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Proliferative Markers in Breast Cancer and Chemotherapy Implications: A Comprehensive Review

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ABSTRACT

Background and Aims: Breast cancer is the most common cancer and a leading cause of cancer-related death among women globally. Determining which patients will benefit from chemotherapy remains challenging. Proliferative markers such as Ki-67, mini chromosome maintenance (MCM) proteins, and proliferating cell nuclear antigen (PCNA) offer valuable insights into tumor growth and treatment response. This review evaluates their clinical roles, with a focus on chemotherapy implications and emerging digital pathology techniques for marker quantification.

Methods: A narrative review was conducted by searching PubMed, Scopus, and Google Scholar for studies related to Ki-67, MCM, PCNA, breast cancer, and chemotherapy. Studies were thematically categorized into five areas. A bibliometric analysis of publications from 2000 to April 2023 was performed using the Bibliometrix R package and VOSviewer to assess research trends and thematic evolution.

Results: Eighty studies were included in the narrative synthesis. Ki-67 is the most commonly used marker, particularly useful in predicting response to neoadjuvant chemotherapy (NAC). MCM proteins show promise for identifying proliferative potential across tumor grades, while PCNA is associated with aggressive tumor features and poor prognosis. Post-chemotherapy changes in Ki-67 levels are linked to survival outcomes. Bibliometric analysis revealed a shift in research focus from basic mechanisms to clinical applications and digital quantification.

Conclusion: Proliferative markers play an essential role in breast cancer management. Ki-67 remains a key predictor of chemotherapy response, while MCM and PCNA offer complementary prognostic insights. Integration of these markers with digital pathology and AI-driven tools may enhance diagnostic accuracy and personalized treatment strategies. Standardization of assessment methods is crucial for broader clinical application.

1 | Introduction

Breast cancer is the most commonly diagnosed cancer worldwide, with an estimated of 2.26 million incident cases in 2020. It is also the leading cause of cancer mortality in women globally. Disparities in survival rates can be attributed to various factors, including delays in diagnosis and limited access to effective treatment [1]. While adjuvant chemotherapeutic and hormonal agents have improved mortality rates, identification of patients who will derive significant benefits from these treatments, as well as those at risk of experiencing treatment-related side effects, continues to pose a clinical challenge. To address this

issue, proliferative markers have emerged as a valuable tool in evaluation of breast cancer diagnosis, prognosis, therapeutic response, and disease surveillance during and after treatment. Therefore, a comprehensive discussion regarding the relevance of these markers in the management of breast cancer patients and the current recommendation for their clinical application can assist clinician in making informed decisions about the most suitable treatment approaches [2].

A biomarker is an objectively measurable characteristic that serves as an indicator of normal biologic function, pathogenic processes, or responses to therapeutic interventions. In the context of cancer, a biomarker refers to a substance or process that could indicate the presence of cancer in the body [3]. Biomarkers include molecules secreted by tumors or specific responses of the body to the presence of cancer. Among biomarker types, protein-based markers carry greater significance compared to their DNA- or RNA-based counterparts. This heightened importance arises from the central role that protein molecules play in the molecular pathways of both healthy and transformed cells, rendering proteomic markers more directly associated with the onset and advancement of diseases. It's worth noting that, as of now, the only FDA-approved biomarkers available for clinical applications fall within the category of protein molecules [3].

Table 1 summarizes the use of Ki-67, proliferating cell nuclear antigen (PCNA), and mini chromosome maintenance (MCM) proteins in assessing the growth fraction (proliferative rate) of a cell population. Although the precise function of Ki-67, a commonly used proliferation marker, remains uncertain, PCNA and MCM proteins have been recognized as key contributors to DNA replication. All three proteins are exclusively expressed during cell division in both normal and neoplastic cells. Given that these proliferative markers are present in various malignant tumors, their prognostic and predictive value has been assessed to determine their relevance in cancer diagnosis [9].

This study aims to review the application of each marker in breast cancer, with a particular emphasis on chemotherapy. Additionally, the latest methods of measuring Ki-67, PCNA, and MCM proteins in digital pathology will be reviewed to provide insight into the future of marker quantification.

2 | Materials and Methods

2.1 | Narrative Review Search Strategy and Selection

We conducted extensive searches across Scopus, PubMed, and Google Scholar to find relevant studies on January 28, 2023. The search strategy for each database involved querying for ("KI67" OR "MCM" OR "PCNA" OR "proliferative markers" OR "proliferation factors") AND ("breast cancer" OR "breast carcinoma") AND ("chemotherapy"). Two reviewers selected studies on chemotherapy and grouped them into themes, which were extracted from the selected articles. An update of the search was conducted on April 24, 2023. Supporting Information S1: Table S1 presents the full search query and

TABLE 1 | Summary of three indices for assessing cell proliferation including ki67, PCNA, and MCM.

Marker	Explanation	Function	Scoring and methodology
Ki67	The Ki67 antigen was originally identified by Gerdes and colleagues in the early 1980s, by use of a mouse monoclonal antibody against a nuclear antigen from a Hodgkin's lymphoma-derived cell line. This non-histone protein was named after the researchers' location, Ki for Kiel University, Germany, with the Ki67 label referring to the clone number on the 96-well plate [4].	Studies have identified the involvement of Ki67 in the early steps of polymerase I dependent rRNA synthesis; although it seems that the protein has an important function in cell division its exact role is still obscure and there is little published work on its overall function [5].	In daily practice, Ki67 is most often measured on paraffin sections by an immunohistochemical method, using the MIB-1 antibody [6].
PCNA	The human sliding clamp protein known as proliferating cell nuclear antigen (PCNA) orchestrates DNA-replication and -repairs and as such is an ideal therapeutic target for proliferative diseases, including cancer [7].	For the replication of the entire genome, PCNA forms a trimeric ring encircling the DNA double helix and functioning as the scaffolding protein of the DNA synthesis machinery at the replication forks (85).	Scoring after staining and using PCNA immunohistochemical methods (86).
MCM	Minichromosome maintenance (MCM) protein complex which consists of six highly conserved proteins (MCM2-7). Cancers arising in different anatomic sites are also associated with MCM2, MCM4, and MCM6 overexpression [8].	Collectively interacting to bring about initiation of DNA replication and DNA unwinding due to its replicative helicase activity. MCM2-7 proteins are present in proliferating cells [8].	Counting is done using anti-MCM2, anti-MCM4 and anti-MCM6 monoclonal antibodies [8].

results. Cross-reference searches were employed to find relevant articles that were missed in the initial search process. Among studies with repetitive results, newer and more comprehensive ones were selected for the narrative review. The total number of selected papers is reported in the Results section.

2.2 | Bibliometric Analysis

For the bibliometric review, we employed a broader search strategy aimed at retrieving all articles related to the utilization of proliferative markers in breast cancer within the PubMed database from 2000 to April 24, 2023 (search terms represented at Supporting Information S1: Table S2). The bibliometric analysis was performed using the Bibliometrix library of the R software [10]. The results are presented as "Contributing Journals, Authors, Countries, and Institutions,", "Trend of Publication," and "Concept Map." Seventy-eight words with more than 20 frequencies in keyword plus terms were reviewed to identify synonymous terms and eliminate those that lacked meaningful or conceptual relevance. The removed terms and synonymous terms can be found in Supporting Information S1: Table S2. For thematic evolution Walktrap clustering method was used weighted by "word occurrence" to find themes evolved during 2000–2007, 2007–2012, 2012–2017, and 2017–2023.

VOSviewer [11] was used to investigate keyword associations using keyword plus terms with a minimum occurrences of 10. Out of 2056 keywords, 158 met the threshold. The following keywords were excluded to improve the output of clustering and network since they do not provide practical information for clustering in this study: breast, therapy, women, recommendations, highlights, index, multicenter, cancer, impact, prediction, predictor, consensus, benefit, association, outcomes, disease, features, decisions, and mechanisms. An experienced pathologist assigned themes to each cluster. The setting of VOSviewer was tuned to achieve the best distinctive network, with parameters set as follows: normalization method - fractionalization; attraction - 1; repulsion - 0; random start - 80; iterations - 100; initial step size - 1.00; step size reduction - 0.50; random seed - 30; resolution - 2; and minimum cluster size - 5.

