

How to Reduce the Ki-67 Variability by Jointly Evaluating the Histological Grade and Hormonal Receptors

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ABSTRACT

Background: The proliferative activity of the tumor-measured immunohistochemically by Ki-67 has a high variability. Clinical use can be improved if its measurement is considered with the histological grade

Methods: Ki-67 was studied in 566 breast cancers from 2007 to 2013 in our institution using the monoclonal antibody MIB1. The histological grade and the status of the hormonal receptors were also evaluated.

Results: In 293 (51.7%) tumors the histological grade was I, in 219 (38.7%) tumors was histological grade II and III in 54 (16.8%) tumors. The estrogen receptor was positive in 166 (29.5%) tumors and the progesterone receptor was positive in 95 (16.8%) tumors. None of the tumors with a histological grade III had a Ki-67 value of less than 10%. Only in 1 case (7%) of tumors with histological grade I had a Ki-67 greater than 25%.

Conclusion: Ki-67 values higher than 25% should be repeated and confirmed in those cases a histological grade I, and Ki-67 values lower than 10 in cases with histological grade III.

Keywords

Ki-67, Cell cycle, Immunohistochemistry, Breast cancer.

Introduction

Ki-67 is a non-histone nuclear protein, the Ki-67 gene is located on the long arm of chromosome 10 [1], which is present in all active phases of the cell cycle (G1, S, G2 and mitosis), but is absent in resting cells. It is a marker to determine the fraction of cell growth, measures cell proliferation using immunohistochemistry (IHC) techniques [2]. It correlates with other markers of cell proliferation such as the mitotic index and the tyrosine kinase [3].

High levels of Ki-67 are associated with a higher probability of recurrence in early stages independently of axillary involvement [4]. In the 2013 Sant Gallen Consensus, the Ki-67 level allows differentiation of the Luminal A molecular subtype of Luminal B [5].

The Ki-67 has acquired a relevant importance as a prognostic and predictive factor in breast cancer, but its great limitation is its high variability when assessed by immunohistochemically techniques

[6], which has motivated the present study that establishes Ki-67 values according with the histological grade and the estrogen receptors.

There is an inverse relationship between the values of Ki-67 and the levels of expression of estrogen receptors [7,8]. It correlates with the histological grade [9,10], in a study by J Gerdes et al. [11], breast cancer GH 1 Ki-67 was 9%, in GH 2 it was 15% and in GH 3 it was 26% ($p < 0.001$).

The expression levels of Ki-67 are associated with a risk of recurrence, disease-free survival and overall survival [4,12,13]. Ki-67 is measured in paraffin sections by an immunohistochemically method, using the MIB-1 antibody; the Ki-67 score is defined as the percentage of the total number of tumor cells with nuclear staining.

There is an important correlation between Ki-67 and the response to chemotherapy, since cytostatics only act on the cells that proliferate [14-20]. Each laboratory that evaluates Ki-67 in invasive breast

cancer must perform an internal study, correlating its Ki-67 values with the histological grade and the estrogen receptor. The study and evaluation of the histological grade and the estrogen receptor have a long and long tradition in their study with little variability in their evaluation. Being an essential quality control that each center must perform for the evaluation of Ki-67 by means of immunohistochemistry and therapeutic and prognostic decision making.

Material and Methods

During the period 2007-2013, Ki-67 was studied in 566 breast cancers that have been evaluated in the Committee of Gynecologic Oncology and Mastology of Dexeus University Hospital.

The Ki-67 is determined with the monoclonal antibody MIB 1 (Ventana anti Ki-67 30-9), classifying it in <10, 10-25 and > 25. The estrogen receptor (ER) (Ventana SP1) and the progesterone receptor (RP) (Ventana, 1E2) are determined.

The correlation between Ki-67 with the histological degree and Ki-67 with the hormonal receptors is studied. The histological grade is established according to the classification of Scarff-Blom Richardson modified by Elson. The number of cases of Ki-67 values is collected according to the histological grade and the hormonal receptors, in order to determine in which cases Ki-67 should be repeated.

It is considered that there is a statistical correlation when $p < 0.05$.

Results

The Ki-67 value, in the 566 patients, was less than 10% in 152 (26.85%), between 10 and 25% in 336 (59.36%) and greater than 25% in 78 (13.78%) (Table 1).

Ki-67	n	%
<10	152	26.85
10-25	336	59.36
>25	78	13.78

Table 1: n=566 patients.

The study of the histological grade (GH) with the Ki-67, shows that with GH III there is no case with a Ki-67 less than 10%, but there were 29 cases (37.2%) with a Ki-67 greater than 25%. With GH I there were 112 (73.7%) cases with Ki-67 <10%, and 13 cases (16.7%) with Ki-67 greater than 25%. Ki-67 is associated with the histological degree. ($p < 0.001$) (Table 2).

Ki-67	GI		GII		GIII		
	n	%	n	%	n	%	
<10	112	73.7	40	26.3	0	0	$p < 0.001$
10-25	168	50	143	42.6	25	7.4	
>25	13	16.7	36	46.2	29	37.2	

Table 2: Histologic Grade – Ki-67.

