RESEARCH

Different Methods of Pretreatment Ki-67 Labeling Index Evaluation in Core Biopsies of Breast Cancer Patients Treated with Neoadjuvant Chemotherapy and Their Relation to Response to Therapy

András Vörös · Erika Csörgő · Bence Kővári · Péter Lázár ·
Gyöngyi Kelemen · Orsolya Rusz · Tibor Nyári · Gábor Cserni

Abstract Increased proliferation activity of breast cancer cells evaluated by Ki-67 immunohistochemistry, i.e. a high Ki-67 labeling index (Ki-67 LI), may predict better tumor regression in case of neoadjuvant chemotherapy. Despite recommendations for the evaluation of Ki-67 LI, there are variations in methodology. We assessed the effect of different evaluation methods on the Ki-67 LI in patients with different response to neoadjuvant chemotherapy. Thirty pretreatment core-biopsy samples of patients receiving neoadjuvant docetaxel-epirubicin chemotherapy with or without capecitabine were evaluated for their Ki-67 LI. Pathologic regression was categorized as no regression, partial regression and complete regression, with 10 cases in each category. Three antibodies (MIB1, B56, SP6), 4 observers and 4 methods (counting or estimating on glass slides and counting or estimating on representative digital images) were compared. The Kruskal-Wallis test and analyses of variance were performed to investigate the differences in Ki-67 LIs between different clinical outcomes (tumor regression categories). Breast carcinomas with pathological complete regression had a higher mean Ki-67 LI than tumors not achieving complete regression with any methods, observers and antibodies investigated, although there was a variation between different evaluations in what may represent high proliferation. Estimating the Ki-67 LI on digital images representing the highest proliferation in the core biopsy seemed the best in separating complete responders from non-responders. High Ki-67 LI values were more likely associated with pathological complete regression independently of the method of evaluation used, although the definition of high proliferation is problematic. Estimating the Ki-67 LI may be an adequate method of evaluation.

Keywords Breast carcinoma · Evaluation methods · Ki-67 · Neoadjuvant therapy · Tumor regression

A. Vörös · E. Csörgő · B. Kővári · P. Lázár · G. Cserni
Department of Pathology, University of Szeged, Allomás u 2,
6725 Szeged, Hungary

G. Kelemen · O. Rusz
Department of Oncotherapy, University of Szeged, Korányi fasor 12,
6720 Szeged, Hungary

T. Nyári
Department of Medical Physics and Informatics, University of
Szeged, Korányi fasor 9, 6720 Szeged, Hungary

G. Cserni
Department of Pathology, Bács-Kiskun County Teaching Hospital,
Nyiri ut 38, 6000 Kecskemét, Hungary

A. Vörös (✉)
Department of Pathology, University of Szeged, Állomás u. 2.,
6720 Szeged, Hungary
e-mail: andrasvoros@libero.it

Introduction

Proliferation activity is of prognostic importance in breast cancer and the higher the proliferation, the faster the progression of the disease [1–4]. One component of the classical grading system is counting the mitotic figures in 10 high power fields [5]. Many other techniques are available to assess tumor cell proliferation beside mitotic counts, including flow cytometry, which examines the proportion of cells being in the S phase of the cell cycle, determination of thymidine-labeling index, or immunohistochemistry with antibodies to proliferating cell nuclear antigen (PCNA), or cyclins E and D [6]. However Ki-67 immunostaining is the most popular in current

everyday practice. The reasons for this are the simplicity of the method and the fact, that this protein is expressed during the whole cell cycle except the G0 phase, therefore nuclear staining is present in all cells in the cycle [7, 8]. Although cells in the cycle may reflect the proliferative fraction, some of the cells may also undergo apoptosis rather than mitosis.

Expression of this protein has not only prognostic value, but may also be predictive of the response to neoadjuvant therapy as suspected by Fasching et al. [9] and by Yerushalmi et al. [4], who analyzed the results of different studies looking for the predictive value of the Ki-67 labeling index (LI: i.e. the proportion of Ki-67 stained tumor cells expressed as a percentage) in case of neoadjuvant chemotherapy. In these studies, better response (complete pathological response / pCR or complete clinical response / cCR) has been reported in breast cancers with higher proliferative activity treated with neoadjuvant chemotherapy [10–15]. Luprosi et al. also found that pCR is in connection with high Ki-67 LI although the predictive value of this marker was not verified [16]. Recent recommendations (including the St. Gallen International Breast Cancer Conference, 2013) contain Ki-67 evaluation as the part of the routine diagnostic procedures although its predictive potential has not yet been proven unequivocally in case of neoadjuvant chemotherapy [17–20].

