Favorable Reproducibility of Ki-67-Labeling Index between Core Needle Biopsy and Surgical Materials in Mammary Carcinoma: Reproducibility Influenced by Hot Spots, a High Ki-67 Labeling Index, and the Total Length of Biopsy Material

Kanako Ogura1*, Toshiharu Matsumoto1, Asumi Sakaguchi1, Hiroko Onagi1, Ayako Ura1, Taijiro Kosaka2, Toshiaki Kitabatake2, Kuniaki Kojima2, Toshio Morizane3

1Departments of Diagnostic Pathology, Juntendo University Nerima Hospital, Tokyo, Japan
2Breast Surgery, Juntendo University Nerima Hospital, Tokyo, Japan
3Japan Council for Quality Health Care, Tokyo, Japan
Email: *kanakogu@juntendo-nerima.jp

Abstract

Aims: The reproducibility of Ki-67 between core-needle biopsies and surgical materials has not been well documented in the literature, although the concordance affects the utility of the Ki-67 labeling index based on the core-needle biopsy materials, which indicates the need for preoperative chemotherapy. The aim of this study was to reveal the reproducibility of Ki-67 between both materials and the cause of discrepancies. Methods and Results: We analyzed 137 cases of invasive carcinoma of the breast and the compared Ki-67-labeling index between core-needle biopsy and surgical materials. The Ki-67-labeling index of biopsy and surgical specimens ranged from 1% to 85% (median: 13%) and 1% to 80% (median: 12%), respectively. The discrepancy of Ki-67-labeling ranged from 0% to 55% (median: 4%) and could be calculated by the tumor size, hot spots of surgical materials, a high Ki-67-labeling index based on the core-needle biopsy materials, and the total length of biopsy material, respectively. Conclusions: The concordance rate of the Ki-67-labeling index between core-needle biopsies and surgical materials was favorable, so we can use each Ki-67-labeling index of core-needle biopsies as a marker for preoperative chemotherapy. Factors affecting the index discrepancy were hot spots, a high Ki-67-labeling index, and the total length of biopsy material. Judgements on the subtypes and clinical procedures of invasive breast carci-
noma could be made comprehensively based on not only the Ki-67-labeling index but also the existence of hot spots and histological grade.

**Keywords**

Ki-67, Core-Needle Biopsy, Breast, Invasive Carcinoma, Hot Spots

---

**1. Introduction**

Ki-67 is a nuclear protein associated with cellular proliferation, and it was originally identified by Gerdes et al. in the early 1980s using a mouse monoclonal antibody directed against a nuclear antigen from a Hodgkin’s lymphoma-descended cell line [1]. Ki-67 has been widely used as a grading marker in non-Hodgkin’s lymphoma or glioma.

In the field of breast cancer, the usefulness of Ki-67 has been focused on over the last decade. It has been documented that the Ki-67 labeling index is well correlated with the histological grade and prognosis [2]. Cheang et al. classified the tumors into luminal A and B based on the gene profile expression using the Ki-67-labeling index and they set the cutoff value at 13.25 [3]. After that, it was proposed that the cutoff value should be set at 14.0% for the classification between luminal A and B [4].

However, the reproducibility of the Ki-67-labeling index has become a major problem, and there have been some reports on this. The optimal cut-off value of the Ki-67-labeling index has been controversial, although there have been a number of reports on it [5] [6] [7] [8]. At the 2015 St. Gallen Consensus Conference, there was no optimal cut point of the Ki-67-labeling index. Recently, the cutoff value has been set for each laboratory [9].

Although there is a possibility that alternative procedures such as new genetic tests will be established in the future, the decision on the therapeutic strategy will continue to be made for some time based on the Ki-67-labeling index, with its cutoff value set for each laboratory.

Thus, the reproducibility of Ki-67 between core-needle biopsies and surgical materials has not been well documented in the literature, although there have been some reports about interobserver reproducibility. It has been decided whether preoperative chemotherapy should be performed based on the result of the Ki-67-labeling index of core-needle biopsy. We must reconsider the judgement of preoperative chemotherapy based only on the Ki-67-labeling index of core-needle biopsy if the concordance rate of the index between core-needle biopsy and surgical materials is low.

