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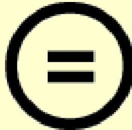

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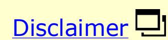

The value of Phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers

(With an emphasis on comparisons to Ki-67)

Ji-Ye Kim

Department of Medicine
The Graduate School, Yonsei University

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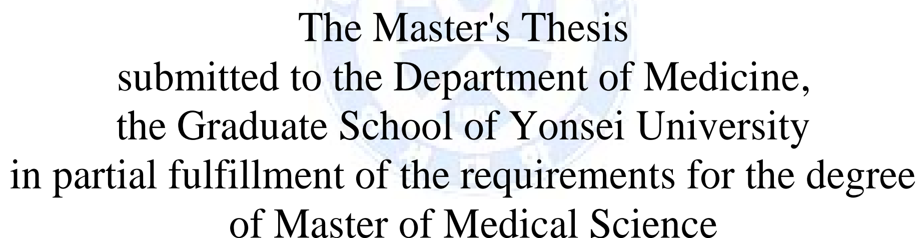
Directed by Professor Ja Seung Koo



The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Ji-Ye Kim

December 2015

This certifies that the Master's Thesis
of Ji-Ye Kim is approved.

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December 2015

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December, 2015

Ji-Ye Kim

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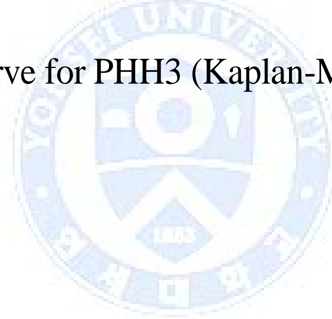

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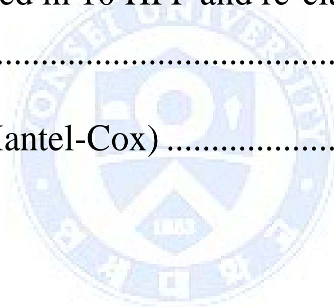

ABSTRACT

The value of Phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers

(With an emphasis on comparisons to Ki-67)

Ji-Ye Kim

Department of Medicine,
The Graduate School, Yonsei University

(Directed by Professor Ja Seung Koo)

INTRODUCTION Ki-67 is a widely used marker of proliferation, but controversial due to problems of non-specificity and lack of reproducibility. In comparison, Phosphohistone H3 (PHH3) is specific marker for mitosis with good reproducibility. We compare the two markers using surgical slides of 220 breast cancer cases diagnosed from 2012 to 2013.

METHODS The most representative sections of invasive breast cancer surgical cases were immunohistochemically stained for Ki-67 and PHH3.

RESULTS Ki-67 and PHH3 had a good positive correlation between each other. PHH3 was tested for inter-rater agreement between two raters using two methods. First method was assessment under a microscope while the second method was assessment with taken

photographs. Both demonstrated nearly perfect intra-class correlation coefficient (0.996, 0.977). PHH3 examined for 10HPF had a tendency to over-grade compared to H-E mitotic index (29 cases, evenly distributed). PHH3 examined at low power (objective 10x) correlated well with scores of 10HPF evidencing ability to accurately identify mitotically active areas(r = 0.999). Finally, PHH3 significantly correlated with recurrence-free survival (P = 0.006), while Ki-67 did not (P = 0.053).

CONCLUSION The problems of Ki-67 for lack of reproducibility and low specificity to measure proliferation may be overcome by the use of PHH3 in diagnosis of breast cancer.

Keywords Ki-67, PHH3, proliferation, invasive breast cancer, reproducibility

The value of Phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers
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I. Introduction

Proliferative activity is an important diagnostic criteria in breast cancers.[1] In practice, proliferative activity of breast cancer is routinely evaluated through assessment of histological grade as the mitotic activity index (MAI), which is largely responsible for the prognostic value of tumor grade.[2] Breast cancer classification based on molecular subtypes also depends on cancer cell proliferative activity, as measured by Ki-67.[3,4] Even in commercially available prognostic molecular assays, such as Oncotype Dx, cancer proliferative activity through the use of Ki-67 is an important determinant factor.[5]

