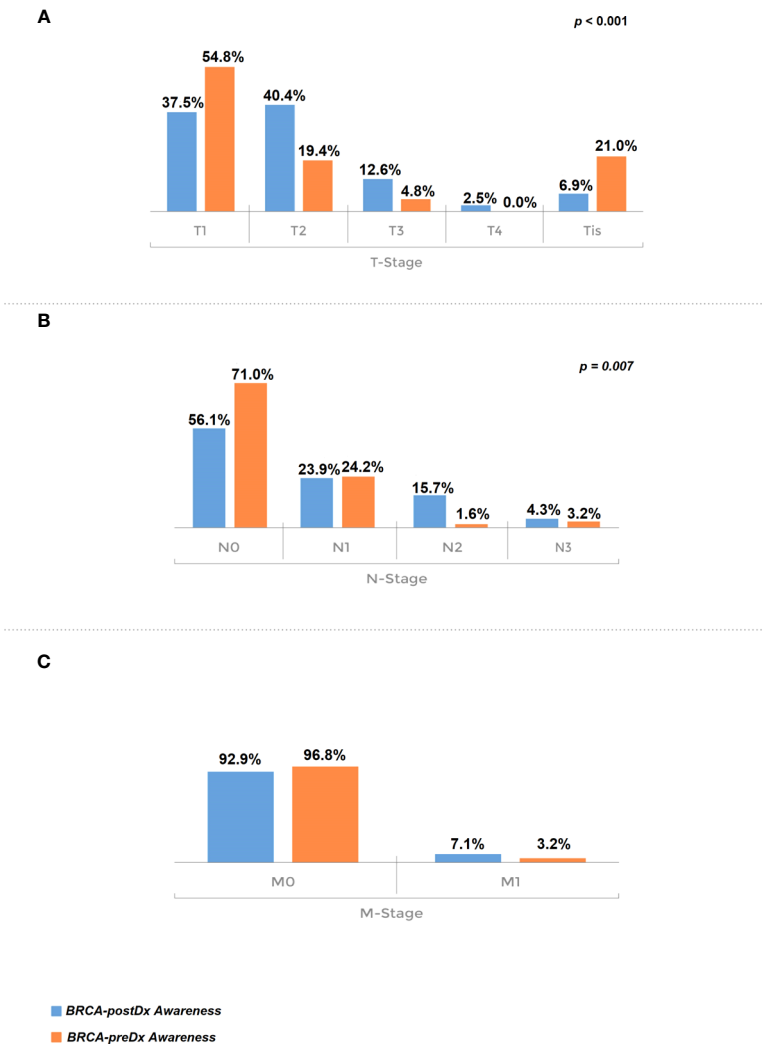

FIGURE 2 | Tumor stage at diagnosis. **(A)** T stage, comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group. **(B)** N stage: comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group. **(C)** M stage: comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group.

TABLE 3 | Therapeutic procedures among the study population.

Location	Procedure	BRCA-pre Dx awareness (*N* = 62)	BRCA-post Dx awareness (*N* = 284)	*P* value
Breast	Lumpectomy	38.6 (22/57)[a]	48.2 (135/280)[b]	**<0.001**
	Unilateral mastectomy	10.5 (6/57)[a]	25 (70/280)[b]	
	Bilateral mastectomy	50.9 (29/57)[a]	19.6 (55/280)[b]	
	Inoperable	3.4 (2/59)[a]	7.1 (20/280)[b]	
Axilla	Sentinel	36.8 (21/57)[a]	29.3 (82/280)[b]	**<0.001**
	Dissection	8.8 (5/57)[a]	38.6 (108/280)[b]	

[a]*Missing data of Procedure was missing for five patients.*
[b]*Missing data of Procedure was missing for four patients.*
(Right) All patients. (Left) Comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group. Unless otherwise indicated, data are percentages (number of patients/total with known variable).

guidelines (7, 14) and previous studies that have shown that MRI has superior sensitivity in the *BRCA1/2* carrier population (15, 16). Second, we show that more *in situ* pathology was found in the BRCA-preDx awareness group. Moreover, invasive tumors' TNM staging was significantly more favorable in the BRCA-preDx awareness group: the tumors were smaller, less axillary involvement and importantly, axillary surgeries were more conservative. These findings are in line with the scientific

TABLE 4 | Outcomes following inclusion of MRI in the national health services.

Characteristic	Before 2009		*P* value	After 2009		*P* value
	BRCA-pre Dx awareness (*N*=29)	BRCA-post Dx awareness (*N*=183)		BRCA-pre Dx awareness (*N*=33)	BRCA-post Dx awareness (*N*=101)	
Pathology[a]			0.516			0.005
DCIS						
Positive	(3/29) 10.3	5.5 (10/181)		31.3 (10/32)	8.1 (8/99)	
Invasive disease						
Positive	89.7(26/29)	94.5 (171/181)		68.8 (22/32)	91.9 (91/99)	
Mode of cancer detection[b]			<0.001			<0.001
Breast self exam	37.9 (11/29)	73.4 (124/169)		15.6 (5/32)	69.4 (68/98)	
Mammography	34.5 (10/29)	24.9 (42/169)		21.9 (7/32)	26.5 (26/98)	
US	3.4 (1/29)	0.6 (1/169)		0 (0/32)	0 (0/98)	
MRI	13.8 (4/29)	0 (0/169)		59.4 (19/32)	1 (1/98)	
Clinical breast exam	6.9 (2/29)	1.2 (2/169)		3.1 (1/32)	3.1 (3/98)	
Prophylactic Mastectomy	3.4 (1/29)	0 (0/169)		0 (0/32)	0 (0/98)	
Procedure[c]			0.091			<0.001
Lumpectomy	46.4 (13/28)	48 (86/179)		31 (9/29)	48.5 (49/101)	
Mastectomy	14.3 (4/28)	29.1 (52/179)		6.9 (2/29)	17.8 (18/101)	
Bilateral mastectomy	39.3 (11/28)	19.6 (35/179)		62.1 (18/29)	19.8 (20/101)	
Inoperable	0 (0/28)	3.4 (6/179)		6.1 (2/33)	13.9 (14/101)	
Axilla			0.004			0.011
Sentinel	39.3 (11/28)	21.2 (38/179)		34.5 (10/29)	43.6 (44/101)	
Dissection	17.9 (5/28)	51.4 (92/179)		0 (0/29)	15.8 (16/101)	
Stage at diagnosis						
T stage[d]			0.079			<0.001
Tis	(3/29)10.3	5.6 (10/180)		30.3 (10/33)	9.3 (9/97)	
T1	55.2 (16/29)	40 (72/180)		54.5 (18/33)	33 (32/97)	
T2	34.5 (10/29)	37.8 (68/180)		6.1 (2/33)	45.4 (44/97)	
T3	0 (0/29)	14.4 (26/180)		9.1 (3/33)	9.3 (9/97)	
T4	0 (0/29)	2.2 (4/180)		0 (0/33)	3.1 (3/97)	
N stage[e]			0.166			0.051
N0	72.4 (21/29)	53.6 (97/181)		69.7 (23/33)	61.2 (60/98)	
N1	24.1 (7/29)	26.5 (48/181)		24.2 (8/33)	19.4 (19/98)	
N2	3.4 (1/29)	14.9 (27/181)		0 (0/33)	16.3 (16/98)	
N3	0 (0/29)	5 (9/181)		6.1 (2/33)	3.1 (3/98)	
M stage[f]			0.364			0.73
M0	100 (29/29)	94.5 (171/181)		93.9 (31/33)	90.1 (91/101)	
M1	0 (0/29)	5.5 (10/181)		6.1 (2/33)	9.9 (10/101)	

[a]*Missing data of Pathology was missing for five patients.*
[b]*Missing data of Mode of cancer detection was missing for eighteen patients.*
[c]*Missing data of Procedure was missing for five patients.*
[d]*Missing data of T stage made was missing for seven patients.*
[e]*Missing data of N stage made was missing for five patients.*
[f]*Missing data of M stage made was missing for two patients.*
(Left) Comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group before the inclusion of MRI. (Right) Comparison of the BRCA-preDx awareness group with the BRCA-postDx awareness group after the inclusion of MRI. DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; LCIS, lobular carcinoma in situ; ILC, invasive lobular carcinoma; MRI, magnetic resonance imaging; US, ultrasound. Unless otherwise indicated, data are percentages (number of patients/total with known variable).

background on which the current guidelines were established (7): MRI is more sensitive than mammography for women at high risk of developing breast cancer (17–19). The use of combined screening modalities elevates the sensitivity of the examination (15, 19) and can lead to detection at favorable stages compared with mammography alone (20). Additionally, mammography alone has a higher false-negative rate in *BRCA* PVcarriers (21), in high-density breast tissue (21–23) and in rapidly growing aggressive tumors (24, 25). All of these are more frequent characteristics among high-risk young women (7). To strengthen our findings, sub-analysis using the year of the beginning of widespread use of MRI made the above-mentioned differences even more apparent (e.g., improved staging and more *in situ* tumors after 2009).

Even though studies show that the addition of MRI is superior to mammography alone in *BRCA1/2* carriers in detecting breast cancer, the currently available data has not shown a clear survival benefit from many of the above screening recommendations (16,

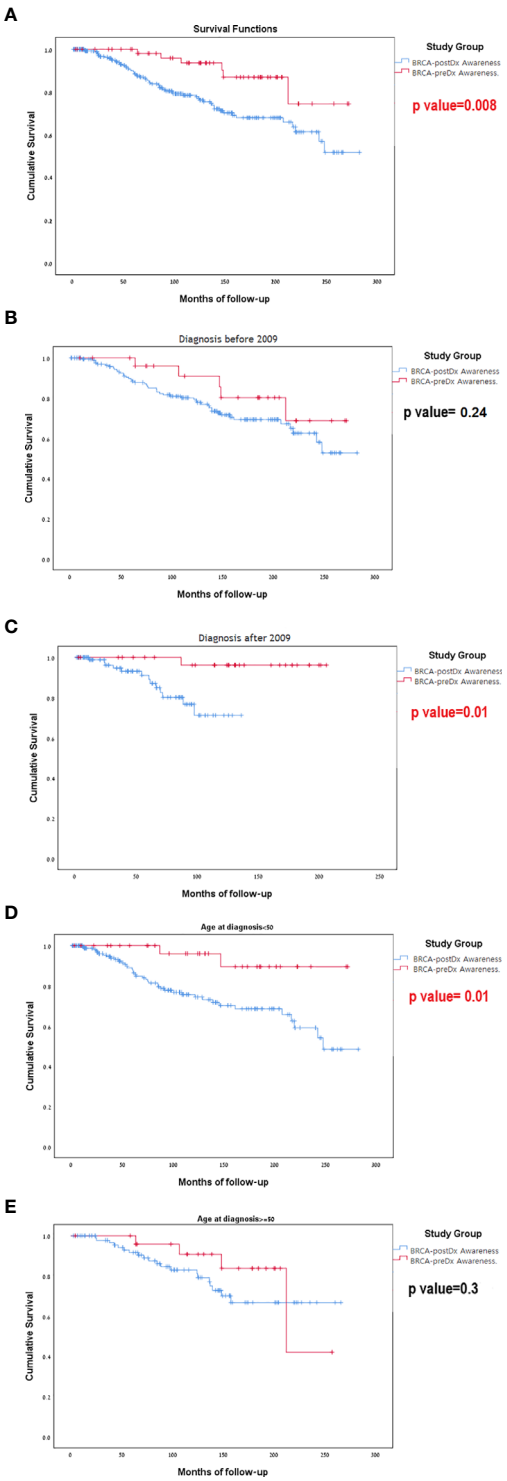

FIGURE 3 | Kaplan-Meier curves for overall survival. **(A)** BRCA-preDx awareness cohort and BRCA-postDx awareness cohort. **(B)** BRCA-preDx awareness cohort and BRCA-postDx awareness cohort before 2009. **(C)** BRCA-preDx awareness cohort and BRCA-postDx awareness cohort after 2009. **(D)** BRCA-preDx awareness cohort and BRCA-postDx awareness cohort before the age of 50 years. **(E)** BRCA-preDx awareness cohort and BRCA-postDx awareness cohort after the age of 50 years.

26–28). For example, the starting age and the age limit for high-risk screening are not well-known and based on limited observational data (26, 28). The interval time between each screening is also not well established, and there is only partial comparative information regarding different interval durations based on age (15).

A small hint of improved efficacy of high-risk screening was shown in a recent paper by Hadar et al. (29) The authors described a limited population (42 out of 105 *BRCA1/2* carriers) who knew that they were *BRCA* carriers prior to cancer diagnosis, were under high-risk screening, and had better outcomes with a possible survival advantage. Our work shows an interesting improvement in survival in young women who underwent high-risk screening. Taken together, our data imply that the introduction of MRI-based screening was the probable driver behind this survival gain due to its ability to detect smaller tumors.

Our study has several limitations. The compliance with high-risk screening in the BRCA-preDx awareness group patients is largely unknown. We do not have additional information on subsequent procedures (e.g. salpingo-oophorectomy) or therapies such as chemotherapy and hormonal therapies. Additionally, we lack data about cancer recurrence rates in both groups. However, we believe that the long follow-up period compensates for the above-mentioned drawbacks and adds important insights to those from published cohorts (16).

In summary, our data emphasize the importance of high-risk MRI-based screening among *BRCA1/2* PV carriers. This study shows a favorable effect on staging for women who have had awareness of their *BRCA* status before cancer diagnosis and had participated in intensified screening. Further studies are needed to refine the optimal screening protocols to maximize the survival benefit from high-risk screening programs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hadassah Medical Center IRB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: SS, ALG, TS, and TP. Collection and assembly data: SS, ALG, TH, YC, LK, OM,YA-L, GZ, AG, BM, EC, VM, TS, and TP. Data analysis and interpretation: SS, ALG, TH, YC, and TP. Manuscript writing: SS, ALG, AZ, and TP. SS

and ALG had equally contributed to this manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.683656/full#supplementary-material

Supplementary Figure 1 | How the first diagnosis was made with regard to age. BRCA-preDx awareness group (right) and BRCA-postDx awareness group (left). MRI, magnetic resonance imaging; US, ultrasound.

Supplementary Figure 2 | Survival analysis following lead-time bias correction. BRCA-preDx awareness cohort and BRCA-postDx awareness cohort.

