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Review Article

Practical Issues of Ki-67 Evaluation in Breast Cancer Clinical Practice

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Abstract

For the past several decades, markers of cellular proliferation in breast cancer have been postulated to indicate prognosis and predict benefits from antineoplastic therapies. The most common method to measure cellular proliferation by Ki-67 is immunohistochemistry (IHC) based assays. However, analytical issues have hindered the widespread adoption of these measures in patient care. The recent monarch E clinical trial prospectively investigated Ki-67 as a biomarker of cyclin-dependent kinase inhibitor (CDKI), Abemaciclib in the adjuvant setting. It established the benefit of CDKI in high-risk ER-positive breast cancer patients with Ki-67 expression >20%, which promoted the increased clinical demand for routine Ki-67 testing in pathology laboratories. This review summarizes some recent developments and practical issues for Ki-67 evaluation.

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Introduction

For the past several decades, markers of cellular proliferation in breast cancer have been seen to indicate prognosis and predict benefits from antineoplastic therapies. The most common method to measure cell proliferation is using immunohistochemistry (IHC) assays to measure Ki-67.

Ki-67 is a nuclear protein and a biomarker of cellular proliferation. A MIB-1 monoclonal antibody is used to assess Ki-67 by IHC. Clinically, breast cancers are categorized into three major groups based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). There are luminal (ER+ and HER2-), HER2-positive (ER+ or -, and HER2+), and triple-negative (TN) breast cancers (ER- and HER2-). As a prognostic marker, Ki-67 was used to differentiate luminal A and luminal B breast cancer molecular subtypes with a cutoff of 14%, which was changed to >20% in 2013 by the breast expert at the St Gallen International Breast Cancer Conference.[1–3] This review summarizes some recent developments and practical issues for Ki-67 evaluation. This review aimed to provide insights into these developments, enhancing our understanding and application of Ki-67 as a reliable biomarker for breast cancer prognosis and therapy management.

Ki67 is useful in determining prognosis in ER+, HER2- breast cancer to identify those who can avoid adjuvant chemotherapy

The percentage of Ki-67 positive tumor cells is used to estimate prognosis in early-stage breast cancer regarding whether further adjuvant chemotherapy is warranted. The phase III POETIC trial investigated long-term prognostic outcomes from Ki67 after perioperative endocrine therapy in postmenopausal women with the hormonal receptor (HR)+ early breast cancer. It demonstrated that patients with a Ki-67 index <10% are known to have a lower recurrence risk and could avoid neoadjuvant endocrine therapy.[4] The ADAPT trial bolstered evidence for short-course neoadjuvant endocrine therapy based on the Ki-67 index which can identify patients who can be spared intensive adjuvant chemotherapy.[5,6] In addition, it has been used as a predictive biomarker while monitoring patients during or following administration of neoadjuvant endocrine or chemotherapy to determine treatment efficacy.

Breast cancer is evaluated from tumor characteristics such as its histopathologic type, grade, size, lymph nodal status, and distant metastasis. The 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual integrates traditional anatomic staging with biological factors, including ER, PR, HER2, and multigene assays, to create a Clinical Prognostic Stage Group.[7] Multigene assays are used in clinical decision-making in determining whether to give chemotherapy in ER+, HER2- breast cancer. There are a variety of prognostic essays, among which are the Oncotype DX Recurrence Score (RS), Prosigna (PAM50), Risk of Recurrence (ROR), EndoPredict (EP), and Breast Cancer Index (BCI). Oncotype Dx RS is the most commonly used multigene assay in the United States. Despite the many genes present in these assays, recurrence risk scores are weighted and most heavily correlated with HR status and the Ki-67 proliferation index.[8]