One of the best known ways to measure tumor proliferative activity is assessment of Ki-67 labeling index through immunohistochemical staining

with the MIB-1 antibody. Ki-67 has been reported to be a strong predictive factor in invasive ductal carcinoma without pre-operative chemotherapy treatment[6,7] and used as a prognostic predictor in post-chemotherapy invasive ductal carcinomas. However, Ki-67 is a DNA-binding nuclear protein that is expressed in all actively dividing cells, except for G0 resting phase cells. Because Ki-67 is expressed in all non-mitotic phase cells of G1, S, and G2 phase, it is criticized to be non-specific for proliferation.[5]

Limitations of Ki-67 may be overcome through the use of Phosphohistone H3 (PHH3). PHH3 is a specific marker of mitotic phase cells. Histone H3 is a nuclear core histone protein of the DNA chromatin, where it plays an important role in chromosome condensation and cell-cycle progression during cell mitosis and meiosis after phosphorylation of the serine-10 and serine-28 terminus. Such activating phosphorylation occurs during late G2 phase to early prophase, while dephosphorylation occurs slowly from late anaphase to early telophase of mitosis. Therefore in metaphase, the cell is always heavily phosphorylated, while interphase cells are unstained or minimally stained.[8]

Immunodetection of phosphohistone H3 has been reported in multiple studies concerning various human tumors (colorectal adenocarcinoma, ovarian serous adenocarcinoma, pulmonary neuroendocrine carcinoma, uterine smooth muscle tumors, astrocytomas and meningiomas),

each highlighting its sensitive and specific role as a marker of mitotic figures and correlated well with outcome.[9] In a study investigating the use of PHH3 in breast cancer, H & E mitotic index strongly correlated with PHH3.[10] In that study, the authors concluded that PHH3 can be used in breast grading because PHH3 allows better accuracy in detection of mitotic figures. More recently there has also been a report that PHH3 is a better than Ki-67 as a prognostic factor of breast cancer. However, these studies were conducted through TMA samples, not representative of the problems of tumor heterogeneity or limited in their number of cases (less than 100 cases).[3]

Our study improves on the previous studies because it is conducted on more than 200 cases of surgical whole slides of the most representative tumor section. Our purpose was to compare the most commonly used proliferation marker, Ki-67 with PHH3 and evaluate the value of each in search for the most adequate marker for breast cancer proliferation activity.

II. Materials and Methods

1. Patient selection and histologic evaluation

220 tissue samples from 218 donor patients who had invasive ductal cancer, Not Otherwise Specified, diagnosed and surgically resected at Severance Hospital from January 2012 to December 2013 were analyzed. Those cases that had been treated with pre-operative chemotherapy or radiation therapy were excluded from the study. The study was approved by the Institutional Review Board of Yonsei University Severance Hospital. IRB exempted the informed consent from patients. Hematoxylin & Eosin (H&E)-stained slides from all cases were reviewed by a breast pathologist (Koo JS). Histological grade was assessed using the Nottingham grading system.[11] Clinicopathologic parameters evaluated in each case included patient age at initial diagnosis, lymph node metastasis, tumor recurrence, distant metastasis, and patient survival.

2. Immunohistochemistry

Antibodies used for immunohistochemistry are listed in Table 1. All immunohistochemistry was performed with formalin-fixed, paraffin-embedded tissue sections. The most representative section of the tumor was selected for immunohistochemial staining. Briefly, 5-μm-thick sections were

obtained with a microtome, transferred onto adhesive slides, and dried at 62℃ for 30 minutes. After incubation with primary antibodies, immunodetection was performed with biotinylated anti-mouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3′-diaminobenzidine chromogen as the substrate. The primary antibody incubation step was omitted in the negative control. Positive control tissue was used as per the manufacturer's recommendation. Slides were counterstained with Harris hematoxylin.

Table 1. Antibody sources, clones, and dilutions

Antibody	**Clone**	**Dilution**	**Vendor**
ER	SP1	1:100	Thermo Scientific, CA, USA
PR	PgR	1:50	DAKO, Glostrup, Denmark
HER-2	Polyclonal	1:1500	DAKO, Glostrup, Denmark
Ki-67	MIB1	1:100	DAKO, Glostrup, Denmark
PHH3	Polyclonal	1:100	Cell Marque, Rocklin, CA, USA

3. Interpretation of immunohistochemical staining

All immunohistochemical markers were accessed by light microscopy. A cut-off value of 1% or more positively stained nuclei was used to define ER and PR positivity.[12] HER-2 staining was analyzed according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining, less than 10% of tumor cells; 2+ = complete membranous staining, either uniform or weak in at least 10% of tumor cells; and 3+ = uniform intense membranous staining in at least 30% of tumor cells.[13] HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed, whereas cases with 0 to 1+ were regarded as negative. Cases showing 2+ HER-2 expression were evaluated for HER-2 amplification by fluorescent *in situ* hybridization (FISH).

