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EDITED BY

Edward Van Der Horst,
Sensei Biotherapeutics, United States

REVIEWED BY

Jyothi S. Prabhu,
St. John's Research Institute, India
Adana A. M. Llanos,
Columbia University, United States

*CORRESPONDENCE

Kristina D. Rinker
✉ kdrinker@ucalgary.ca

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Status of breast cancer detection in young women and potential of liquid biopsy

Maya Stibbards-Lyle^{1,2}, Julia Malinovska^{1,2}, Selem Badawy^{1,2},
Pepper Schedin³ and Kristina D. Rinker^{1,2,4,5*}

¹Department of Biomedical Engineering, Schulich School of Engineering, University of Calgary, Calgary, AB, Canada, ²Cellular and Molecular Bioengineering Research Lab, University of Calgary, Calgary, AB, Canada, ³Knight Cancer Institute, Oregon Health and Science University, Portland, OR, United States, ⁴Arnie Charbonneau Cancer Institute, University of Calgary, Calgary, AB, Canada, ⁵Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada

Young onset breast cancer (YOBC) is an increasing demographic with unique biology, limited screening, and poor outcomes. Further, women with postpartum breast cancers (PPBCs), cancers occurring up to 10 years after childbirth, have worse outcomes than other young breast cancer patients matched for tumor stage and subtype. Early-stage detection of YOBC is critical for improving outcomes. However, most young women (under 45) do not meet current age guidelines for routine mammographic screening and are thus an underserved population. Other challenges to early detection in this population include reduced performance of standard of care mammography and reduced awareness. Women often face significant barriers in accessing health care during the postpartum period and disadvantaged communities face compounding barriers due to systemic health care inequities. Blood tests and liquid biopsies targeting early detection may provide an attractive option to help address these challenges. Test development in this area includes understanding of the unique biology involved in YOBC and in particular PPBCs that tend to be more aggressive and deadly. In this review, we will present the status of breast cancer screening and detection in young women, provide a summary of some unique biological features of YOBC, and discuss the potential for blood tests and liquid biopsy platforms to address current shortcomings in timely, equitable detection.

KEYWORDS

breast cancer, young women, postpartum, involution, liquid biopsy, molecular diagnostics, early detection

Introduction

New evidence suggests incidence rates are increasing for breast cancer in young patients (aged 25–39 years) and outcomes are worse compared to older patients (1). Early-stage detection is a strong determinant of survival and quality of life, and the earlier a cancer is diagnosed, the lower the overall cancer-associated health care costs (2, 3). However, breast cancer screening has been limited to women over 50 or those at elevated risk of developing breast cancer. This review will present an overview of young onset breast cancer (YOBC), with a focus on postpartum breast cancer, current gaps in screening and diagnosis, and technologies relevant to early detection, including blood tests and liquid biopsy.

YOBC has typically been associated with poor outcomes due to lack of early diagnoses, poor clinicopathological features, and dense breast tissue affecting mammography sensitivity (1, 4–15). More specifically, this includes a higher proportion of aggressive cancer like triple negative breast cancer (TNBC), high risk of local recurrence, metastasis and lymph node involvement and larger tumor size (5, 6, 8, 10). Equitable care for breast cancer patients requires a deeper understanding of the specific mechanisms associated with breast cancer in young patients, specifically during the postpartum period, which will allow for development of improved diagnostic options for these groups. This review will examine some barriers postpartum women face when accessing breast cancer screening and early diagnosis, as well as the potential role for liquid biopsy in expanding access.

Young onset breast cancer: clinical and biological aspects

Although breast cancer is typically viewed as a disease of older women, women under 45 account for a considerable portion of overall breast cancer patients, ranging from 5% to 25%, with estimates varying based on study, country, and ethnicity (1, 16–26). The incidence and mortality rates for all early-onset cancers (women under 45) have increased over the past decade, with breast cancer leading the way (20). Despite improving outcomes in older patients through screening and better therapies, these advances have not improved outcomes in young patients. While there were improvements for young patients through the late 20th century, these trends have recently slowed (27) or even reversed (28). In addition, developing breast cancer under the age of 45 doubles the risk for metastasis and mortality, as compared to patients older than 45 (1, 29). This age-based discrepancy in outcome suggests that there is an unmet need in the care of patients under 45 with breast cancer.

Defining young-onset breast cancer (YOBC) necessitates considering the significant differences in hormone shifts with the start of menarche, pregnancy, postpartum and involution as well as perimenopause, menopause, and post-menopause (27, 30, 31). Women begin the shift from a reproductive to non-reproductive state in their mid- to late 40s, with an overall mean age of menopause at 49.9 (32, 33). Moreover, factors like hysterectomy

(20% of women undergo by age 55), menopause hormone therapy (~20–35% in peri- and postmenopausal individuals) and use of oral contraceptive (62% of reproductive age women use worldwide) add layers of complexity to understanding the role of hormones (both natural and pharmaceutical), on breast cancer development and its detection (34–38). To encompass the most studies available, this review classifies YOBC as women under the age of 45 (37, 39).