3 | Results

3.1 | Studies Selected for Narrative Review

A total of 712 studies have been retrieved from four search queries of PubMed database. Two evaluators (SAASN and ASN) evaluated studies and finally selected 80 studies for narrative review. Selected articles had been grouped into five themes entitled: The role of markers in the clinical management; Comparison of markers and their value; Value of markers before chemotherapy; The effect of chemotherapy on markers; Digital pathology and quantification of proliferative indexes.

3.2 | Bibliometric Analysis

A total of 1078 articles from 7554 authors and 344 sources were retrieved. Annual growth rate of publication was 6.97% with 21.15% international coauthorship rate. The quality of meta data for bibliometric analysis was good and is presented in Supporting Information S1: Figure S1.

3.3 | Bibliometric Analysis: Contributing Journals, Authors, Institutions, and Countries

The "Breast Cancer Research and Treatment," "Clinical Cancer Research," "BMC Cancer," "PLOS ONE," "Breast," "British Journal of Cancer," "Oncotarget," "Breast Cancer," "Breast Cancer Research," and "Annals of Oncology" were the main sources publishing studies on proliferative markers of breast cancer. "Breast Cancer Research and Treatment" published 8.19% ($N = 89$) of the studies, followed by Clinical Cancer Research ($N = 36$), and BMC Cancer ($N = 32$). Figure 1 and Figure 2 present the most relevant sources, affiliations, and authors, as well as their production over time.

Top three relevant institutes were Unicancer ($N = 164$), Udice French Research Universities ($N = 124$), and University of Texas ($N = 70$). Mitch Dowsett from Breast Cancer Now Toby Robins Research Centre ($N = 35$), GEORGE FOUNTZILAS from Aristotle University of Thessaloniki ($N = 17$), and Carsten Denkert from Philipps-University Marburg ($N = 16$) were the top 3 relevant authors. Figure 3 shows the most relevant countries along with their collaboration network. USA ($N = 793$), Italy ($N = 690$), and China ($N = 620$) had the highest scientific production, and USA ($N = 5396$), UK ($N = 4492$), and China ($N = 2491$) had the highest citations.

3.4 | Bibliometric Analysis: Concepts and Evolution of Themes

Figure 4 shows the major concepts in "breast cancer proliferative markers" research, and Figure 5 presents the evolution of these themes over time. The understanding of genetic signatures and molecular mechanisms, particularly regarding Ki-67 marker, represents earlier concepts, while recent research has shifted its focus towards clinical trials and the utilization of these markers in the management of breast cancer.

3.5 | Bibliometric Analysis: Historiography

The exploration of proliferative markers in breast cancer has undergone a transformative evolution over the past decades, and we explored top 20 studies in the evolution of this journey. Beginning in the early 2000s, studies such as "Relationship between Tumor Shrinkage and Reduction in Ki-67 Expression after Primary Chemotherapy in Human Breast Cancer" (2001) laid the foundation by investigating the relationship between tumor size reduction and Ki-67 expression changes following chemotherapy [12]. As the field progressed, studies like "Early Changes in Apoptosis and Proliferation following Primary Chemotherapy for Breast Cancer" (2003) delved deeply into the intricate interplay between apoptosis and proliferation, setting the stage for understanding the dynamic response to therapies [13].

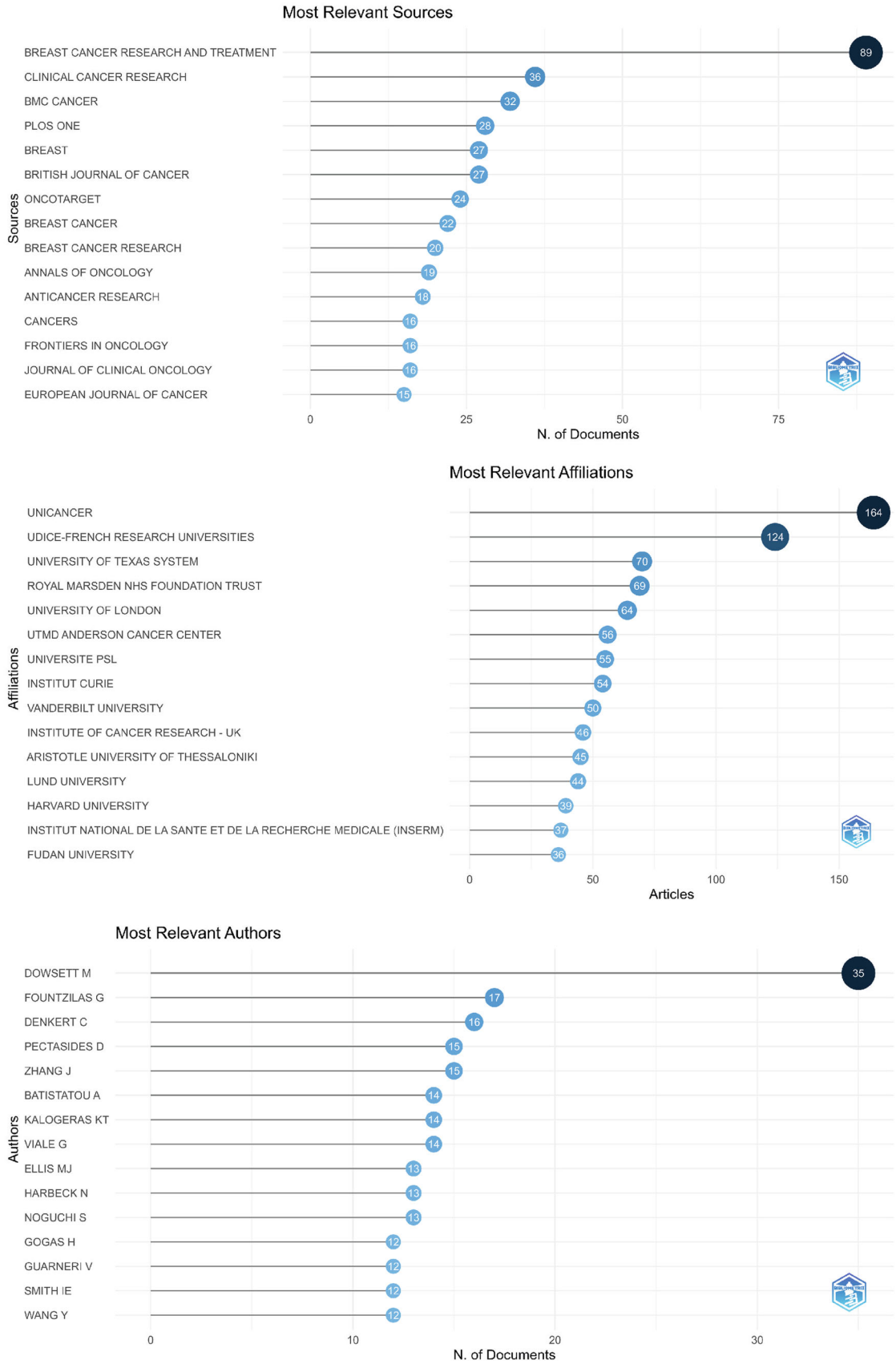


FIGURE 1 | Most relevant sources, affiliations, and authors contributing to proliferative marker research in breast cancer.