The aim of this study was to clarify the reproducibility of Ki-67 between core-needle biopsies and surgical materials in breast cancer and the cause of discrepancies. We examined the concordance rate of the Ki-67-labeling index between core-needle biopsies and surgical materials in invasive cancers of more than 1 cm in patients who had not received preoperative chemotherapy.
2. Materials and Methods

2.1. Patient Selection

One hundred and eighty-nine cases of invasive carcinoma larger than 10 mm were obtained from the database at our laboratory in a period of three years. All cases were diagnosed based on the textbook of the WHO Classification Tumours of the Breast (4th edition) [10]. Fifty-two cases were excluded from the study: preoperative chemotherapy was performed in 49 cases, only the ductal component was identified in the biopsy material in one case, and biopsy samples were too small to make a definite diagnosis of carcinoma in two cases. As a result, we analyzed 137 cases of invasive ductal carcinoma. We compared immunohistochemistry for Ki-67 in both core needle biopsies and surgical specimens (total or partial mastectomies). The age of the study subjects ranged from 28 to 88 years (median: 59 years) (Figure 1), and the size ranged from 10 to 100 mm (median: 19 mm) (Figure 2).

2.2. Light Microscopy and Immunohistochemistry

Tissue samples (both core-needle biopsies and surgical specimens) were fixed in 15% formalin. The time needed for fixation in both core-needle biopsies and surgical specimens ranged from 20 to 24 and 20 to 48 hours, respectively. Especially in the cases of total mastectomy, the time of the fixation had a tendency to be longer because of thicker materials. Hematoxylin and eosin staining was performed on 3.5-μm-thick sections of formalin-fixed paraffin-embedded tissue.

Immunohistochemistry for Ki-67 (MIB-1, Dako, Tokyo, Japan), ER (SP1, Roche, Tokyo, Japan), PR (1E2, Roche, Tokyo, Japan), and HER2 (4B5, Roche, Tokyo, Japan) was performed on sections from each case by the Labelled Streptavidin-Biotin method using LT Benchmark (Ventana, Yokohama, Japan).

Ki-67 antibody was used at a dilution of 1:200. ER, PR, and HER2 were antibodies that had already been diluted. Specimens were treated by incubating them with ethylenediamine tetraacetic acid buffer (10 mmol/L sodium-citrate monohydrate, pH 8.5) at 100˚C for 30 min. After washing in 0.01 mol/L phosphate-buffered saline, endogenous peroxidase activity was blocked by treating for 20 min with 0.3% aqueous hydrogen peroxidase. Visualization was performed with diaminobenzidine (Dako Japan).

2.3. Assessment of Light Microscopy and Immunohistochemistry

We examined 137 cases of invasive carcinoma microscopically based on the histological subtypes, nuclear and histological atypia, tissue sample degeneration, and hot spots. We judged tissue sample degeneration to be present when tumor cells tended to be incohesive because of poor fixation (Figure 3). Tissue sample degeneration tended to occur in thick breast specimens obtained by mastectomy, and no degeneration was noted in any core-needle samples.

The Ki-67-labeling index was defined as the percentage of Ki-67-positive cells among all nuclei counted in a section of confirmed invasive carcinoma [11]. At
Figure 1. Age distribution (n = 137 cases).

Figure 2. Size distribution (n = 137 cases).

Figure 3. Tissue sample degeneration (H-E stain, ×100).
least 500 invasive carcinoma cells in a “hot spot” lesion were counted for each case by the same pathologist experienced in counting Ki-67 in breast carcinoma. A Ki-67-labeling index being different for each high-power field up to 20% was defined as a hot spot in surgical materials (Figure 4).

2.4. Statistical Analysis
Multivariate analysis using multiple logistic regression was performed. In the multivariate analysis, dependent variables were defined as Ki-67-labeling indexes of biopsy and surgical materials, and independent variables included histological subtypes, nuclear and histological atypia, hormone receptors, HER2, surgical method (partial or total mastectomy), tissue sample degeneration, hot spots, and the number and total length of core needle biopsies.