YOBC often exhibits aggressive tumor biology and late-stage diagnosis, correlating with poor patient outcomes. Breast cancer in young women presents with higher prevalence of hormone receptor negative, triple negative and HER2+ tumors (29, 40), elevating risk of recurrence and metastases (41). Moreover, characteristics such as larger tumor diameter (>20mm), increased proliferation/Ki-67 expression, lymphovascular invasion and lymph node involvement are common and correlate to increased mortality (10, 42–44). In addition, factors relevant to young women, such as older age at first birth and not breastfeeding may contribute to increased risk of certain aggressive subtypes of YOBC, such as estrogen receptor negative (ER-) (45). These dynamics may be further amplified based on racial and ethnic predisposition. There is also increasing evidence that certain racial/ethnic groups, such as Black women, are at high risk of TNBC and represent a disproportionate number of cases diagnosed in young patients (15). In particular, young Black women (<50 years old) have a higher breast cancer incidence than young white women, a trend which reverses around menopause (46). Furthermore, Black women are more than twice as likely, and Hispanic women 1.2 times as likely, to be diagnosed with metastatic disease than white women in the US (47). Of the TNBCs diagnosed in young patients, cancers are often of a higher grade, are diagnosed at stage III or later, and have elevated Ki-67 as compared to their older counterparts. Young women diagnosed with Stage I/II cancer exhibit worse prognosis and higher mortality rates compared to their older counterparts, regardless of subtype (48). This may be further exacerbated by social and structural barriers, such as limited access to healthcare, which patients face in accessing a timely diagnosis, as reviewed extensively elsewhere (49–51). Globally, the average risk of dying from breast cancer before 40 years old is similar across continents except for Africa, which has more than double the risk (52). Approximately half of all young patients harbor a germline mutation in BRCA1, BRCA2 or TP53 that increases the risk of developing breast cancer (53, 54). As in older women, most breast cancers are invasive ductal carcinoma (IDC) as compared to invasive lobular carcinoma (ILC) (41). Epigenetic factors are also relevant, appearing to contribute to breast cancer risk in a manner dependent on ethnic and epidemiological factors as reviewed elsewhere (55, 56).

Postpartum breast cancer

Within YOBC, cases can be subdivided into broad categories. Breast cancer occurring in never-pregnant (nulliparous) patients and cases diagnosed during pregnancy, known as pregnancy-related breast cancer (PrBC), are associated with similar outcomes (57). In contrast, breast cancers diagnosed within 5, and up to 10 years postpartum have increased metastasis and mortality compared to

diagnoses in nulliparous and PrBC patients (12, 44, 58–61). Women with PPBC are a unique and vulnerable population and like YOBC, defining a specific age range for this group poses challenges. Typically, ≤ 45 years has been used as a benchmark, but it is crucial to recognize that this may shift due to increasing age at first childbirth (62, 63). Notably, first pregnancy after 35 years old (classified as geriatric pregnancy or advanced maternal age) is considered a risk factor for breast cancer, with 50% increased risk compared to pregnancy at 20 years old (64, 65). It is stipulated that this is due to older women already possessing cancer-causing mutations or abnormal cells by the time of pregnancy and involution, thereby not benefiting from protective effects seen in younger pregnancies, and instead contributing to metastasis (64). The mechanism of the protective effect seen in pregnancies under 35 years old, remains unknown but is hypothesized to involve changes in hormone levels and the mechanical forces in the mammary gland (66–68). The poor outcomes of PPBC patients as compared to nulliparous and pregnant patients suggest that there are unique processes occurring in the breast after childbirth requiring further investigation.

The mammary gland is a unique and dynamic organ, as it largely develops postnatally and only reaches a mature state with lactation (69). During the time of puberty, the mammary gland undergoes cyclic proliferation, differentiation and death corresponding to hormone changes of the menstrual cycle (70, 71). Significant tissue expansion occurs with pregnancy and lactation, followed by regression at weaning (72). The cessation of lactation begins the process of involution, a remodeling of the mammary gland back to pre-pregnancy state. However, the immune signature developed during involution has been shown to persist up to 10 years post-childbirth (12, 73). The plasticity of the mammary gland is a key factor to influencing its vulnerability to the carcinogenesis process and has been reviewed elsewhere (74–76).

Breastfeeding has shown potential in reducing the risk of some breast cancer subtypes, but the mechanism of protection remains under-investigated (77, 78). One meta-analysis has shown breastfeeding associated with 10% risk reduction in estrogen receptor (ER) negative and progesterone receptor (PR) negative breast cancer, and a 20% risk reduction in TNBC (78–80). The risk of ER+ cancers also appears to be decreased in women who breastfed (80, 81). Additionally, the effectiveness of lactation may be reduced by the decline of breastfeeding duration and breastfeeding overall, with only 35.6% of females exclusively breastfeeding for at least 6 months in Canada as per WHO and Health Canada recommendations (82). Although a temporary increase in breast cancer risk follows childbirth (13, 65), it is succeeded by a long-term protective effect and reduced risk. However as discussed, this positive impact diminishes with a later age at first childbirth, posing a unique and significant challenge as the trend toward delayed pregnancies and increased child-bearing age continues to increase (13, 83). As women delay pregnancy and the age of first childbirth increases, with a historically high average age of first childbirth of 27.3 years old in the US in 2021, 29.4 years old in Europe in 2019 (84), 29.7 in Asia in 2003 (85), the incidence of PPBC is expected to rise, leading to a subsequent rise in

morbidity rates (65, 86). These trends necessitate an increased focus on early diagnosis for patients with PPBC.