Scoring method for Ki-67 and PHH3 was as follows. After scanning the tumor area at low power field, four HPFs (objective 40x) that best represents the overall tumor were selected from the invasive front of the tumor. When hot spots were present, these were included for the overall average score. Each field of examination was photographed in order to assure consistency of the field of examination at one time. Then cells were counted manually using the counter application provided by the publicly available image analysis program, Image J. The same tumor area on each stain was assessed in order to eliminate the influence of intratumoral heterogeneity.

Tumor cells were considered positive if there was any nuclear signal above background. Consistent with previous studies,[14,15] intact nuclei with fine granular staining of phosphohistone H3 were not counted as these cells are not in G2 phase. Ki-67 counted for all positively nuclear stained tumor cells. A score was generated by dividing the number of positively stained cells by the total number of cells counted. A final score was taken from the average of four fields.

4. Tumor phenotype classification

In this study, we classified breast cancer phenotypes according to the immunohistochemistry results for ER, PR, HER-2, Ki-67 and FISH results for HER-2 as follows:[16] *luminal A type*, ER or/and PR positive, HER-2 negative and Ki-67 LI < 14%; *Luminal B type*, (HER-2 negative) ER or/and PR positive, HER-2 negative and Ki-67 LI. ≥ 14%; (HER-2 positive) ER or/and PR positive and HER-2 overexpressed or/and amplified; *HER-2 overexpression type*, ER and PR negative and HER-2 overexpressed or/and amplified; *TNBC type*: ER, PR, and HER-2 negative.

5. Inter-rater agreement

For inter-rater agreement, two different methods were assessed. The first method involved a total of 31 PHH3 slides selected in random. These were scored for 10 HPF by two separate raters by individual counting of positive cells under a microscope. Both raters used a web-based counter application while scoring the glass slide under a microscope. A second method involved a total of 220 randomly selected PHH3 photographs and assessed separately by two raters.

6. Statistical analysis

Data were analyzed using SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA). For determination of statistical significance, Student's *t* and Fisher's exact tests were used for continuous and categorical variables, respectively. In the case of analyzing data with multiple comparisons, a corrected p-value with the application of the Bonferroni multiple comparison procedure was used. Correlation between Ki-67 and PHH3 score was analyzed through linear regression and Spearman correlation coefficient. For inter-rater agreement, intra-class correlation coefficient and κ statistics were used. For comparison of dichotomous labelling indices for Ki-67 and phosphohistone H3 with recurrence, an optimal cut-off point was determined

by the use of ROC curves and Youden's index. Ki-67 and PHH3 scores were calculated as both continuous variable and categorical variables. Statistical significance was set to $P < 0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival. Multivariate regression analysis was performed using the Cox proportional hazards model.

III. Results

1. Baseline characteristics of breast cancer

Clinicopathologic characteristics of patients are summarized in Table 2. Of the 220 breast cancer cases evaluated, 218 patients were involved. Median follow up was 31 months (range, 1-93months). During follow up, there were no deaths with 8 local recurrence/metastases.

Table 2. Clinicopathologic characteristics of patients

Parameters	Total N= 220 (%)	Ki-67 LI Mean (%) ± SD	Ki-67 LI *P*	PHH3 Mean (%) ± SD	PHH3 *P*	PHH3/Ki-67 ratio Mean ± SD	PHH3/Ki-67 ratio *P*
Age (years)			0.742		0.883		0.661
≤50	89 (40.5)	25.3± 18.2		0.38± 0.51		0.0136± 0.0176	
>50	131 (59.5)	26.2± 20.1		0.37± 0.48		0.0149± 0.0253	
Nuclear grade			0.074		0.212		0.910
1	18 (8.2)	26.0± 18.3		0.44± 0.65		0.0135± 0.0191	
2	143 (65)	23.8± 18.7		0.33± 0.44		0.0149± 0.0256	
3	59 (26.8)	30.6± 20.5		0.46± 0.54		0.0135± 0.0137	
Histologic grade			**0.009**		0.133		0.636
I	62 (28.2)	25.81 ±20.4		0.31± 0.47		0.0126± 0.0242	
II	102 (46.4)	22.32 ±17.0		0.36± 0.46		0.0159± 0.0253	
III	56 (25.4)	32.15 ±20.6		0.48± 0.55		0.0136± 0.0134	
Tumor stage			0.694		0.802		0.670
T1	150 (68.2)	26.38 ±20.1		0.37± 0.48		0.0136± 0.0200	
T2	66 (30)	24.26 ±16.9		0.38± 0.49		0.0164± 0.0277	
T3	4 (1.8)	29.90 ±29.4		0.53± 0.85		0.0104± 0.0173	
Nodal metastasis			0.671		0.466		0.219