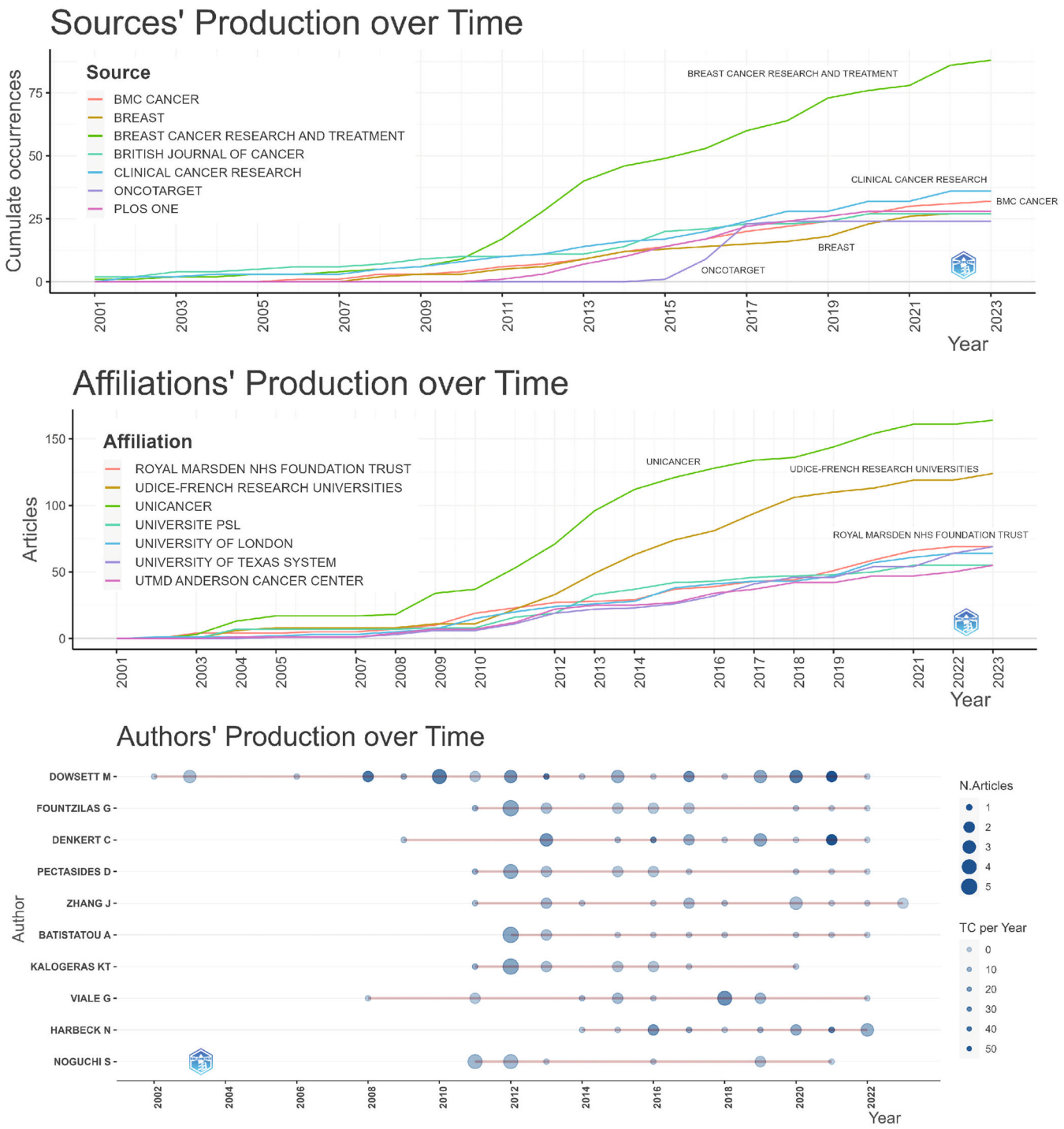


FIGURE 2 | Publication trends over time for key sources, affiliations, and authors.

Predictive and prognostic implications gained prominence with works like "Outcome Prediction for Estrogen Receptor-Positive Breast Cancer based on Postneoadjuvant Endocrine Therapy Tumor Characteristics" (2008), emphasizing the role of post-neoadjuvant endocrine therapy characteristics in predicting outcomes [14]. This was further reinforced by "The Prognostic Significance of Ki-67 before and after Neoadjuvant Chemotherapy in Breast Cancer" (2009) and "Ki-67 Expression and Docetaxel Efficacy in Patients with Estrogen Receptor-Positive Breast Cancer" (2009), which highlighted Ki-67's importance as a prognostic marker and its relevance in treatment response, respectively [15, 16]. The integration of molecular subtyping and proliferative markers emerged with "A Comparison of PAM50 Intrinsic Subtyping with Immunohistochemistry and Clinical Prognostic Factors in Tamoxifen-Treated Estrogen Receptor-Positive Breast Cancer" (2010), demonstrating the evolving role of molecular profiling in treatment decisions [17].

Advancements in methodology were mirrored by studies like "Ki-67, Chemotherapy Response, and Prognosis in Breast Cancer Patients Receiving Neoadjuvant Treatment" (2011), exploring the connection between Ki-67, chemotherapy response, and overall

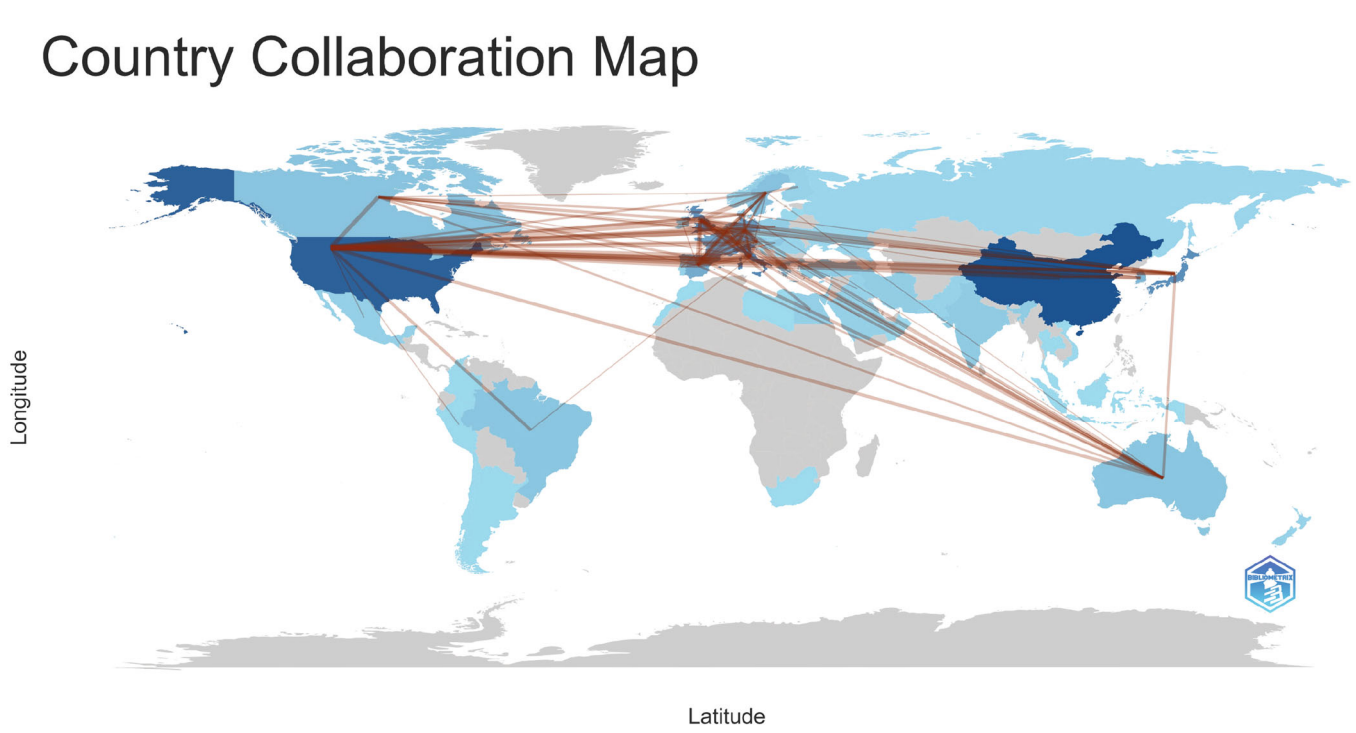


FIGURE 3 | Global collaboration network of the most prolific countries in breast cancer proliferative marker research.

prognosis [18]. The convergence of personalized treatment approaches and molecular subtyping was evident in "Randomized Phase II Neoadjuvant Comparison between Letrozole, Anastrozole, and Exemestane for Postmenopausal Women with Estrogen Receptor-Rich Stage 2 to 3 Breast Cancer" (2011), which emphasized the tailored therapy decisions guided by molecular subtyping [19]. These findings paved the way for studies such as "Ki-67 is a Prognostic Parameter in Breast Cancer Patients: Results of a Large Population-Based Cohort of a Cancer Registry" (2013), affirming the clinical utility of Ki-67 in broader patient populations [20].