Supplementary Figure 3 | Survival analysis according to *BRCA1* and *BRCA2* status. BRCA-preDx and BRCA-postDx awareness cohort separated according to *BRCA1/2*.

Supplementary Table 1 | Univariate and multivariate analyses of overall survival.

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REVIEW
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Ovarian Function Suppression With Luteinizing Hormone-Releasing Hormone Agonists for the Treatment of Hormone Receptor-Positive Early Breast Cancer in Premenopausal Women

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Chemotherapy and endocrine therapies are mainstays of treatment for early and advanced hormone receptor-positive (HR+) breast cancer. In premenopausal women with HR+ tumors, the benefits of adding ovarian function suppression (OFS) to endocrine therapy have been debated. Consequently, for many years, tamoxifen monotherapy has been the standard of care for endocrine treatment in the adjuvant setting. Recent studies have, however, provided new evidence that, in some premenopausal patients, OFS in combination with tamoxifen or aromatase inhibitors (AIs) can significantly increase survival *versus* tamoxifen alone. Luteinizing hormone-releasing hormone agonists (LHRHa), including goserelin, triptorelin, and leuprorelin, achieve OFS through sustained suppression of the release of follicle-stimulating hormone and luteinizing hormone from the pituitary. In turn, this suppresses production and secretion of estradiol, an ovarian hormone that supports cancer cell growth, survival, and proliferation. In this review, we discuss the clinical evidence supporting the addition of LHRHa to adjuvant endocrine therapies, including tamoxifen and AIs, for premenopausal women with breast cancer. We also discuss the role of LHRHa use in combination with adjuvant chemotherapy to preserve ovarian function and fertility in young patients with breast cancer. Finally, we discuss important practical aspects of the use of LHRHa in breast cancer treatment, including side-effects, patient adherence to treatment, and the use of slow-release, long-acting drug formulations.

Keywords: premenopausal, breast cancer, ovarian function suppression (OFS), ovarian function preservation, endocrine therapy, luteinizing hormone releasing hormone

INTRODUCTION

Breast cancer is one of the most frequently diagnosed malignancies worldwide. According to the World Health Organization, in 2020 an estimated 2.26 million cases were diagnosed and 685,000 deaths resulted from the disease (1). Although most breast cancer cases occur in postmenopausal women, a substantial proportion occur in premenopausal women under the age of 50 years; estimates range from approximately 20% of all breast cancers in some developed countries, such as the USA, to as many as 50% of all breast cancers in less economically developed countries and some developed countries in Asia (2, 3). This makes breast cancer the most frequently diagnosed malignancy and the leading cause of cancer-related death worldwide in women under 40 years of age (4).

Younger age at diagnosis has long been recognized as a factor associated with higher risk of disease recurrence and death (5, 6), and in premenopausal women, breast cancer is often characterized by tumors with aggressive pathological phenotypes. Evidence from the UK-based Prospective Study of Outcomes in Sporadic and Hereditary breast cancer (POSH) revealed that, at diagnosis, in women aged 18–40 years, median tumor diameter was 22 mm, 58.9% of patients had grade 3 tumors, 50.2% had lymph node-positive disease, and 33.7% had estrogen receptor-negative (ER–) tumors (7). These values are notably higher than those reported in studies of older, postmenopausal women. In line with the more aggressive tumor features, the 5-year overall survival (OS) of patients in the POSH study was worse than that of contemporary patients aged 40–69 years in the UK (81.9% *versus* 89.1–90.4%) (7, 8). Further evidence from retrospective analysis of the Surveillance, Epidemiology and End Results (SEER) database, of over 200,000 patients diagnosed between 1988 and 2003 also showed that women aged under 40 years at diagnosis (n = 15,548) had tumors that were more likely to be larger in size, higher grade, lymph node positive and hormone-receptor-negative (HR–) (9) than those who were older. Thus, the prognosis for young women diagnosed with breast cancer is, in many cases, worse than for older women even though younger patients are often given more intensive treatments (10).

Growing evidence suggests that tumor biology and genetics play a primary role in determining the relatively poorer outcomes in premenopausal women compared with postmenopausal women (6). Numerous studies have identified that tumors in younger patients frequently have different expression patterns of key biomarkers, including HRs, human epidermal growth factor receptor 2 (HER2), and proliferation markers compared with tumors in older, postmenopausal patients (5, 6). In premenopausal women, data from Western countries show that approximately 65–80% of tumors are luminal-type HR-positive (HR+) tumors (5, 7, 11). However, younger patients in these countries have a higher proportion of more aggressive basal-like tumors (also known as triple-negative breast cancer; TNBC) that are ER–, progesterone-receptor-negative (PR–), and HER2-negative (HER2–), as well as a higher proportion of HER2-overexpressing tumors (that are ER–/PR–) than older patients (11–13). A key point to note is that breast cancer in young Asian women has distinctive clinicopathological features that differ from those seen in Western women and therefore it requires different treatment guidelines (14, 15); for example, the probability of being diagnosed with TNBC has been shown to decrease with age in patients from the USA but not in patients from East Asia (15). Nonetheless, HR+ disease remains the most common breast cancer diagnosis in premenopausal women and these patients are, therefore, good candidates for treatment with endocrine therapy in the adjuvant setting.

ADJUVANT TREATMENT OPTIONS FOR PREMENOPAUSAL PATIENTS WITH BREAST CANCER

Adjuvant chemotherapy and endocrine therapy are integral to the treatment of early breast cancer and can significantly reduce the risk of death and relapse. In premenopausal breast cancer patients with aggressive TNBC, treatment options are limited, and prognosis is poor relative to patients with HR+ cancers. In these patients, whose tumors are resistant to endocrine therapy and HER2-targeting treatments, cytotoxic chemotherapy remains the only well-validated and approved treatment in the adjuvant setting following surgery (16); although the development of immune checkpoint inhibitors, including programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1), is changing the treatment landscape for these patients (17).

In HR–/HER2-positive (HER2+) patients, the anti-HER2 agent trastuzumab has transformed disease outcomes and has become the standard of care given as a monotherapy or in combination with other drugs including paclitaxel (16). Newer agents, including pertuzumab (18), trastuzumab emtansine (19), and neratinib (20), have also shown effectiveness in the treatment of patients with HER2+ tumors.

For patients with luminal-type HR+ tumors, adjuvant endocrine therapy is typically the preferred option. Tamoxifen, a selective ER modulator (SERM) (21), has been standard for adjuvant endocrine therapy in both premenopausal and postmenopausal women for many decades (22, 23). By blocking ERs, tamoxifen reduces the mitogenic effects of the ovarian hormone estradiol (E2), helping to prevent cancer cell growth and proliferation (21) (**Figure 1A**). Early trials of tamoxifen, including the Nolvadex Adjuvant Trial Organization (NATO) trial (24, 25), the Cancer Research Campaign Adjuvant Breast (CRCAB) trial (26), and the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial (27, 28), demonstrated clear reductions in risk of disease recurrence and death in patients receiving the drug for between 2 and 5 years in the adjuvant setting. The clinical effectiveness of tamoxifen has been subsequently confirmed by a large meta-analysis (n = 21,457 patients in 20 trials) performed in 2011 by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG). In 10,645 patients with ER+ disease, 5 years of adjuvant tamoxifen treatment *versus* no adjuvant tamoxifen reduced recurrence rates (RRs) by nearly 50% (RR 0.53) during

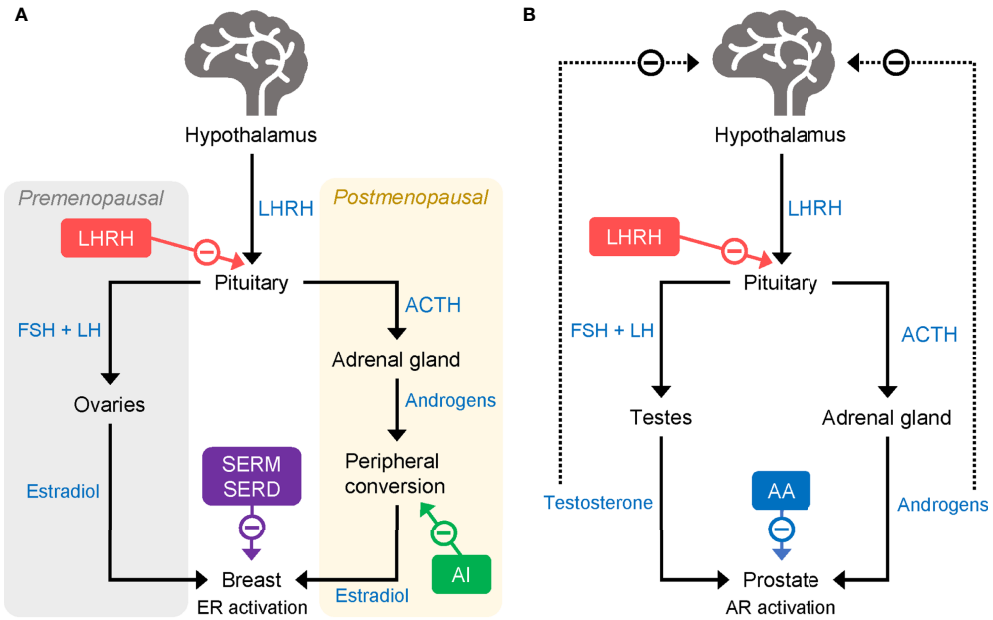

FIGURE 1 | Mode of action of LHRHa in **(A)** breast cancer and **(B)** prostate cancer. AA, abiraterone acetate; ACTH, adrenocorticotropic hormone; AI, aromatase inhibitor; AR, androgen receptor; ER, estrogen receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRHa, luteinising hormone-releasing hormone agonist; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator.

years 0–4 and 30% (RR 0.68) in years 5–9 of follow up; breast cancer mortality was reduced by approximately 30% during the 15-year follow-up period (22).

Aromatase inhibitors (AIs), including the third-generation compounds letrozole, anastrozole, and exemestane, are a class of endocrine-based therapies commonly used in the adjuvant setting in postmenopausal patients (29). AIs reduce the production of estrogens by suppressing the activity of aromatase enzymes. In premenopausal women, most circulating estrogens are produced in the ovaries, but following menopause aromatases found in fat and muscle tissues are responsible for most estrogen production (**Figure 1A**) (29). Unlike postmenopausal women, premenopausal women have a large amount of ovarian estrogen production under the strong influence of pituitary gonadotropins. AI administration markedly increases gonadotropin release and promotes estrogen-dependent aromatase activity which, in turn, counteracts the effectiveness of AIs in reducing ovarian estrogen production. Thus, in premenopausal women, AIs have limited ability to reduce circulating estrogen and are not typically given without combination with another treatment to suppress ovarian function.

Ovarian Function Suppression and LHRH Agonists

Ovarian function suppression (OFS) or ablation has been studied in breast cancer for many decades. The relationship between ovarian function and breast cancer was recognized as early as 1882, with the first reported evidence of cancer regression after menopause, and since the 1890's it has been known that surgical removal of the ovaries in premenopausal patients with breast cancer has the ability to reduce the likelihood of remission (30, 31). Following these pivotal findings, numerous further studies have demonstrated that early menopause, either naturally induced or induced by bilateral oophorectomy, is associated with a substantial reduction in risk of breast cancer (32–34). Furthermore, in young patients with early breast cancer, chemotoxic damage to the ovaries associated with systemic chemotherapy carries a high risk of amenorrhea and early menopause which is believed to provide benefit in terms of cancer outcomes; this benefit comes, however, at the cost of reduced fertility. More recently, a meta-analysis by the EBCTCG demonstrated definitively that ovarian ablation as a single intervention reduces risk of recurrence for women aged less than 50 years with axillary node-positive and node-negative disease (15-year survival was 52.4% for those undergoing ovarian ablation *versus* 46.1% in those who did not) (35). Thus, there is a clear link between the reduction of ovarian function, with corresponding reduction in circulating estrogens, and improved outcomes in breast cancer.

In the modern clinical setting, as well as complete surgical ovarian ablation, OFS can be achieved through the administration of luteinizing hormone (LH)-releasing hormone (LHRH) agonists (LHRHa; also known as gonadotropin-releasing hormone [GnRH] agonists, GnRHa) (36) or *via* radiation therapy. LHRH [also known as GnRH and gonadorelin (37)] is released from the hypothalamus and acts on G protein-coupled receptors (GnRH receptor type 1, GnRHR1) in the pituitary to increase the production of follicle-stimulating hormone (FSH) and LH, which, in turn, stimulates the release of E2 by the ovaries (38). LHRHa act by mimicking the effects of LHRH at the GnRHR1 (**Figure 1A**). Owing to their specific affinity for LHRH receptors, when first administered LHRHa initially produce a surge in

ovarian hormones that can be accompanied by adverse effects, such as hot flashes. However, long-term administration of LHRHa reduces ovarian hormone production and secretion by causing a downregulation and desensitization of LHRH receptors in pituitary gonadotropic cells (39). The resulting reduction of circulating estrogens slows the growth of HR+ tumors.

Initially, development of clinically useful LHRHa was complicated by their short half-life, but by modification of several amino acids found in the human LHRH peptide, long-acting agonists have been successfully developed and have become useful agents in the treatment of both prostate and breast cancer. The most used LHRHa are the GnRHR1 agonists goserelin (Zoladex®) (40), triptorelin (Decapeptyl®) (41), and leuprorelin (Lupron®) (**Table 1**).

Efficacy of OFS Combined With Adjuvant Endocrine Therapy

In a 2005 EBCTCG review examining 10- and 15-year disease recurrence rates and mortality in 7,601 women aged less than 50 years, benefits of OFS (via ovarian ablation or suppression with LHRHa) were observed only when OFS was given in the absence of other systemic treatments (42) and OFS did not add further benefit to that of adjuvant tamoxifen alone. However, studies included in this review may have been confounded by clinical selection criteria; some trials covered by this analysis included patients with HR− tumors and women receiving adjuvant chemotherapy, which can, on its own, produce OFS capable of masking the effects of specific OFS treatments. In contrast, a meta-analysis by the LHRH-agonists in Early Breast Cancer Overview group in 2007 found that LHRHa given alone did not significantly decrease disease recurrence (28.4% relative reduction; 95% confidence interval [CI] −50.5%, 3.5%) or death after recurrence (17.8%; 95% CI −52.8%, 42.9%) but LHRHa given in combination with tamoxifen, chemotherapy or both reduced disease recurrence and death after recurrence *versus* those therapies alone (36). In contrast to that finding, an Adjuvant Breast Cancer Trials Collaborative Group (ABCTCG) trial found no significant benefit of the addition of OFS to 5-years of tamoxifen treatment in premenopausal patients with early breast cancer (43) and the Zoladex in Pre-menopausal Patients (ZIPP) trial showed no significant difference between 2 years of treatment with tamoxifen plus goserelin *versus* 2 years of tamoxifen alone (44).