(continued)

Parameters	Total N= 220 (%)	**Ki-67 LI**		**PHH3**		**PHH3/Ki-67 ratio**	
		Mean (%) ± SD	***P***	**Mean (%) ± SD**	***P***	**Mean ± SD**	***P***
Absent	168 (76.4)	26.11 ±19.3		0.36± 0.45		0.0132± 0.0205	
Present	52 (23.6)	24.81 ±19.4		0.42± 0.60		0.0183± 0.0277	
Estrogen receptor			**0.009**		0.095		0.966
Negative	48 (21.8)	32.23 ±21.4		0.48± 0.56		0.0145± 0.0177	
Positive	172 (78.2)	24.01 ±18.3		0.34± 0.46		0.0144± 0.0237	
Progesterone receptor			0.167		0.624		0.289
Negative	106 (48.2)	27.67 ±19.5		0.39± 0.47		0.0127± 0.0147	
Positive	114 (51.8)	24.07 ±19.0		0.36± 0.51		0.0159± 0.0279	
HER-2 status			0.108		0.234		0.975
Negative	186 (84.5)	24.91 ±19.2		0.36± 0.48		0.0144± 0.0237	
Positive	34 (15.5)	30.70 ±19.2		0.47± 0.53		0.0143± 0.0144	
Molecular subtype			0.075		0.102		0.673
Luminal A	105 (47.7)	23.99 ±20.3		0.29± 0.43		0.0130± 0.0242	
Luminal B	67 (30.5)	24.06 ±14.9		0.43± 0.51		0.0165± 0.0229	
HER-2	12 (5.5)	33.54 ±19.2		0.42± 0.53		0.0159± 0.0194	
TNBC	36 (16.3)	31.79 ±22.4		0.50± 0.58		0.0102± 0.0105	

2. Ki-67 was more frequently stained in tumor cells than PHH3

PHH3 was stained for a significantly lower percentage than Ki-67 LI overall. The mean for PHH3 was 0.3763 while Ki-67 was 25.81 (Table 3), while the range for Ki-67 was wide, varying from 0 to 89. In comparison, PHH3 range was much narrow, ranging from 0 to 2.22. While Ki-67 stained in a variety of intensities and in other extraneous cells, other than tumor cells, PHH3 stained in only a few, in strong intensities (Figure 1). At low power, it was easy to distinguish areas of high mitosis and therefore count in those areas of maximum mitosis.

Table 3. Basic analytical comparison between Ki-67 LI and PHH3

Parameters	Mean	Range	Standard Deviation
Ki-67	25.81	0-89	19.29
PHH3	0.3763	0.00-2.22	0.49

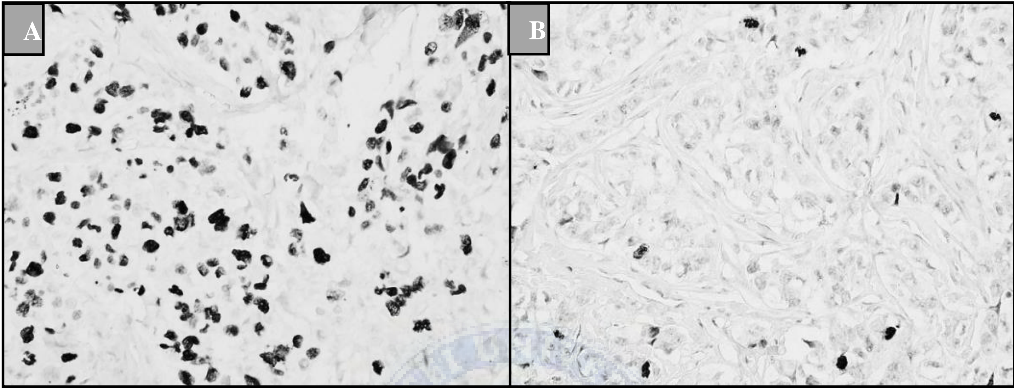

Figure 1. Ki-67 stained in various intensities (A) while the same area for PHH3 stained a few specific mitotically active tumor cells (B).

