RESEARCH ARTICLE

Correlation between Ki67 and Histological Grade in Breast Cancer Patients Treated with Preoperative Chemotherapy

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Abstract

Background and Aim: Breast cancer (BC) is a heterogeneous disease and cell proliferation markers may help to identify subtypes of clinical interest. We here analyzed the correlation between cell proliferation determined by Ki67 and HG in BC patients undergoing preoperative chemotherapy (PCT). Materials and Methods: We obtained clinical/pathological data from patients with invasive BC treated at our institution from 1999 until 2012. Expression of estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor type 2 (HER2) and Ki67 were determined by immuno-histochemistry (IHC). Clinicopathological subtypes were defined as: Luminal A, ER and/or PR positive, HER2 negative, HG 1 or 2; Luminal B, ER and/or PR positive, HER2 negative or positive and/or HG 3; triple negative (TN), ER, PR and HER2 negative independent of HG; HER2 positive, ER, PR negative and HER2 positive, independent of HG. By using Ki67, a value of 14% separated Luminal A and B tumors, independently of the histological grade. We analyzed correlations between Ki67 and HG, to define BC subtypes and their predictive value for response to PCT. Results: 1,560 BC patients were treated in the period, 147 receiving PCT (9.5%). Some 57 had sufficient clinicopathological information to be included in the study. Median age was 52 years (26-72), with 87.7% invasive ductal carcinomas (n=50). We performed IHC for Ki67 in 40 core biopsies and 50 surgical biopsies, 37 paired samples with Ki67 before and after chemotherapy being available. There was no significant correlation between Ki67 and HG (p=0.237), both categorizing patients into different subtypes. In most cases Ki67 decreased after PCT (65.8%). Only 3 patients had pathologic complete response (cPR). Conclusions: In our experience we did not find associations between Ki67 and HG. Determination of clinicopathological luminal subtypes differs by using Ki67 or HG.

Keywords: Breast cancer - Ki67 index - biological markers - molecular subtypes

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Introduction

BC is the leading cause of death from malignancies in Chilean women (Acevedo et al., 2013). The widespread use of screening methods, resulting in increased rates of early diagnosis (Berry et al., 2005), have contributed to improved its prognosis. New drugs, better chemotherapy (CT) schemes, and the use of monoclonal antibodies on HER2 over expressing tumors (Burstein et al., 2012) explain major advances on its treatment. Knowledge of BC heterogeneity has also enabled personalize the treatment, thus BC subtype (Goldhirsch et al., 2011) affects the prognosis of the disease, and the possibility of response to endocrine therapy (ET) and CT. Intrinsic subtypes defined initially through genetic-molecular studies, are associated with certain subtypes characterized by classic histopathological parameters (Paik et al., 2004; Cuzick et al., 2011; Goldhirsch et al., 2011; Tang et al., 2011; The Cancer Genome Atlas Research Network 2012) and defined as: Luminal: phenotypically characterized by ER expression; HER2 positive: showing HER2 over expression without ER expression and TN: negative for ER, PR and HER2 (Goldhirsch et al., 2011; Reis-Filho et al., 2011). 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 IHC, also allows to subdivide the Luminal subtype in A and B (Pathmanathan et al., 2013) (Table 1). The lack of standardization of the technique, interpretation and associated costs, do not make this a routine test in the BC pathology reports worldwide. The objective of this study is to evaluate the correlation between the expression of Ki67 and determination of HG, the latter often used to determine subtypes from routine BC biopsies reports; we also analyzed the subtype's determination with and without the use of Ki67. As an exploratory objective we

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assessed the predictive value of Ki67 and HG in patients undergoing PCT in order to determine its predictive value for pCR.

Materials and Methods

This was a retrospective study performed at the Universidad Catolica de Chile Cancer Center. This study was approved by the Scientific Ethics Committee of our University.