Keywords: Breast cancer; Ki-67; Immunohistochemistry; CDKI; ER-positive.
Abbreviations: AI, artificial intelligence; ASCO, American Society of Clinical Oncology; CDKI, cyclin-dependent kinase inhibitor; DIA, digital image analysis; ER, estrogen receptor; IKWG, International Ki-67 in Breast Cancer Working Group; IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2; HR, hormonal receptor; PR, progesterone receptor; TN, triple-negative.
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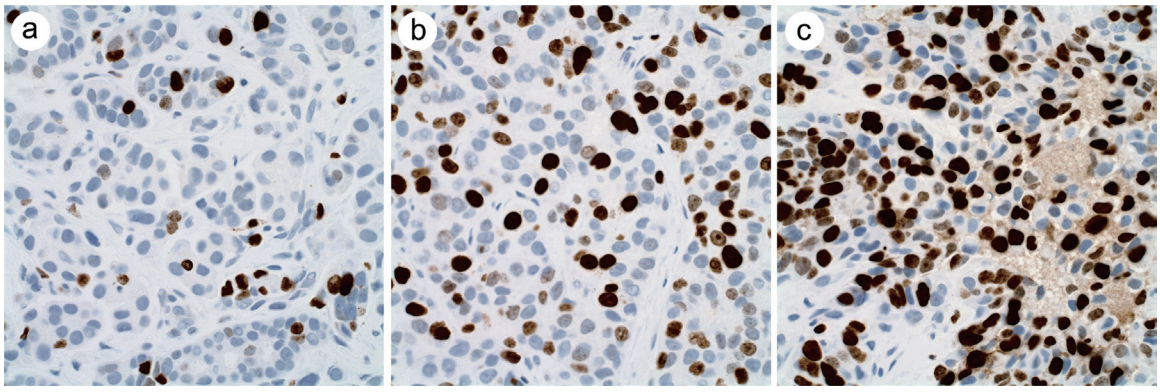

Fig. 1. Ki-67 IHC was variably percentage and intensity. (a) 10% Ki-67 immunolabeling, (b) 30% Ki-67 immunolabeling, (c) 80% Ki-67 labeling. Photomicrographs were taken with 40× objectives at 400× magnification.

High-risk ER+ breast cancer with Ki-67 >20% is beneficial for using CDKI in the adjuvant setting

The recent monarchE clinical trial was the first to prospectively investigate Ki-67 as a biomarker in a phase III trial of cyclin-dependent kinase inhibitor (CDKI) abemaciclib in the adjuvant setting.[5] It established the benefit of a CDKI for adjuvant treatment of adult patients with HR+, HER2-, node-positive, early-stage breast cancer at high risk of recurrence with a Ki-67 score ≥20%. An updated monarch E trial reported that the abemaciclib benefit can extend beyond 2-years post-treatment, and a Ki-67 ≥20% correlated well with prognosis in conjunction with the overall clinicopathological profile to identify patients with a high risk of recurrence. However, while Ki-67 is prognostic, the study also found that the use of adjuvant abemaciclib in combination with endocrine treatment benefited high-risk patients based on clinicopathologic features regardless of Ki-67.[9]

IKWG established a standardized visual scoring method for clinical assessment

With a lack of consensus regarding the definition of low versus high expression or an appropriate cutoff point for positivity and tremendous observer variability in the clinically relevant 10–20% range, the evaluation of Ki-67 has not currently received a recommendation by either the American Society of Clinical Oncology (ASCO) or the National Comprehensive Cancer Network (NCCN). Recent attempts to integrate Ki-67 workflows are supported by data from several clinical trials suggesting its potential for guiding therapeutic decisions.

The recent trials, particularly monarch E, promoted clinical demand for Ki-67 testing in routine pathology practice. The *International Ki-67 in Breast Cancer Working Group (IKWG)* has established a standardized visual scoring method for clinical assessment,[10,11] which addresses the detail of preanalytic and analytic issues in laboratory practice, such as preanalytic variables and delays in fixation which can lead to decreased labeling. Analytic validity for expression of <5% to >30% is generally consistent; however, substantial inter-observer/laboratory variability is observed in the range of >5 to <30%.[10] In the range of >5 to <30% by IHC, multigene expression assay such as Oncotype Dx is recommended by ASCO.

Ki-67 analysis by digital pathology

Advances in artificial intelligence (AI) and the growing digitization in pathology are shouldering new opportunities for computational approaches to biomarker quantification.[12,13]

Previous publications have used virtual dual staining with cytokeratin and Ki-67 to quantify positive and negative tumor cells.[14,15] Koopman *et al*. evaluated 154 consecutive invasive breast cancers which underwent dual staining for Ki-67 and cytokeratin 8/18 with IHC and compared scoring by digital image analysis (DIA) and manual analysis.[14] Spearman's correlation coefficients for the inter-observer agreement were 0.94 between manual analysis and platform A, 0.93 between manual analysis and platform B, and 0.96 between DIA platforms. Importantly, higher inter-platform agreement for DIA platforms over manual analysis supports its use in clinical practice.