Based on the results of multivariate analysis, bivariate analysis was also performed.

The current study was approved by the Research Ethics Committee of Jun-tendo University Nerima Hospital.

3. Results
We summarized the clinicopathological features of 137 cases of breast carcinoma in Table 1.

The Ki-67-labeling index of biopsy and surgical specimens ranged from 1 to 85% (median: 13%) and 1% to 80% (median: 12%), respectively. The discrepancy of the Ki-67-labeling index between biopsy and surgical specimens ranged from 0% to 55% (median: 4%) (Figure 5).

Based on bivariate analysis, the discrepancy of the Ki-67-labeling index between biopsy and surgical materials can be calculated by the tumor size (p = 0.05), hot spots of surgical materials (p < 0.01), a high Ki-67-labeling index based on the core-needle biopsy materials (p < 0.01), and the total length of core needles (p = 0.02), respectively (Figure 6).

Furthermore, based on multivariate analysis, hot spots (p < 0.01), a high Ki-67-labeling index in biopsy materials (p < 0.01), and the total length of core needles (p = 0.04) were respectively correlated with the discrepancy of the Ki-67-labeling index between biopsy and surgical specimens (Table 2).

There were 8 cases (6%) with a discrepancy of the Ki-67-labeling index of more than 20%. We summarized these cases in Table 3. These cases showed tendencies such as a higher Ki-67-labeling index of core-needle biopsies (p < 0.01), a higher histological grade (p = 0.02), and more frequent hot spots (p < 0.01). In only one case (case #4), the tumor subtype was different based on the Ki-67-labeling index between core-needle biopsy and surgical materials.

4. Discussion
The use of Ki-67 as a predictive and prognostic marker in breast cancer has been widely investigated. The panel of experts at the St. Gallen Consensus in 2009
Table 1. Clinicopathological findings of invasive carcinoma of breast (all 137 cases).

| Clinicopathological Findings | Results |
|------------------------------|---------|
| Age                          | 28 - 88 (median: 59) |
| Size (mm)                    | 10 - 100 (median: 19) |
| Histology                    |         |
| NST                          | 109 (80.6%) |
| ILC                          | 13 (9.5%)  |
| TC                           | 2 (1.5%)   |
| MUC                          | 9 (6.6%)   |
| Micropap                     | 1 (0.7%)   |
| Metaplastic                  | 2 (1.5%)   |
| Small                        | 1 (0.7%)   |
| Grade                        |         |
| nuclear                      |         |
| NG1                          | 20 (17.7%) |
| NG2                          | 88 (77.9%) |
| NG3                          | 15 (13.3%) |
| histological                 |         |
| HG1                          | 42 (37.2%) |
| HG2                          | 67 (59.3%) |
| HG3                          | 14 (12.4%) |
| Subtypes                     |         |
| Luminal A                    | 69 (50.4%) |
| Luminal B                    | 50 (36.5%) |
| Luminal-HER2                 | 6 (4.4%)   |
| Triple-negative              | 9 (6.6%)   |
| HER2                         | 3 (2.2%)   |
| Surgical method              |         |
| Total mastectomy             | 64 (46.7%) |
| Partial mastectomy           | 73 (53.3%) |
| Tissue degeneration          |         |
| +                            | 34 (24.8%) |
| -                            | 103 (75.2%) |
| Hot spots                    |         |
| +                            | 56 (40.9%) |
| -                            | 81 (59.1%) |
| number of cores              |         |
| total length of core needle materials (mm) | 1-21 (median 2) |

1) NST: invasive carcinoma of no special type, 2) ILC: invasive lobular carcinoma, 3) TC: tubular carcinoma, 4) MUC: mucinous carcinoma, 5) Micropap: invasive micropapillary carcinoma, 6) Metaplastic: Metaplastic carcinoma of no special type, 7) small: small cell carcinoma (neuroendocrine carcinoma, poorly differentiated), 8) +: positive.
### Table 2. Multivariate analysis using multiple logistic regressions including hot spots, Ki-67 of core-needle biopsy, and core-needle length as explanatory variables.