We reviewed medical record from BC patients receiving PCT according to their physician indication in the period between 1999 and 2012. Inclusion criteria were: (1) Patients who received at least one cycle of PCT, (2) availability of sufficient information to evaluate the response to treatment and pathological data, (3) availability of tissue sample for additional IHC and determination of Ki67. Pathologic report includes 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). The status of ER, PR and HER2 were determined by IHC. The cutoff value to determine if ER and PR were positive was ≥ 1% of tumor cells. Tumors with HER2 3 + on IHC were considered HER2 positive. If the degree of expression of HER2 was 2 +, a FISH (inmunofluorescence in situ) assay for HER2 was performed. 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 a set of photomicrographs at high magnification (400x) of the tumor sites with higher number of cells with positive reaction ("Hot-Spots") was obtained. The cell count for each image was performed manually using the Image J software 1.42q (National Institute of Health, USA). The data were recorded in Microsoft Excel 2003 program, were the percentage was calculated. Low Ki67 was defined less than 14%. The Ki67 analysis was performed on core biopsies (core tissue sample taken under radiological guide before the preoperative treatment) and in surgical specimens (partial or total mastectomy performed post CT). The tumor stage at diagnosis was determined according to the TNM 2010 (American Joint Committee on Cancer Staging Manual, 7th Edition) system. Pathological/Clinical Subtypes: The tumors were classified into 4 subtypes according to two classifications: 1) According to classical markers, HG 1 and 2 were combined and considered as low proliferation tumors: Luminal A (ER-positive and / or PR positive, HG 1-2, HER2 negative), Luminal B (ER positive and / or PR positive, HG 3 and / or HER2 positive), TN (ER, PR and HER negative), HER2-enriched (RE and PR negative, HER2 positive). 2) According to St Gallen 2013 classification (Goldhirsch et al., 2013), which considers the Ki67 value to define luminal subtypes A and B (Table 2).

Tumor Response: The cPR was defined as the absence of invasive tumor in the breast (independent of the presence of residual carcinoma in situ) and axillary lymph nodes.

Statistical analysis: Descriptive statistics were used. Categorical variables were evaluated with Chi-square or Fisher exact test. Pearson correlation coefficient (r) was used to assess correlation and McNemar´s test to assess Ki67 changing before and after PCT. Kappa concordance rate between the 2 forms proposed to determine the histological subtype (HG vs Ki67) was assessed. p values< 0.05 was considered as to be significant. All data were analyzed using version 15 IBM ® SPSS ® program.

Table 1. BC Clinico-Pathological Subtypes Base on IHC Biomarkers

Subtype	IHC expression
Luminal A	ER+; PR+; HER2-;Ki67<14%
Luminal B	ER+; PR±; HER2±; Ki67>14%
HER2 +	ER-; PR-; HER2+.
Triple negative	ER-; PR-; HER2-.

*IHC: immuno-histochemistry; ER: estrogen receptor; PR: progesterone receptor; HER2: epidermal growth factor receptor type 2

Results

1560 patients with BC were treated at our institution from 1999 to 2012, 147 received PCT (9.5%). 57 were included for analysis. Eight were excluded because they were still in treatment; in 6 patients we did not obtain complete clinical-pathological data, and in 76 cases there were not enough available biopsy material for Ki67 study. The median age at diagnosis was 52 +/- 10.8 years (26-72 years). Lump was the chief complaint in 96.5% (n=55) of the cases; the tumor size on physical examination was 7 +/- 3.3 cm (2-15 cm). Clinical stage was stage II in 35% (n=20), stage III in 45.6% (n=26) and stage IV 19.3% (n=11). The major histological type was invasive ductal carcinoma: 87.7% (n=50) of the cases. IHC for Ki67 was performed on 95 samples; they corresponded to 40 core biopsies (before CT) and 55 to surgical biopsies (after CT). To determine the association between HG and Ki67, chi-square test of independence was performed on 95 samples processed; we observed that Ki67 and HG are independent variables and there was no relationship between them (X^2=0.187, DF=1, p=0.665). Neither correlation between HG and Ki67 value was observed (rP=0.076, p=0.237) (Table 3). In 38 patients the value of Ki67 in the core biopsy and also in the surgical specimen

Table 2. Clinico-pathological Subtypes According to St Gallen 2013

Luminal A	ER and PR positive, HER2 Negative, Ki67 <14%
Luminal B	HER2 negative: ER positive, HER2 negative and: Ki67 high (>14%) and/or PR negative or low (<20%) HER2 positive: ER positive, HER2 over-expression, any Ki67 and PR
HER2 positive	ER and PR negatives , HER 2 over-expression
Triple Negative	ER, PR and HER2 negative

*ER: estrogen receptor; PR: progesterone receptor; HER2: epidermal growth factor receptor type 2

was obtained. We observed significant changes in Ki67 value after preoperative treatment ($p<0.001$) (Figure 1). There were 25 (65.8%) cases where the Ki67 value decreased, 11 (20%) where it was increased and 2 cases (5.2%) where Ki67 did not change (Figure 1). In patients with available core biopsy tissue (n=40), 29 (72.5%) tumors were classified as Luminal subtype and 11 (22.5%) as No Luminal subtype (TN 7 cases and HER2 positive). Regarding the Luminal subtypes classification, when classical IHC parameters were applied (using HG to define subtype), 12 cases (30%) were Luminal A and 17 (42.5%) Luminal B. Using Ki67 value, instead of HG, to define Luminal subtypes, 5 cases were classified as Luminal A (12.5%) and 24 (60%) Luminal B. The level of agreement between the two forms to define subtypes was negligible (Kappa 0.145) (Figure 2). Only 3 patients (7.5%) had cPR (1 Luminal B, 1 TN and 1 HER2 positive). The sample size and low rates of cPR obtained do not allow deeper analysis to evaluate the Ki67 role as a predictor of pathologic response versus classical IHC markers (HG).