The estrogen receptor (ER) is <10 in 151 (26.72%) cases, between

10-25 in 336 (59.47%) cases and > 25 in 78 (13.80%) cases.

The study of the progesterone receptor (RP) with Ki-67 shows that with a higher Ki-67 there is a lower RP. With a Ki-67 > 25% 24 cases (30.8%) have an RP > 70, while with Ki-67 <10% there are 77 (51.7%) cases with RP > 70. ($p < 0.001$) (Table 3).

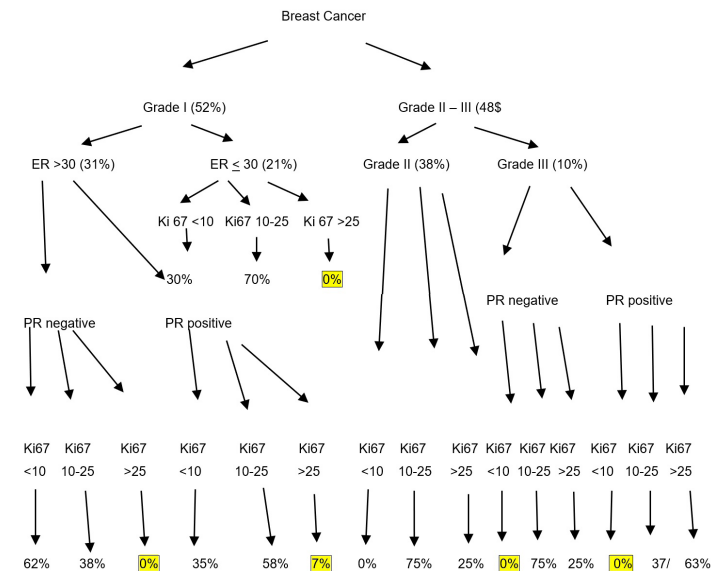
Ki-67	RP										$p < 0.001$
	neg		<10		10-29		30-70		>70		
	n	%	n	%	n	%	n	%	n	%	
<10	33	22.1	0	0	22	14.8	17	11.4	77	51.7	
10-25	87	26	7	2.1	25	7.5	32	9.6	184	54.9	
>25	36	46.2	3	3.8	8	10.3	7	9	24	30.8	

Table 3: Progesterone Receptor – Ki-67.

The study of the estrogen receptor with Ki-67 does not show any statistically significant relationship ($p = ns$) (Table 4). The GH I is in 294 (52%) and GH II-III in 272 (48%). In the 119 (21%) breast cancers GH I with estrogen receptor (ER) negative there is none with a Ki-67 > 25% and the 175 (31%) with ER positive, when the RP is negative there is no case with a Ki-67 > 25, only when the RP is positive (7%) the Ki-67 is > 25% (Figure 1).

Ki-67	RE									
	neg.		<10		10-29		30-70		>70	
	n	%	n	%	n	%	n	%	n	%
<10	7	4.6	5	3.3	6	4.0	21	13.9	46	30.5
10-25	52	15.5	8	2.4	8	2.4	29	8.6	95	28.3
>25	20	25.6	3	3.8	1	1.3	8	10.3	11	14.1

Table 4: Estrogen Receptor – Ki-67.



Discussion

Ki-67 levels have become very important in the study of breast cancer, contributing to the evaluation of risk and contributing to the choice of adjuvant medical treatment. The value of the Ki-67 is decisive to differentiate the Luminal A subtype of the Luminal B

HER2 negative, allowing to establish a different evaluation of the risk and therefore of the prognosis. This entails advising a different adjuvant medical treatment [21].

Recently, the study of Ki-67 is also predictive of the response to adjuvant hormone therapy, as shown by the results of the PEPI (Preoperative Endocrine Prognostic Index) study [22] allowing the selection of patients with positive hormone receptors, negative HER2 that will respond to hormonal therapy adjuvant and which are not, with adjuvant chemotherapy being necessary.

The multiple applications of Ki-67 in the clinical management of breast cancer can be compromised by the variability in its determination, and should be a concern of every laboratory that evaluates the Ki-67, its internal validation of its results correlating them with other values already established historically [23,24].

The International Ki-67 in Breast Cancer Working Group recommends that the Ki-67 evaluation be done visually on a glass slide, where the cut-off points for prognosis, prediction and monitoring must have been validated.

The variability existing in the interlaboratory evaluation requires being very careful in making therapeutic decisions based only on the Ki-67 of another laboratory [25]. Each laboratory must perform a validation of the Ki-67 evaluation, comparing it with other validated histological parameters [24].

The reason for the present study was to analyze the evaluation of Ki-67 in our laboratory, validating it with other histological parameters (GH, RE, RP).

Conclusion

Each laboratory must carry out a study of their own results of Ki-67 correlating them with the histological grade and the estrogen and progesterone receptors, establishing their own values of normality, and in which cases the Ki-67 study should be repeated.

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