Current detection technologies and barriers to detection

The primary obstacle to detecting YOBC and PPBC is the lack of diagnostic technology and regular screening procedures with demonstrated efficacy in this population. Commonly used metrics for evaluating performance of breast cancer screening/diagnostic tests, such as mammography and MRI, include clinical sensitivity and specificity. Clinical sensitivity is the ability of the test to accurately detect cancer when present; a low sensitivity indicates a higher rate of missed cancers. Clinical specificity is the ability to correctly determine a patient as disease-free when cancer is not present; low specificity can lead to unnecessary downstream procedures and patient anxiety. These parameters are determined in clinical studies using a “gold standard” or longitudinal follow-up of participants to capture the true positives and true negatives. Comparing the performance of different technologies remains difficult. For review see Hollingsworth (2019) (87). Further, the population recruited in a study may have different characteristics including varying breast density, racial and ethnic predisposition, and more, that could affect performance. This produces performance metrics that may be higher or lower than other published studies, depending on the benchmark and population used. The gold standard for breast screening sensitivity is MRI. When compared to MRI, mammography sensitivity is lower ($\leq 40\%$) (88, 89) due to MRI’s lower limit of detection (ability to find smaller tumors) and effectiveness of contrast agent in enhancing visualization of breast cancer, including lobular breast cancers. The difficulty in comparing technologies is a barrier in development and clinical implementation of effective screening and/or diagnostic tools for YOBC.

Mammography is the standard for breast cancer screening in many countries but has limited clinical utility in YOBC. Specifically, the sensitivity and specificity of mammography is reduced in young women due to increased prevalence of high breast density (15). Figure 1 compares the sensitivity of mammography to the breast density of women under and over 45. Elevated breast density [heterogeneously dense (C) or very dense (D)], decreases the sensitivity and specificity of mammography screening (92), resulting in detection when tumors are larger (94–96). Breast density is elevated in over 50% of women, and this is associated with a 2 to 5 times greater risk of breast cancer (90, 91, 97–100). Furthermore, Black women have the highest breast densities across all age groups (101, 102). Breast ultrasonography is often employed in young women with dense breast tissue; however, lower specificity and increased rates of operator error have hindered wider deployment as a stand-alone first screen (103, 104). MRI is used for screening in young women with elevated risk of breast cancer, however the need for contrast agent, cost, and access challenges limit participation and availability. Together, this evidence points to young patients being the most at risk due to high breast density but having limited screening options.

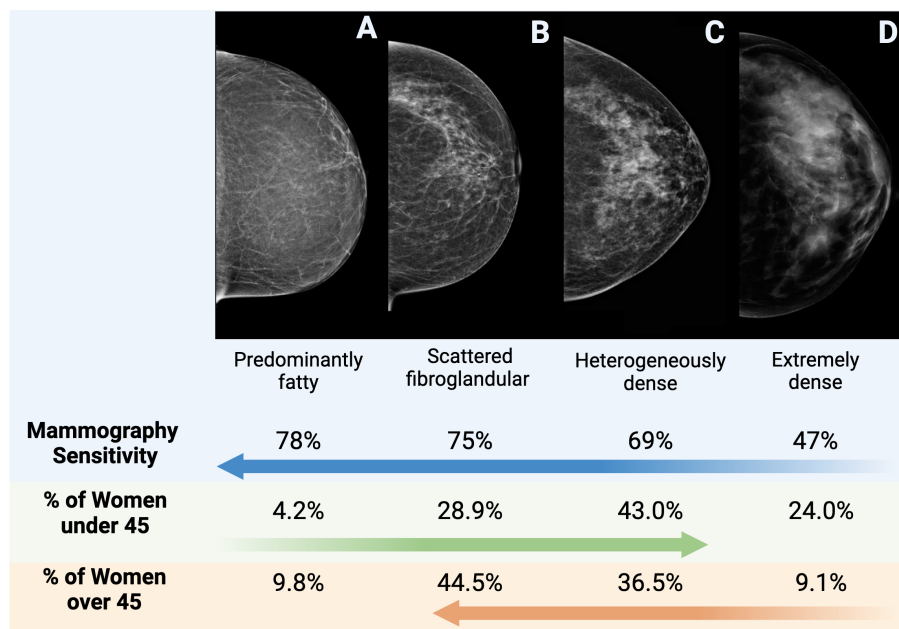


FIGURE 1
Comparison of BI-RADS classification to mammography sensitivity and percent of women under/over 45. The Breast Imaging Reporting and Data System (BI-RADS) uses mammographic density for classification of breasts based on percent of fibroglandular tissue. The four categories are (A) predominantly fatty ($\leq 25\%$), (B) scattered fibroglandular (26–50%), (C) heterogeneously dense (51–75%), and (D) extremely dense (76–100%). The distribution of mammographic density was adapted from Checka et al. (90) and sensitivity from Lynge et al. (91). The sensitivity of mammography is decreased with dense tissue which is predominantly found in young women (92). The density of breast tissue decreases with age making mammography a suitable option for older women but presents a gap in screening of young women. Mammographic images originally open access published by Pawlak et al. (2023) (93).

US breast cancer screening guidelines reflect the higher performance of mammography and higher incidence of breast cancer in older women, with a gap in the screening and diagnosis of YOBC. A summary of the guidelines and statistics can be found in Table 1. Overall, breast cancer guidelines remain mostly consistent among 21 high-income countries (including the US, UK, Canada, etc.), with most countries recommending screening every 2 years between 50 to 69 years old for women of average risk (112). Women 40 to 49 may participate in screening based on individualized needs and a physician recommendation (111). Women with elevated risk

may begin screening at earlier ages, typically using MRI. MRI has high screening sensitivity but is not viable for broader implementation due to high equipment and personnel costs, low availability, and lower specificity rates resulting in higher rates of follow-up procedures (113). The National Comprehensive Cancer Network (NCCN) guidelines recommend women begin MRI screening at age 25 to 40, depending on family history (first-degree relative with breast cancer) and genetic predisposition (BRCA1/2, p53 or pTEN mutations) (114). Similarly, the Canadian Task Force on Preventive Health Care recommends for women 40–49 years old

TABLE 1 Comparison of groups at risk for young onset breast cancer.