| Explanatory Variable | Estimate | Standard Error | t-value | Pr (>|t|) |
|----------------------|----------|----------------|---------|-----------|
| (Intercept)          | 0.66026  | 1.46407        | 0.451   | 0.652743  |
| Hot spots            | 4.1177   | 1.19623        | 3.442   | 0.000772  |
| Ki-67_biopsy         | 0.10828  | 0.03714        | 2.915   | 0.004173  |
| Needle_length        | 0.10363  | 0.05194        | 1.995   | 0.048073  |

### Table 3. The 8 cases with discrepancy of the Ki-67-labeling index accounting for more than 20%.

| Case | Age (mm) | Size (mm) | Histology | Ki-67-labeling index (%) | Grade | Surgical | Surgical | Tissue degeneration | Hot spots | Number of cores | Total length of cores (mm) |
|------|----------|-----------|-----------|--------------------------|-------|----------|----------|---------------------|-----------|------------------|----------------------------|
| 1    | 63       | 38        | NST       | 45                      | 25    | 20       | 22       | Luminal B            | m\(^1\)    | +               | 1                          | 20                         |
| 2    | 60       | 19        | NST       | 75                      | 55    | 20       | 33       | Triple-negative      | p\(^2\)    | +               | 2                          | 24                         |
| 3    | 59       | 20        | NST       | 25                      | 14    | 20       | 22       | Luminal B            | p          | +               | 1                          | 20                         |
| 4    | 41       | 17        | NST       | 27                      | 6     | 21       | 22       | Luminal A            | p          | -               | 3                          | 34                         |
| 5    | 61       | 25        | NST       | 60                      | 36    | 25       | 22       | Triple-negative      | m          | -               | 1                          | 21                         |
| 6    | 44       | 75        | ILC       | 45                      | 18    | 27       | 22       | Luminal-HER2         | m          | +               | 6                          | 66                         |
| 7    | 78       | 32        | NST       | 20                      | 48    | 28       | 33       | Triple-negative      | m          | +               | 1                          | 20                         |
| 8    | 43       | 25        | NST       | 20                      | 75    | 55       | 22       | Luminal B            | p          | +               | 2                          | 32                         |

\(^1\) m: total mastectomy, \(^2\) p: partial mastectomy.

### Figure 4. A Case with Hot Spots of the Ki-67-labeling Index. (a) A case of invasive carcinoma of no special type (H-E stain, \(\times\)200); (b) the Ki-67-labeling index was counted for 25% in the periphery of the tumor (Ki-67 stain, \(\times\)200); (c) the Ki-67-labeling index was counted for 1% in the center of the tumor (Ki-67 stain, \(\times\)200).
Figure 5. Ki-67-labeling Index. (a) core-needle biopsy materials; (b) surgical materials; (c) the discrepancy of the Ki-67-labeling index between core-needle biopsy and surgical materials.
considered the Ki-67-labelling index to be important for selecting the addition of chemotherapy to endocrine therapy in hormone receptor-positive breast cancer patients [4].

Nishimura et al. reported that Ki-67 values before neoadjuvant treatment could be used to predict the disease-free survival of patients [12].

However, the most important problems of using the Ki-67-labeling index for patients with breast cancer have been reproducibility, objectivity, and quantitative capability for Ki-67 assessment in breast cancer. Kontzoglou K. et al. re-