Owing to these and other contrasting findings (45), the utility of OFS as an adjuvant therapy in combination with other endocrine agents in premenopausal patients has long been contested. In recent years, several trials have sought to provide clarity over the question of whether the addition of OFS to tamoxifen or AIs provides real added benefit in the adjuvant setting for premenopausal patients with HR+ breast cancer.

Tamoxifen Plus OFS *Versus* Tamoxifen Alone

The phase 3 Eastern Cooperative Oncology Group (ECOG) 3193 trial (E-3193; INT-0142) (**Table 2**) comparing standard 5-year tamoxifen treatment with 5 years of tamoxifen plus OFS (surgical ablation, radiation, goserelin, or leuprolide acetate) in premenopausal women with node-negative, HR+ breast cancer

TABLE 1 | Luteinizing hormone releasing-hormone agonists.

Generic name	Brand name	IUPAC condensed sequence	Dosing frequency	Formulations[a]	Needle characteristics
Gonadorelin (hGnRH)		H-Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2			
Goserelin acetate	Zoladex®	H-Pyr-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NHNHCONH2	1- or 3- monthly	SC slow-release solid implants containing goserelin acetate equivalent to 3.6 mg and 10.8 mg goserelin, respectively	14 or 16 gauge
Triptorelin acetate	Decapeptyl SR®	H-Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH2	1-, 3-, or 6-monthly	Powder and solvent to be reconstituted for IM injection containing triptorelin pamoate equivalent to 3.75 mg, 11.25 mg, and 22.5 mg triptorelin, respectively	19–21 gauge
Leuprorelin acetate	Eligard® Lupron® Lupron Depot® Prostap 3® Leuprorelin Sandoz®	H-Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt	1-, 3-, 4-, or 6-monthly	Powder and solvent to be reconstituted for SC or IM depot injection containing leuprorelin acetate equivalent to 7.5 mg, 22.5 mg, and 45 mg leuprorelin, respectively SC slow-release solid implants containing leuprorelin acetate equivalent to 3.6 mg and 5 mg leuprorelin, respectively	14 gauge (implant) 21–23 gauge (SC) 21 gauge (IM)

[a]*Not all formulations listed are licenced for use in both prostate and breast cancer. Not all available formulations are listed.*

hGnRH, human gonadotropin-releasing hormone; IM, intramuscular; IUPAC, International Union of Pure and Applied Chemistry; SC, subcutaneous.

found no significant difference between tamoxifen alone and tamoxifen plus OFS in the primary endpoints of disease-free survival (DFS; 5-year rate: 87.9% *versus* 89.7%) and OS (95.2% *versus* 97.6%) (46). However, this trial may have been confounded by its size, the relatively low-risk population it included, and the unknown HER2 status of most enrolled patients.

The ASTRRA trial (**Table 2**) also evaluated the efficacy of adding OFS (goserelin) to 5 years of adjuvant tamoxifen, this time in patients with HR+ breast cancer who retained or regained premenopausal status following neoadjuvant/adjuvant chemotherapy (55). In these patients, who had a higher risk of disease recurrence and previous chemotherapy, the addition of OFS to tamoxifen resulted in a significant improvement in 5-year DFS (91.1% *versus* 87.5% with tamoxifen alone). A significant improvement in OS was also observed in the OFS plus tamoxifen group (99.4% *versus* 97.8%) (47), although this finding is confounded by the small number of events (four in the tamoxifen plus OFS group and 14 in the tamoxifen only group).

Conflicting results were observed in the initial analysis of the Suppression of Ovarian Function Trial (SOFT) (**Table 2**), conducted by the International Breast Cancer Study Group (IBCSG). In SOFT, premenopausal patients with HR+ early breast cancer were randomized to receive exemestane plus OFS (bilateral oophorectomy, ovarian radiation, or triptorelin), tamoxifen plus OFS, or tamoxifen alone (48, 49, 56). In the primary analysis, performed at 5.6 years follow-up, no significant difference was observed between patients who received tamoxifen plus OFS and those who received tamoxifen alone for DFS (5-year event rate 86.6% *versus* 84.7%) or OS (5-year event rate 96.7% *versus* 95.1%) (48). Thus, at primary analysis, no benefit of adding OFS to tamoxifen was observed in the overall patient group, which included premenopausal women of all ages and all prior chemotherapy statuses. However, in SOFT, 90% of the deaths occurred in patients who had received prior chemotherapy, which may have confounded the overall results. Indeed, in patients who had not received prior chemotherapy, 5-year OS rates exceeded 99% in both treatment groups (48), whereas in patients who had received prior chemotherapy, tamoxifen plus OFS led to a significant improvement in OS versus tamoxifen alone (94.5% *versus* 90.9%, hazard ratio [HR]: 0.64, 95% CI 0.42–0.96). Thus, the initial analysis of SOFT demonstrated some benefit of the addition of OFS to tamoxifen, but only in terms of OS for patients who had received prior chemotherapy.

An updated analysis of SOFT, with 8-years of follow-up, subsequently showed a significant improvement in both DFS (8-year rate 83.2% *versus* 78.9%) and OS (8-year rate 93.3% *versus* 91.5%) for all patients who received tamoxifen plus OFS *versus* tamoxifen alone (49). While the relative benefits of tamoxifen plus OFS were similar regardless of prior chemotherapy, the absolute benefits were greater in those patients who remained premenopausal having received prior chemotherapy (49). Clinico-pathological features in these patients, including younger age, may have contributed to a higher risk of disease recurrence. Indeed, DFS in this cohort was 5.3% higher in patients who received tamoxifen plus OFS than in patients who received tamoxifen alone.

The most recent data from SOFT therefore support the addition of OFS to tamoxifen in the adjuvant setting for higher-risk women who remain premenopausal after receiving adjuvant chemotherapy. A recent Cochrane Library systematic review and meta-analysis conducted by Bui and colleagues of 11 studies including 10,374 women supports this conclusion, having demonstrated that addition of OFS to tamoxifen resulted in a significant reduction in mortality (HR: 0.86, 95% CI 0.78–0.94) (57).

Tamoxifen Plus OFS *Versus* OFS Alone

The ECOG 5188 trial (E5188, INT-101) (**Table 2**) compared 5 years of adjuvant tamoxifen plus OFS (goserelin), OFS alone, or no adjuvant endocrine therapy in premenopausal women with node-positive, HR+ breast cancer who had previously received cyclophosphamide, doxorubicin, fluorouracil (CAF) chemotherapy (50). The addition of tamoxifen to OFS significantly improved 9-year DFS (68% *versus* 60%; $P < 0.01$) but not 9-year OS (76% *versus* 73%; $P = 0.21$) compared with OFS in the overall population. Results of a retrospective subgroup analysis also showed that combining tamoxifen and OFS seemed to provide superior DFS outcomes *versus* OFS alone both in women aged less than 40 years (64% *versus* 55%) and those aged 40 years and older (69% *versus* 62%) (50).

Anastrozole Plus OFS *Versus* Tamoxifen Plus OFS

The Austrian Breast and Colorectal Cancer Study Group (ABCSG)-12 (**Table 2**) trial compared 3 years of treatment with either the AI anastrozole plus OFS (goserelin) or tamoxifen plus OFS in premenopausal women with stage 1–2 HR+ breast cancer and a low risk of disease recurrence (51, 58, 59). Although there was no significant difference in DFS between treatment groups, a higher risk of death was observed for patients who received anastrozole than for those who received tamoxifen (53 *versus* 33 events; HR: 1.63, 95% CI 1.05–2.52; $P = 0.03$). Therefore, although this study did not compare the benefits of either tamoxifen or AI plus OFS versus tamoxifen alone, the data suggest that combining OFS with tamoxifen provides greater benefit than combining it with AIs.

In contrast, the phase 3 STAGE study comparing anastrozole plus OFS (goserelin) with tamoxifen plus OFS, given in the neoadjuvant setting to a premenopausal HR+/HER2− Japanese patient cohort, found a significantly higher tumor response rate for anastrozole plus OFS *versus* tamoxifen plus OFS (70.4% *versus* 50.5%). However, this study was relatively small ($N = 204$ patients) and, compared with a typical adjuvant treatment duration of 5 years, the neoadjuvant treatment period was short (24 weeks) (52).

Exemestane Plus OFS *Versus* Either Tamoxifen Plus OFS or Tamoxifen Alone

Further conflicting evidence regarding whether OFS is more effective when combined with an AI *versus* with tamoxifen comes from two studies that compared exemestane plus OFS with tamoxifen plus OFS – the SOFT trial and the contemporaneous phase 3 Triptorelin and Exemestane Trial (TEXT) (56). A combined

TABLE 2 | Overview of trials evaluating the addition of OFS to adjuvant endocrine therapy in premenopausal women with HR+ breast cancer.

Trial name	Randomized patients, N	Clinical characteristics	Follow-up, years	Age	Treatment arms	Outcomes
E-3193 (46) (INT-0142) Phase 3	345	Premenopausal Node-negative HR+ BC	9.9	Median age, 45 years	Tamoxifen *vs.* tamoxifen plus OFS (radiation therapy, surgical ablation, or goserelin 3.6 mg or leuprolide 3.75 mg acetate, 4-weekly) Adjuvant treatment duration 5 years	5-year DFS tamoxifen, 87.9% 5-year DFS tamoxifen plus OFS, 89.7% DFS HR 1.17 (95% CI 0.64–2.12) 5-year OS tamoxifen, 95.2% 5-year OS tamoxifen plus OFS, 97.6% OS HR 1.19 (95% CI 0.52–2.70)
ASTRRA (47)	1,282	HR+ BC Retained or regained premenopausal status for 24 months after ending neoadjuvant or adjuvant chemotherapy	5	Median age, 40 years	Tamoxifen alone *vs.* tamoxifen plus OFS (3.6 mg goserelin, 4-weekly) Adjuvant tamoxifen for 5 years plus 2 years OFS	5-year DFS tamoxifen, 87.5% 5-year DFS tamoxifen plus OFS, 91.1% DFS HR 0.69 (95% CI 0.48–0.97); P = 0.033 5-year OS tamoxifen, 97.8% 5-year OS tamoxifen plus OFS, 99.4% OS HR 0.31 (95% CI 0.10–0.94); P = 0.029
SOFT (48) Phase 3	3,066	Premenopausal HR+ early BC	5.6	Median age, 43 years	Tamoxifen *vs.* tamoxifen plus OFS (bilateral oophorectomy, ovarian radiation, or triptorelin 3.75 mg, 4-weekly) *vs.* exemestane plus OFS Adjuvant treatment duration 5 years	5-year DFS tamoxifen, 84.7% 5-year DFS tamoxifen plus OFS, 86.6% DFS HR 0.83 (95% CI 0.66–1.04); P = 0.10 5-year OS tamoxifen, 95.1% 5-year OS tamoxifen plus OFS, 96.7% OS HR 0.74 (95% CI 0.51–1.09); P = 0.13
SOFT (49) Phase 3	3,066	Premenopausal HR+ early BC	8	Median age, 43 years	Tamoxifen *vs.* tamoxifen plus OFS (bilateral oophorectomy, ovarian radiation, or triptorelin 3.75 mg, 4-weekly) *vs.* exemestane plus OFS Adjuvant treatment duration 5 years	8-year DFS tamoxifen, 78.9% 8-year DFS tamoxifen plus OFS, 83.2% DFS HR 0.76 (95% CI 0.62–0.93); P = 0.009 8-year OS tamoxifen, 91.5% 8-year OS tamoxifen plus OFS, 93.3% OS HR 0.67 (95% CI 0.48–0.92); P = 0.01 8-year DFS exemestane plus OFS, 85.9% DFS HR *vs.* tamoxifen alone 0.65 (95% CI 0.53–0.81) 8-year OS exemestane plus OFS, 92.1% OS HR *vs.* tamoxifen alone 0.85 (95% CI 0.62–1.15)
E-5188 (50) (INT-101) Phase 3	1,503	Premenopausal Node-positive HR+ BC	9.6	<40 years, 438 (29%) ≥40 years, 1,065 (71%)	CAF chemotherapy alone *vs.* CAF chemotherapy followed by OFS (goserelin 3.6 mg, 4-weekly) *vs.* CAF followed by OFS plus tamoxifen Adjuvant treatment duration 5 years	9-year DFS CAF alone, 57% 9-year DFS CAF plus goserelin, 60% 9-year DFS CAF plus goserelin and tamoxifen, 68% DFS HR CAF plus goserelin *vs.* CAF plus goserelin plus tamoxifen 0.74 (95% CI 0.60–0.91); P < 0.01 DFS HR CAF *vs.* CAF plus goserelin 0.93 (95% CI 0.76–1.12); P = 0.22 9-year OS CAF alone, 70% 9-year OS CAF plus goserelin, 73% 9-year OS CAF plus goserelin and tamoxifen, 76% OS HR CAF plus goserelin *vs.* CAF plus goserelin plus tamoxifen 0.91 (95% CI 0.71–1.15); P = 0.21 OS HR CAF *vs.* CAF plus goserelin 0.88 (95% CI 0.70–1.11); P = 0.14

(Continued)