Group	PrBC	PPBC	Black Women	Women under 45
Prevalence	<ul style="list-style-type: none"> • 0.04% of pregnancies (105) • 0.2–3.8% of all newly diagnosed breast cancer (106) 	<ul style="list-style-type: none"> • 50% of breast cancers arising within 10 years of last childbirth (107) 	<ul style="list-style-type: none"> • Higher incidence rates of women under 40 compared to White women (42, 108) 	<ul style="list-style-type: none"> • 5–25% (1, 16–26)
5-year OS	<ul style="list-style-type: none"> • Increased risk of death, pooled hazard ratio of 1.45 (95% CI 1.30–1.63) (58). 	<ul style="list-style-type: none"> • 77.5% (44) 	<ul style="list-style-type: none"> • 75% (109) 	<ul style="list-style-type: none"> • 72–84% (25)
Stage at diagnosis	<ul style="list-style-type: none"> • Advanced stages 	<ul style="list-style-type: none"> • Advanced stages • 2-fold increase in metastasis (107, 110) 	<ul style="list-style-type: none"> • Advanced stages • High risk of TNBC (15) 	<ul style="list-style-type: none"> • Advanced stages • Excess risk at early stages (8)
Current US guidelines	<ul style="list-style-type: none"> • Mammography only if an underlying malignancy is suspected or has been proven by tissue biopsy 	<ul style="list-style-type: none"> • No specific guidelines 	<ul style="list-style-type: none"> • No specific guidelines 	<ul style="list-style-type: none"> • Biennial mammography for women >40 (111) • MRI screening in women with elevated risk

PrBC, pregnancy related breast cancer; PPBC, postpartum breast cancer; OS, overall survival; TNBC, triple negative breast cancer.

to not screen with mammography and only undergo screening based on the “relative value a woman places on the possible benefits and harms from screening” (115). There are currently no recommendations for screening of women with dense breasts, leaving clinical diagnosis to occur following self-detection, indicating advanced disease progression, and contributing to unfavorable outcomes due to delayed intervention. Current guidelines do not address broad groups of individuals at risk of YOBC, including those with high breast density, recent childbirth, or racial/ethnic genetic predisposition.

Globally, there are population disparities for breast cancer incidence, diagnosis, access to new technologies, and consequent outcomes. Lebanon, for example, has the highest incidence of breast cancer in the Middle East, with diagnosis occurring at a younger age than its Western counterparts (52 years compared to 63 years respectively), as well as more aggressive and fatal outcomes (116). Access to and participation in screening technologies is a contributing factor to late detection in some geographical regions. Approximately 80% of deaths from breast cancer occur in low to middle income countries according to the World Health Organization (WHO), prompting the formation of the Global Breast Cancer Initiative Framework (117). Notably, among young women, regions across the world with comparable incidence rates have very different mortality rates, which is not the case for older women, where greatly different incidence rates have comparable mortality rates (52). There could be many reasons for this including differences in the availability of screening, healthcare and treatments (118). Current literature is limited regarding characterization, screening and treatment of YOBC, and the role of race and ethnicity. This is compounded by studies focusing on a specific demographic or being too broad as well as adopting varying definitions of YOBC and PPBC, making it difficult to compare patient outcomes (6, 8, 10, 26, 58, 119–121). More diversified studies using a population under 45 with information on parity status, breast density and ethnicity would highly benefit research within this area.

There are many barriers to implementation of screening in young women including physical access, procedural costs, and post-diagnosis care expenses. The deterrents for not seeking postpartum/postnatal care and breast cancer screening overlap, and depending on location, commonly include public transportation access, distance to facility, travel time, lack of trained professionals and lack of awareness (122–124). In higher income countries, immigrant and refugee women, in particular, face significant hurdles in access resulting in increased risk of mortality and morbidity related to pregnancy as compared to the rest of the population (125, 126). Furthermore, women of all racial/ethnic backgrounds living in rural areas have higher breast cancer mortality than women living in urban areas, illustrating the critical impact of accessibility on care and screening (47). Racial disparities in access also persist with Black women having three times the maternal mortality of White women in the United States (127). While it is difficult for early diagnosis and screening to eliminate these barriers, emphasis on community resources and education have been identified as an effective approach (128). Exploring the incorporation of early breast cancer screening alongside postnatal

and postpartum care, which is widely implemented in many countries, could potentially enhance accessibility.

Early detection and liquid biopsy

Blood tests or liquid biopsies offer the potential to address many of the gaps in early detection of breast cancer for young women such as increased accessibility, higher participation, and complementarity to imaging. Liquid biopsy involves the use of a body fluid (such as blood, breast milk, nipple aspirate fluid or urine) for identifying the molecular characteristics of the disease. Typically, liquid biopsy tests have been used for treatment selection and risk monitoring of recurrence, but emerging multi-biomarker blood-based tests are focusing on early detection (129–137). Key criteria to consider in the implementation of liquid biopsy tests for early detection of breast cancer include analytical and clinical performance metrics in targeted patient populations, accessibility, and scalability. Detection technologies used in first pass screening typically have high diagnostic specificity with the intention of limiting the number of false positives and unnecessary downstream procedures. However, diagnostic sensitivity is also important due to the inevitable risk of false negatives. In most cases, stringent specimen collection and handling requirements have a significant effect on sample stability, integrity, and overall performance of the test. Molecular processing of samples in the laboratory is usually performed by certified professional laboratory users or automated solutions, with an effect on the cost and ability to scale operations.