3. PHH3 and Ki-67 LI had a positive linear correlation

There was a tendency for highly stained Ki-67 LI cases to be also relatively high in PHH3 staining. On Pearson's correlation, $r = 0.504$ ($P < 0.001$), demonstrates a clear positive relationship between the two (Figure 2).

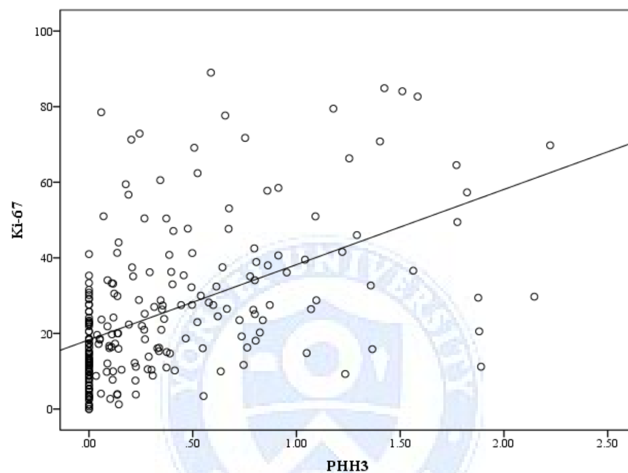

Figure 2. Positive correlation between Ki-67 LI and PHH3 stain percentage, correlation coefficient, $r = 0.504$ ($P < 0.001$).

4. PHH3 had inter-rater agreement between raters using two different methods

Two different methods were used to assess agreement for PHH3 rating. First method involved 31 randomly selected cases, in which PHH3 evaluated through the microscope and counted for ten consecutive high power fields between two raters (Table 4 and Figure 3A, $\kappa = 0.331$). To avoid variations by assessment in varied microscopic fields and evaluate the variation in threshold of immunointensity interpreted as positive by different pathologists, the second method involved 220 randomly selected cases of photographs taken at HPF. These were rated between two raters (Table 4 and Figure 3B). Results for both methods yielded significant κ values ($P < 0.001$) with the method scored with photographs having a much higher agreement than the method scored through the microscope (κ, $0.331 < 0.711$). The intra-class correlation coefficient (ICC) was also assessed in consideration of the ordinal, continuous nature of values. ICC showed a nearly perfect agreement for both methods of microscope (ICC 0.996, 95% CI: 0.991-0.998) and photographs (ICC 0.977, 95% CI: 0.970-0.982).

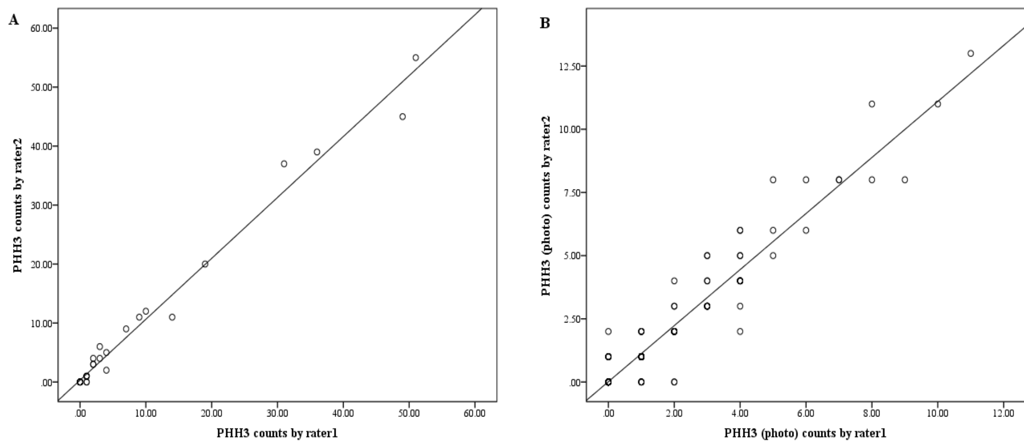

Figure 3. Scatter plot demonstrating agreement between two raters for PHH3. (A) 31 PHH3 stained slides evaluated under the microscope by two independent raters show a positive correlation, $r = 0.992$ ($P < 0.001$). (B) 220 PHH3 photographed cases were evaluated by two independent raters show a positive correlation $r = 0.963$ ($P < 0.001$).