Table 3. Correlation of Ki67 and HG in 90 BC Samples

Histological grade (HG)	Ki67 Low (Ki 67< 14%)	Ki67 High (Ki67³ 14%)	Total
Low (HG 1 y 2)	11	21	32
High (HG 3)	16	42	58
Total	27	63	90

Table 4. Luminal Subtypes Based on HG and Ki67 Luminal Subtypes Based on ki67

Luminal subtypes based on HG	Luminal A	Luminal B	Total
Luminal A	3	9	12
Luminal B	2	15	17
Total	5	24	29

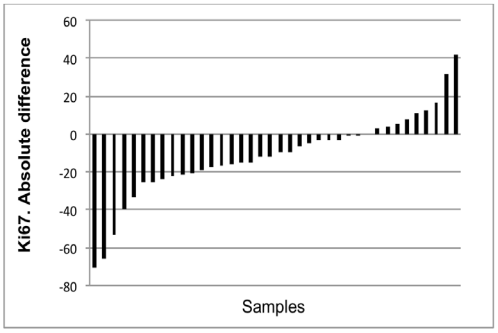

Figure 1. Ki 67 Changes After Preoperative chemotherapy in 38 Patients. (Final Ki67 minus initial Ki67)

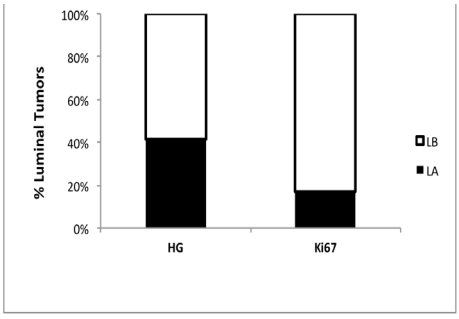

Figure 2. Luminal Breast Cancer Subtypes Using HG or Ki67

Discussion

Clinical interest in knowing and identifying BC subtypes is based on its association with prognosis and prediction of treatment response (Goldhirsch et al., 2013; Khokher et al., 2013). Tumors "Luminal A" (more endocrine-sensitive, indolent and with better prognosis) and "Luminal B" (less endocrine-sensitive and aggressive) can be distinguished by their gene expression and IHC (Goldhirsch et al., 2013; Shim et al., 2014). An algorithm based on semiquantitative evaluation of the ER, PR, HER2 expression and Ki67 value (called 'IHC-4' score) has shown similar results to those achieved by genetic risk models on ER positive BC (Yoo et al., 2012; Pathmanathan and Balleine, 2013).

Based on this information the St. Gallen consensus suggests the use of IHC to determine BC subtypes, using proliferation assessment, trough Ki67 study, as a way to differentiate Luminal's tumors (Goldhirsch et al., 2011). There is a tendency for a reduction on Ki67 value after CT, and a greater decrease, better response to treatment has been reported (Kumaki et al., 2011). High levels of Ki67 are associated with poor prognosis (Azambuja et al., 2007; Viale et al., 2008; Haroon et al., 2013; Kilickap et al., 2014. Tanriverdi et al., 2014).

In the BIG 1-98, a randomized phase III study comparing two types of ET, a high baseline Ki67 was the only factor in the univariate analysis predicting the benefit of a drug over another (Viale et al., 2008). The initial value of Ki67 and its dynamic changes post treatment have predictive and prognostic importance. However, the routine use of Ki67 for prognostic evaluation in BC, it is not even considered a standard practice. Its cost and the lack of standardization in its measurement limit their use and are not part of the usual pathological report in all pathology labs. In clinical practice classical parameters related to cell proliferation reported routine breast biopsy are used, such as HG and mitotic index (MI), which have shown correlation with Ki67, and one of them is part of the Nottingham Index (Galea et al., 1992). In the HG assessment are combined nuclear grade, tubes formation and mitosis. Both MI and Ki67 are cell proliferation markers, however Ki67 is expressed in all cell cycle phases except in G0 (or resting stage) and therefore would be a superior prognostic marker (Yerushalmi et al., 2010). While the MI is a component in the evaluation of HG, not all studies have found a correlation between them (Stumpp et al., 1992).

In our study we observed no association between Ki67 values and HG. We observe a decrease in Ki67 value after PCT, as it has been widely reported in the literature, but given the small number of patients with cPR in our study, it was not possible to assess their predictive value for tumor response. This study was limited by the low availability of tissue sample for Ki67 IHC and also by the small number of patients with cPR. However, the data obtained allow us to suggest the necessity of a routine use of Ki67 in order to determine BC subtypes. Actually we have six more cases and we are collecting more BC samples in order to increase our sample size and statistical power in future investigations.