Early liquid biopsy tests encompassed analysis of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) or other genetic material such as micro-RNAs from plasma for use in prognosis and treatment selection [for review see (129, 130, 132, 136, 137)]. Some tests have obtained FDA clearance including the CellSearch test by Veracyte that is indicated for cancer prognosis and the Guardant360DX test for treatment selection. The FoundationOne Liquid CDx (Foundation Medicine) test is an FDA-approved test indicated for breast cancer gene profiling for treatment selection (138, 139). As the CellSearch test is based on the expression of epithelial cell adhesion molecule (EpCAM) on CTCs and with EpCAM demonstrated to be downregulated in most aggressive breast cancer cells undergoing epithelial-to-mesenchymal transition (EMT), the ability of the test to accurately detect disease even in advanced stages might be limited (140, 141). First generation screening tests have shown limited sensitivity for breast cancer, particularly in the early stages, given the limited ctDNA and CTC that is shed from the breast tumor among other technical limitations (142–144). As an example, the ctDNA based multicancer early detection (MCED) screening blood test Galleri (Grail) showed limited detection of early breast cancer as part of breast screening. However the test identified cases of recurrence (145). TruCheck (Datar Genetics) is a blood test that measures 5 markers by immunofluorescence microscopy of CTCs isolated and expanded from a blood sample. Clinical performance results based on 141 participants (112 cancers, 29 no cancer) from women aged 18–81 revealed a specificity of 93.1% and a sensitivity of 94.64% (146). The Syantra DXTM Breast Cancer test (Syantra Inc.) is

based on qPCR and machine-learning based software analysis of a proprietary panel of 12 target mRNA markers from whole blood. Clinical performance for this test revealed an overall accuracy of 92% (specificity of 94% and sensitivity of 79%) in blinded, independent clinical studies on a test set of 695 women (aged 30–75) screening for breast cancer (147).

To date, a limited number of studies have examined liquid biopsy technologies for breast cancer detection in young women. Lourenco et al. (148) examined the utility of a proteomic biomarker assay (Videssa Breast) to rule out breast cancer in women with inconclusive or suspicious imaging findings. The study focused on women under 50 with high breast density (BI-RADS 3 or 4), and reported sensitivity of 87.5% and specificity of 83.8% in a cohort of 545 women (148). The Syantra DXTM Breast Cancer test also evaluated clinical performance in women under 50, for which enhanced performance was reported with clinical sensitivity and specificity rates of 91.7% and 99.0%, respectively (147). Further clinical studies in this area will expand populations of women evaluated and evidence to support use in younger women.

Research-based approaches for breast cancer detection are demonstrating potential. Nipple aspirate fluid has been used for biomarker detection at the earliest stages of breast cancer, prior to a visible tumor mass (149). However, this strategy exhibits low yield, requires a local anesthetic, and cannot be collected from pregnant or lactating women. As a result, it is a promising option for the screening of young women in general, but not PrBC and PPBC. Jang et al. (150) demonstrated that miRNA multiplex analysis from plasma may be useful for diagnosing women under 50 with dense breasts. Most recently, Saura et al. (2023) demonstrated that breast milk contains ctDNA and surpasses the yield found in plasma (151). Interestingly, the samples with the highest ctDNA concentration demonstrated a loss-of-function variant of E-cadherin, consistent with decreased tumor cell junction tightness due to mutations in this gene. They also presented two cases in which breast cancer was detected via breast milk prior to diagnosis by imaging. Testing of breast milk did not produce any false positive results, though the numbers were small – limiting statistical analysis (n<30). These studies indicate that screening technologies for breast cancer in young women is an emerging field with great promise.

Artificial intelligence (AI) is expanding in use in existing imaging modalities and emerging detection tests (152). Published in 2023, an AI model “Sybil” was developed to analyze low-dose CT scans and predict future lung cancer risk, achieving a success rate over 86% (153). This capability to predict an individual’s future cancer risk from a single scan supports personalized screening and monitoring. Improved and automated image analysis is a primary application of AI (154–156). Radiologists assisted with AI have improved sensitivity and specificity in making clinical decisions than either approach alone (157). Despite the progress in this area, challenges in cancer detection and clinical adoption persist, including model bias, data security, data size limitations and variable methodology standards, as discussed elsewhere (158). Additionally, AI assisted image analysis remains constrained to the sensitivity and specificity of current imaging modalities and has limited enhancement ability. Liquid biopsy tests often incorporate machine learning and advanced data analytics for performance

enhancement. Advances in AI and data analytic technology hold promise for enhancing detection and management of breast cancer.

Biomarkers for screening/diagnosis of postpartum breast cancer

Previous research has identified PPBC as a unique population of breast cancer patients based on molecular phenotype and has suggested that this distinct phenotype may persist for up to 10 years (12, 59). As a result, biomarker identification and validation are important in this population. PPBC represents an opportunity to identify specific and sensitive biomarkers suitable for screening/diagnosis, based on processes solely associated with the postpartum period that are thought to promote breast cancer progression.

