RESEARCH ARTICLE

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Can Histological Grade and Mitotic Index Replace Ki67 to Determine Luminal Breast Cancer Subtypes?

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Abstract

Introduction: Breast cancer can be classified into subtypes based on immunohistochemical markers, with Ki67 expression levels being used to divide luminal BC tumors in luminal A and B subtypes; however, Ki67 is not routinely determined due to a lack of standardization. **Objective:** To evaluate histological grade and Eliminate: the mitotic index to determine if they can be used as an alternative method to Ki67 staining for luminal subtype definition. **Methods:** We evaluated estrogen receptor positive breast cancer tissue samples. Pathological analysis included determination of Ki67. A low level of Ki67 was defined as <14% positive cells. **Results:** We evaluated 151 breast cancer samples; 24 (15,9%) were classified as I; 74 as HG II (49%), and 53 (35,1%) as HG III. The median value for Ki67 was 13% (range: <1% - 82%) and for MI was 2 (0-12). Histological grade I tumors exhibited Ki67 values significantly lower than HG II and III tumors (Anova, Tamhane test p=0,001). A higher Ki67 value was related to a higher MI (Rho Sperman p=0,336; R2= 0,0273). ROC curve analysis determined that a MI ≥ 3 had a sensibility of 61.9% and specificity of 66.7% in predicting a high Ki67 value (≥14%) (area under the curve: 0,691; p =0,0001). A HG I tumor or HG II-III with MI ≤2, had a high probability of corresponding to a LA tumor (76,3%), as defined using Ki67 expression, while the probability of a LB subtype was higher with HG II-III and a MI ≥3 (57.4%). Global discrimination was 68.1%. **Conclusions:** For the LA subtype, our predictive model showed a good correlation of HG and MI with the classification based on Ki67<14%. In the LB subtype, the model showed a weak correlation; therefore Ki67 determination seems to be needed for this group of patients.

Keywords: Breast neoplasms- Ki67 antigen- mitotic index- histology

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Introduction

Breast cancer (BC) is the major cause of death from malignancies in Chilean women (Acevedo et al., 2016). During the last decades, the widespread use of screening methods had resulted in increased rates of early diagnoses (Berry et al., 2005) contributing in better prognosis. New drugs, better chemotherapy (CT) schemes, and the use of monoclonal antibodies on Epidermal Growth Factor receptor 2 (HER-2) over expressing tumors (Coates et al., 2015) explain major advances on the treatment of BC. Knowledge of BC heterogeneity has also enabled to personalize the treatment, thus BC subtype (Goldhirsch et al., 2013) affects the prognosis of the disease and the possibility of response to endocrine therapy (ET) (Rugo et al., 2016) and CT. Intrinsic subtypes, defined initially through genetic-molecular studies, are associated with BC subtypes characterized by classic histo-pathological parameters (Ma and Ellis, 2013; Dowsett et al., 2013) and are defined as: Luminal: phenotypically characterized by estrogen receptor (ER) expression; HER2 (human epidermal growth factor receptor type 2) enriched: showing HER2 over expression without ER expression; and triple negative (TN): negative for ER, progesterone receptor (PR) and HER2 expression (Coates et al., 2015) Ki67 is a nuclear protein, expressed by proliferating cells in late G1, S and G2 / M cell cycle phases; reflecting the proportion of proliferating cells, and has been used as a predictor of response to ET and in recent studies, to CT. Ki67 expression, as determined by immunohistochemistry (IHC), also allows to subdivide the Luminal subtype into A and B (Pathmanathan et al., 2014) (Table 1). Due to a lack of standardization of the technique, interpretation and associated costs, this is not a

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routine test in BC pathology reports worldwide (Tashima et al., 2015).

Our group has previously published preliminary results about the association between Ki67 and HG, determined through IHC in 57 patients treated with neoadjuvant CT. This work showed that the use of Ki67 changed the classification in LA or LB BC subtypes (Table 1) obtained after using only HG. Sample size and lack of information about other parameters related to cell proliferation, as MI, may explain a bias in our results (Petric et al., 2014).