During Ki-67 immunohistochemical examinations, the proportion of positive (stained) cancer cells is determined in percentage. This evaluation is based on the pathologist's own decision. Some pathologists use estimation, while others count the positive and negative cells in an area with a certain size or average the values of many different areas. Several published recommendations exist regarding the evaluation techniques of Ki-67, however there is no uniformly accepted one [16–18, 21, 22]. It is also notable that different detailed counting methods (e.g. counting 500 or 1,000 tumor cells) are time consuming and can be difficult to use in the everyday practice of centers managing significant number of patients. In our previous studies we found that different evaluation methods have different reproducibility [23]. Our previous results suggested that the use of representative digital images in combination with visual evaluation increased the reproducibility of Ki-67 assessment [24]. Reproducibility is an important factor, as it may contribute to the predictive value of Ki-67. In our previous studies we analyzed only the reproducibility of Ki-67 evaluation on core-biopsy samples of patients who underwent neoadjuvant chemotherapy on the basis of their locally advanced stage. We also had the information on the therapeutic response, as the group originally studied included equal numbers of cases from each of the pathological complete response, partial response and no response categories.

The aim of the present study was to compare different therapeutic response categories with different Ki-67 evaluation methods in the cases previously analyzed for

reproducibility [23, 24]. We intended to know if there was an evaluation method (among the previously used ones) which could be particularly recommended or rejected in everyday practice. Another important issue is the usability of the cut-off values in the different recommendations assorting breast carcinomas into low- and high proliferation groups [16–18]. In the present paper we also aimed to know whether we could identify a cut-off value which can be considered as separation point between tumors having high or low proliferation activity before neoadjuvant therapy and is worth of further studies.

Materials and Methods

Thirty core-biopsy samples of patients with operable T2≥3 cm or T3-4 and/or N1-2 and M0 breast cancer candidate for neoadjuvant chemotherapy were retrospectively analyzed for their Ki-67 LI. All patients received combination chemotherapy containing docetaxel and an anthracycline (epirubicin for 29 and doxorubicin for one patient) with or without capecitabine [25]. Details on clinical and pathologic information of patients are summarized in Table 1. Core-biopsy samples were chosen by considering regression of the primary tumor after neoadjuvant chemotherapy. Three categories were made: no regression (or progression), partial regression (histological signs of regression) and pathological complete regression (no residual invasive tumor). Each category included different types of breast carcinoma regarding histological subtype, grade (G), hormone receptor (HR) and HER-2 status (Table 1). Histologic response was assessed according to the European guidelines for quality assurance in breast cancer screening and diagnosis [26]. Each group consisted of 10 samples.

Samples were immunostained for Ki-67 with the following 3 antibodies: SP6 (monoclonal rabbit antibody, Hisztopatologia Kft., Pécs, Hungary), B56 (monoclonal mouse antibody, Hisztopatologia Kft., Pécs, Hungary) and MIB-1 (monoclonal mouse antibody, Dako, Glostrup, Denmark) [23, 24], and the original HR and HER-2 staining was taken into consideration during the study [25]. Four different investigational methods were used to evaluate the Ki-67 LI.

In Method 1 (counting on glass slides) all samples were assessed independently by 3 pathologists at high-power magnification (×400). Each pathologist was asked to evaluate the slides according to his/her daily routine practice. The ratio of Ki-67 positive cells was evaluated with 5 % accuracy. Each participant, following daily practice, evaluated the LI on the basis of one hundred tumor cells. Owing to the 5 % precision, only values ending with 5 or 0 were recorded. To avoid bias arising from remembering the LI of a given sample, the observers first assessed all cases stained with one antibody and this was followed by the evaluation of all cases stained

Table 1 Clinical and pathological data of patients selected for the study in the three regression groups