**Figure 6.** Bivariate Analysis (the discrepancy of the Ki-67-labeling index between core-needle biopsy and surgical materials. (a) Size and Discrepancy (Correlation coefficient: 0.1677879, p-value = 0.05002); (b) hot Spots and Discrepancy (Correlation coefficient: 0.3042665, p-value = 0.0003006); (c) Ki-67 Biopsy and Discrepancy (Correlation coefficient: 0.2969948, p-value = 0.0004247); (d) total Length of Core Needles and Discrepancy (Correlation coefficient: 0.1919794, p-value = 0.02461).
ported that further studies are needed in order to establish Ki-67 as a standard prognostic marker in breast cancer, although most studies have established an association between Ki-67 and overall and disease-free survival [13]. Pathmanathan N. and Balleine R.L. reported that pathologists must establish a standardized framework for scoring Ki-67 and communicating the results to a multidisciplinary team [14]. Polly M.Y.C. et al. reported that Ki-67 values and cutoffs for clinical decision-making cannot be transferred between laboratories without standardizing the scoring methodology because the analytical validity is limited [15]. On the other hand, Varga et al. showed that region analysis and individual review on light-microscopy yielded the highest inter-observer reliability [16]. They documented that these results are a slight improvement on previously published data on poor reproducibility, and thus might offer a practical-pragmatic method routinely assess the Ki-67 index in Grade 2 breast carcinomas.

Thus, there are some reports on the reproducibility analysis of Ki-67 in breast cancer, and there was no optimal cutoff point of the Ki-67-labeling index at the 2013 St. Gallen Consensus Conference [17]. The cutoff value has been set for each laboratory based on the 2015 St. Gallen Consensus Conference.

However, there are few reports on the reproducibility of the Ki-67-labeling index between core-needle biopsy and surgical materials in breast cancer. The accuracy of the Ki-67-labeling index in core-needle biopsy materials is very important because it is used to judge the necessity of preoperative chemotherapy. Acs B. et al. suggested that both the Ki-67-labeling index and subtype showed a significant correlation with the pathological response [18]. Additionally, their data also suggested that if a tumor did not respond to neoadjuvant therapy, increased Ki-67 was a poor prognostic marker. In this study, we examined the reproducibility of Ki-67 between core-needle biopsies and surgical materials and the cause of discrepancies.

The rate of discrepancies of the Ki-67-labeling index between core-needle biopsies and surgical materials was about 4%. The concordance rate of the index was favorable, so we can use each Ki-67-labeling index of core-needle biopsies as a marker of preoperative chemotherapy. Based on multivariate analysis using multiple logistic regression, the discrepancy of the Ki-67-labeling index can be calculated by hot spots of surgical materials, a high Ki-67-labeling index in biopsy materials, and the total length of core needles. Furthermore, the tumor size also tended to influence the discrepancy.

Niikura et al. tried to identify the causes of discrepancies in Ki-67-labeling index measurements by different observers under different conditions using breast cancer samples [19]. They reported that the most common reasons for a discrepancy in scores were the selection of the area for counting and the quality of nuclear staining. Shui R. et al. revealed that an overall average assessment across the whole section including hot spots may be a better method of Ki-67-labeling index analysis [20]. So, in our study, the correction rate of hot spot lesions by core-needle biopsy may have been markedly influenced if the tumor size was larger because the materials by core-needle biopsy were more localized. Fur-
thermore, the proliferative activity of a tumor with a high Ki-67-labeling index might easily fluctuate. However, few reports have documented the fluctuation of the Ki-67-labeling index. Horimoto et al. suggested that Ki-67 expression varied in the same estrogen receptor-positive breast carcinoma patients according to the menstrual cycle phase [21]. In our study, there was no correlation between the hormone receptor status and the discrepancy of the Ki-67-labeling index between core-needle samples and surgical specimens. There have been interlaboratory reproducibility studies on the immunohistochemical assessment of Ki-67 [22]. They found that preanalytical factors such as fixation and the method of immunohistochemistry decrease the reproducibility of the Ki-67-labeling index. Although we strictly observed the fixation time for materials, the surgical materials, especially those obtained by total mastectomy, had a tendency to require a much longer fixation time because they were thicker. There were 34 cases of surgical materials (25%) that degenerated. However, degeneration was not correlated with the discrepancy of the Ki-67-labeling index between core-needle and surgical materials.