Collectively, this journey underscores the transformative role of proliferative markers in breast cancer research, transitioning from basic correlations to integrated molecular insights that have shaped personalized treatment paradigms. The emphasis on Ki-67's predictive and prognostic potential, along with its convergence with molecular subtyping, has redefined the landscape of breast cancer management, enhancing patient outcomes and contributing to a deeper understanding of the disease's complexity. The consensus achieved in "Assessment of Ki-67 in Breast Cancer: Updated Recommendations from the International Ki-67 in Breast Cancer Working Group" (2021) reflects the ongoing commitment to refining assessment methodologies and harnessing the power of proliferative markers in clinical practice [21].

4 | Discussion

Ki-67, MCM, and PCNA are proliferation markers that are being investigated extensively for their clinical use as potential prognostic and predictive indicators in breast cancer [8, 21, 22]. While Ki-67, a well-established biomarker for assessing tumor proliferation, aids clinicians in tailoring therapy for each patient, it is not without its limitations, such as its absence in cells with the potential to enter the G1 phase of the cell cycle and the lack of universally accepted cutoff values [23]. MCM proteins have some advantages over Ki-67, including being detectable in cells that are in the resting phase and having stable expression during the cell cycle [24]. Additionally, MCM family such as MCM2, MCM4-7, MCM10 have shown potential roles as diagnostic and prognostic biomarkers, as well as potential targets for therapeutic interventions in breast cancer [8]. MCM6 exhibit a superior capacity to classify breast tumors based on histologic and mitotic grades more precisely when compared to Ki-67 [25]. Further to the above, PCNA and its phosphorylation status have been associated with poor outcomes, and the presence of PCNA+ tumor-associated macrophages (TAMs), is related to high-grade, hormone receptor (HR)-negative breast cancer and an elevated risk of recurrence [26]. PCNA can distinguish between actively proliferating cells and those that are not, and it plays a crucial role in DNA repair. Nevertheless, immunohistochemical studies vary considerably, and its expression can be induced by growth factors or in response to DNA damage even when the cell is no longer active in the cell cycle.

Despite the advantages and limitations of each marker, Ki-67 is still the most commonly used marker due to its specificity for the proliferation of tumor cells and cost-effectiveness. The Ki-67 index is a useful biomarker to assess the effectiveness of neoadjuvant chemotherapy (NAC) in breast cancer. A reduction of Ki-67 levels after NAC is associated with better survival outcomes and a higher chance of achieving a pathological complete response (pCR) [27, 28]. Patients with high Ki-67 expression before NAC, who are subsequently experiencing a reduction of over 30% in their Ki-67 levels following chemotherapy demonstrate notably improved disease-free survival (DFS) rates [29]. However, there are controversies regarding the association between Ki-67 levels, pCR rates, and survival outcomes. Specifically, Ki-67 may be a better reflection of tumor response to chemotherapy in patients with hormone receptor

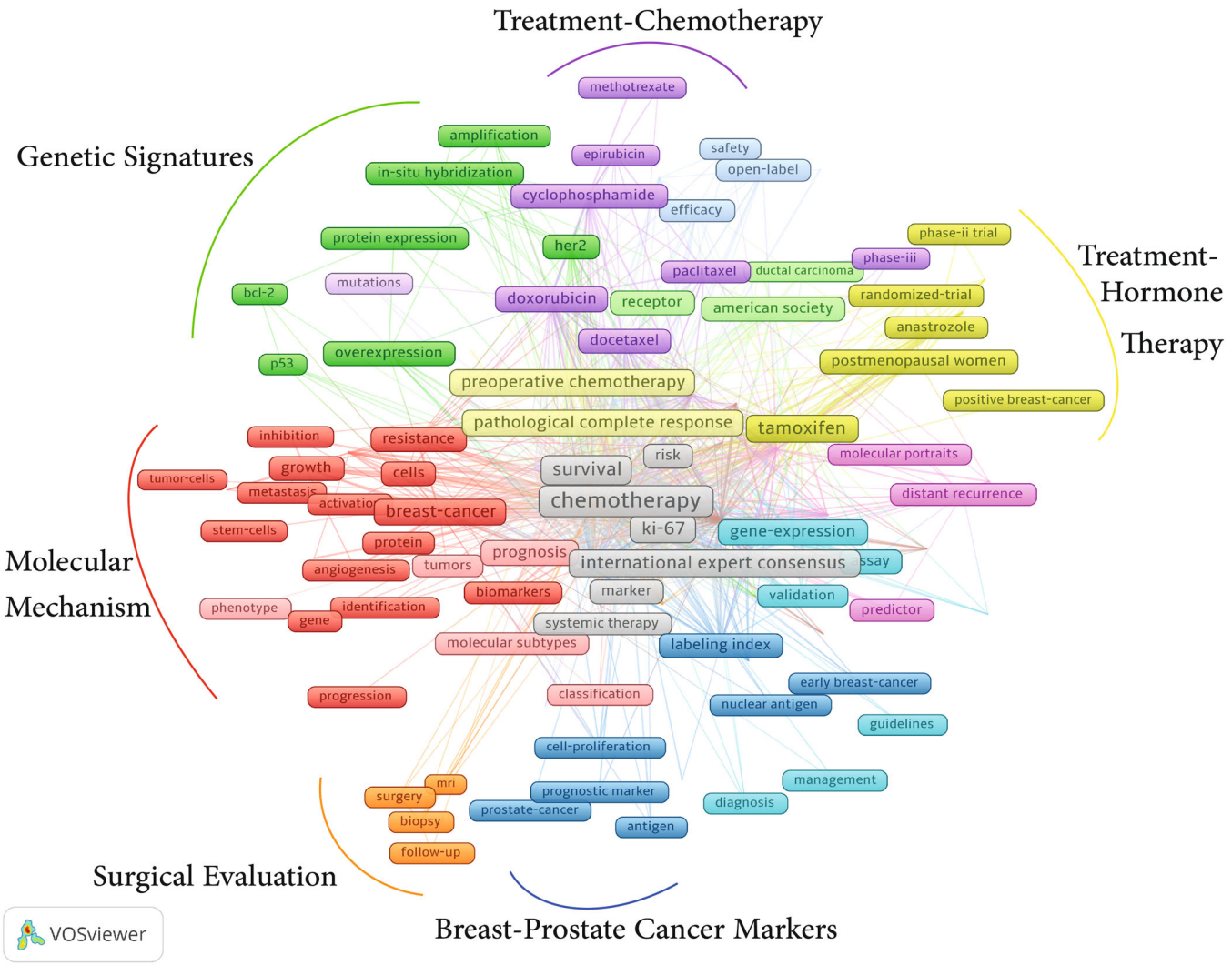


FIGURE 4 | Conceptual structure of research on proliferative markers in breast cancer.

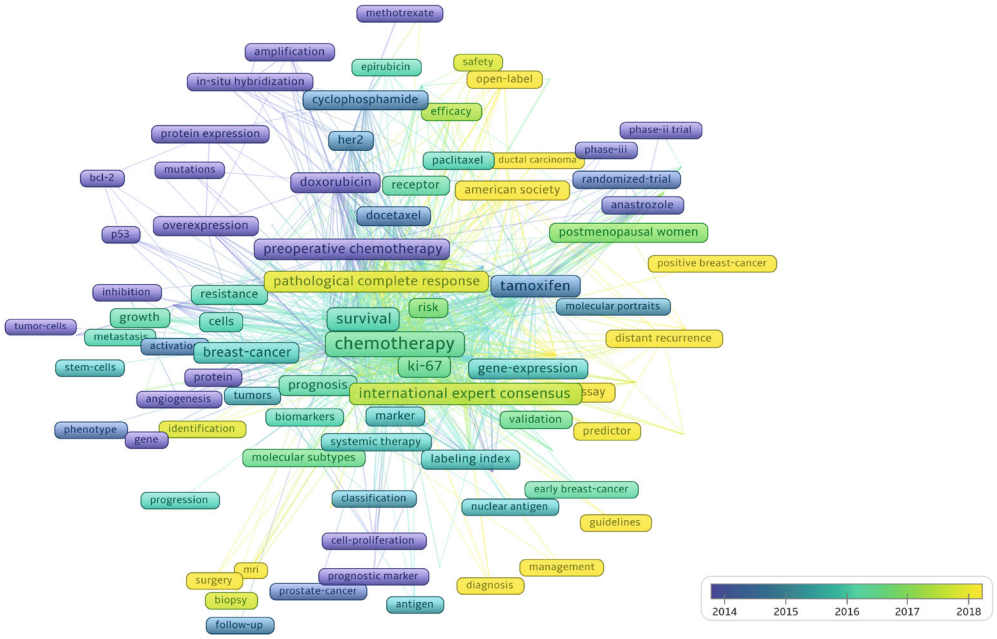
(HR)-negative breast cancer, whereas in HR-positive tumors, high Ki-67 levels may indicate a poor prognosis [30, 31]. Therefore, re-examination of biomarkers expression after NAC could be helpful in optimizing the adjuvant systemic chemotherapy for each patient.