TABLE 2 | Continued

Trial name	Randomized patients, N	Clinical characteristics	Follow-up, years	Age	Treatment arms	Outcomes
ABCSG-12 (51) Phase 3	1,803	Premenopausal Stage 1–2 HR+ BC and low risk of disease recurrence	7.9	Median age, 45 years	Tamoxifen plus OFS (goserelin 3.6 mg, 4-weekly) plus zoledronic acid *vs.* anastrazole plus OFS plus zoledronic acid Adjuvant treatment duration 3 years	7.9-year DFS tamoxifen plus goserelin, 117 events 7.9-year DFS anastrozole plus goserelin, 134 events DFS HR, 1.13 (95% CI 0.88–1.45); P = 0.335 7.9-year OS tamoxifen plus goserelin, 33 events 7.9-year OS anastrozole plus goserelin, 53 events OS HR, 1.63 (95% CI 1.05–2.52); P = 0.030
STAGE (52) Phase 3	204	Premenopausal HR+ early BC	0.5		Tamoxifen plus OFS (goserelin 3.6 mg, 4-weekly) *vs.* anastrozole plus OFS Neoadjuvant treatment duration 24 weeks	Overall (complete or partial) tumor response rate tamoxifen plus goserelin, 50.5% Overall tumor response rate anastrozole plus goserelin, 70.4% Difference between groups, 19.9% (95% CI 6.5–33.3%); P = 0.004
TEXT (49) Phase 3	2,672	Premenopausal HR+ early BC	8	Median age, 44 years	Tamoxifen plus OFS (bilateral oophorectomy, ovarian radiation or triptorelin 3.75 mg, 4-weekly) *vs.* exemestane plus OFS Adjuvant treatment duration 5 years	See combined SOFT + TEXT analysis
SOFT + TEXT (53) Phase 3	4,690[b]	Premenopausal HR+ early BC	5.7	Median age, 43 years	Tamoxifen *vs.* tamoxifen plus OFS (bilateral oophorectomy, ovarian radiation, or triptorelin 3.75 mg, 4-weekly) *vs.* exemestane plus OFS Adjuvant treatment duration 5 years	5-year DFS tamoxifen plus OFS, 87.3% 5-year DFS exemestane plus OFS, 91.1% DFS HR 0.72 (95% CI 0.60–0.85); P < 0.001 5-year OS tamoxifen plus OFS, 96.9% 5-year OS exemestane plus OFS, 95.9% OS HR 1.14 (95% CI 0.86–1.15); P = 0.37
SOFT + TEXT[a] (49), Phase 3	4,690[b]	Premenopausal HR+ early BC	8	Median age, 43 years	Tamoxifen *vs.* tamoxifen plus OFS (bilateral oophorectomy, ovarian radiation, or triptorelin 3.75 mg, 4-weekly) *vs.* exemestane plus OFS Adjuvant treatment duration 5 years	8-year DFS tamoxifen plus OFS, 82.8% 8-year DFS exemestane plus OFS, 86.8% DFS HR 0.77 (95% CI 0.67–0.90); P < 0.001 8-year OS tamoxifen plus OFS, 93.3% 8-year OS exemestane plus OFS, 93.4% OS HR 0.98 (95% CI 0.79–1.22); P = 0.84
HOBOE (54) Phase 3	710[c]	Premenopausal HR+ BC	5.3	Median age, 45 years	Tamoxifen plus OFS (triptorelin 3.75 mg, 4-weekly) *vs.* letrozole plus OFS Adjuvant treatment duration 5 years	5-year DFS tamoxifen plus OFS, 85.4% 5-year DFS letrozole plus OFS, 93.2% DFS HR 0.72 (95% CI 0.48–1.07); P = 0.06 5-year death rate tamoxifen plus OFS, 4.8% 5-year death rate letrozole plus OFS, 3.1% OS HR not reported; P = 0.14

[a]*Includes patients (N = 1014) from the exemestane plus OFS arm of SOFT.*

[b]*Number of patients included in combined analysis after exclusions.*

[c]*The total number of patients randomized in the trial was 1,065. The letrozole plus OFS plus zoledronic acid group (N = 355) is not included.*

BC, breast cancer; CAF, cyclophosphamide, adriamycin, fluorouracil; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; HR+, hormone receptor positive; OFS, ovarian function suppression; OS, overall survival.

analysis of tamoxifen from SOFT and TEXT after a median follow-up of 5.7 years found that DFS was significantly higher for exemestane plus OFS than for tamoxifen plus OFS (5-year DFS 91.1% *versus* 87.3%), but there was no significant difference in OS (**Table 2**) (53). When the duration of follow-up was increased to a median of 8 years, a similar pattern of results was obtained (**Table 2**) (49, 53).

In SOFT, comparisons were also made between the exemestane plus OFS arm and the tamoxifen alone arm. These analyses showed that the 8-year DFS rate was significantly higher with combination therapy than with tamoxifen monotherapy (85.9% and 78.9%, respectively) whereas the OS was similar for both groups (**Table 2**) (49).

Similar to what was observed in the tamoxifen plus OFS *versus* tamoxifen alone arms of SOFT described earlier, the addition of OFS to exemestane provided greater absolute benefits in DFS in patients who had received prior chemotherapy. In this cohort, DFS was 9% higher in patients who had received exemestane plus OFS than in patients who had received tamoxifen alone (49).

The results of SOFT and SOFT/TEXT, after 9 years of follow-up, suggest that the addition of OFS to tamoxifen results in significantly higher rates of DFS and OS than tamoxifen alone, and that addition of OFS to an AI leads to significantly higher rates of DFS than tamoxifen alone, particularly in patients at high risk of disease recurrence who had received prior chemotherapy (48, 49, 60). Addition of OFS to an AI also produced a greater absolute benefit in DFS than did addition of OFS to tamoxifen, but no greater absolute benefit in OS. Therefore, considering the lack of superiority of an AI (plus OFS) over tamoxifen (plus OFS) on OS, the decision to choose an AI plus OFS must be weighed against additional complications of using AIs in premenopausal women (see below).

Letrozole Plus OFS *Versus* Tamoxifen Plus OFS

Further evidence for a lack of difference between AIs over tamoxifen when combined with OFS comes from the phase 3 HOrmonal BOne Effects (HOBOE) trial (61) (**Table 2**). In two arms of the three-arm trial, premenopausal women with HR+ breast cancer were randomized to receive adjuvant letrozole plus OFS (triptorelin) or tamoxifen plus OFS. A numerically greater benefit in 5-year DFS rates was observed for letrozole plus OFS *versus* tamoxifen plus OFS although the difference did not reach statistical significance (54) (**Table 2**). There was no significant difference in 5-year OS between the two groups.

Conclusion

While ECOG 3193 found no benefit in adding OFS to endocrine therapy, other studies have shown that this combination can improve survival outcomes, *versus* either OFS alone (ECOG 5188) or endocrine therapy alone (SOFT, SOFT/TEXT, and ASTRRA). There is also some evidence to suggest that combining OFS with an AI may lead to more favorable outcomes than combining OFS with tamoxifen (STAGE and SOFT/TEXT; **Figure 2**). However, results from other studies indicate that the opposite may be true (ABCSG OS data) or that there is no difference between the two endocrine therapies (ABCSG DFS data and HOBOE). Overall, the most recent available evidence suggests that OFS added to either tamoxifen or AIs can provide significant benefit in premenopausal patients with less favorable clinicopathological characteristics, such as those who have received previous chemotherapy. Since ER– tumors are not sensitive to ovarian E2 secretion, American Society for Clinical Oncology guidelines state that there is no role for OFS as adjuvant therapy in ER– breast cancers (62).

Efficacy of OFS Combined With Adjuvant Chemotherapy for Fertility Preservation in Premenopausal Women With Breast Cancer

Premenopausal women diagnosed with early breast cancer associated with unfavorable clinico-pathological features are candidates for treatment with systemic chemotherapy. In this group of patients, adjuvant chemotherapy has demonstrated clinical effectiveness in reducing risk of breast cancer relapse and death (16). However, in these, typically younger, women there is a risk of long-lasting and impactful toxicities associated with chemotherapy. One such risk is of cytotoxic damage to the ovaries and therapy-induced amenorrhea which can be permanent and can cause infertility. Estimates of the rate of chemotherapy-induced amenorrhea, for regimens including cyclophosphamide, vary between 20% and 70% in premenopausal women aged under 40 years but can rise to near 100% in older premenopausal patients (63). This is of increasing concern because, in many countries, the age of childbearing is increasing; a change that is accompanied by an increased risk of developing breast cancer. The potential for loss of fertility due to treatment can have serious psychological effects on women and can, therefore, influence treatment decisions taken at diagnosis.

Temporary OFS, using LHRHa, during adjuvant chemotherapy has been developed as a potential option to prevent chemotherapy-mediated gonadotoxicity and premature ovarian failure (POF) to maintain fertility in women of childbearing age undergoing breast cancer treatment. This approach is also recommended by the European Society for Medical Oncology in female patients who wish to preserve ovarian function and/or fertility while undergoing cancer treatment (64). In the past 15 years, several randomized clinical trials have attempted to answer the question of whether LHRHa administration during chemotherapy is effective in preventing POF and preserving fertility. The three largest phase 3 studies to date (> 200 patients each) are the Prevention of Early Menopause Study (POEMS) (65, 66), the Prevention of Menopause Induced by Chemotherapy: A Study in Early Breast Cancer Patients-Gruppo Italiano Mammella 6 (PROMISE-GIM6) trial (67, 68), and the Anglo Celtic OPTION trial (69). In contrast with the use of OFS as an adjuvant therapy, in the POEMs and OPTION trials, patients with ER– breast cancer were enrolled. This strategy was adopted in these trials owing to concerns about the ability of concurrent endocrine therapy to reduce the efficacy of chemotherapy. However, recent results in the TEXT trial suggest LHRHa are likely to be suitable for use concurrently with chemotherapy in women for HR+ breast cancer.

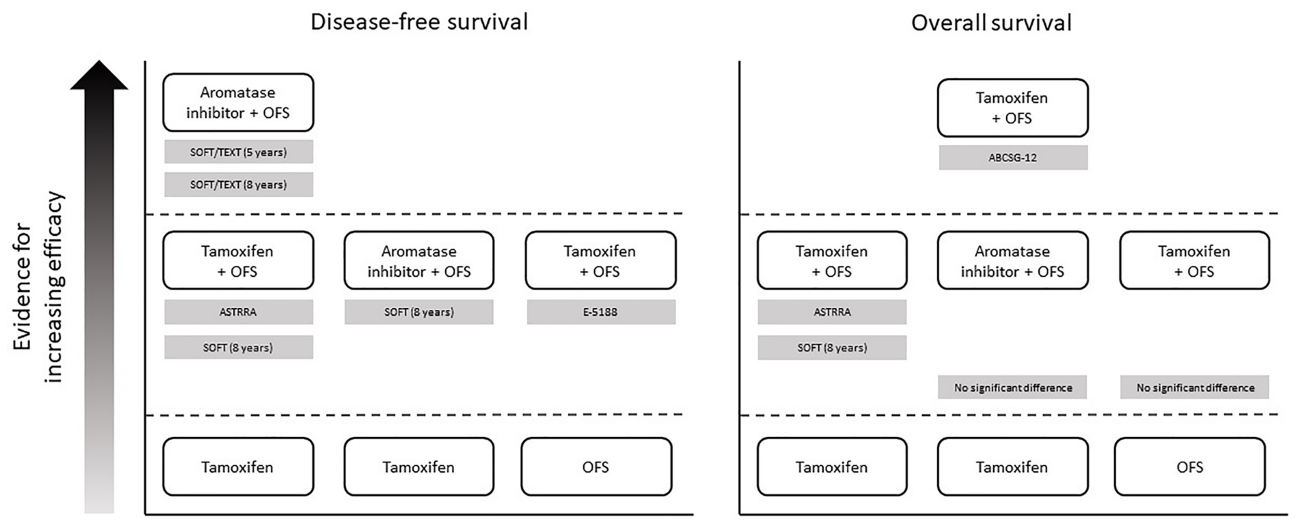

FIGURE 2 | Overview of evidence supporting the addition of OFS to endocrine therapy in premenopausal women with HR+ breast cancer. In each panel, treatments that are positioned higher up in the figure have been shown to be more effective than treatments positioned lower down (directly below). Supporting studies are detailed in gray boxes. Note that the figure does not present data from studies demonstrating equivalent efficacy between treatments – see main text and **Table 2** for full results of all studies. ABCSG, Austrian Breast and Colorectal Cancer Study Group; HR+, hormone receptor-positive; OFS, ovarian function suppression; SOFT, Suppression of Ovarian Function Trial; TEXT, Triptorelin and Exemestane Trial.

In the POEMS trial (**Table 3**), patients received either adjuvant/neoadjuvant chemotherapy alone or chemotherapy plus OFS (goserelin). After 2 years of follow-up, the POF rate was significantly lower in the group that received OFS (8%) *versus* the group that received only chemotherapy (22%) (65). At 5 years of follow-up, the rate of pregnancies was also significantly higher in the OFS-treated group (5-year cumulative incidence: 23.1% *versus* 12.2%) (66).

The PROMISE-GIM6 trial (**Table 3**) was an Italian study that randomized premenopausal breast cancer patients to receive either chemotherapy alone or chemotherapy plus OFS (triptorelin, starting 1 week prior to chemotherapy). The rate of POF 12 months after the end of chemotherapy was significantly higher in the chemotherapy alone group than in the chemotherapy plus OFS group (25.9% *versus* 8.9%) (67). After a median follow-up of 7.3 years, the benefit of OFS was retained, with a 5-year cumulative incidence of menstrual resumption of 64.0% *versus* 72.6%, and a 5-year incidence of pregnancy of 1.6% *versus* 2.1% (68).

Further reassuring evidence regarding the safety of this strategy come from the recently published final analysis of the study (median follow-up of 12.4 years), in which the 10-year cumulative pregnancy incidence was 3.2% in patients receiving chemotherapy alone, compared with 6.5% in patients receiving chemotherapy plus OFS. Importantly, 80% of the trial population had HR+ disease, yet no interaction between treatment effect and HR status was observed (70).

In the Anglo Celtic Group OPTION trial (**Table 3**), patients with early-stage breast cancer were randomized to receive either chemotherapy alone or chemotherapy plus OFS (goserelin). In the primary analysis, the prevalence of amenorrhea was significantly reduced with the addition of OFS, from 38% in the chemotherapy alone group to 22% in the chemotherapy plus OFS group (P = 0.015) (69). POF was also higher in the chemotherapy alone group (34.8% *versus* 18.5%), while the number of pregnancies was lower (six *versus* nine) (69).

Thus, the three largest trials to date support addition of LHRHa to chemotherapy to reduce POF and to help in maintaining fertility in premenopausal women. Importantly, the addition of LHRHa to achieve preservation of ovarian function does not have detrimental effects on the effectiveness of the chemotherapy. For example, in the PROMISE-GIM6 trial, the estimated 5-year DFS rates were 80.5% (95% CI 73.1–86.1) for chemotherapy plus triptorelin *versus* 83.7% (95% CI 76.1–89.1) for chemotherapy alone (HR: 1.17, 95% CI 0.72–1.92, P = 0.519) (71). Several smaller trials, including the GBG73 ZORO trial (72), a study by Badawy and colleagues (73), and several meta-analyses that support this conclusion were recently summarized in detail in a comprehensive review by Lambertini and colleagues (74).