The current objective of our study is, with a bigger sample size, to establish a correlation between Ki67 expression, HG and MI in order to determine if HG and MI could be used as an alternative to the Ki67 value in BC subtype determination. If we validate the utility of HG or MI and its correlation with Ki67, the use of those simple, routinely used and validated biomarkers, which do not require any staining, could be used in any pathology lab, with no associated extra cost or time consumed.

Materials and Methods

This was a retrospective, non-interventional study performed at the Pontificia Universidad Catolica de Chile Cancer Center and approved by the Scientific Ethics Committee of our University. We reviewed medical record from ER+, BC patients who underwent surgery between 1999 and 2014. Pathologic reports include tumor size, lymph node involvement and histological type. Aiming to evaluate the correlation between the expression of Ki67 and determination of HG, the methodology was carried out as follows: HG was determined according to Elston and Ellis (Elston and Ellis, 2002) and the status of ER, PR and HER2 were determined by immunohistocheminstry (IHC). The cutoff value to determine if ER and PR were positive was ≥ 1% of tumor cells. Tumors with HER23 + on IHC were considered HER2 positive. If HER2 expression was 2+, a fluorescent in situ hybridation (FISH) (immunofluorescence in situ) assay for HER2 was performed. For Ki 67 evaluation, the analysis was done using the Ki67 antibody Ki67 clone MIB-1 (Dako) and Envision Flex kit link High pH (Dako) as the visualization and disclosed. For each case, we obtained a set of photomicrographs at high magnification (400x) of the tumor sites with the highest number of cells with positive reaction ("Hot-Spots"). The cell count for each image was performed manually using the Image J software 1.42q (National Institute of Health, USA). Data were recorded in Microsoft Excel 2003, where the percentage was calculated. Low Ki67 was defined as less than 14%. The Ki67 analysis was performed on core biopsies (core tissue sample taken under radiological guide) and in surgical specimens (partial or total mastectomy). The same pathologist assessed all Ki67 analyses.

Samples obtained from patients that received neoadjuvant treatments were not included in our study. The mitotic figures were counted in the highest cellular area at the periphery of the tumor from 10 consecutive high-power fields (hpf) (35). Mitotic counting was carried out using a standard laboratory microscope (objective, ×40; field diameter, 0.65 mm).

The tumor stage at diagnosis was determined according to the TNM 2010 (American Joint Committee on Cancer Staging Manual, 7th Edition) system. Estrogen receptor positive tumors were classified into 4 subtypes according to two classifications: 1) According to classical bio-markers, HG I and II were combined and considered as low proliferation tumors: Luminal A (ER-positive and / or PR positive, HG I-II, HER2 negative), Luminal B (ER positive and / or PR positive, HG III and / or HER2 positive), 2) According to St Gallen 2013 classification (Coates et al., 2015), which considers the Ki67 value to define luminal subtypes A and B (Table 2).

Statistical analysis

In order to estimate the relation between Ki67, HG and MI using a spearman correlation, we determined a sample size of 150 patients, with a α =0.05, power =0.88 and an r-value of at least 0.4. Descriptive statistics was used to present data as central tendency ± standard deviation. To evaluate the relation between HG, Ki67 and MI we performed ANOVA followed by Tamhane post-hoc tests. To determine the relation between MI and Ki67 we calculated a Spearman coefficient of correlation.

We made a receiver operating characteristic (ROC) curve to determine the best MI cutoff to predict a Ki67 ≥14%. We performed a lineal and logistic predictive model to determine the correlation between Ki67 values, HG and MI to categorize BC subtypes as LA or LB.

Categorical variables were evaluated with Chi-square or Fisher exact test.All data were analyzed using version 15 IBM ® SPSS ® program.

Results

We evaluated 151 patients with ER+, BC. Average age was 56,8 ± 12,5 years old. Average tumor size was 2,2 cm ± 1,7 cm. The most frequent histological subtype was ductal carcinoma in 144 cases (95,4%). We summarize the main patient´s characteristics in Table 2.