There were 8 cases (6%) whereby the discrepancy of the Ki-67-labeling index accounted for more than 20%. Three cases were triple-negative cancers and the other 5 cases were luminal A (one case), B (3 cases), and luminal-HER2 (one case) types. These cases showed tendencies such as a higher Ki-67-labeling index of core-needle biopsies, higher histological grade, and more frequent hot spots. So, there is a possibility over- or under-diagnosis in such cases because of the low reproducibility of the Ki-67-labeling index. In only one case of the luminal type (case #4), the Ki-67-labeling index was 27% and 6% in core needle biopsy and surgical materials, respectively. The case involved the possibility of changing the subtype even with a cut-off value of 14 or 20%. Shui R et al. showed that the concordance was relatively low in an intermediate Ki-67-labeling index group (11% - 30%) compared with low (<10%) and high (>30%) Ki-67-labeling index groups [20]. So, it might be necessary to pay close attention counting Ki-67, especially in the intermediate Ki-67-labeling index group of luminal-type cancer. Further study will be needed focused on this group.

We revealed the reproducibility of Ki-67 between core-needle biopsies and surgical materials in breast cancer in our laboratory. The discrepancies of Ki-67-labeling were about 4% and the concordance rate of the index was favorable, so we might be able to use each Ki-67-labeling index of core-needle biopsies as a marker of preoperative chemotherapy. The discrepancy tended to occur in cases with a higher Ki-67-labeling index of core-needle biopsies, higher histological grade, and more frequent hot spots. Decisions on the subtypes and clinical procedures can be made comprehensively based on not only the Ki-67-labeling index but also the existence of hot spots and histological grade.

**Acknowledgements**

The authors thank Mr. Mrozek (Medical English Service, Kyoto, Japan) for or-
organizing the English revision of this article.

A Conflict of Interest Statement

None.

References

[1] Gerde, J., Schwab, U., Lemke, H. and Stein, H. (1983) Production of a Mouse Monoclonal Antibody Reactive with a Human Nuclear Antigen Associated with Cell Proliferation. International Journal of Cancer, 31, 13-20. https://doi.org/10.1002/ijc.2910310104

[2] Inwald, E.C., Klinkhammer-Schalke, M., Hofstadter, F., et al. (2013) Ki-67 Is a Prognostic Parameter in Breast Cancer Patients: Results of a Large Population-Based Cohort of a Cancer Registry. Breast Cancer Research and Treatment, 139, 539-552. https://doi.org/10.1007/s10549-013-2560-8

[3] Cheang, M.C., Chia, S.K., Vodc, D., et al. (2009) Ki67 Index, HER2 Status, and Prognosis of Patients with Luminal B Breast Cancer. Journal of the National Cancer Institute, 101, 736-750. https://doi.org/10.1093/jnci/djp082

[4] Goldhirsch, A., Wood, W.C., Coates, A.S., et al. (2011) Panel Members. 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. Annals of Oncology, 22, 1736-1747. https://doi.org/10.1093/annonc/mdr304

[5] Bustreo, S., Osella-Abate, S., Cassoni, P., et al. (2016) Optimal Ki67 Cut-Off for Luminal Breast Cancer Prognostic Evaluation: A Large Case Series Study with a Long-Term Follow-Up. Breast Cancer Research and Treatment, 157, 363-371. https://doi.org/10.1007/s10549-016-3817-9

[6] Petrelli, F., Viale, G., Cabiddu, M., et al. (2015) Prognostic Value of Different Cut-Off Levels of Ki-67 in Breast Cancer: A Systematic Review and Meta-Analysis of 64,196 Patients. Breast Cancer Research and Treatment, 153, 477-491. https://doi.org/10.1007/s10549-015-3559-0

[7] Alco, G., Bozdogan, A., Selamoglu, D., et al. (2015) Clinical and Histopathological Factors Associated with Ki-67 Expression in Breast Cancer Patients. Oncology Letters, 9, 1046-1054. https://doi.org/10.3892/ol.2015.2852

[8] Ono, M., Tsuda, H., Yunokawa, M., et al. (2015) Prognostic Impact of Ki-67 Labeling Indices with 3 Different Cutoff Values, Histological Grade, and Nuclear Grade in Hormone-Receptor-Positive, HER2-Negative, Node-Negative Invasive Breast Cancers. Breast Cancer, 22, 141-152. https://doi.org/10.1007/s12282-013-0464-4

[9] Coates, A.S., Winer, E.P., Goldhirsch, A., et al. (2015) Tailoring Therapies-Improving the Management of Early Breast Cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Annals of Oncology, 26, 1533-1546. https://doi.org/10.1093/annonc/mdv221

[10] Lakhani, S.R., Ellis, I.O., Schnitt, S.J., et al. (2012) WHO Classification of Tumours of the Breast. 4th Edition.