However, standardization of techniques and scoring methods is necessary for the integration of Ki-67 into everyday practice. Tumor biomarker tests can be influenced by several factors, from specimen collection to reporting. Thus, performing Ki-67 immunohistochemistry test in patient care and decision making depends on analytical validity and clinical utility, similar to other biomarker tests [21, 32, 33]. Overall, Ki-67, MCM proteins, and PCNA usage as biomarkers can support the selection of appropriate therapeutic strategies for individuals and improve clinical outcomes in breast cancer.

4.1 | The Role of Markers in the Clinical Management

There is a robust correlation between the phase S of cell cycle and Ki-67 and it has been substantiated that a quantitative assessment of Ki-67 can provide an accurate estimation of the tumor proliferation index. The proliferation index is categorized as low or negative when fewer than 14% of nuclei are stained, and as positive or high when more than 14% of nuclei exhibit staining [34]. Biological markers capable of predicting clinical or pathological responses to primary systemic therapy in its early stages during a chemotherapy cycle can hold significant clinical value [35].

It is demonstrated that high expression of Ki-67 in axillary lymph nodes, rather than breast tissue, is significantly associated with shorter patient survival. Consequently, patients with increased proliferative activity in lymph node metastases may necessitate more intensive therapeutic approaches and closer clinical monitoring of their condition [34]. This biomarker can be regarded as a potential prognostic factor for therapeutic decision-making; however, standardizing techniques and scoring methods is essential for its integration into routine clinical practice. Low proliferation is typically associated with the luminal A subtype, whereas the luminal B subtype exhibits higher proliferation rates. Over time, the St. Gallen Panel has established criteria to define standard values, including an All-Active-Quotient (AAQ) of ≤ 15% for low proliferation, 16%–30%



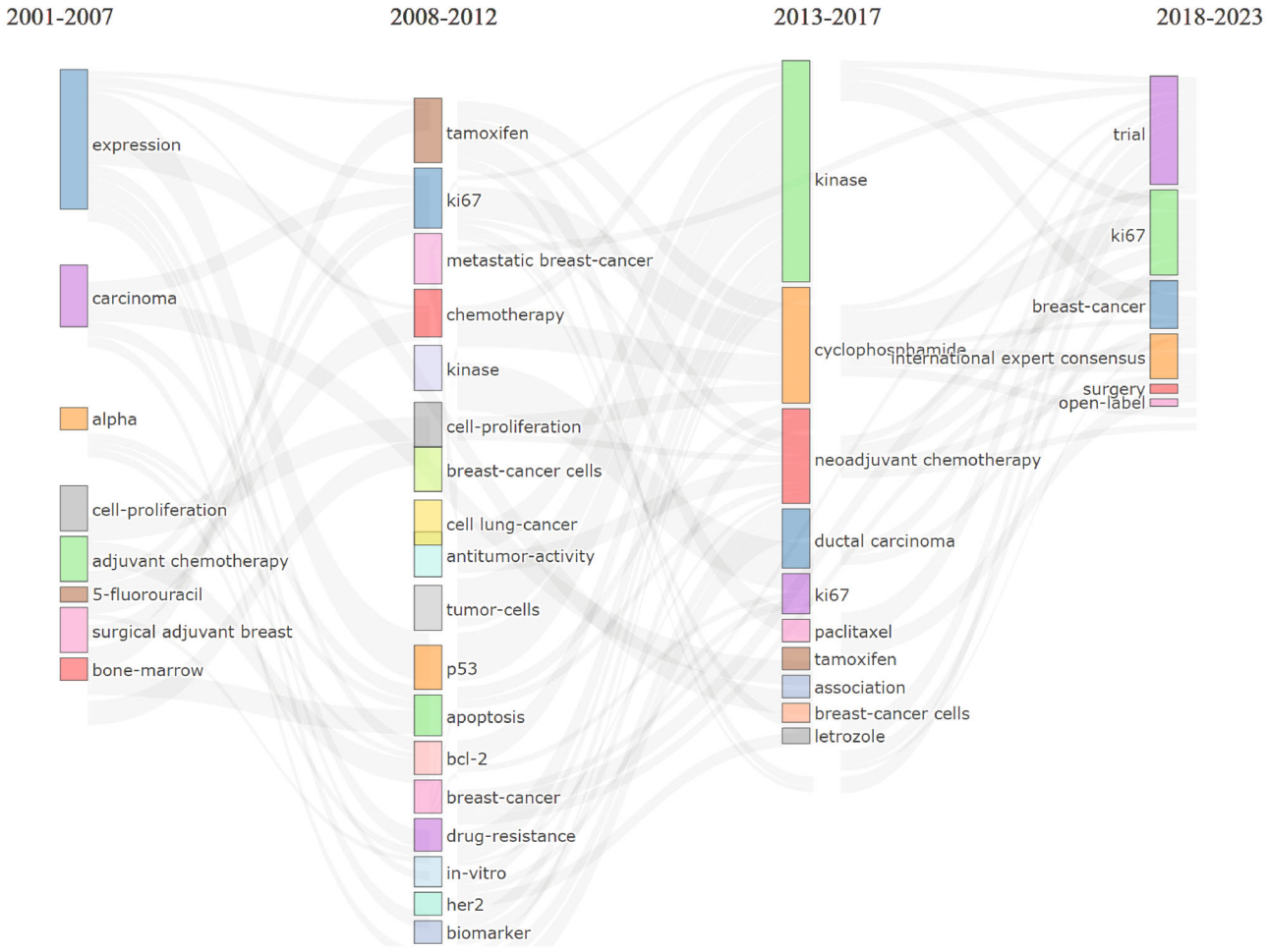


FIGURE 5 | Thematic evolution of proliferative marker research in breast cancer from 2000 to 2023.

for intermediate levels, and > 30% for high proliferation. Additionally, specific cutoff points for Ki-67-positive tumor cells, such as 14%, 20%, and 30%, have been proposed to differentiate between luminal A and luminal B subtypes. However, due to significant inter-laboratory variability in Ki-67 measurement, these cutoff values should be interpreted in the context of local laboratory standards [36].

In recent years, PCNA has demonstrated promising potential in clinical practice. This marker exists in two distinct forms: a replication-competent, chromatin-bound form and a chromatin-unbound form that is not involved in DNA synthesis. However, the regulatory mechanisms governing these two PCNA populations remain unclear. In various tumors, PCNA levels have been linked to mitotic activity and tumor grade. Its role in signal transduction significantly influences the growth regulation of breast cancer cells and is associated with poor overall survival. Notably, recent studies have identified the phosphorylation of PCNA at tyrosine 211 (Y211) as a promising therapeutic target for breast cancer treatment. The findings from Zhao et al [37] suggested that targeting phospho-Y211 PCNA could emerge as an effective strategy in the treatment of breast cancer, potentially holding promise for future therapeutic approaches [35].