Table 4. Inter-rater agreement for PHH3 and Ki-67

	ICC	95% CI		κ	*P*
Microscope	0.996	0.991	0.998	0.331	< 0.001
Photograph	0.977	0.97	0.982	0.711	< 0.001

5. Breast cancer cases reclassified under Nottingham's criteria for M grade

PHH3-immunostained MFs were counted based on the number of mitotic figures per 10 contiguous high power fields (HPF) in the area of highest mitotic activity, the same way as in hematoxylin and eosin- stained sections. Anti-PHH3-labeled MFs were easily seen and permitted quick identification of the areas of highest mitotic activity. PHH3 counting yielded greater sensitivity and in total of 46 cases suggested a change in grade. Cases which were downgraded from PHH3 counting were 17 cases, predominantly from older blocks, suggesting a loss of antigen preservation. Cases which were upgraded from PHH3 counting were 29 cases, which were evenly distributed amongst the years, thereby prove to yield greater sensitivity for detecting mitosis.

Table 5. PHH3 counted in 10 HPF and re-classified according to M grade

Parameters	Number of cases	Percent (%)
under-graded	16	7.2
over-graded	29	13.0
concordance	175	78.5
Total	220	98.7

6. PHH3 permitted quick identification of the areas of highest mitotic activity

In order to demonstrate the efficacy of PHH3 in identifying areas of mitotically active hot spots in heterogeneous tumors, PHH3 labeled mitotic figures were counted at low power (objective 10x) and compared with counts on ten high power fields. The correlation between PHH3 mitotic index (MI) and PHH3 MI at lower power was high (correlation coefficient, r = 0.999; regression coefficient R^2 = 0.999; P = 0.001). Because there was no change in M grade through both methods of counting, κ statistics reveals a perfect fit of these methods (κ = 1).

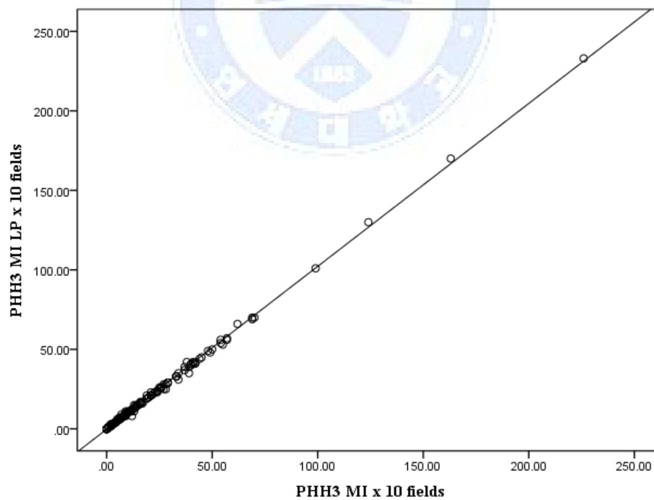

Figure 4. Strong linear correlation between PHH3 MI and PHH3 MI LP (correlation coefficient, r = 0.999; regression coefficient R^2 = 0.999; P = 0.001).

7. PHH3 outperformed Ki-67 LI as a prognostic indicator

Despite the short follow up time (median 31 months), PHH3 was significantly associated with recurrence free survival by Log-rank test (Table 6, $P = 0.006$) while Ki-67 LI failed to be a prognostic indicator ($P = 0.053$). All variables were evaluated for COX-multivariate regression, however neither Ki-67 LI nor PHH3 was a significant factor. Only the number of metastatic lymph nodes was shown to be of significance ($P = 0.005$).