Practicalities of Using LHRHa in Treatment of Breast Cancer

When determining the most appropriate adjuvant endocrine therapy for individual patients, the potential benefits of the addition of LHRHa must be weighed against increased rates of side effects and practical aspects of using these drugs. The effects of addition of OFS to tamoxifen or AIs on adverse events and patient-reported outcomes have been reported in the E-3193, SOFT, ZIPP, and OPTION trials (46, 75–78). Overall, evidence from these trials suggests that, in premenopausal patients, addition of LHRHa to tamoxifen is associated with worse

TABLE 3 | Overview of largest trials evaluating the addition of OFS to adjuvant chemotherapy for fertility preservation in premenopausal women with breast cancer.

Trial name	Randomized patients, N	Clinical characteristics	Follow up, years	Median age	Treatment arms	Outcomes
POEMS/SWOG (65, 66) Phase 3	218	Premenopausal Early-stage BC	2 (POF) 5 (pregnancy)	Overall, 38 years Chemotherapy alone, 38.7 years Chemotherapy plus OFS, 37.6 years	Neoadjuvant/adjuvant cyclophosphamide-containing chemotherapy alone *vs.* chemotherapy plus 3.6 mg 4-weekly goserelin	POF defined as amenorrhea for the preceding 6 months and FSH levels in the postmenopausal range at 2 years[a] POF rate chemotherapy alone, 22% POF rate chemotherapy plus OFS, 8% POF OR: 0.30 (95% CI 0.09–0.97); P = 0.04 5-year cumulative incidence of pregnancy: chemotherapy alone, 12.2% chemotherapy plus OFS, 23.1% OR: 2.34 (95% CI 1.07–5.11); P = 0.03
PROMISE-GIM6 (67, 68) Phase 3	281	Premenopausal BC	1 (POF) 7.3 (menstrual resumption; pregnancy)	39 years	Chemotherapy alone *vs.* chemotherapy plus 3.75 mg 4-weekly triptorelin	POF defined as no resumption of menstrual activity or the presence of postmenopausal levels of FSH and E2 for 1 year after the end of chemotherapy POF rate chemotherapy alone, 25.9% POF rate chemotherapy plus OFS, 8.9% Absolute difference: −17%; P < 0.001 5-year cumulative incidence of menstrual resumption: chemotherapy alone, 64.0% chemotherapy plus OFS, 72.6% 5-year incidence of pregnancy: chemotherapy alone, 1.6% chemotherapy plus OFS, 2.1%
Anglo Celtic Group OPTION (69)	227	Early-stage BC	1–2	Chemotherapy alone, 38.8 years Chemotherapy plus OFS, 37.9 years	Cyclophosphamide- and/or anthracycline-containing chemotherapy alone *vs.* chemotherapy plus 3.6 mg 4-weekly goserelin	Primary outcome was amenorrhea at 12–24 months after end of chemotherapy Amenorrhea rate chemotherapy alone, 38% Amenorrhea rate chemotherapy plus OFS, 22% (P = 0.015) POF defined as the presence of amenorrhea and elevated FSH (> 25 IU/L) POF rate chemotherapy alone, 34.8% POF rate chemotherapy plus OFS, 18.5%

[a]*POF was evaluated in 135 patients for whom data were available at 2 years.*

BC, breast cancer; CI, confidence interval; E2, estradiol; FSH, follicle-stimulating hormone; IU, international units; OFS, ovarian function suppression; OR, odds ratio; POF, premature ovarian failure.

endocrine symptoms and sexual function, which is particularly problematic in the population of younger women who gain most benefit from these drugs. Analysis of the combined SOFT and TEXT showed that when OFS was added to tamoxifen or exemestane, reported grade 3 or 4 adverse events increased (tamoxifen 24.6% *versus* tamoxifen plus OFS 31.0% *versus* exemestane plus OFS 32.3%) and patients reported considerable worsening from baseline in key endocrine symptoms (including hot flushes, depression, sweating, fatigue, and insomnia). No overall difference in quality of life was found between tamoxifen and AI (49, 79). In the combined analysis of SOFT and TEXT, 19% of patients overall stopped treatment with LHRHa earlier than the 5-year planned treatment duration (tamoxifen plus LHRHa 19.6%, exemestane plus LHRHa 18.3%) (49); this increased to 23% at 4-years in the most high-risk patient group aged under 35 years (80). In the patients receiving tamoxifen plus LHRHa, 8.1% had an adverse event related to a reaction at the drug injection site; this was 7.5% in those receiving exemestane plus LHRHa compared with 0.4% in those receiving only tamoxifen (49). Rates of early discontinuation of oral endocrine therapy in SOFT and TEXT were 21.5% overall, with discontinuation higher in those assigned exemestane plus OFS (23.7%) than in those receiving tamoxifen plus OFS (19.3%) (49). However, recently reported results from the OPTION trial suggest that when goserelin is added to chemotherapy to provide ovarian function protection in premenopausal women with early breast cancer the detrimental effects experienced on quality of life are short-lived. Within 24 months, the majority of patient-reported outcomes in individuals receiving goserelin with chemotherapy did not differ from those receiving chemotherapy alone (78).

Another potential problem when adding LHRHa to AIs is the risk associated with incomplete estrogen suppression. AIs are more effective than tamoxifen in postmenopausal women and, when combined with OFS in premenopausal women, have been associated with greater improvements in DFS and OS *versus* tamoxifen plus OFS for some patients. However, in premenopausal women, most estrogen production occurs in the ovaries. For AIs to be fully effective, unlike with tamoxifen, complete suppression of ovarian function is required. Numerous studies have demonstrated the ability of LHRHa to significantly suppress E2 levels, but it remains unclear whether the degree of E2 suppression is sufficient to permit combination with AIs in some high-risk premenopausal women. In fact, the SOFT-Estrogen (SOFT-EST) prospective study, which measured E2 levels in 116 patients, found that between 17–25% of patients receiving exemestane plus triptorelin had E2 levels above the threshold target level of ≤ 2.72 pg/mL during the 12-month study (81). Thus, when deciding to use an AI combined with LHRHa to treat those patients with the worst prognostic features, clinicians should be aware of the potential for incomplete OFS and closely monitor patient E2 levels during treatment. In real-world situations in which E2 monitoring is not available, tamoxifen plus LHRHa should be considered for patients at higher risk of incomplete OFS, including younger women, those who have not received prior chemotherapy, and those with a high body mass index (BMI). Indeed, in the ABCSG-12 trial, secondary analysis showed that patients with a BMI ≥ 25 kg/m^2 receiving anastrozole plus goserelin had a 50% increased risk of disease recurrence (HR: 1.49, 95% CI 0.93–2.38) and 3-fold increase in risk of death (HR: 3.03, 95% CI 1.35–6.82) compared with those receiving tamoxifen plus goserelin (82). This may be due to incomplete OFS in patients with higher BMI but could also be the result of increased ER activation by other factors, such as insulin/insulin-like growth factor, that are increased in overweight patients. The confounding factors in the worse prognosis for overweight patients require further exploration in future randomized control trials.

One approach to overcoming some of the problems associated with the use of LHRHa in the clinic has been the introduction of long-acting drug formulations (83). In prostate cancer, in which LHRHa including goserelin, triptorelin, and leuprorelin are used to reduce circulating androgens (**Figure 1B**), long-acting drug formulations have been shown to be clinically effective and well tolerated and have been used extensively for several years (84, 85). Several long-acting formulations have been approved for use in prostate cancer, allowing dosing at 1-, 2-, 3-, 4-, 6-, and 12-month intervals (**Table 1**). Goserelin acetate (Zoladex®) is available as a slow-release solid implant injected subcutaneously on a monthly or three-monthly basis. Leuprorelin acetate is available in several different formulations that allow for 1-, 3-, 4-, or 6-monthly administration, including a slow-release solid implant (83). Triptorelin acetate (Decapeptyl® SR) can be administered intramuscularly at 1-, 3-, or 6-monthly dosing intervals (83). Experience from use in prostate cancer has shown several advantages of solid implant formulations, including being ready to use with no need for reconstitution and the ability to be stored without refrigeration (83). Moreover, the exact dose given is known whereas for other formulations, issues can arise when reconstitution is performed incorrectly, potentially resulting in insufficient dosing (86). A final advantage of solid implants *versus* gel-like or reconstituted powder injections is the ability to remove the implant in the event of severe adverse effects of the medication. Long-acting formulations are also be preferred by patients (83).

In breast cancer, long-acting LHRHa formulations remain less commonly used than short-acting alternatives and fewer different formulations are approved for use in a smaller number of countries. For premenopausal patients with HR+ breast cancer, leuprorelin is available as a 1-, 3-, and 6-month depot formulation (Lupron®, Prostap 3). Early studies showed that 3-monthly administration of leuprorelin was as effective, as well tolerated, and provided similar E2 suppression as monthly administration (87). A retrospective analysis of SOFT and TEXT found that, in 201 patients randomized to receive an AI plus either 7.5 mg leuprorelin monthly or 22.5 mg leuprorelin 3-monthly, the ability to achieve ovarian ablation (defined as an E2 concentration < 40 pg/mL and an FSH concentration 23–116 mU/mL) was the same with both formulations (88). In 167 premenopausal patients with HR+ breast cancer randomized to receive either 11.25 mg leuprorelin 3-monthly or 22.5 mg leuprorelin 6-monthly, the rate of E2 suppression (to ≤ 30 pg/mL) was found to

be 1.2% higher in the group using the longer-acting formulation (3-monthly formulation 96.4%; 6-monthly formulation 97.6%) without significant differences in adverse events (89). In a retrospective study by Lee and colleagues of 318 women who had previously undergone surgery for breast cancer, post-surgery treatment with 3-monthly leuprorelin acetate (11.25 mg) successfully reduced E2 levels below 30 pg/mL (mean: 4.9 pg/mL) in all patients demonstrating the effectiveness of this formulation (90).

Goserelin (Zoladex®) is used in premenopausal patients with breast cancer as a 3.6 mg solid implant and has more recently been approved for use as a 3-monthly 10.8 mg implant in Japan, Taiwan, Ukraine, South Korea, Indonesia, Singapore and Malaysia. Several studies have demonstrated the non-inferiority of 3-monthly *versus* monthly goserelin. In an open-label, randomized study conducted in Japan, E2 levels were measured in 170 premenopausal patients with ER+ early breast cancer who were randomized to receive either goserelin 10.8 mg given 3-monthly or goserelin 3.6 mg given monthly. After 24 weeks of treatment, serum E2 levels were 18.95 pg/mL (n = 84) for goserelin 3.6 mg and 18.32 pg/mL (n = 86) for goserelin 10.8 mg (91), demonstrating comparable OFS with both formulations; no clinically important differences in safety and tolerability were found. In a further trial conducted in India, Japan, Republic of Korea, Philippines, Thailand, and Taiwan in 222 patients with ER+ advanced breast cancer, progression-free survival (PFS) and overall response rates (ORRs) after 24 weeks were similar with goserelin 10.8 mg given 3-monthly and 3.6 mg given monthly (PFS: 10.8 mg, 61.5%; 3.6 mg, 60.2%; ORR: 10.8 mg, 23.9%; 3.6 mg, 26.9%). Similar to the previous study, E2 levels at 24 weeks were also suppressed equally by 3-monthly (10.8 mg, 20.3 pg/mL) and monthly (3.6 mg, 24.8 pg/mL) administration (92). A third study conducted in Russia and Ukraine also found non-inferiority of 3-monthly *versus* monthly goserelin, with similar PFS, ORR, and E2 suppression observed for both formulations. Finally, an ongoing phase 3 study in China, due for completion in November 2021 (NCT03658213), is investigating the non-inferiority of 3-monthly 10.8 mg goserelin in ER+/HER2− early breast cancer patients.

CONCLUSIONS

As treatment options have rapidly expanded, management of adjuvant treatment of premenopausal women with early and advanced breast cancer has become more complicated. The most recent evidence suggests that addition of LHRHa to adjuvant endocrine therapy, with both tamoxifen and AIs, can provide significant benefits in some premenopausal patients who are at high risk of recurrence and have poor prognostic characteristics. Longer-acting depot and implant LHRHa formulations may help to overcome some of the barriers to adding OFS to endocrine therapy in the adjuvant setting in premenopausal women.

AUTHOR CONTRIBUTIONS

Y-SL, AW, and H-JK contributed equally to writing this review. All authors contributed to the article and approved the submitted version.

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Factors Associated With the Discussion of Fertility Preservation in a Cohort of 1,357 Young Breast Cancer Patients Receiving Chemotherapy

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Purpose: Female breast cancer (BC) patients exposed to gonadotoxic chemotherapy are at risk of future infertility. There is evidence of disparities in the discussion of fertility preservation for these patients. The aim of the study was to identify factors influencing the discussion of fertility preservation (FP).

Material and Methods: We analyzed consecutive BC patients treated by chemotherapy at Institut Curie from 2011-2017 and aged 18-43 years at BC diagnosis. The discussion of FP was classified in a binary manner (discussion/no discussion), based on mentions present in the patient's electronic health record (EHR) before the initiation of chemotherapy. The associations between FP discussion and the characteristics of patients/tumors and healthcare practitioners were investigated by logistic regression analysis.

Results: The median age of the 1357 patients included in the cohort was 38.7 years, and median tumor size was 30.3 mm. The distribution of BC subtypes was as follows: 702 luminal BCs (58%), 241 triple-negative breast cancers (TNBCs) (20%), 193 $HER2^+/HR^+$ (16%) and 81 $HER2^+/HR^-$ (6%). All patients received chemotherapy in a neoadjuvant (*n*=611, 45%) or adjuvant (*n*= 744, 55%) setting. A discussion of FP was mentioned for 447 patients (33%). Earlier age at diagnosis (discussion: 34.4 years *versus* no discussion: 40.5 years), nulliparity (discussion: 62% *versus* no discussion: 38%), and year of BC

diagnosis were the patient characteristics significantly associated with the mention of FP discussion. Surgeons and female physicians were the most likely to mention FP during the consultation before the initiation of chemotherapy (discussion: 22% and 21%, respectively). The likelihood of FP discussion increased significantly over time, from 15% in 2011 to 45% in 2017. After multivariate analysis, FP discussion was significantly associated with younger age, number of children before BC diagnosis, physicians' gender and physicians' specialty.