Even without dual staining, DIA has been demonstrated by Acs *et al*. to have outstanding reproducibility, both within and between different platforms, including an open-access platform (QuPath).[16] Computational methods have been proposed to assess intratumoral heterogeneity of proliferation rate. Plancoulaine *et al*. utilized a hexagonal grid to compute the bimodality and spatial entropy of Ki-67 to assess intratumoral heterogeneity.[17] Studies that followed have demonstrated that bimodality of the intratumoral proliferation rate, not the level itself, was the best independent predictor of survival.[18,19] With this in mind, too much emphasis may be placed on the concordance of Ki-67 by DIA with manual analysis.

Practical issues in routine laboratory testing for Ki-67

Here we shed light on practical issues regarding the laboratory reporting of Ki-67. Careful attention to these issues can improve both the reproducibility and robustness of the results obtained.

Among the controversies surrounding the Ki-67 assay are different counting methodologies. The most significant issue for Ki-67 evaluation is the differences in staining between individual cases. Tumor cells also exhibit variability in the intensity of nuclear staining with apparent intratumoral "hot" and "cold" spots (Fig. 1). The *IKWG* has endorsed global or average quantification as opposed to "hot spot" quantification, where only target areas with the highest proliferative activity are evaluated. Hot spot quantification has been associated with higher variability.[20] In our practice, we follow the recommendations of the *IKWG*. An average Ki-67 is reported with a global assessment of the percentage of "hot" and "cold" spots. This practice is hypothesized to grant better agreement amongst breast pathologists and possibly

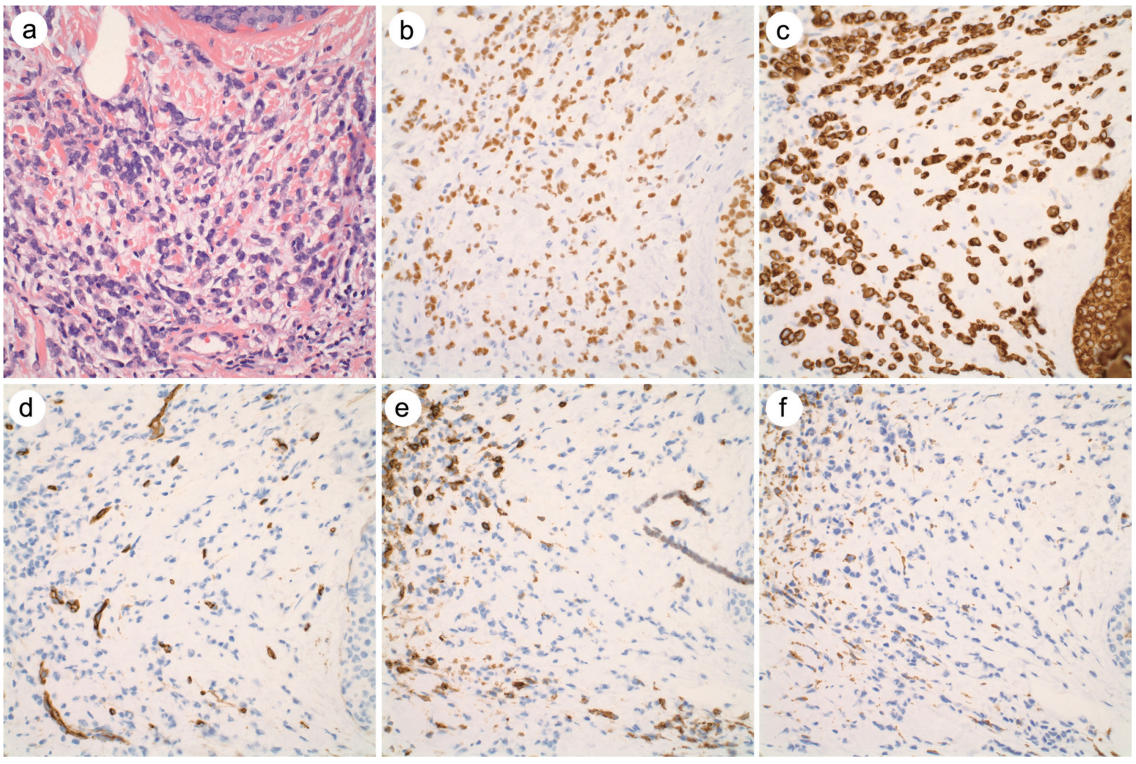

Fig. 2. An example of invasive lobular carcinoma with high background of non-tumor cells. (a) Invasive lobular carcinoma H&E. (b) ER, if the ER is considered 100%, as a surrogate for tumor cell baseline, the Ki-67 can be over-estimated; if ER expression is read as 70%, the Ki-67 can be under-estimated. (c) Cytokeratin AE1/AE3 highlighted tumor cells (d) CD34 highlighted stromal fibroblasts. (e) CD45 highlighted infiltrating lymphocytes. (f) CD68 highlighted macrophages. Photomicrographs were taken with 20× objectives at 200× magnification.

a better correlation with both DIA and the Oncotype Dx RS. Reporting a "Ki-67 = 15% with focal up to 30%" could be misleading for the clinician and lead to difficulty in making an appropriate treatment decision.