References

Acevedo F, Herrera ME, Madrid J, Sánchez C (2013). Neoadyuvant endocrine therapy in breast cancer. *Revista Médica de Chile*, **141**, 367-74.

Berry DA, Cronin KA, Plevritis SK, et al (2005). Effect of screening and adjuvant therapy on mortality from breast cancer. *New Engl J Med*, **353**, 1784-92.

Burstein HJ, Piccart-Gebhart MJ, Perez EA (2012). Choosing the best trastuzumab-based adjuvant chemotherapy regimen: should we abandon anthracyclines? *J Clin Oncol*, **30**, 2179-82.

Cuzick J, Dowsett M, Pineda S, et al (2011). Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the genomic health recurrence score in early breast cancer. *J Clin Oncol*, **29**, 4273-78.

De Azambuja E, Cardoso F, De Castro G Jr (2007). Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. *Br J Cancer*, **96**, 1504-13.

Elston CW, Ellis IO (2002). Pathological prognostic factors in breast cancer. i. the value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*, **41**, 154-61.

Galea MH, Blamey RW, Elston CE, Ellis IO (1992). The nottingham prognostic index in primary breast cancer. *Breast Cancer Res Treat*, **22**, 207-19.

Goldhirsch A, Winer EP, Coates AS (2013). Personalizing the treatment of women with early breast cancer: highlights of the st gallen international expert consensus on the primary therapy of early breast cancer. *Ann Oncol*, **24**, 2206-23.

Goldhirsch A, Wood WC, Coates AS, et al (2011). 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. *Ann Oncol*, **22**, 1736-47.

Jin SH, Kim SH, Kang BJ, et al (2014). Breast cancer recurrence according to molecular subtype. *Asian Pac J Cancer Prev*, **15**, 5539-44.

Khokher S, Qureshi MU, Mahmood S, Nagi AH (2013). Association of immunohistochemically defined molecular subtypes with clinical response to presurgical chemotherapy in patients with advanced breast cancer. *Asian Pac J Cancer Prev*, **14**, 3223-28.

Kilickap S, Kaya Y, Yucel B, et al (2014). Higher ki67 expression is associated with unfavorable prognostic factors and shorter survival in breast cancer. *Asian Pac J Cancer Prev*, **15**, 1381-85.

Kumaki N, Umemura S, Tang X, et al (2011). Alteration of immunohistochemical biomarkers between pre- and post-chemotherapy: hormone receptors, HER2 and Ki-67. *Breast Cancer,* **18**, 98-102.

Paik S, Shak S, Tang G, et al (2004). A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *New England J Med*, **351**.

Pathmanathan N, Balleine RL (2013). Ki67 and proliferation in breast cancer. *J Clin Pathol*, **66**, 512-16.

Reis-Filho J, Pusztai L (2011). Gene expression profiling in breast cancer: classification, prognostication, and prediction. *Lancet*, **378**, 1812-23.

Rinat Y, Woods R, Ravdin PM, Hayes MM, Gelmon KA (2010). Ki67 in breast cancer: prognostic and predictive potential. *Lancet Oncol*, **11**, 174-83.

Saroona H, Hashmi AA, Khurshid A, et al (2013). Ki67 index in breast cancer: correlation with other prognostic markers and potential in Pakistani patients. *Asian Pac J Cancer Prev*, **14**, 4353-58.

Stumpp J, Dietl J, Simon W, Geppert M (1992). Growth fraction in breast carcinoma determined by Ki-67 immunostaining: correlation with pathological and clinical variables. *Gynecol Obstet Invest*, **33**, 47-50.

Gong T, Shak S, Paik S, et al (2011). Comparison of the prognostic and predictive utilities of the 21-gene recurrence score assay and adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat*, **127**, 133-42.

Tanriverdi O, Meydan N, Barutca S (2014). Reconsideration of clinical and histopathological prognostic factors in breast cancer patients: A single center experience. *Asian Pac J Cancer Prev*, **15**, 807-12.

The Cancer Genome Atlas Research Network (2012). Comprehensive molecular portraits of human breast tumours. *Nature*, **490**, 61-70.

Viale Giuseppe, Anita Giobbie-Hurder, Meredith M Regan, et al (2008). Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from breast international group trial 1-98 comparing adjuvant tamoxifen with letrozole. *J Clin Oncol*, **26**, 5569-75.

Yoo C, Ahn J-H, Jung KH, et al (2012). Impact of immunohistochemistry-based molecular subtype on chemosensitivity and survival in patients with breast cancer following neoadjuvant chemotherapy. *J Breast Cancer*, **15**, 203-10.