Involution may involve processes promoting tumor cell dissemination and upregulation of molecular markers associated with poor prognosis. In the initial phase of involution, there is an upregulation of acute immune response genes including STAT3 and interleukins (159–162). Leukocyte chemoattractants are also upregulated during this time, leading to the recruitment of large numbers of macrophages (163, 164). Mammary epithelial cells enter apoptosis and are further responsible for the continuation of this inflammatory, albeit immune regulatory phenotype. In the later phase of involution, there is an active T-cell presence, followed by T-cell exhaustion/suppression and resulting immune avoidance (59). Cyclooxygenase-2 (COX2), a well-known inflammatory mediator, has been shown to mediate persistent lymphangiogenesis up to 10 years postpartum, suggesting that the unique immune signature present during mammary gland involution persists long after involution has concluded (12, 73). The increased lymphatic density observed during mammary gland involution is consistent with increased rates of lymph-node metastasis in PPBC, as compared to nulliparous cases.

Dynamics of the extracellular matrix (ECM) are equally important to the pro-tumorigenic effects of involution (Figure 2). Culturing of tumor cells on ECM isolated from involuting rat mammary glands leads to disruption of cell-cell adhesion junctions and loss of apical-basal polarity, consequently enhancing the invasive capacity of breast cancer cells. Furthermore, there is an increase in collagen, tenascin C, and proteolysis of collagen, fibronectin, and laminin (165–168). Increased fibrillar collagen density and radial alignment of collagen are observed in invasive breast tumors and observed in physiologically normal breast involution and in PPBC (169–171). These ECM-based mechanisms inherently improve the motility and invasiveness of breast cancer cells, however, there are also well-documented interactions between ECM dynamics and the immune system, suggesting further downstream effects of the local mechanical involution environment (172, 173). Beyond ECM dynamics, there is a distinct role for direct mechanical forces in regulating involution. Some groups have hypothesized that milk accumulation induces a stretching force on cells lining breast lobules, triggering the release of STAT3 and subsequent initiation of involution (174). Furthermore, there are likely changing fluid dynamics between interstitial fluid flow and inflammation, and the

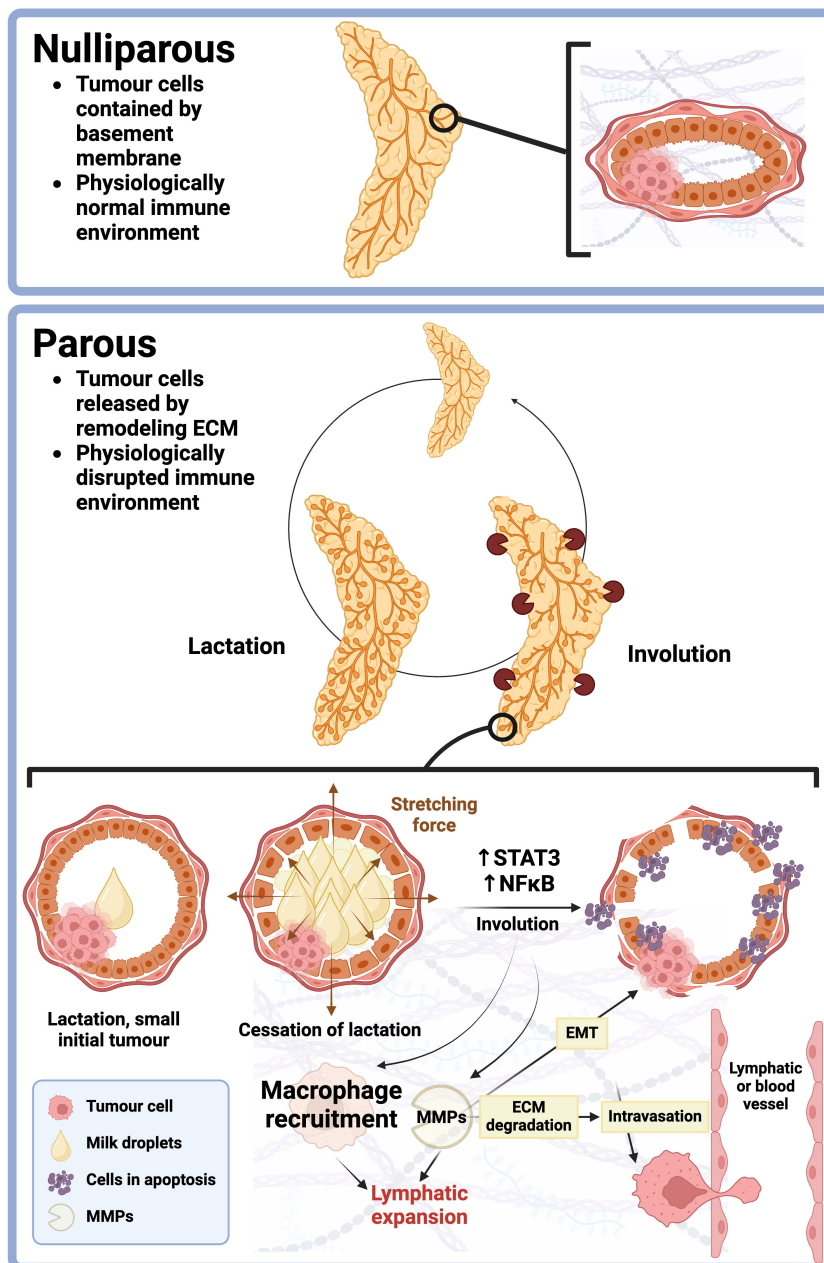


FIGURE 2
Visual representation of differences in the microenvironment of nulliparous Y0BC and PrBC or PPBC. The top panel represents full view and ductal view of normal, undifferentiated (nulliparous) mammary gland, prior to pregnancy. As indicated, the basement membrane and basic structure of the duct remains intact, compared to the parous state. In the bottom panel, a full view and ductal view of the parous mammary gland is presented. The mammary gland undergoes cyclical remodeling prior and post-childbirth. Involution is a remodeling process that occurs post-lactation. It involves process such as matrix metalloproteinase (MMP) activation, immune cell recruitment and dysregulation, lymphatic expansion, extracellular matrix (ECM) degradation, epithelial-to-mesenchymal transition (EMT) and other activities that allow breast cancer cells to escape the primary tumor more easily, migrate into circulation and establish secondary sites in other locations.