Conclusion: FP discussion rates are low and are influenced by patient and physician characteristics. There is therefore room for improvement in the promotion and systematization of FP discussion.

Keywords: breast cancer, fertility preservation, discussion, chemotherapy, oncofertility

1 INTRODUCTION

Breast cancer (BC) is the most frequent cancer in women (1), and about 7% of BC diagnoses concern women under the age of 40 years (2). Survival rates are continually improving, thanks to advances in early detection and treatment. Mean age at first pregnancy is continuing to increase, due to changes in society, and the question of fertility and pregnancy after BC is therefore being raised increasingly frequently (3).

Oncological treatments may impair the fertility of premenopausal patients with BC. Chemotherapy may induce premature ovarian failure, depending on the woman's age and the drugs used, their dose and the duration of treatment (4). Adjuvant endocrine therapy, which is generally recommended for five years in patients with hormone-responsive cancers, can also delay parenthood, due to the potential teratogenicity of the treatment (5).

A number of fertility preservation (FP) techniques are available, and the freezing of embryos or oocytes after controlled stimulation for future *in vitro* fertilization procedures is the most frequently used (6). If this is unfeasible or if ovarian stimulation is contraindicated, ovarian tissue cryopreservation of oocyte/embryo vitrification after the *in vitro* maturation of oocytes recovered from small antral follicles may be used as an alternative (7).

Previous studies have suggested that many BC patients are interested in maintaining their future fertility at the time of diagnosis. However, they do not systematically receive information about the fertility risks of treatment and fertility preservation options (8), with such discussion occurring in 30 to 70% of patients (9, 10). The American Society of Clinical Oncology recommends that physicians question newly diagnosed cancer patients as soon as possible about their desire for future fertility, and that interested patients be immediately referred to specialists in fertility preservation techniques, when appropriate (6). In France, the National Cancer Plan 2014-2019 highlighted the need for systematic and appropriate information on fertility preservation and promoted the concept of oncofertility (11).

Publications to date on the factors predictive of FP discussion in BC are mostly limited to small qualitative studies. Disparities in referral patterns and access to FP have been observed with respect to the demographic, clinical and socioeconomic characteristics of patients. A few studies have shown that patient age, and parity, the type of treatment, type of center and physician characteristics may affect the likelihood of FP discussion (9, 10, 12).

The objective of this study was to identify the factors associated with FP discussion in a population of women receiving chemotherapy for BC to improve patient counselling and timely access to FP services.

2 MATERIALS AND METHODS

2.1 Study Design

We analyzed a cohort of female patients with invasive BC aged between 18 and 43 years at the time of BC diagnosis, treated by chemotherapy at Institut Curie between January 1, 2011 and September 30, 2017. The upper limit of 43 years was chosen as this is the maximum age for reimbursement of assisted reproductive technology in France. In the study, we also used the 37 years as a cut-off point, as it has been shown that the age of 37 years is correlated with an accelerated disappearance of ovarian follicles in mid-life (13, 14). The study was conducted at two centers: the Institut Curie centers at Paris and Saint Cloud.

The cohort was constructed with the ConSore (15) search engine, a next-generation data analysis program developed by UNICANCER and allowing both requests with structured criteria and natural language processing for semantic searches (flow chart in **Supplementary Table 1**).

The exclusion criteria were another cancer before BC, distant metastases at diagnosis or within six months of diagnosis, bilateral breast cancer, refusal of treatment, hysterectomy, tubal sterilization or bilateral ovariectomy performed before diagnosis, patient refusal of the use of their data. We did not include patients who did not receive chemotherapy because in the 2 institutions in which the patients were treated that we analyzed, patients without chemotherapy were not offered fertility preservation procedures at the time of the study. All medical charts were manually verified from September 2017 to March

2018. The study was approved by the Breast Cancer Study Group of Institut Curie and was conducted in accordance with institutional and ethical rules concerning research on tissue specimens and patients.

The objective of this study was to identify factors associated with discussion of FP in this population, and discussion of FP was used as the primary endpoint.

Under French regulations, written informed consent from patients was not required for this study. This study is a part of the young breast cancer project (YBCP), an institutional project aiming at characterizing BC care pathways in young women. It was approved by the breast cancer group and institutional board (approval 29th, April 2019, reference cri-data DATA190136).

2.2 Patients

The data collected included age, parity and body mass index (BMI) at diagnosis, date of first consultation at Institut Curie, date of first biopsy showing malignant histological features, date of first chemotherapy, date of surgery, and BRCA status, when available. The date of the first consultation at Institut Curie was taken as the date of diagnosis.

2.3 Tumors

We retrieved the following tumor characteristics from the patients' medical records: clinical T (size) stage and clinical N (nodal) status, immunohistochemical characteristics, such as the detection of estrogen receptors (ER), progesterone receptors (PR), HER2 status, Ki67 and histological grade. Cases were considered estrogen receptor (ER)- or progesterone receptor (PR)-positive (+) if at least 10% of the tumor cells expressed estrogen and/or progesterone receptors (ER/PR), in accordance with the guidelines used in France (16). HER2 expression was assessed by immunohistochemistry, with scoring according to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines. Scores of 3+ were considered positive, scores of 1+/0 were considered negative (-). Tumors with scores of 2+ were subjected to further testing by FISH. HER2 gene amplification was defined according to ASCO/CAP guidelines (17). Based on immunohistochemical surrogates, pathological breast cancer subtypes were defined as follows: tumors positive for either ER or PR and negative for HER2 were classified as luminal; tumors positive for HER2 were considered HER2-positive BC; tumors negative for ER, PR, and HER2 were considered triple-negative BC (TNBC). Histological grade was determined according to the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system (18).

2.4 Treatments

Patients were treated according to national guidelines. Treatments were decided after multidisciplinary consultation meetings considering the characteristics of the patients and prognostic factors. For patients receiving neoadjuvant chemotherapy, surgery was performed four to six weeks after the end of chemotherapy. Trastuzumab was used in an adjuvant and/or neoadjuvant setting for *HER2*-positive breast cancer, in accordance with national guidelines. Most patients received adjuvant radiotherapy. Endocrine therapy (tamoxifen, aromatase inhibitor, and/or GnRH agonists) was prescribed when indicated. Every patient included in our study received chemotherapy (neoadjuvant and/or adjuvant).

2.5 Discussion About Fertility Preservation

Discussion about FP (FP discussion) — *i.e.* the delivery of information about the existence of fertility preservation procedure before chemotherapy — was assessed from electronic health records (EHR) as a binary variable (discussion/no discussion), and through a two-way process. Any discussion on damages on fertility induced by chemotherapy counted as "FP Discuss". Only files with no information on fertility risks were classified as "FP No-Discuss". We first extracted specific string character patterns by text mining (TM), using specific key words associated with a high likelihood of FP discussion having occurred ("oncofertility", "IVM", "frozen oocytes", "frozen embryos", "(fertility)", "ov* fragment preservation", "ov* cryopreservation", "ov* cryoconservation"), making it possible to identify the keyword concerned directly in the EHR. This text recognition method was developed and validated on two independent datasets and has been shown to have a better performance than the manual rereading of medical records to identify pregnancies (19). For patients for whom none of the keywords sought was found, we then manually checked all medical consultations between BC diagnosis and chemotherapy, from June to October, 2018.

2.6 Physicians

For any consultation with a medical doctor occurring between BC diagnosis and chemotherapy, demographic information about the physician was collected: sex (male *versus* female), age at consultation (junior < 45 years old *versus* senior > 45 years old) and type of specialty (surgeon, oncologist or radiotherapist), together with the rank of healthcare provider (ranging from 1 to 3). Once FP had been discussed with a healthcare provider, subsequent consultations were censored.

2.7 Fertility Preservation Procedures

Specific data concerning the procedures were retrieved from the three partner fertility preservation centers in the Parisian region: Jean Verdier Hospital in Bondy, Antoine Beclere Hospital in Clamart and Port Royal Hospital in Paris. We collected the following information: the final choice of the patients or the physician concerning FP procedures, recorded as a binary variable (yes/no), and the method used (oocyte or embryo vitrification after IVM or after controlled ovarian stimulation (COS), cortex cryopreservation).

2.8 Statistical Methods

The study population was described in terms of frequencies for qualitative variables, or medians and associated ranges for quantitative variables. For the comparison of continuous variables between groups, Wilcoxon-Mann-Whitney tests were used for groups including fewer than 30 patients, and for variables with multimodal distributions, and Student's *t* tests were performed otherwise. Associations between categorical variables were assessed in chi-squared tests, or with Fisher's exact test if at least one category included fewer than three

patients. A value of $P < 0.05$ was considered significant. Data were processed and statistical analyses performed with R software version 3.1.2 [www.cran.r-project.org, (R Foundation for Statistical Computing, 2009)].

Data were evaluated using multiple correspondence analysis (MCA). This method involves a multivariate analysis of categorical data and allows joint observation of a vast number of variables. By grouping various characteristics, it attempts to establish a profile capable of suggesting a predisposition to specific situations. Analysis was conducted with the package library (FactoMineR), which performs various mathematical procedures to define the best organization of variables and allocate variables into a four-quadrant plot divided by two axes. Results are interpreted by observation of clusters formed by variables. These clusters represent relations between the variables; the closer they are on the plot, the greater the frequency of their co-occurrence. The two axes separate variables plotted on the left upper quadrant from those in the right lower quadrant and those in the right upper quadrant from those in the left lower quadrant, establishing groups of variables with opposing profiles. It gives a representation of the absolute contribution of each variable according to its distance from the axis, both towards the positive and towards the negative side; the greater the distance, the greater its significance in the interpretation of results.

We used a mixed model combining mixed effects and random effects for the multivariate analysis. The fixed effects influence the mean of the variable of interest (FP discussion) and the random effects influence only the variance of that variable. We used this model based on the assumption that the observations in our database are not independent (i.e. that the occurrence of a FP discussion can be the same depending on the characteristics of the doctors and patients). Thus, the residual variance of the model is partitioned into a between two components: patients and doctors.

3 RESULTS

In total,1357 patients were included in the study (**Table 1**). Median age at BC diagnosis was 38.7 years (range: 18-43 years). Most patients had one (21%) or more children (52%) at BC diagnosis, but 27% did not have children. Median tumor size was 30.3 mm, and 58% of the patients had luminal BCs. All patients received chemotherapy (neoadjuvant (45%)/adjuvant (55%) setting). The characteristics of the patients and their tumors differed according to age at BC diagnosis (**Supplementary Table 2**), with a larger number of patients having children (81% *versus* 66%), a larger proportion of luminal tumors (64% *versus* 54%), and a lower likelihood of receiving neoadjuvant as opposed to adjuvant chemotherapy (35% *versus* 52%) in older patients than in younger patients.

3.1 Factors Associated With Fertility Discussion

Some mention of FP discussion was found in the EHRs of 447 (33%) of the 1357 patients, whereas no such mention was not found in the EHRs of 909 patients (67%).

3.1.1 Patient-Related Factors

FP discussion was significantly associated with a younger age at BC diagnosis (median age 34 years *versus* 40 years, $p<0.001$) (**Figure 1A**), and were less likely to occur for obese patients (**Figure 1B**). Her frequency decreased with increasing numbers of children (**Figure 1C**) and increased over the time period of the cohort, reaching a plateau at about 45% after 2015 (**Figure 1D**). Clinical stage ($p=0.05$) and neoadjuvant chemotherapy ($p<0.001$) were also significantly associated with FP discussion (**Figures 1E, F** respectively). Multiple component analysis identified two groups of patients and characteristics associated with fertility discussion (red ellipse: patients aged 37 years or older, with children at diagnosis, for whom there was no FP discussion; and blue ellipse: patients below the age of 37, with no children at diagnosis, for whom FP discussion occurred) (**Figure 1G**).

3.1.2 Doctor- and Center-Related Factors

In total, 2468 pre-chemotherapy consultations were retrieved from the EHRs (with surgeons n=1280; medical oncologists n=1073, and radiotherapy oncologists, n=115) (**Table 2**).

FP discussion was more frequently mentioned during the first pre-chemotherapy consultation, than during the following visits (discussed with the first practitioner n=336; second n=92; third n=19).

Doctors' specialty was significantly associated with the likelihood of FP discussion. Surgeons were more likely to discuss FP with patients (22%) than medical oncologists (14%) and radiation oncologists (10%) (**Figure 2A**). Doctors' age (**Figure 2B**) and sex (**Figure 2C**) were also significantly associated with FP discussion: junior doctors (21%) and female doctors (21%) were slightly more likely to discuss FP than senior doctors (17%) and male doctors (15%), respectively. The site where patients received their treatment (center 1 *versus* center 2) (**Figure 2D**) was not significantly associated with the likelihood of FP discussion ($p=0.14$).

Multiple correspondence analysis identified two groups of physicians and characteristics associated with FP discussion (red ellipse: oncologists and male physicians not discussing FP with patients; blue ellipse: surgeons and female physicians discussing FP with patients) (**Figure 2E**).

3.1.3 Factors Associated With FP Discussion in Multivariate Analysis

After multivariate analysis with the mixed model, fertility discussion was significantly associated with younger age, number of children before BC diagnosis, physicians' gender and physicians' specialty (**Table 3**).

3.2 Factors Associated With the Performance of Fertility Preservation Procedures (FPPs)

FP procedures were performed in 262 of the 1357 patients (19%). Seventeen patients received treatment with LHRH analogs. The main factor associated with the occurrence of FPPs was the occurrence of FP discussion (only three patients underwent FPPs without prior FP discussion).

TABLE 1 | Patient and tumor characteristics (*n*=1357) as a function of the presence or absence of discussion about fertility preservation.