[11] Dowsett, M., Nielsen, T.O., A’Hern, R., et al. (2011) International Ki-67 in Breast Cancer Working Group. Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. Journal of the National Cancer Institute, 103, 1656-1664. https://doi.org/10.1093/jnci/djr393

[12] Nishimura, R., Osaka, T., Okumura, Y., et al. (2010) Clinical Significance of Ki-67 in Neoadjuvant Chemotherapy for Primary Breast Cancer as a Predictor for Che-
mosensitivity and for Prognosis. *Breast Cancer*, **17**, 269-275. 
[13] Kontzoglou, K., Palla, V., Karaolanis, G., *et al.* (2013) Correlation between Ki67 and Breast Cancer Prognosis. *Oncology*, **84**, 219-225. [https://doi.org/10.1159/000346475](https://doi.org/10.1159/000346475)

[14] Pathmanathan, N. and Balleine, R.L. (2013) Ki67 and Proliferation in Breast Cancer. *Journal of Clinical Pathology*, **66**, 512-516. [https://doi.org/10.1136/jclinpath-2012-201085](https://doi.org/10.1136/jclinpath-2012-201085)

[15] Polley, M.Y.C., Leung S.C.Y., McShane, L.M., *et al.* (2013) An International Ki67 Reproducibility Study. *Journal National Cancer Institution*, **105**, 1897-1906.

[16] Varga, Z., Cassoly, E., Li, Q., *et al.* (2015) Standardization for KI-67 Assessment in Moderately Differentiated Breast Cancer. A Retrospective Analysis of the SAKK 28/12 Study. *PLoS ONE*, **10**, e0123435.

[17] Goldhirsch, A., Winer, E.P., Coates, A.S., *et al.* (2013) Personalizing the Treatment of Women with Early Breast Cancer: Highlights of St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Annals of Oncology*, **24**, 2206-2223. [https://doi.org/10.1093/annonc/mdt303](https://doi.org/10.1093/annonc/mdt303)

[18] Acs, B., Zambo, V., Vizkeleti, L., *et al.* (2017) Ki-67 as a Controversial Predictive and Prognostic Marker in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. *Diagnostic Pathology*, **12**, 20. [https://doi.org/10.1186/s13000-017-0608-5](https://doi.org/10.1186/s13000-017-0608-5)

[19] Niikura, N., Sakatani, T., Arima, N., *et al.* (2014) Assessment of the Ki-67 Labeling Index: A Japanese Validation Ring Study. *Breast Cancer*, **23**, 92-100. [https://doi.org/10.1007/s12282-014-0536-0](https://doi.org/10.1007/s12282-014-0536-0)

[20] Shui, R., Yu, B., Bi, R., *et al.* (2015) An Interobserver Reproducibility Analysis of Ki67 Visual Assessment in Breast Cancer. *PLoS ONE*, **10**, e0125131. [https://doi.org/10.1371/journal.pone.0125131](https://doi.org/10.1371/journal.pone.0125131)

[21] Horimoto, Y., Arakawa, A., Tanabe, M., *et al.* (2015) Menstrual Cycle Could Affect Ki67 Expression in Estrogen Receptor-Positive Breast Cancer Patients. *Journal of Clinical Pathology*, **68**, 825-829. [https://doi.org/10.1136/jclinpath-2015-203085](https://doi.org/10.1136/jclinpath-2015-203085)

[22] Pinhel, I.F., Macneill, F.A., Hills, M.J., *et al.* (2010) Extreme Loss of Immunoreactive p-Akt and p-Erk1/2 during Routine Fixation of Primary Breast Cancer. *Breast Cancer Research*, **12**, R76. [https://doi.org/10.1186/bcr2719](https://doi.org/10.1186/bcr2719)