role of mechanical forces in regulating lymphatic expansion during involution. Consistently, many markers associated with the tumorigenic effects of mammary gland involution, such as fibronectin, Semaphorin 7A, matrix metalloproteinases, collagen I and more, have been shown to be flow-regulated by our group and others. Our group has previously demonstrated that fluid shear stress (FSS) upregulates S100 genes and fibronectin, and promotes epithelial-to-mesenchymal transition, motility, and adhesion of

breast cancer cells (175, 176). These studies further highlight the important interactions between fluid dynamics and the immune microenvironment during involution (175, 177–186).

Collectively, these data suggest a unique opportunity to identify biomarkers of PPBC by leveraging knowledge of mammary gland involution, focusing on a distinct inflammatory and wound healing signatures and the mechanical cell environment. Further research regarding the interactions between the mechanical and immune

microenvironments during mammary gland involution present an excellent opportunity to develop diagnostic biomarkers exclusively associated with and aimed at sensitive detection of PPBC.

Accessing liquid biopsy for women in the postpartum period

As discussed above, one barrier to breast cancer screening participation is difficulty in accessing transportation to centralized imaging facilities (187–191). Further, the lack of awareness of the prevalence and risks of PPBC results in a decreased likelihood of women receiving referrals and access to traditional diagnostic methods like more sensitive diagnostic imaging or tissue biopsy. While these concerns cannot be directly addressed without a larger transition to community-based medicine, the existence of an early detection liquid biopsy test would be compatible with integration into a community-based approach and existing postpartum care (Figure 3). During pregnancy and the postpartum period, many patients will have more exposure to the health care system through their OB/GYN provider. This presents an opportunity to integrate early steps in the preventative cancer care pathway. Routine postpartum care already involves blood-based testing, providing an efficient and streamlined approach for incorporating PPBC screening especially with blood collection being available in many locations. Milk samples are also often collected as a part of routine postpartum care, particularly in cases of mastitis etc. Furthermore, if a patient is diagnosed with PPBC, liquid biopsy presents an opportunity for treatment surveillance and minimal residual disease (MRD) assessment (192), which may be beneficial given the unique reproductive concerns related to PrBC and PPBC (1, 107, 193). Additionally, there may be opportunities for integration into annual

OB/GYN care for those who are not pregnant. Implementation of liquid biopsy tests in the context of breast cancer screening/diagnosis benefits from a sensitive imaging modality for downstream referrals in the event of a positive result. MRI is the gold-standard for sensitivity, however contrast-enhanced mammography and other imaging technologies are advancing. As we look to improve women’s health care, there are likely to be many combinations of technologies that may work to address the varied needs of women of different characteristics and life situations. Finally, limitations exist for disadvantaged communities in accessing all types of postpartum care, particularly evident in less developed countries and countries that operate without universal or public health care models, including the US (188). These systemic issues would need to be considered and addressed for liquid biopsy to fulfill its full potential in aiding the detection of YOBC across all communities.

Few studies have examined the feasibility of collecting liquid biopsy specimens in routine, first-line clinical settings. Pilot studies during the pandemic have shown mobile collection of blood based liquid biopsy to be beneficial and cost-effective in delivering cancer care to patients (194, 195). In the case of milk-based liquid biopsies, there is a lack of guidelines for adequate storage and handling, appropriate collection containers, temperature control, and other factors (196). It is likely that immediate freezing would be required to prevent degradation of RNA and miRNA relevant to molecular diagnostics. Though milk-based liquid biopsies may be an attractive alternative to blood-based liquid biopsies for analytic reasons discussed, the practical implementation requires further investigation. For blood-based liquid biopsies, the National Cancer Institute currently recommends maximum storage times from 4 hours for EDTA tubes and up to 3 days for preservative tubes at ambient conditions determined from manufacturer