Variable name	Level *n*	Overall 1357 (100%)	FP discussion 447 (33%)	No FP discussion 909 (67%)	*p*
Age (year)	[0 -30)	95 (7%)	72 (76%)	23 (24%)	**<0.001**
	[30 -35)	246 (18%)	173 (70%)	73 (30%)	
	[35 -40)	460 (34%)	162 (35%)	298 (65%)	
	40+	554 (41%)	40 (7%)	514 (93%)	
Age (mean)		38.7 [34.9, 41.6]	34.4 [31.2, 37.2]	40.5 [37.6, 42.3]	**<0.001**
Number of children	0	373 (27%)	231 (62%)	141 (38%)	**<0.001**
	1	279 (21%)	99 (35%)	180 (65%)	
	More than 1	705 (52%)	117 (17%)	588 (83%)	
BMI	<18.5	78 (6%)	29 (37%)	49 (63%)	**0.002**
	18.5-24.9	811 (65%)	302 (37%)	509 (63%)	
	25-29.9	257 (21%)	88 (34%)	168 (66%)	
	>=30	107 (8%)	20 (19%)	87 (81%)	
BMI (mean)		22.6 [20.4, 25.5]	22.3 [20.3, 24.9]	22.8 [20.7, 25.9]	**0.004**
Treatment center	Curie Paris	818 (60%)	287 (35%)	531 (65%)	**0.047**
	Curie St Cloud	538 (40%)	160 (30%)	378 (70%)	
Year of BC diagnosis	2011	167	23 (14%)	144 (86%)	**<0.001**
	2012	190	31 (16%)	159 (84%)	
	2013	186	51 (27%)	135 (73%)	
	2014	224	69 (31%)	155 (69%)	
	2015	221	109 (49%)	112 (51%)	
	2016	216	96 (44%)	120 (56%)	
	2017	151	68 (45%)	83 (55%)	
Hereditary predisposition	No	547 (80%)	240 (44%)	306 (56%)	0.235
	Yes	140 (20%)	70 (50%)	70 (50%)	
Inflammatory BC	No	1338 (99%)	443 (33%)	895 (67%)	0.469
	Yes	18 (1%)	4 (22%)	14 (78%)	
Clinical tumor size (mm)		30.3 (21.7%)	31.5 (20.3%)	29.7 (22.4)	0.148
Clinical T stage (TNM)	T0-T1	588 (44%)	178 (30%)	410 (70%)	**0.052**
	T2	592 (39%)	217 (37%)	375 (63%)	
	T3-T4	166 (12%)	51 (31%)	115 (69%)	
Clinical N stage (TNM)	N0	854 (63%)	281 (33%)	573 (67%)	0.859
	N1-N2-N3	492 (36%)	165 (34%)	327 (66%)	
SBR grade	Grade I	58 (4%)	16 (28%)	42 (72%)	**0.006**
	Grade II	528 (39%)	150 (28%)	378 (72%)	
	Grade III	760 (57%)	278 (37%)	482 (63%)	
BC subtype	Luminal	702 (58%)	208 (30%)	494 (70%)	**0.021**
	TNBC	241 (20%)	92 (38%)	148 (62%)	
	$HER2^+/HR^+$	193 (16%)	75 (39%)	118 (61%)	
	$HER2^+/HR^-$	81 (6%)	28 (35%)	53 (65%)	
Histological type	NST	1265 (93%)	426 (34%)	839 (66%)	**0.014**
	Lobular	54 (4%)	8 (15%)	46 (85%)	
	Others	36 (3%)	13 (36%)	23 (64%)	
Chemotherapy setting	Adjuvant	744 (55%)	201 (27%)	543 (73%)	**<0.001**
	NAC	611 (45%)	245 (40%)	366 (60%)	
FP procedure	No	1095 (81%)	188 (17%)	906 (83%)	**<0.001**
	Yes	262 (19%)	259 (99%)	3 (1%)	
LHRH Analogs	No	1340 (99%)	242 (18%)	1098 (82%)	**<0.001**
	Yes	17 (1%)	17 (100%)	0 (0%)	

Values in bold are the significant values.

Out of 447 patients who had a FP discussion, 259 patients (58%) had a FP procedure and 188 (42%) didn't have (**Table 4**). The factors significantly associated with the realization of a FPP in FP discussion group were age (**Figure 3A**), and previous children (**Figure 3B**). The type of chemotherapy was not associated with PPF (**Figure 3C**). The MCA clustered patients into two distinct groups, with FP discussion, already having children, and age as the major factors explaining the performance of a FPP (**Figure 3D**).

Most patients (*n*=175) underwent IVM, and one third (*n*=84) had at least one COS. The factors associated with the type of FPP (**Supplementary Table 3**) were mostly related to the chemotherapy setting with COS used in a neoadjuvant setting in only six out of 146 patients.

4 DISCUSSION

This large, real-life study found that the rates of the discussion of fertility preservation (FP) were low (33%) in a consecutive series of 1,357 female breast cancer patients exposed to gonadotoxic chemotherapy. Furthermore, we discussed the correlation between FP discussion and the characteristics of patients/

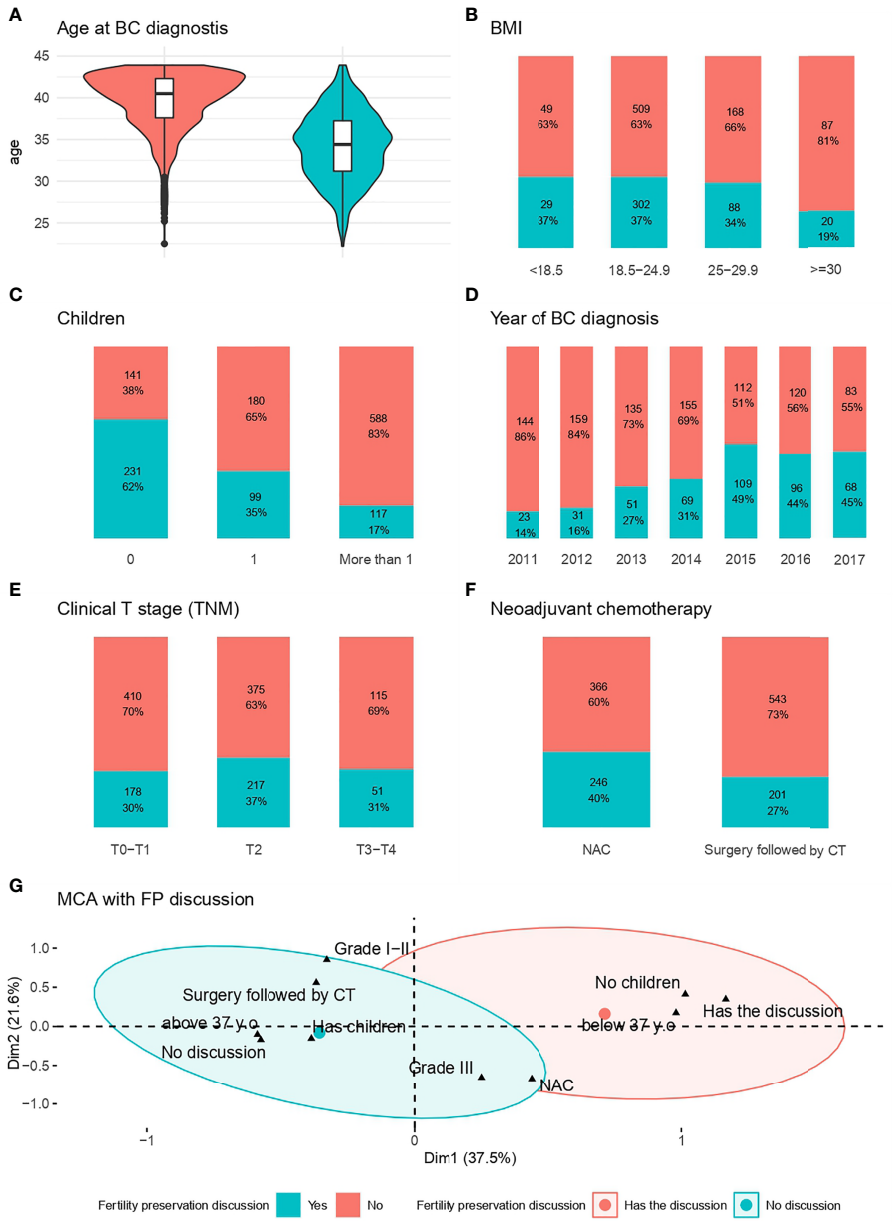

FIGURE 1 | Factors associated with the likelihood of FP Discussion. **(A)** Age at BC diagnosis; **(B)**: BMI; **(C)** Patient with children at the time of diagnosis; **(D)** Year of diagnosis; **(E)** Clinical stage (TNM); **(F)** Neoadjuvant chemotherapy; **(G)** MCA for fertility preservation discussion*. *The red ellipse represents the concentration of people who had no discussion about fertility preservation, whereas the blue ellipse represents the concentration of people who discussed fertility preservation with a physician.

tumors and healthcare practitioners. We found that younger age, number of children before breast cancer diagnosis, physicians' gender and physician's specialty were independent predictors of FP discussion. We also found an increased likelihood of FP discussion over time. In general, the findings in the present study support the above-mentioned conclusion. The results of our study confirm and reinforce previous findings from the literature.

One of its key findings is that FP discussion was mentioned in only one third of EHRs. These rates lie in the lower part of the range of published values, which generally range from 30 to 70% (9, 12, 20). There are several possible reasons for these low rates. First, we included patients up to 43 years old, and patients 40 y.o. or above represented 41% of the cohort. When focusing only in the subpopulation of patients below 40, the discussion rate increased to 51%. Second, this cohort study began in 2011, a

TABLE 2 | Likelihood of FP discussion according to physician characteristics and center (*n*=2468).

Variable name	Level *n*	Overall 2468	FP Discussion 447	No FP Discussion 2021	p
Specialty	Oncologist	1073 (43%)	150 (14%)	923 (86%)	**<0.001**
	Radiotherapy oncologist	115 (5%)	12 (10%)	103 (90%)	
	Surgeon	1280 (52%)	285 (22%)	995 (78%)	
Age	Junior	937 (38%)	193 (21%)	744 (79%)	**0.017**
	Senior	1521 (62%)	254 (17%)	1267 (83%)	
Sex	Female	1292 (52%)	274 (21%)	1018 (79%)	**<0.001**
	Male	1169 (48%)	173 (15%)	996 (85%)	
Treatment center	Center 1	1454 (59%)	287 (20%)	1167 (80%)	0.143
	Center 2	1014 (41%)	160 (16%)	854 (84%)	

Values in bold are the significant values.

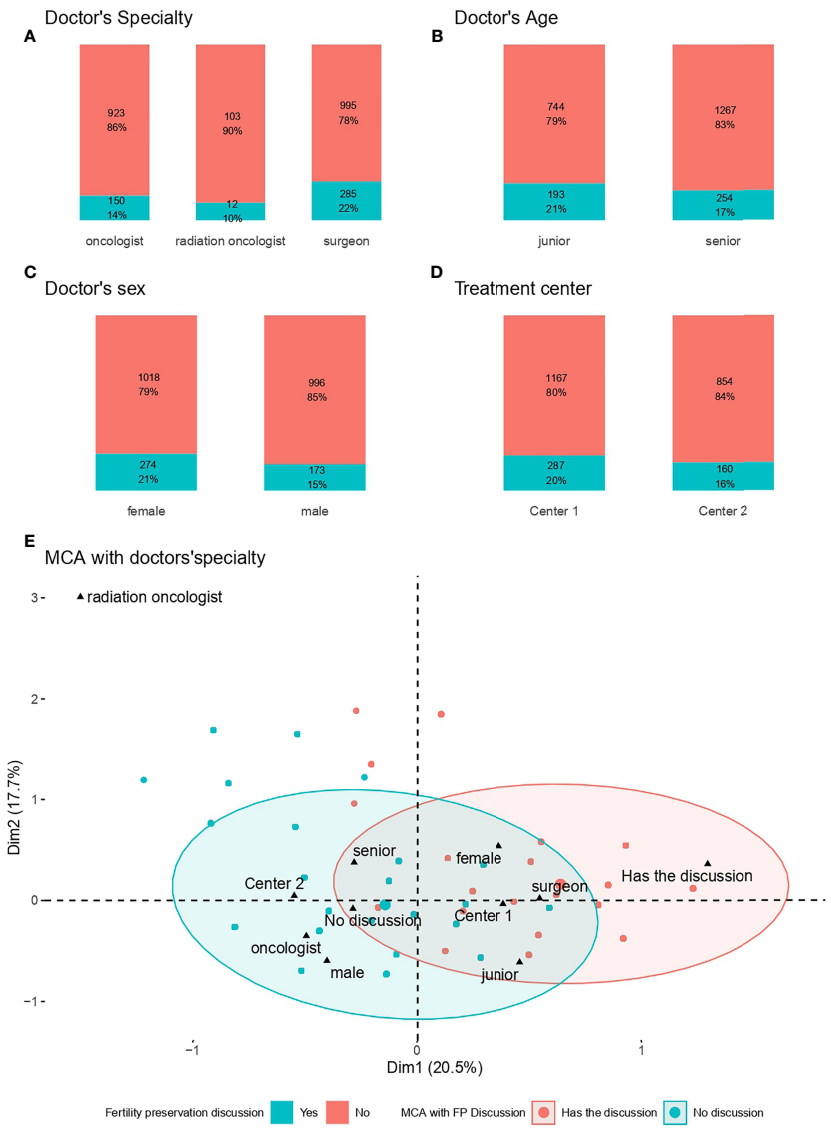

FIGURE 2 | Factors associated with fertility preservation Discussion. **(A)** Doctors' specialty; **(B)** Doctors' age; **(C)** Doctors' sex; **(D)** Treatment Center; **(E)** MCA with FP discussion*. **(E)** The red ellipse represents the concentration of patients who did have discussion about fertility preservation, whereas the blue ellipse represents the concentration of patients who discussed fertility preservation with a physician.

TABLE 3 | Factors associated with FP discussion in multivariate analysis (mixed model).

Variable name	Level	OR (IC 95%)	*p*
Patient characteristics			
Age (year)	[0 -30)	1.00	
	[30 -35)	1.24 (0.77-1.98)	0.375
	[35 -40)	0.38 (0.24 – 0.60)	**p<0.001**
	40+	0.05 (0.03 – 0.09)	**p<0.001**
Number of children	0	1.00	
	0 - 1	0.39 (0.27 – 0.54)	**p<0.001**
	More than 1	0.17 (0.12 – 0.23)	**p<0.001**
Tumor characteristics			
SBR grade	Grade I	1.00	
	Grade II	0.78 (0.39 – 1.59)	0.498
	Grade III	0.76 (0.38 – 1.53)	0.449
Neoadjuvant chemotherapy	No	1.00	
	Yes	1.15 (0.88 – 1.50)	0.298
Physician characteristics			
Sex	Female	1.00	
	Male	0.59 (0.35 – 0.99)	**0.048**
Age	Junior	1.00	
	Senior	0.77 (0.46 – 1.31)	0.336
Specialty	Surgeon	1.00	
	Radiotherapy oncologist	0.22 (0.07 – 0.64)	**0.006**
	Oncologist	0.78 (0.46 – 1.32)	0.352

Values in bold are the significant values.

time at which FP had yet to emerge as a major issue. In addition, since 2011, vitrification can be performed in France, which improves the results of embryo and especially oocyte freezing. The improvement in practices over time indicates an increase in the awareness of healthcare practitioners. A plateau was nevertheless reached in 2015, and the proportion of patients for whom FP was discussed never exceeded 50%. This result is consistent with previous studies (10) indicating a significant, but nevertheless incomplete, improvement in practices. Another possible reason is that the Institut Curie is a specialist cancer center focusing purely on oncology care. Thus, unlike multispecialty clinics, it does not have its own gynecology or reproductive biology department.