Pathologic evaluation and immunohistochemistry

Most tumors were classified as HG II (74 patients, 49%), 53 as HG III (35,1%) and 24 as HG I (15,9%). The average percentage of positivity for ER and PR was 77,5 ± 27,3% and 58,9 ± 37,2%; respectively. Only 3 patients (1,98%) had HER2 positive BC tumors. The median value for Ki67 was 13% (Range <1-82%).

Relation between HG and Ki67

Table 3 summarizes the correlation between HG and Ki67 percentage to classify invasive BC in LA or LB using a cutoff point of 14% for Ki67 and grouping HG into HG I-II and HG III. When the Ki67 value was added to the Luminal subtype (A or B), in the case of LA, 37 of 98 patients (37,8%) were reclassified as LB for having a Ki67 ≥ 14% while 23 of 53 patients initially classified a LB were reclassified as LA for having a Ki67 < 14%. We found differences between HG and Ki67 (ANOVA p=0,001). The Tamhane post hoc test showed that most BC defined as HG I had values for Ki67 significantly lower than HG 2 or 3 tumors (ANOVA, Tamhane test, prueba de Tamhane

Table 1. Breast Cancer Subtypes and its Correlation with Immunohistochemistry (IHC)

Subtype	IHC
Luminal A	ER+, PR+, HER2-, CK8 and 18; Ki67 <14%
Luminal B	ER+, PR±, HER2±, CK8 and 18; Ki67 ≥14%
HER2 +	ER-, PR-, HER2+; high Ki67
Basal	ER-, PR-, HER2-; CK5/6+; EGFR+
Claudin low	ER-, PR-, HER2-; Ki67 intermediate

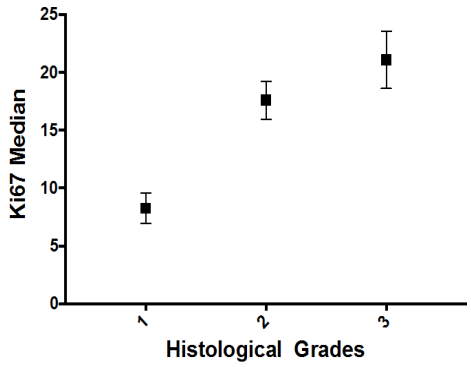

Figure 1. Ki67 Median for Different Histological Grades (HG). Difference given between HG I, and HG II/III (Anova, Tamhane test p=0.001).

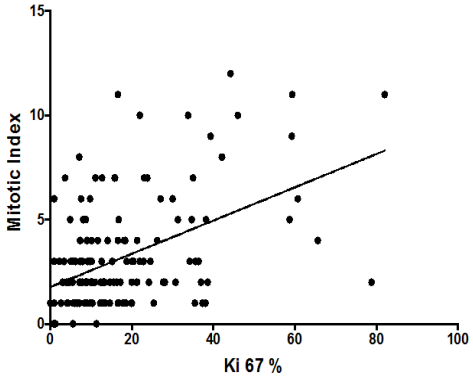

Figure 2. Correlation between Mitotic Index and Ki67 Levels

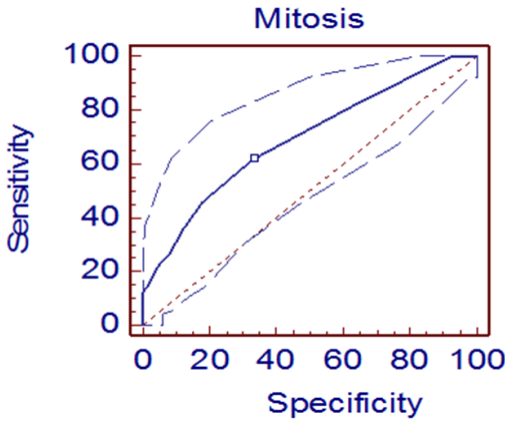

Figure 3. ROC Curve for MI with a Ki67 ≥14%. The circle shows the point of greater sensibility and specificity (61,9 y 66,7% respectively; area under the curve: 0,69, p= 0.001)