Another marker that has been investigated in clinical use is MCM. Alterations in the transcriptional levels of MCM family members, including MCM1–10, have been widely reported in various types of cancer. In breast cancer samples, the mRNA expression of MCM2–8 and MCM10 exhibited a significant upregulation, in contrast to MCM1 and MCM9, which did not show such changes compared to normal samples. The expression levels of MCMs in relation to breast cancer tumor stages were analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) database. Notably, MCM2, MCM3, MCM7, and MCM10 exhibited significant variations across different tumor stages, whereas MCM1, MCM4, MCM5, MCM6, MCM8, and MCM9 groups did not demonstrate significant differences. Consequently, elevated mRNA expression levels of MCM2, MCM4–7, and MCM10 were associated with a shorter relapse-free survival (RFS), while MCM3 and MCM8 showed no such association, signifying their correlation with an unfavorable prognosis among breast cancer patients. Nonetheless, the decreased expression level of MCM1 and MCM9 in breast cancer was significantly related to prolonged RFS, suggesting their correlation with a favorable prognosis among breast cancer patients [38]. Figure 6 summarizes the role of markers in clinical management.

4.2 | Comparison of Markers and Their Value

Ki-67, a widely recognized proliferative marker, is considered a valuable predictor of survival, recurrence, and breast cancer aggressiveness. Additionally, numerous studies have explored the relationship between Ki-67 levels and various tumor characteristics, including grade, stage, lymph node involvement, and estrogen receptor (ER) status [20, 39, 40]. The classification of breast tumors into distinct molecular subtypes based on hormone receptor status, HER2 expression, and Ki-67 levels, as well as the role of Ki-67 in guiding systemic treatment decisions for early-stage breast cancer, is well established. However, Ki-67 has certain limitations. Notably, it is not expressed in cells with the potential to enter the G1 phase of the cell cycle, which may lead to the misidentification of tumor cells. Additionally, its exact functions remain incompletely understood, and, more importantly, there are no universally accepted cutoff values for its assessment [41].

MCM proteins are essential regulators of DNA replication in eukaryotic cells. Compared to Ki-67, MCM family members offer certain advantages; for instance, they can be detected in cells that are in the resting phase of the cell cycle but still retain replication competency. Additionally, their expression remains stable throughout the cell cycle. Consequently, MCM proteins have a greater ability to identify a higher proportion of proliferative cells across different types of neoplasms compared to Ki-67 [40]. In normal breast terminal duct-lobular units (TDLU), MCM expression levels are higher than those of Ki-67, reflecting the large proportion of mammary epithelial cells that express MCM while remaining in a licensed but non-proliferative state [42]. MCM6 can effectively distinguish between histologic grades of invasive ductal carcinoma due to

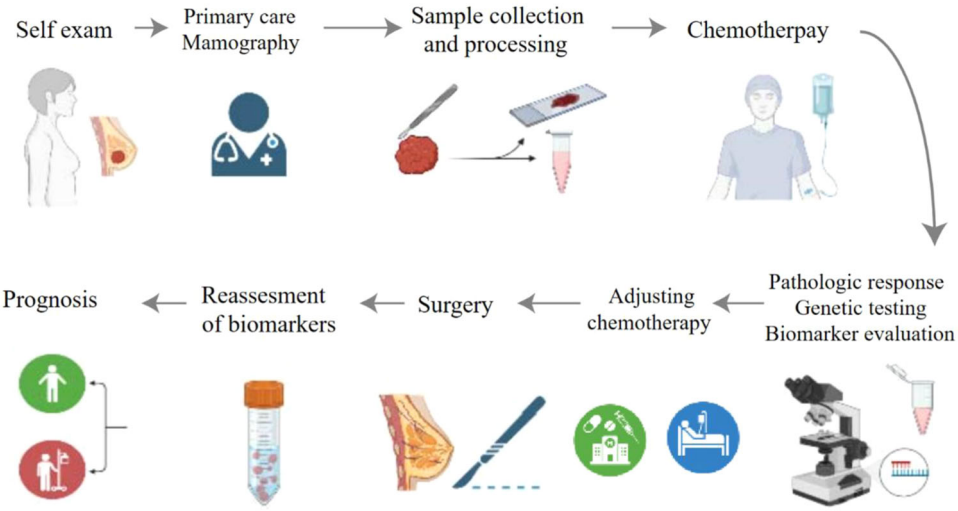


FIGURE 6 | The role and timing of production of production of markers in clinical management.

its strong correlation with differentiation grade, which is even more pronounced than that of Ki-67. This suggests that MCM6 may allow for a more precise classification of breast tumors based on histologic and mitotic grades. Notably, both Ki-67 and MCM6 are associated with ER status, with higher expression observed in HR-negative tumors. The most significant finding is that both markers can differentiate between luminal A and B molecular subtypes, as well as between HR-positive and Triple-Negative Breast Cancer (TNBC), playing a crucial role in guiding appropriate therapeutic strategies [40].

Studies showed nuclei can continue to express PCNA even after completing the cell cycle, which makes it different from Ki-67. In addition, the increase of PCNA levels can be triggered by growth factors or in response to DNA damage, even when the cell has exited the active phase of the cell cycle. PCNA is involved in the excision and replacement of abnormal nucleotides. Consequently, PCNA is also expressed in non-proliferating cells which are undergoing DNA repair [43, 44]. Several factors can influence the immunoreactivity of PCNA in archived paraffinized samples, including the duration and temperature of fixation, as well as the type of fixative used. Additionally, the effects of fixation and processing on the immunorecognition of Ki-67 and PCNA vary, further contributing to differences in their detection [45]. This difference suggests that these antigens may be affected in a different way because they are packaged differently in the various types of cells. Another aspect to take into consideration is the dilution of the antibody: the lower the antibody dilution, the greater the percentage of PCNA expression observed in the nucleus [43]. It is important to recognize that immunohistochemical studies on PCNA exhibit significant variability. These differences may stem from factors such as sample size, the number of cells counted per field, variations in cell counting techniques, and the statistical methods employed. Collectively, these factors may contribute to the higher positivity rate of PCNA compared to other proliferation markers, such as Ki-67 [46].

Some investigations have revealed that Ki-67 remains less susceptible to the influences of both internal and external factors, unlike PCNA, in some studies, Ki-67 is considered to be the more specific marker of cell proliferation [47]. After the discovery of the Ki-67 antigen in all proliferating cells, both normal and tumor, it quickly became clear that this protein serves as an effective marker for evaluating the growth fraction of a cell population. Consequently, antibodies targeting Ki-67 are increasingly utilized as diagnostic tools for various types of neoplasms [48]. The expression of human Ki-67 protein is closely linked to cell proliferation. During interphase, this antigen is exclusively localized within the nucleus, while in mitosis, the majority of the protein relocates to the chromosome surface. Its presence throughout all active phases of the cell cycle (G1, S, G2, and mitosis) and absence in resting cells (G0) make Ki-67 an ideal marker for assessing the growth fraction of a given cell population [49].

4.3 | Breast Cancer Chemotherapy

Chemotherapy has an important effect on reducing the mortality rate. Those patients who have been evaluated as high risk of recurrence, based on pathologic factors (e.g., tumor size, grade, and nodal involvement), biomarkers or multi-gene assay may also receive chemotherapy before surgery (neo-adjuvant) or after surgery (adjuvant) [50]. For several years the administration of NAC has been regarded as a favorable alternative therapy for patients with early-stage breast cancer and a common therapeutic strategy in locally advanced breast cancer [51]. The efficacy of NAC primarily hinges on the clinical and pathologic response of both the breast tumor and lymph nodes, serve as the key endpoints in many clinical trials [52, 53]. Patients receiving NAC could have a higher chance to undergo a breast-conserving surgery with negative margins and without extensive lymph node dissection, due to downsizing the tumor and achieving pCR. Moreover, patients achieving pCR following NAC are most likely to experience better DFS and overall survival (OS). Additionally, NAC can serve as a valuable tool for in vivo assessment of a tumor's response, aiding in the development of novel therapeutic approaches.

4.4 | Value of Markers Before Chemotherapy

The utilization of conventional biomarkers in breast cancer treatment, including human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR) in breast cancer treatment has been developed over the years. Nevertheless, advances in tumor biology have identified multiple additional biomarkers that hold promise for progress in clinical practice. Markers also have been demonstrated as prognostic factors. All these biomarkers studies could navigate clinicians to precision medicine [54].