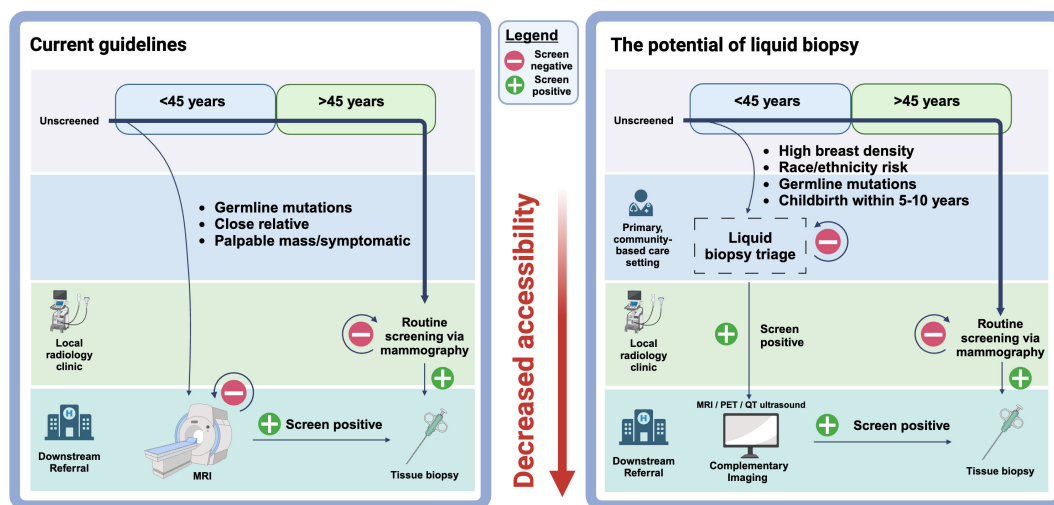


FIGURE 3
The potential of liquid biopsy to improve breast cancer screening accessibility through primary, community-based care. The current breast cancer screening guidelines do not have recommendations for several at-risk populations due to lack of diagnostic technology. Implementation of liquid biopsy early detection tests may allow for breast cancer screening to be accessed through a primary physician instead of a specialized clinic. This would address barriers of distance to facility, travel time and transportation. There is still a need for improvement of imaging modalities such as MRI, PET and QT ultrasound to be used complementary to liquid biopsy.

stability tests (197–199). Longer times are available for samples that can be stored and transported at -20C or -80C. CTC-based assays continue to be limited in this context, with very few suitable collection tubes on the market (200). Current limitations associated with collection tubes could impact remote collection, depending on location, and would require efficient delivery of samples to processing locations. Other options to mitigate these issues include Point-of-Care (lab-on-chip) solutions, which have been reviewed extensively elsewhere (201–203).

Clinician education is critical to the implementation of any new technology into clinical practice, however implementation of liquid biopsy in the primary care setting is further complicated by the number of primary care physicians and their high workload/wide breadth of practice (200, 204). As outlined, liquid biopsy for early cancer detection is an emerging field with different technologies becoming available. Interpreting results within the context of patient characteristics alongside available tests and imaging data adds an additional layer of complexity. Clinicians must not only understand the indicated populations for use of liquid biopsy tests, but also the performance metrics and limitations of standard of care imaging for screening and diagnosis. This comprehensive understanding is essential for effective use of available technologies and facilitating early-stage detection. Many clinical blood tests report the amount of a biomarker, while cancer detection tests usually report a positive or negative signal informing a recommendation for follow-up diagnostic imaging. Appropriate options for downstream referral will also be paramount in providing primary care physicians with options for their patients in the event of a positive result. While patients with PPBC represent a uniquely suitable cohort for liquid biopsy early detection tests, clinician awareness of screening technology limitations is critical to quality care. This is particularly true in situations where OB-GYNs and other primary care providers with less knowledge of oncology are responsible for administering these tests and communicating with patients. Knowledge dissemination to clinicians to support this new aspect of postpartum care will facilitate widespread adoption.

The economics of liquid biopsy for breast screening appear promising but will vary based on target population, test performance, test cost and current standard of care. Emerging early detection liquid biopsy tests will likely enter the market with small target populations and low coverage by payers, and expand populations and coverage with time as more clinical validation and usage data is obtained. Liquid biopsy may benefit from an economy of scale model, wherein centralized labs are able to conduct large numbers of tests from multiple regional facilities (200). This model may lead to decreased costs, however the benefit may not trickle down to individual patients, depending on the market. Preliminary data suggests that liquid biopsies can be a cost-effective option with faster turnaround time, compared to conventional diagnostics (205–207). These estimates, however, can vary greatly, and further research is needed in this area (208). An understanding of the health economics of liquid biopsy for YOBC is currently limited by several factors. As previously discussed, the comparison of screening technology performance parameters, such as sensitivity, is difficult due to inconsistent

“gold standards”. Additionally, when considering the population of women under 50, the availability and participation in screening are limited, resulting in inadequate performance data. Though the prevalence rates of breast cancer are lower in young women, liquid biopsy may be more cost effective as breast density is elevated in over 50% of young women, reducing mammography sensitivity, increasing risk of breast cancer, and delaying diagnosis (65–70). Liquid biopsy would likely improve early stage diagnosis thereby reducing disease burden which is increased with advanced stages (209). However, no studies have examined the cost-effectiveness of liquid biopsy in this specific population. Future studies to address this knowledge gap are imperative if we are to reverse the current trends of increased incidence of lethal breast cancer in young women worldwide.

Discussion/Conclusions

This review highlights the practical, clinical, and technological gaps that currently exist in early detection and screening of YOBC. Lack of detection options for young women, as well as their unique biology, make early-stage detection challenging. Challenges include lack of inclusion in screening recommendations, difficulties accessing screening, low awareness of breast cancer in women under 50 and screening technology limitations for this population. Emerging liquid biopsy tests may be able to address barriers in accessing screening through implementation of community-based (de-centralized) approaches in primary and integrated gynecological/postpartum care. Expanded clinical studies recruiting women under 50 for liquid biopsy early detection tests will support test approvals and reimbursement, increasing availability and awareness. Improved understanding of the mammary gland microenvironment during all stages of development may lead to identification of biomarkers particularly effective for this population for the next generation of tests. Further research investigating the cancer biology of YOBC, tests utilizing biological advancements, and clinical studies focused on YOBC and PPBC are needed to advance the available technologies and address the current gaps in care of these underserved populations.

Author contributions

MS-L: Writing – original draft, Writing – review & editing. JM: Writing – original draft, Writing – review & editing. SB: Writing – original draft, Writing – review & editing. PS: Writing – review & editing. KR: Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Supervision.

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Conflict of interest

Author KR is a co-founder, director, and shareholder of the company Syantra, Inc.

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