Our findings confirm that several patient-related factors are associated with the likelihood of FP discussion, as summarized in **Supplementary Table 4** (BC) and **Supplementary Table 5** (all cancer types). Earlier age at diagnosis was significantly associated with a greater likelihood of FP discussion (8, 9). The frequency of FP discussion was 35% in women aged 35 years or older, falling to 7% in women over the age of 40 years. The mixed model of our study confirms the impact of age on FP discussion. Age at diagnosis is a well-known, important factor associated with FP discussion, and this association has been found to be significant in most studies. This finding is nevertheless a matter of concern, because the proportion of women diagnosed with BC increases steadily with age, and most "young" BC patients are already at least 37 years old at BC diagnosis. There are currently no guidelines specifying that such discussion is dispensable for women over the age of 37 years. For the use of vitrified oocytes, French guidelines consider that it is imperative to take into account obstetrical morbidity, which increase with age (after 45 years, pregnancy is at high risk of complications and even more after the age of 50 years) (21). Not all patients will be eligible for FP, but it is essential to have a discussion with them about their options and about post-cancer infertility.

Consistent with another study (20), we found that nulliparity was significantly associated with FP discussion. Such discussion took place for only 17% for patients who already had more than one child at diagnosis. Thewes et al. reported that about 70% of 228 BC patients under the age of 45 years wished to have a child after their treatment was completed (8). Marklund et al. (22), analyzed a cohort of 1275 BC patients and found that 171 patients (33%) had a live birth after the end of treatment, and that 63% of these patients already had at least one child at diagnosis.

In our study, no factor related to BC disease (clinical T stage, lymph node status, SBR grade, BC subtype, histological type) was found to be significantly associated with the likelihood of FP discussion. Conflicting results have been reported (8), but several studies (10) have suggested that early-stage disease is more frequently associated with FP discussion. We did not include bilateral breast cancer which makes more complex statistical analyses as it requires the use of multilevel models, and it causes difficulties in attributing relapse to one or to the other side. Furthermore, it is very unlikely that the results are biased because synchronous bilateral breast cancers represent 1-3% (23). In terms of treatment, FP was more frequently discussed in the group of patients receiving neoadjuvant chemotherapy than in patients receiving adjuvant chemotherapy, but this was highly probably due to age acting as a confounding factor, because it was very significantly associated with the chemotherapy setting. We did not include patients who did not receive chemotherapy, but we must highlight that the patients who did not receive chemotherapy represent a very minority in this age group (15 to 20%). We found no impact of type of chemotherapy, hormone therapy, or radiation therapy, consistent with the findings of other studies (9, 24). However, FP is an important subject in this context, because hormone therapy can delay pregnancy plans by at least two to three years.

Several practitioner-related factors were associated with the likelihood of FP discussion, including specialty in particular. Surgeons were the most likely to discuss FP with their patients, followed by medical oncologists and then radiotherapists. We also identified the sex and age of the medical practitioner as significantly associated with the likelihood of FP discussion. Korkidakis et al. (20) analyzed a cohort of 4,452 breast cancer patients aged 15-39 years before chemotherapy treatment and obtained similar results, with female physicians and surgeons the most likely to discuss FP with their patients. Covelli et al. (25) investigated the barriers to physicians discussing fertility and found that physicians often assigned responsibility for fertility counselling to other clinicians and felt a lack of confidence in their ability to initiate FP discussion. Patel et al. (26) found that multi-specialty clinics had lower rates of FP counseling concerning fertility risk than single-specialty clinics.

TABLE 4 | Performance of fertility preservation procedures (FPPs) as a function of patient with FP discussion characteristics (n = 447).

Variable name	Level *n*	Overall 447	FP Procedure 259 (58%)	No FP Procedure 188 (42%)	*p*
Age (year)	[0 -30)	72	63 (88%)	9 (12%)	**<0.001**
	[30 -35)	173	118 (68%)	55 (32%)	
	[35 -40)	162	75 (46%)	87 (54%)	
	40+	40	3 (8%)	37 (92%)	
Age (mean)		34.2 (4.1)	32.7 (3.7)	36.3 (3.7)	**<0.001**
Number of children	0	231	176 (76%)	55 (24%)	**<0.001**
	1	99	51 (52%)	48 (48%)	
	More than 1	117	32 (27%)	85 (73%)	
BMI	<18.5	29	15 (52%)	14 (48%)	0.543
	18.5-24.9	302	181 (60%)	121 (40%)	
	25-29.9	88	46 (52%)	42 (48%)	
	>=30	20	12 (60%)	8 (40%)	
BMI (mean)		22.3 [20.3, 24.9]	22.0 [20.3, 24.5]	22.6 [20.4, 25.1]	0.327
Treatment center	Curie Paris	287	167 (58%)	120 (42%)	0.967
	Curie St Cloud	160	92 (57%)	68 (42%)	
Year of BC diagnosis	2011	23	10 (43%)	13 (57%)	0.063
	2012	31	22 (71%)	9 (29%)	
	2013	51	36 (71%)	15 (29%)	
	2014	69	33 (48%)	36 (52%)	
	2015	109	62 (57%)	47 (43%)	
	2016	96	60 (62%)	36 (38%)	
	2017	68	36 (53%)	32 (47%)	
Hereditary predisposition	No	240	156 (65%)	84 (35%)	1.000
	Yes	70	46 (66%)	24 (34%)	
Clinical tumor size (mm)		31.5 (20.3)	32.2 (20.2%)	30.6 (20.4)	0.428
Clinical T stage (TNM)	T0-T1	178	94 (53%)	84 (47%)	0.168
	T2	217	135 (62%)	82 (38%)	
	T3-T4	51	29 (57%)	22 (43%)	
Clinical N stage (TNM)	N0	281	163 (58%)	118 (42%)	1.000
	N1-N2-N3	165	95 (58%)	70 (42%)	
SBR grade	Grade I	16	11 (69%)	5 (31%)	0.212
	Grade II	150	94 (63%)	56 (37%)	
	Grade III	278	153 (55%)	125 (45%)	
BC subtype	Luminal	208	120 (58%)	88 (42%)	0.609
	TNBC	92	54 (59%)	38 (41%)	
	$HER2^+/HR^+$	75	48 (64%)	27 (36%)	
	$HER2^+/HR^-$	28	14 (50%)	14 (50%)	
Histological type	NST	426	246 (58%)	180 (42%)	0.591
	Lobular	8	6 (75%)	2 (25%)	
	Others	13	7 (54%)	6 (46%)	
Neoadjuvant chemotherapy	No	201	115 (57%)	86 (43%)	0.853
	Yes	246	144 (59%)	102 (41%)	

Values in bold are the significant values.

One possible reason for this difference may be a lack of clear designation of the doctor responsible for discussing the infertility risk associated with chemotherapy. Multicenter studies have identified regional disparities in information about FP, and differences between oncology centers (10), but we found no significant differences between the cancer centers in our study.

Finally, we confirm the crucial importance of FP discussion for favoring the performance of FPPs. Only three of the 262 patients who underwent PF procedures had not previously discussed FP with their doctors. Our data therefore indicate that a lack of discussion about FP during in-house consultations severely impedes patient choice as to whether to undergo FPPs. However, almost one third (188/447) of the patients who received information about FP chose not to undergo FPPs, or were not eligible for the procedures. We found the same factors associated with the FP procedure in the group of patients who had a FP discussion: age and parity. Previous studies analyzing annual income or health insurance as possible factors influencing discussion about FP found no association with these factors (9), which can be ruled out in our study because all the patients were covered by a universal social security system guaranteeing the full reimbursement of FP fees, up to 43 years.

Our study has several strengths, in particular, the inclusion of a large number of patients and doctors, allowing an analysis of a multitude of variables. However, it also has limitations, such as its retrospective nature, in particular. Information on the FP discussion was retrospectively obtained from the patient's electronic health record. Since some doctors may not record their discussion with patients about FP in the electronic health record system, results from this study may underestimate the rates of FP discussion. The healthcare providers play an important role in the discussion of fertility preservation. However, less than 50% of the patients had FP

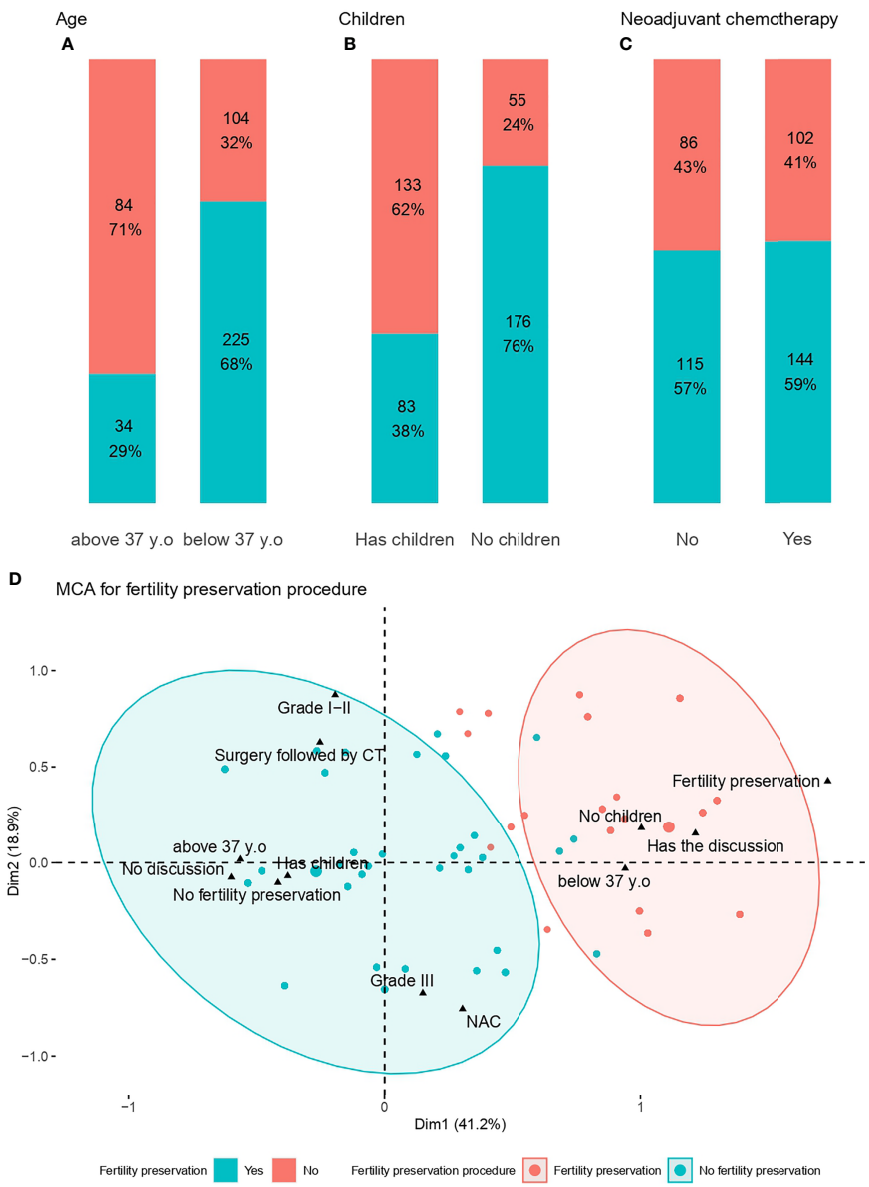

FIGURE 3 | Factors associated with fertility preservation procedures. **(A)** Age at diagnosis; **(B)** Children; **(C)** Neoadjuvant chemotherapy; **(D)** MCA for fertility preservation procedures*. *The red ellipse represents the concentration of patients who did not undergo fertility preservation procedures, whereas the blue ellipse represents the concentration of patients who underwent fertility preservation procedures.

with their doctors and only 19% of the patients had FP procedures. More characteristics of the healthcare providers are recommended to be analyzed and discussed, such as their knowledge about FP procedures, or how much time spent for each communication on FP with patients would be of major interest to further understand determinants associated with physician's related barriers and facilitators.

This work has several clinical implications and identifies areas in which there is room for improvement. It highlights a patient population with unmet needs regarding information on FP (patients in their late 30s who already have children). It also calls for better training for healthcare providers to raise awareness on this topic, particularly among male doctors, through seminars (27), joint training with reproductive medicine experts (28), or the development of FP networks (29).

Prestructured fields in the EHR may be pertinent tools for preventing omissions and could provide an alert in real time, prompting such discussion. Alerts of this type have already proved effective for preventing drug interactions and are currently used in this context (30, 31). A similar reminder could be issued for all women of childbearing age receiving gonadotoxic treatment, to improve oncofertility practices in

cancer care. To clear the delineation of who is responsible for discussing the infertility risk associated with chemotherapy, the discussion could be done at the first consultation, which would facilitate a better systematization of the information. Finally, providing patients with information directly, *via* posters or flyers in waiting rooms, patient advocacy, and communities could help to increase the proportion of patients who are informed and empowered, and able to decide independently whether or not they wish to undergo FPPs if it is possible, before receiving gonadotoxic treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization, AH, AT, FR, and A-SH. Methodology, FC and A-SH. Software, JB, A-SH. Validation, AH, AT, VC, CaS, A-SH, and FR. Formal analysis, A-SH. Investigation, AH, AT, VC, J-YP, and J-GF. Data curation, AH, AT, CaS, and A-SH. Writing—original draft preparation, AH and A-SH. Writing—review and editing, AH, AT, CaS, EL, BG, AT, FC, EDu, EDa, J-YP, J-GF, FR, and A-SH. All authors contributed to the article and approved the submitted version.

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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.2021.701620/full#supplementary-material

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