	No regression $n=10$	Partial regression $n=10$	Complete regression $n=10$
Type of chemotherapy			
TE	3	2	5
TEX	7	8	4
TAC	0	0	1
cT			
1	0	1	1
2	1	6	5
3	6	2	2
4	3	1	2
cN			
0	2	3	1[a]
1	2	1	6
2	6	5	2
3	0	1	1
FNA (lymph node)			
Positive	4	4	3
Not performed	6	6	7
Pathological data			
ER - PR - HER-2 +	1	0	3
ER + PR + HER-2 -	4	6	1
ER + PR -	1	0	0
ER - PR -	4	4	6
Grade 2	3	3	1
Grade 3	7	7	9
Invasive ductal	10	8	10
Invasive lobular	0	2	0

[a] The patient had a pretreatment sentinel lymph node biopsy yielding 2 metastatic sentinel nodes

with the second antibody and finally the cases stained with the third antibody were analyzed [23].

In another study (Method 2 and Method 3), four investigators analyzed representative areas of the same 30 samples [24]. Microphotographs were taken of each immunostained core biopsy sample at the same magnification (200×). The hot-spot area was photographed in all cases where such a hot spot could be identified. A single digital photo of each biopsy sample was selected for the study, and the pictures were entered in a Microsoft PowerPoint file. In this study, Method 2 was applied (counting on digital images): a uniform grid composed of equidistant parallel horizontal lines was laid on all digital images (Fig. 1), previously used for estimation. The observers were asked to count the tumor cells crossed by the lines or touching the lines. The lines of such a grid can be followed and the touching or crossed cells can be recorded (counted) continuously without the doubt of double counting or omitting single cells. Both immunohistochemically negative and positive nuclei were counted. Non-cancerous cells (stromal elements, lymphocytes etc.) were ignored as much as possible. The ratio of positive cells was derived from these values.

Method 3 (estimation on digital images) was also used in this second study: before counting the proportion of Ki-67 stained cells with the help of the overlaid grid, investigators determined the Ki-67 LI by estimating the proportion of stained cells with 5 % accuracy in the same areas (i.e. the same digital image displayed on a screen without the grid). No counting was involved in this assessment.

For the present study, the previously mentioned three methods were completed with a fourth, in which the proportion of Ki-67 labeled cells was estimated by eyeballing on the glass slides (Method 4). The same four investigators estimated the proportion of positive tumor cells on the slides by quick inspection of the slides, without counting.

The average Ki-67 LIs of the respective 10 cases of all observers, antibodies and assessment methods were used for comparisons of clinical outcomes (tumor regression categories). The Kruskal-Wallis test and analyses of variance (one-way and two-way ANOVA) were performed to investigate the differences in Ki-67 LIs between different clinical outcomes. Receiver operating characteristic (ROC) curve analyses were applied to broadly approach a distinction between Ki-67 values of cases with complete response to therapy and those without it. The possible connection between ER receptor status and type of tumor regression (omitting the cases with partial regression) following neoadjuvant chemotherapy was also analyzed applying Fisher's exact test. All analyses were performed with the statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois).

Because of the retrospective nature of the study, anonymous handling of the available data and the lack of influence on patient outcome, no ethical approval was deemed necessary according to local regulations. The institutional data safety monitor (Bács-Kiskun County Teaching Hospital) was consulted and made no objections to the anonymous handling of the data.

Results

Altogether 1,350 evaluations were analyzed in our study (as one investigator did not take part in the first count, i.e. Method 1). The overall mean value of the Ki-67 LI (with all assessment methods, antibodies and observers considered) was 54.22 (95 % CI: 53.13–55.31).