One of the main proliferation markers is the famous Ki-67 that expressed in all of the cell cycle phases, except G0 phase, as previously mentioned. At this time there are three application for Ki-67 immunohistochemistry; as a prognostic factor in early-stage of breast cancer regarding whether adjuvant chemotherapy is required, as a predictor whether chemotherapy is useful, and to determine whether regimen of NAC or endocrine therapy is sufficient or an alternative should be replaced [21]. A large multicentric study (8088 patients from 10 study groups) revealed that automated Ki-67 scoring provides prognostic information in breast cancer due to the potential of standardization and reproducibility, as a reliable alternative to visual scoring. This is mostly relevant for ER-positive and node-negative tumors, to support decisions on planning adjuvant chemotherapy [55]. Therefore, this marker could be helpful to choose patients who will benefit from NAC. For instance, low expression of Ki-67 may be an indicator of poor benefit of cytotoxic chemotherapy [56]. A systematic review and meta-analysis of 53 studies and 10,848 breast cancer patients, with a wide variation of patients characteristics, NAC regimens, molecular subtypes, Ki-67 cutoff points, and pCR definitions, showed that those patients who have higher Ki-67 before NAC, are more likely to benefit from therapy. They also suggested that 15% or 20% might be suitable cutoff for Ki-67, still, there is a need for efforts to establish appropriate cutoff points [23].

A strong association exists between Ki-67 expression and histologic grading since both correlated with proliferation [57]. Furthermore, tumor subtypes are identified by grade and proliferative ratio which mostly evaluated by Ki-67 immunostaining [58].

The first valuable study of applying Ki-67 quantitative visual immunohistochemistry scores to breast cancer biological subtypes, have reported that tumors with Ki-67 > 14% remarkably had higher histological stage and grade, PR negativity and HER2 positivity. According to this, the cutoff point will likely be more efficient in other cohorts with different treatment regimens and diverse risk distributions [59]. The association between Ki-67 index and intrinsic subtypes show that triple-negative breast cancer have the highest Ki-67 index, and mostly appear with a high proliferation index (> 50%) [60]. Among other subtypes, the HER2-positive, luminal B, and luminal A subtypes exhibited correspondingly lowest Ki-67 indices. The panel also determined that the Ki-67 labeling index is mainly important for distinguishing between the "luminal A" and "luminal B" subtypes, by a cutoff value of 14% [61]. Among histologic subtypes, metaplastic and medullary showed remarkably higher Ki-67 index [62].

In another study, patients with locally advanced tumor were treated with fluorouracil + epirubicin + cyclophosphamide and then randomized into two groups of docetaxel + capecitabin or docetaxel monotherapy. The pCR rate was higher in patients with a higher Ki-67 expression (Ki-67 > 10%) [63]. Therefore, the detection of Ki-67 marker before NAC should be considered. Noteworthy, the 13th St Gallen International Breast Cancer Conference Expert Panel confirmed the high Ki-67 as a inclusion factor of chemotherapy [61].

MCMs is another biomarker consisting of ten peptides (MCM1–10) with important roles in many aspects of genome stability and the regulatory process of DNA replication. MCM expression initiates as cells enter the G1 phase, preceding active replication. Hence, they can be detected as a hallmark for early cancer diagnosis due to the elevation in non-cycling cells [64]. Actually, rising MCM proteins levels is related with malignant cells and may also identify precancerous cells [65].

MCM2, MCM4, and MCM6 have prognostic and diagnostic values in breast cancer. Evidence shows that they can distinguish between luminal A and B subtypes and also are related to higher histological grades and more aggressive subtypes such as triple-negative, luminal B, and HER2-positive. Also, there is a significant correlation between these three markers, Ki-67, and the histologic grade. Higher expression of these markers is correlated to shorter relapse-free survival (RFS) [8]. However, in univariable analysis, MCM2 had more highly association with breast cancer specific survival rather than Ki-67 [66]. Analysis showed that HR-negative tumors have higher expression of MCM6 and Ki-67. Additionally, both MCM6, and Ki-67 can discriminate between luminal A and B subtypes, as well as between HR-positive and triple-negative tumors, which has a key role in choosing the suitable therapeutic strategies. In contrast to Ki-67, MCM also helps to determine cells that are in resting or inactive phase [24]. Therefore, MCM can identify a greater number of proliferative cells compared to Ki-67 [67]. As a whole, MCM6 might be a more effective discriminator of tumor grade due to its better scoring than Ki-67 in all three parameters of the Nottingham Score [68]. However, efficacy of this marker in routine clinical practice instead of Ki-67 has still a lot more unknown features for evaluating in future studies.

Recently, it has been reported that increased miRNA expression levels of MCM2, MCM4–7, and MCM10 were also remarkably related to shorter RFS and could be used as diagnostic markers. Findings supported that MCM10 has a capacity of prognostic marker and could be a therapeutic target. Identifying the overexpression of MCM10 can be detected as an early indicator of poor prognosis and invasive potential of breast carcinoma by deactivating Wnt/β-catenin signaling. As a consequence, investigating potential therapeutic approaches involving MCM10 holds substantial promise for advancing treatment strategies [69]. Additionally, the reduced expression levels of MCM1 and MCM9 showed a significant correlation with prolonged RFS, suggesting their potential as markers for favorable prognosis in breast cancer patients.

In summary, expression levels of all MCM complex proteins are significantly associated with each other, and could imply breast cancer prognosis as their co-overexpression might be a superior prognostic factor and a valuable therapy indicator compared to individual MCMs alone. Additionally, tumors with overexpression of multiple MCMs, showed a more favorable response to treatment [70].

Another biomarker is PCNA, with significant roles in the nucleic acid metabolism. It performs a crucial role in DNA replication and also participates in DNA repair, RNA transcription, control of cell cycle, and chromatin assembly. Notably, the nuclear EGFR is responsible for phosphorylating the chromatin-bound PCNA at tyrosine 211 (Y211), which is essential for maintaining PCNA functions. Studies have revealed that PCNA Y211 phosphorylation is usually higher in triple-negative breast cancer cells. In every triple-negative cell line tested, phosphorylation suppression inhibited cell activity and caused cell death. Alternatively, PCNA Y211 phosphorylation might be responsible for regulating cell viability during cell proliferation. Distinguishing between these mechanisms will clarify more information about the signaling pathway in PCNA mediated cell death [71]. The findings from an exploratory study demonstrated that a substantial presence of PCNA +TAMs is related to an unfavorable prognosis in breast cancer cases treated with NAC, particularly in the absence of an immune microenvironment [22]. A subpopulation of TAMs which express PCNA are correlated with high grade, HR-negative breast cancer and increased risk of recurrence [26]. More investigations of PCNA+ TAMs could eventually provide detecting patients who may benefit from targeted therapy [72].

4.5 | The Effect of Chemotherapy on Markers

The pathological response to NAC is useful for improving adjuvant chemotherapy regimens and predicting the possibility of relapses and survival outcomes. The results demonstrate that it is essential to evaluate the Ki-67 index of tumors both in pre-NAC core needle biopsy samples and in surgical specimens following NAC. Alteration of Ki-67 index may carry essential clinical indication for adjuvant chemotherapy particularly when a patient's Ki-67 status shifts from above 20% to ≤ 20% [73].

The post-chemotherapy Ki-67 value is a strong predictor of survival in patients who do not achieve a pCR [74]. Higher

Ki-67 reveals a higher chance of pCR rate before NAC and lower Ki-67 values after NAC indicate a greater prognosis and DFS [75]. Evidence proved that high expression of Ki-67 in posttreatment tumors was significantly associated with DFS and OS regardless of the subtype. It has been reported that patients without a reduction in Ki-67 in residual tumors after NAC have poor prognosis and outcomes [27]. Patients with high Ki-67 tumor expression were identified as responsive to NAC (anthracycline-based ± taxane regimen), resulting in a significant reduction in the Ki-67 index post-chemotherapy. Especially, patients experiencing a reduction of more than 30% in Ki-67 levels after chemotherapy achieved significantly improved DFS rates [76].