Table 2. Clinical pathological characteristics of 151 patients with invasive BC

Variable	
Age (years)	56
Tumor size	2.2 cm
	Number of patients
Histological type	
Ductal	144
Mucinous	2
Lobular	2
Ductolobular	1
Others	2
Tumor Size (T)	
pT1	78
pT2	58
pT3	12
pT4	3
Lymph node status (N)	
N0	81
N1	39
N2	19
N3	12
Metastatic disease (M)	
M0	148
M1	3
Stage(AJCC 2010)	
I	59
II	58
III	31
IV	3

p=0,001). Figure 1 displays Ki67 values for different HG.

Relation between MI and Ki67

We found a significant correlation between MI and Ki67. A higher value was correlated to higher mitotic figures on the examined samples (Rho Sperman p=0,336, R^2= 0.0273) (Figure 2).

A ROC curve analysis (Figure3) determined that an MI >2 has a sensibility of 61,9% and specificity of 66,7% to determine a Ki67≥14% (Area under curve: 0,691, p =0.0001).

Relation between HG and MI

We saw differences between HG and MI (ANOVA p=0.001). The post hoc T2 Tamhane test showed this difference between HG 1 and HG 3 tumors (ANOVA, Tamhane test, p=0.035). Figure 4 shows the average MI in all different HG.

In the Logistic regression analysis we found that samples with Ki67 <14%, presented a higher proportion of HG I and MI ≤ 2, generating a predictive model to characterize Luminal BC A and B, using IHC determinations for reference. Based on these BC subtypes classifications (Table 1), a HG I or HG II-III tumor,

Table 3. Correlation between Histological Grade and Ki67 Value in the Determination of Luminal BC Subtype

Histological grade	Ki 67<14%	Ki 67 ≥14%	Total
HG I-II (Luminal A)	61	37	98
HG III (Luminal B)	23	30	53
Total	84	67	151

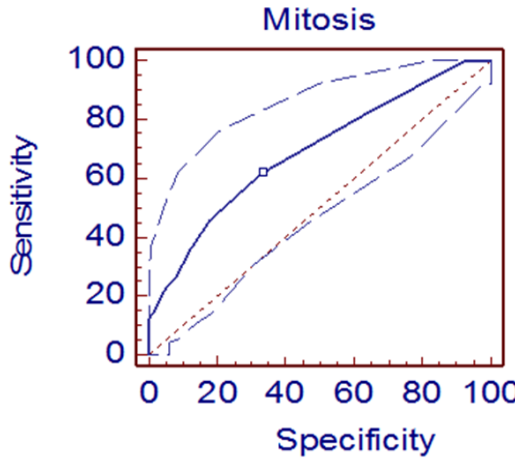

Figure 4. Correlation between MI Levels and Histological Grade (HG). There was a significant difference for HG I and III (Anova, Tamhane test p=0.001).

with MI ≤2 has high possibility to correspond to a LA BC tumor, while the probability to have a LB tumor, is higher in HG II-III tumors with MI ≥3. The model has a discrimination for LA and LB, BC subtypes, of 76, 3% and 57,4%; respectively, with a global discrimination of 68.1%.

Discussion

Breast cancer is a heterogeneous disease. Its adequate evaluation and classification into subtypes based on genetic testing is recommended in order to determine its prognosis and define the treatment (Sgroi and Brufsky, 2016). However, its elevated cost and low access to molecular –genetic studies in the clinical practice limits its use; therefore, the use of IHC markers to define BC subtypes is more frequent. The use of routine BC biomarkers (ER, PR, HER2), basal cytokeratins (CK5/6, CK17), low weight cytokeratins (CK7, CK8, CK18, etc.), Ki 67 expression and epidermal growth factor receptor type 1 (EGFR) expression, permit the classification of BC in subtypes in an equivalent way to those based on genetic profiling (Dowsett et al., 2011; Ma and Ellis, 2013; Dowsett et al., 2013). The IHC based classification uses biomarkers routinely available in the pathology lab, and can be applied over archived tissue samples.