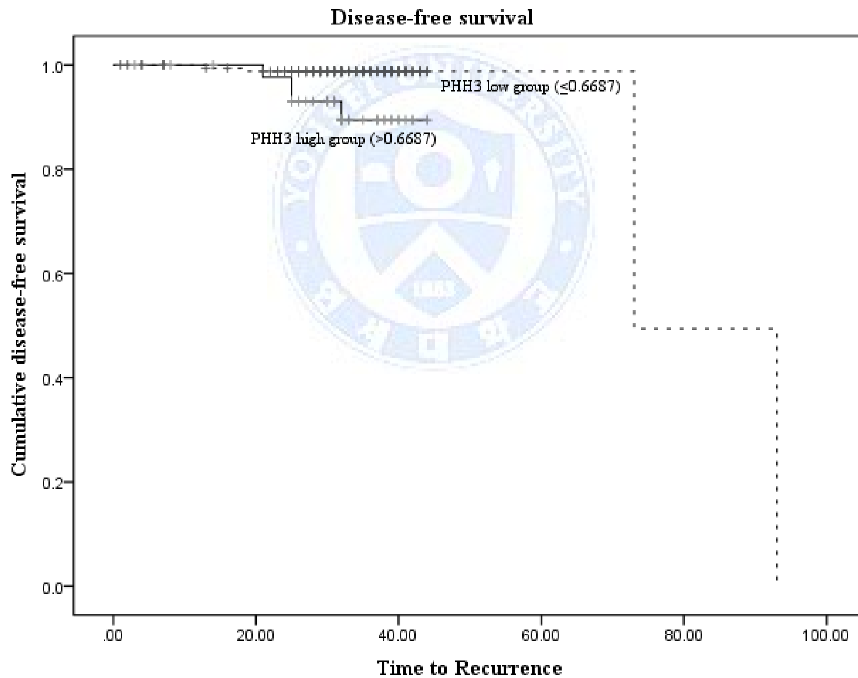

Figure 5. Recurrence-free survival in 218 patients for PHH3 low and high groups (dashed line ≤ 0.6687; solid line > 0.6687). Higher PHH3 values was significantly associated with worse outcome.

Table 6. Log rank (Mantel-Cox) shows significant association of low PHH3 with recurrence free survival

Parameters	Total number/recurrence	Disease-free survival	
		Mean survival (95% CI) months	*P*
PHH3			
Low	174/4	82.19 (66.32-98.05)	**0.006**
High	46/4	42.15 (40.42-43.88)	
Ki-67			
Low	179/5	81.90 (66.62-97.18)	0.053
High	41/3	40.46 (38.69-42.23)	

IV. Discussion

Between the two proliferation markers, PHH3 is specific for mitosis in comparison to Ki-67. This was evident from the significant difference in staining percentage between the two. As evidenced by the positive linear relationship, the two markers proved to be related in their expressions, recapitulating the idea that both PHH3 and Ki-67 to some degree have an overlap in quantification of proliferative activity.

The use of Ki-67 LI as representation of proliferation has long been under contention amongst experts, mainly due its lack of reproducibility. Arguably the important reason for this lack of reproducibility is due to tumor heterogeneity.[17] In selection of different fields amongst raters will inevitably result in different scores for the same tumor. There is also the problem of lack of a consensus on the method of scoring. Some advocate the selective use of hot-spots in assessment of Ki-67,[11,17] while others are in favor of taking the average of the advancing edge.[18] In the recommendation published by the international Ki-67 in breast cancer working group in 2011, the method of overall average of the tumor, including areas of the hot spots was advocated for consistency among raters. Yet even with these recommendations, reproducibility and agreement amongst raters is a problem reported consistently.[17] In the study assessing the reproducibility of Ki-67 amongst institutions and pathologists, there were 32% patients misclassified in terms of

low versus high Ki-67 levels, when two laboratories were compared.[19] Another study reported high interobserver variabilities amongst 15 pathologists who each assessed three breast carcinoma cases (κ = 0.04-0.14).[20] In the present study, the inter-rater agreement on microscopic estimates for PHH3 was 0.996 in ICC, which is better than those reported for Ki-67(ICC 0.92, 95% CI: 0.88–0.96).[21] For the low κ statistic, this was most likely due to the small number of cases (31) assessed through pair-wise comparison in this study. Kappa statistics is likely to improve with more cases to fairly evaluate the reproducibility of PHH3. Cui et al reported that the inter-rater agreement for PHH3 for 97 invasive breast cancer cases, the κ statistics amongst three pathologists were 0.87, 0.79, 0.76, demonstrating good and reliable concordance. Enumeration of PHH3 on taken photographs, the κ statistics was 0.711, which was a significant improvement from the microscopic evaluation.[10]

PHH3 also demonstrated better sensitivity for detecting mitosis than H-E mitotic index evaluation. This was evidenced by the up-graded tendencies through PHH3 enumeration. Low power assessments were also well correlated with those of high power field enumerations, therefore evidencing that mitotically active areas can be identified by low power, facilitating in accurate measurement of mitosis. Another similar tumor that also relies on mitosis for grading, is meningioma and studies demonstrated that those qualities of PHH3 for identification of mitotically active areas was

valuable in rapid, reliable grading of meningiomas.[22,23]

In evaluable for recurrence-free survival, PHH3 proved to be a better prognostic marker than Ki-67. Gerring et al. had already published that PHH3 out-performed Ki-67 in prognostic value through multivariate Cox-regression.[3] However this study had been conducted with TMA samples, therefore in disagreement of the actual practice of assessing Ki-67 and PHH3. Our study is better representative of the actual practice because of our assessment of surgical slides for analysis.