It is important to mention that IHC protocols, such as choice of primary antibody (Ab) clone, format, and staining platform can influence IHC assays and Ki-67 results. Røge *et al*.[21]

demonstrated mean Ki-67 scoring on the Ventana BenchMark ULTRA platform to be 11.9% higher than the mean, while Ab 30.9 RTU on the Ventana platform was 10.4% above the mean. MIB1 Ready-To-Use (Dako Autostainer Link 48) and MM1 Ready-To-Use (Leica Bond) were 8.6% and 12.5% below the mean, while MIB1 concentrated and SP6 concentrated on the Dako Autostainer, and Leica Bond provided results near the mean. Pathologists need to consider these differences when reporting Ki-67.

We also noted a more problematic Ki-67 reading when the tumors morphologically showed the following features: lack of tubule formation/lobular feature, pauci-cellularity, low nuclear grade, and inflammatory cell infiltration (Fig. 2). These features could potentially over-or underestimate the proliferation index, create disagreement, and confound pathologists during manual analysis. These problems may be not easily resolved with digital pathology as training AI models for Ki-67 detection requires ground truth annotations by human observers and properly distinguishing invasive lobular carcinoma from background negative cells. Non-tumor cells such as fibroblasts, lymphocytes, and macrophages are indistinguishable from the non-glandular forming tumor cells found on the IHC slide. Since tumors with these features are relatively uncommon, we found that adding a cytokeratin stain is most helpful when encountering such cases. With refined technology, and awareness of salient issues, high-quality routine laboratory reporting of Ki-67 is achievable.

Conclusions

We currently still mostly rely on manual analysis of immunohistochemistry for the evaluation of Ki-67 in clinical practice. Improved reproducibility may be achieved through future approaches in digital pathology. However, robust, well-designed applications must be developed which are intuitive for pathologists to use while considering the holistic issues related to laboratory reporting of Ki-67.

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Conflict of interest

SH is the co-founder of CloudPath Diagnostics LLC, New York. YW has been an editorial board member of the *Journal of Clinical and Translational Pathology* since May 2021. The authors have no other conflicts of interest to disclose.

Author contributions

SH and YW drafted, read, revised, and approved the final submission. All authors have made a significant contribution to this manuscript.

References

[1] Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, *et al*. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101(10):736–750. doi:10.1093/jnci/djp082, PMID:19436038.

[2] Feeley LP, Mulligan AM, Pinnaduwage D, Bull SB, Andrulis IL. Distinguishing luminal breast cancer subtypes by Ki67, progesterone receptor or TP53 status provides prognostic information. Mod Pathol 2014;27(4):554–561. doi:10.1038/modpathol.2013.153, PMID:24051696.

[3] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ, *et al*. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22(8):1736–1747. doi:10.1093/annonc/mdr304, PMID:21709140.

[4] Smith I, Robertson J, Kilburn L, Wilcox M, Evans A, Holcombe C, *et al*. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. Lancet Oncol 2020;21(11):1443–1454. doi:10.1016/S1470-2045(20)30458-7, PMID:33152284.

[5] Harbeck N, Rastogi P, Martin M, Tolaney SM, Shao ZM, Fasching PA, *et al*. Adjuvant abemaciclib combined with endocrine therapy for high-risk early breast cancer: updated efficacy and Ki-67 analysis from the monarchE study. Ann Oncol 2021;32(12):1571–1581. doi:10.1016/j.annonc.2021.09.015, PMID:34656740.

[6] Kuemmel S, Gluz O, Nitz U, Braun M, Christgen M, Luedtke-Heckenkamp K, *et al*. Abstract GS4-03: Neoadjuvant nab-paclitaxel weekly versus dose-dense paclitaxel followed by dose-dense EC in high risk HR+/HER2- early BC by: Results from the neoadjuvant part of ADAPT HR+/HER2- trial. Cancer Res 2021;81(4_Supplement):GS4–03. doi:10.1158/1538-7445.SABCS20-GS4-03.