On the other hand, there are controversies about the relation between Ki-67, pathological response, and survival rates. Ki-67 has been shown a clear association with pCR rate in triple-negative subtype; in a meta-analysis higher Ki-67 leads to a 3.4-fold increase in pCR rate [77]. Accordingly, triple-negative showed high Ki-67 expression and high percentage of pCR [78]. A retrospective study illustrated association of Ki-67 with patient survival outcomes among subtypes and independent prognostic predictor for long-term outcomes in luminal B and triple-negative subtypes. Since pCR is not a reliable predictor for luminal B subtype, ΔKi-67% status could be assisting with estimating the efficacy of NAC and providing evidence for adjuvant therapy modification [79].

In a recent retrospective analysis with a 2-year follow-up period, it was observed that achieving a higher pathological response rate was more common in patients with HER2-positive and triple-negative subtypes, which appears to be closely linked to reduction in Ki-67 expressions after NAC [28]. Analysis of another retrospective study of patients with HR-negative breast cancer stage II and III who received NAC (anthracycline and/or taxane-based) between 2004 and 2011, showed that the Ki-67 labeling index may be a better indicator of tumor response to chemotherapy for patients with HR-negative breast cancer compared to other markers. Moreover, a high Ki-67 index ($>30\%$) after NAC in residual tumors was the only factor that strongly associated with poorer DFS, but not OS [80].

According to a clinical trial of 1198 patients, adjuvant taxane chemotherapy showed improvement of OS and DFS in ER-positive patients with high Ki-67 ($>14\%$), while no improvement was seen in low Ki-67 group. They also performed a descriptive analysis of four randomized prospective studies (including their own) suggesting the advantages of taxane exclusively in patients with ER-positive/high Ki-67, indicating a high proliferation rate. Consequently, these patients may obtain significant benefit from adjuvant docetaxel therapy [81]. Interestingly, in another meta-analysis, it was illustrated that patients with high Ki-67 or ER-positive status have more sensitivity to chemotherapy [30]. Consistently, another multivariate analysis of locally advanced breast cancer represented that ER status and Ki-67 level after NAC are prognostic factors which associated with OS and DFS. Furthermore, a constantly elevated Ki-67 level ($>15\%$) and ER-negative status following NAC can determine patients with poor survival [31].

Overall, re-examination of biomarker after NAC can be beneficial for prognosis prediction and treatment guidance. In particular, this practice may result in treatment alteration, in addition to optimizing adjuvant systemic chemotherapy [82].

4.6 | Limitations of Proliferative Biomarkers in Clinical Practice

Ki-67, PCNA, and MCM are essential biomarkers for assessing cellular proliferation in cancer diagnostics and prognostics, yet they each present significant limitations that impact their clinical utility and reliability. Ki-67, commonly assessed via IHC, suffers from considerable interobserver variability, leading to inconsistent results across different laboratories and pathologists, which can impact clinical decisions [83]. Additionally, the lack of standardized cutoffs and scoring methodologies further complicates Ki-67's integration into clinical practice, as the absence of universally accepted thresholds for interpretation prevents its broad adoption [84]. Although Ki-67 is a valuable prognostic marker, its utility is hampered by the need for precise cutpoint definitions that remain inconsistent. PCNA, a marker linked to DNA replication, also faces limitations, particularly due to its lack of specificity; it is expressed in both cancerous and normal proliferating cells, reducing its effectiveness as a dedicated cancer biomarker [4, 5]. Technical variability in PCNA assessments similarly affects reproducibility across settings. MCM proteins, while promising, present challenges in terms of interpretive complexity, as their expression can be influenced by various biological factors, complicating clinical application [4]. Additionally, MCM proteins lack the extensive clinical validation that Ki-67 has achieved, limiting their routine use in clinical practice [4]. Although these markers offer valuable biological insights, these limitations underscore the necessity for improved standardization and validation efforts. Future innovations in genomic technologies and biosensor accuracy may help address these challenges, potentially enhancing the clinical reliability of proliferative biomarkers.

4.7 | Digital Pathology and Quantification of Proliferative Indexes

With the advent of artificial intelligence (AI), a promising transformation has emerged in the field of medicine. AI-driven automation and the pattern recognition capabilities of machine learning (ML) have the potential to revolutionize various aspects of pathology, including the assessment of proliferative markers in breast cancer chemotherapy. The application of AI and ML extends beyond the quantification of traditional proliferation indices; it also holds promise in predicting neoadjuvant chemotherapy (NAC) outcomes, metastasis likelihood, and relapse risk [6, 7]. These technologies can particularly streamline the assessment of markers like Ki-67, MCM, and PCNA. By automating the marker counting process, the burden of interobserver variability is alleviated, enhancing their utility in clinical settings.

In this context, digital pathology has emerged as a pivotal tool, integrating AI-driven automation and image analysis algorithms. This approach involves digitizing histological slides, enabling computational tools to precisely delineate regions of interest within tissue samples. Through intricate pattern recognition techniques, these algorithms identify and quantify

cells expressing markers like Ki-67, MCM, and PCNA. The synergy between AI and machine learning not only refines the quantification process but also has the potential to shed light on spatial distribution patterns, revealing nuances of tumor heterogeneity. Nevertheless, challenges loom, including the need to ensure the robustness of algorithms across diverse patient populations and tissue preparation methods [7]. Moreover, the accessibility of AI-driven systems and the development of robust models for selecting regions of interest and counting procedures represent crucial avenues for future research and implementation.

4.8 | Bibliometric Analysis

The bibliometric analysis of breast cancer research trends and publications offers a compelling insight into the evolving landscape of this critical field. The steady growth of publications over the years reflects the sustained interest and commitment of the scientific community to address the multifaceted challenges posed by breast cancer. The prominence of key research topics such as biomarkers, treatment strategies, and molecular subtypes underscores the concerted efforts to unravel the complexities of breast cancer and tailor interventions for improved patient outcomes. The upward trajectory of collaborative research networks signals a global endeavor to pool expertize and resources, transcending geographical boundaries to confront this global health concern collectively. Furthermore, the increasing utilization of advanced methodologies such as machine learning and genomics mirrors the integration of cutting-edge technologies into breast cancer research, fostering innovative avenues for diagnosis, prognosis, and treatment. As research continues to evolve, this bibliometric analysis serves as a compass, guiding researchers toward areas of unmet need, propelling the field toward novel breakthroughs and a future marked by enhanced understanding and management of breast cancer.

5 | Conclusion

In conclusion, the utilization of proliferation markers in breast cancer management represents a transformative shift in treatment paradigms. These markers, including Ki-67, MCM proteins, and PCNA, hold the key to predicting treatment responses, guiding therapeutic decisions, and enhancing patient outcomes. The dynamic changes in marker expressions following chemotherapy provide valuable insights into tumor behavior and prognosis. Moreover, the convergence of traditional marker assessment with digital pathology and AI-driven methodologies promises to revolutionize diagnostic accuracy and standardization. As we stride forward into an era of personalized oncology, the integration of proliferation markers and cutting-edge technologies underscores our commitment to refining treatment strategies and offering renewed hope to breast cancer patients worldwide.

Author Contributions

Aryan Salahi-Niri: conceptualization, formal analysis, writing – original draft, visualization, project administration. **Paniz Zarand:** writing – original draft. **Fatemeh Shojaeian:** writing – review and editing. **Negar Mansouri:** writing – review and editing. **Omid Yazdani:** writing – review and editing. **Romina Esbati:** writing – review and editing. **Seyed Amir Ahmad Safavi-Naini:** methodology, visualization, writing – original draft, software, formal analysis, supervision. **Behnaz Jahanbin:** supervision, conceptualization, writing – review and editing.

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During the preparation of this manuscript, and following multiple revisions by the authors, author ASN utilized the GPT-4 language model to correct grammatical errors and refine the English language. The prompt given was as follows: "Act as an English language editor. Suggest improvements for grammar and English style of this text. Avoid adding or removing claims. This text is part of a scientific article aiming to be reliable and accountable for the statements with a neutral tone." ASN and coauthor SAASN reviewed the suggestions provided by GPT-4 and incorporated the relevant corrections, ensuring that the original concepts remained unaltered and no new conclusions were introduced. All authors have read and approved the final version of the manuscript and assume full responsibility for its content.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Transparency Statement

The lead author Seyed Amir Ahmad Safavi-Naini, Behnaz Jahanbin affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.