The limitation of this study was the short follow up time with a mean follow up of 31 months and the longest time recorded at 93 months. Gerring et al[3] had reported on the prognostic value after following up on patients for the maximum length of 191 months with a median follow up of 85months. Due to the long duration of follow up, they were able to accumulate enough data of patient survival (a total of 54 deaths out of a total of 108 patients).[3] Their data had allowed for a significant value of PHH3 on multivariate cox regression, proving that PHH3 to be a better prognostic marker than Ki-67.

V. Conclusion

In this study, we present a possibility to utilize PHH3 as reinforcing weaknesses of Ki-67. PHH3 is a marker specific for mitosis that is expressed in all stages of mitosis. It therefore reliably discriminates actually proliferating cells from mimickers that are difficult or impossible to discriminate on H-E mitotic index or Ki-67 LI. It also has an advantage in heterogeneous tumors that identifies mitotically active areas allowing for a good reproducibility amongst raters. Despite the problems of reproducibility and no better known alternative, Ki-67 LI has long been the mode of evaluation of breast cancer proliferative activity in many pathology centers. The findings of our study advocate the routine use of PHH3 in diagnostics to reinforce the weaknesses of Ki-67.

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ABSTRACT (IN KOREAN)

Invasive breast cancer의 증식력을 평가하는 도구로서
Phosphohistone H3 의 유용성:
Ki-67와의 비교를 중심으로

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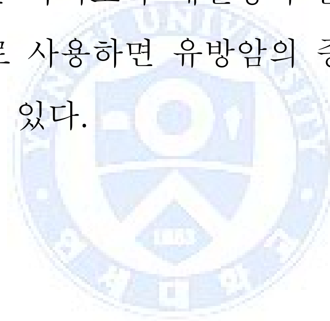
Ki-67은 종양의 증식력을 평가하는데 있어 널리 사용하는 표지자로서 유방암에서 환자의 항암치료의 반응 정도를 예측하는 인자로서 사용되고 있다. 그러나 Ki-67은 증식력을 반영하는데 있어 비특이적이고 재현성이 낮다는 문제가 있다. 대조적으로 Phosphohistone H3 (PHH3)는 증식력으로 정의되는 유사분열에 대해 특이적이고 재현성 높은 표지자로 알려져 있다. 본 연구는 유방암의 증식력을 평가하는데 있어 두 표지자의 유용성을 비교하였다.

연구방법: 2012년도부터 2013년 까지 유방암으로 수술한 환자의 FFPE 중 가장 대표성 있는 단면에 Ki-67과 PHH3의 IHC 염색을 하여 비교하였다.

결과: Ki-67과 PHH3은 서로간의 양의 상관관계가 있었다. PHH3의 재현성을 확인하기 위해 두 가지 방법을 통해 두 명의 평가자 간의 평가 일치도를 확인하였다. 첫 번째 방법은,

동일 슬라이드를 현미경에서 관찰하여 평가하였고, 두 번째 방법은 동일 영역의 사진을 평가하게 하였다. 두 방법 모두 거의 완벽한 급내 상관 계수(0.996, 0.977)를 나타냈다. PHH3로서 저배율에서 유사분열이 가장 많은 영역을 발견할 수 있는지 알아보기 위해 PHH3를 저배율로 평가한 수치와 고배율로 10개의 영역을 평가한 수치를 비교하였을 때 높은 상관성을 보였다(r = 0.999). 마지막으로, PHH3는 recurrence-free survival(P = 0.006) 와 유의한 관련성이 있었으나, Ki-67 은 그렇지 않았다(P = 0.053).
결론: Ki-67의 낮은 특이도와 재현성의 문제를 보완하기 위해 PHH3를 보조적으로 사용하면 유방암의 증식력을 보다 정확하게 평가할 수 있다.



핵심되는 말: Ki-67, PHH3, 유방암, 증식력, 신뢰도

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