[7] Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, *et al*. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 2017;67(2):93–99. doi:10.3322/caac.21388, PMID:28094848.

[8] Sahebjam S, Aloyz R, Pilavdzic D, Brisson ML, Ferrario C, Bouganim N, *et al*. Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score. Br J Cancer 2011;105(9):1342–1345. doi:10.1038/bjc.2011.402, PMID:21970880.

[9] Johnston SRD, Harbeck N, Hegg R, Toi M, Martin M, Shao ZM, *et al*. Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR+, HER2-, Node-Positive, High-Risk, Early Breast Cancer (monarchE). J Clin Oncol 2020;38(34):3987–3998. doi:10.1200/JCO.20.02514, PMID:32954927.

[10] Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, *et al*. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 2021;113(7):808–819. doi:10.1093/jnci/djaa201, PMID:33369635.

[11] Leung SCY, Nielsen TO, Zabaglo L, Arun I, Badve SS, Bane AL, *et al*. Analytical validation of a standardized scoring protocol for Ki67: phase 3 of an international multicenter collaboration. NPJ Breast Cancer 2016;2:16014. doi:10.1038/npjbcancer.2016.14, PMID:28721378.

[12] Ibrahim A, Gamble P, Jaroensri R, Abdelsamea MM, Mermel CH, Chen PC, *et al*. Artificial intelligence in digital breast pathology: Techniques and applications. Breast 2020;49:267–273. doi:10.1016/j.breast.2019.12.007, PMID:31935669.

[13] Yousif M, van Diest PJ, Laurinavicius A, Rimm D, van der Laak J, Madabhushi A, *et al*. Artificial intelligence applied to breast pathology. Virchows Arch 2022;480(1):191–209. doi:10.1007/s00428-021-03213-3, PMID:34791536.

[14] Koopman T, Buikema HJ, Hollema H, de Bock GH, van der Vegt B. Digital image analysis of Ki67 proliferation index in breast cancer using virtual dual staining on whole tissue sections: clinical validation and inter-platform agreement. Breast Cancer Res Treat 2018;169(1):33–42. doi:10.1007/s10549-018-4669-2, PMID:29349710.

[15] Røge R, Riber-Hansen R, Nielsen S, Vyberg M. Proliferation assessment in breast carcinomas using digital image analysis based on virtual Ki67/cytokeratin double staining. Breast Cancer Res Treat 2016;158(1):11–19. doi:10.1007/s10549-016-3852-6, PMID:27283833.

[16] Acs B, Pelekanou V, Bai Y, Martinez-Morilla S, Toki M, Leung SCY, *et al*. Ki67 reproducibility using digital image analysis: an inter-platform and inter-operator study. Lab Invest 2019;99(1):107–117. doi:10.1038/s41374-018-0123-7, PMID:30181553.

[17] Plancoulaine B, Laurinaviciene A, Herlin P, Besusparis J, Meskauskas R, Baltrusaityte I, *et al*. A methodology for comprehensive breast cancer Ki67 labeling index with intra-tumor heterogeneity appraisal based on hexagonal tiling of digital image analysis data. Virchows Arch 2015;467:711–722. doi:10.1007/s00428-015-1865-x, PMID:26481244.

[18] Zilenaite D, Rasmusson A, Augulis R, Besusparis J, Laurinaviciene A, Plancoulaine B, *et al*. Independent Prognostic Value of Intratumoral Heterogeneity and Immune Response Features by Automated Digital Immunohistochemistry Analysis in Early Hormone Receptor-Positive Breast Carcinoma. Front Oncol 2020;10:950. doi:10.3389/fonc.2020.00950, PMID:32612954.

[19] Laurinavicius A, Plancoulaine B, Rasmusson A, Besusparis J, Augulis R, Meskauskas R, *et al*. Bimodality of intratumor Ki67 expression is an independent prognostic factor of overall survival in patients with invasive breast carcinoma. Virchows Arch 2016;468(4):493–502. doi:10.1007/s00428-016-1907-z, PMID:26818835.

[20] Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, *et al*. An international Ki67 reproducibility study. J Natl Cancer Inst 2013;105(24):1897–1906. doi:10.1093/jnci/djt306, PMID:24203987.

[21] Røge R, Nielsen S, Riber-Hansen R, Vyberg M. Impact of Primary Antibody Clone, Format, and Stainer Platform on Ki67 Proliferation Indices in Breast Carcinomas. Appl Immunohistochem Mol Morphol 2019;27(10):732–739. doi:10.1097/PAI.0000000000000799, PMID:31460869.