This classification of BC subtypes based on IHC markers is widely accepted as a tool to differentiate ER+ BC in LA and LB subtypes (Goldhirsch et al., 2013) (Table 1).

Values of Ki67 are not routinely measured in all BC biopsies, and the correlation between this marker and others usually described in pathology reports, especially those that do not require additional staining as the HG and

Table 4. Correlation between Histological Grade/Mitotic Index and Ki 67% to Determine Luminal Breast Cancer Subtype

Histological grade	Ki 67<14%	Ki 67 ≥14%	Total
GH I- GH II o III and MI ≤2 (Luminal A)	63	31	94
GH II o III and MI ≥3 (Luminal B)	21	36	57
Total	84	67	151

MI, has been contradictory (Pathmanathan and Balleine, 2013; Dowsett et al., 2011).

The nuclear protein Ki67, is a nuclear protein, expressed in proliferative cells in the late phase of G1, S and G2/M reflects the proportion of cells proliferating, being used as a predictive factor. In addition, high Ki67 values are mostly related to a bad prognosis (Dowsett et al., 2011; Jonat and Arnold, 2011).

The HG evaluation includes histological features that determine tumor aggressiveness. Three parameters are used for its determination: tubule formation, nuclear grade and MI (Elston and Ellis, 2002).

The MI measures cell proliferation directly on histological samples. The mitotic activity is measured as the number of mitosis in pre-defined major optical fields (usually 10) in samples stained routinely. One of the main advantages is that it requires no extra stains to be evaluated. Several studies have shown that a higher MI is correlated with bad prognosis (Aaltomaa et al., 1991; Aaltomaa et al., 1992).

In this study we examined the relation between Ki67, HG and MI, classic biomarkers for cell proliferation, routinely reported on the BC pathology report. We found a significant correlation with a weak statistical significance for all studied factors.

Regarding HG, we observed that HG I is different from HG II and III, the former showing lower Ki67 values. Only 4 cases (16,6%) with HG I showed Ki67 values of ≥14%, while in patients with HG II and III, 44,6% and 56,6% respectively, had Ki67≥14%. It is known that a population with HG II may include the existence of at least two entities with different biology and clinical behavior. Aleskandarany (2011) in a study with 1550 BC patients, using a cutoff for Ki67 of 10%, concluded that patients with HG II and Ki67 <10%, have better prognosis than those with Ki67 ≥10%.

Considering MI, the most frequent cutoff value used to define a higher proliferation rate is ≥10 mitotic cells (Van Diest et al., 1992). Several series have shown that MI has an important prognostic role in BC patients (Michels et al., 2004; Baak et al., 2005). A retrospective study recently published (Jobsen et al., 2015) evaluated the prognostic role of MI in invasive BC patients and negative lymph nodes, showing that in patients younger than 55 years old, the distant disease free survival was better in patients with MI ≤10 ($p < 0.001$). In our series, only 7 patients (4,6%) showed MI ≥10 and the cut off point of MI>2 was the one which presented major sensibility and specificity to determine a Ki67 ≥14% (Area under curve:

0,691, p =0,0001).

Until now we have commonly used HG to classify luminal BC subtypes in A (HG I-II) or B (HG III), but without considering MI.

Classifying Luminal subtypes A and B using HG or MI had a low concordance with those obtained using the Ki67 values. However, this concordance improved when both factors were combined, according to the created predictive model (Table 4). Several studies have shown that MI is the most important constituent of HG (Baak et al., 2005; Jobsen et al., 2015; Bertucci et al., 2013), which could explain their complementarity.

In conclusion, this study suggests that a correlation between HG and MI with Ki67 in luminal tumors exist. Our predictive model showed a good correlation with Ki67 definition of LA tumor, however, the model showed a weak correlation for LB tumors. It suggests the need to measure Ki67, due to the modifications in treatment that can be generated based on this classification.

Statement conflict of Interest

We have no conflict of interest to declare.

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