According to the statistical analyses, values of Ki-67 LI were significantly different in the different regression groups: values of the group without regression were significantly lower than the values of the group showing complete
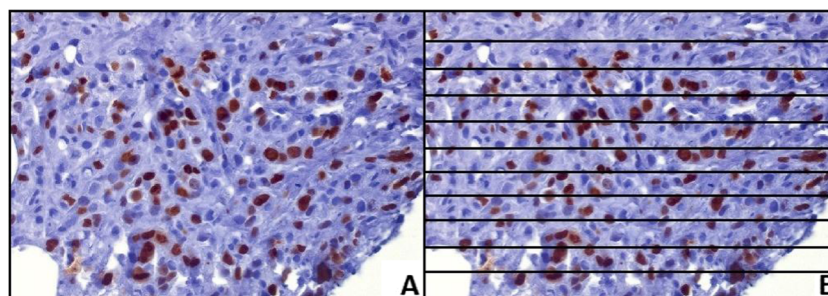
Fig. 1 An example of the digital pictures analyzed. **a** image used for estimating the Ki-67 LI by eyeballing (Method 3); **b** the same image with *parallel grid lines* laid over delineates the stained and unstained cells (those touching the lines or crossed by them) to be considered when counting (Method 2)

regression ($p<0.0001$). The mean values of Ki-67 LI taking into account all methods of assessment were 66.61 (95 % CI: 64.71–68.50), 51.32 (95 % CI: 49.43–53.21) and 44.72 (95 % CI: 42.83–46.62) for the groups with complete, partial and no regression, respectively.

The two way analysis of variance using the methods of evaluation and the response categories as factors showed significant differences in mean Ki-67 values according to both factors. The mean KI-67 value was the lowest in Method 1 and highest in Method 3. Mean Ki-67 LI values were 46.63 (95 % CI: 44.21–49.05), 54.62 (95 % CI: 52.52–56.72), 61.59 (95 % CI: 59.49–63.69) and 54.04 (95 % CI: 51.94–56.17) in case of Methods 1 through 4, respectively.

We found similar results after taking the pathologically assessed response into consideration. Mean value of Ki-67 LIs by Method 1 were 58.72 (95 % CI: 54.53–62.92), 46.39 (95 % CI: 42.19–50.59) and 34.78 (95 % CI: 30.58–38.98) in case of complete, partial and no regression, respectively, whereas these values with Method 3 were 77.18 (95 % CI: 73.54–80.18), 56.59 (95 % CI: 52.95–60.13) and 51.00 (95 % CI: 47.37–54.64), respectively.

Figure 2 remarkably illustrates that the red and blue fields representing the mean Ki-67 LIs of cases with pCR and no regression, respectively are best separated from each other in case of Method 3, the estimation on single digital images and this is in accordance with the mean Ki-67 LIs described above. It is also clearly demonstrated that the yellow fields, which show partial regression are mostly located between the red and blue fields, slightly closer to the blue ones; sometimes they are fused with them (green). These patterns can be observed in case of all investigational methods, observers and antibodies.

By applying an ROC curve analysis to the mean Ki-67 data, the distinction between cases showing complete regression versus cases showing no regression (and omitting the cases with partial regression) on the basis of Ki-67 values (gained by any method) gives an area under the curve (AUC) of 0.969, whereas comparing complete regression values with the cases with partial regression and those showing no regression aggregated together, the AUC turns to 0.93 (Fig. 3). Here the suggested best cut-off value to predict complete regression

would have been around 56 % LI (sensitivity: 0.89; specificity: 0.81).

Figure 4 gives an insight into the distribution of individual Ki-67 LIs behind the mean values shown in Method 3 related part of Fig. 2. Although the mean values shown in Fig. 2 are obviously higher (more to the right) for cases with pCR (red fields) than for the rest of the cases, it is clear that the individual cases show considerable overlaps. Although most red fields fall to the right and above 50 %, there are a few blue and yellow fields (representing no regression and partial pathological response, respectively) falling among the red fields and a few red fields falling among the other colors and below 50 %. Similar overlaps could have been represented for all methods investigated.

According to the Fisher's exact test cases showing complete pathological regression were more likely negative for ER receptor immunohistochemistry ($p=0.024$).

Discussion

Ki-67 immunohistochemistry is one of the most common methods to prognosticate biologic behavior of breast carcinomas. Precise evaluation of the Ki-67 LI is important for both pathologists and clinicians because this factor is also considered in therapeutic decision making, especially when chemotherapy is indicated in addition to hormonal treatment [4, 9–15]. Although Ki-67 immunohistochemistry provides more or less obvious nuclear positivity in the cells within the cell cycle, including proliferating tumor cells, it is still a real challenge for pathologists to perform an objective evaluation. A precise and exact evaluation is complicated by numerous factors like tumor heterogeneity, intensity of staining to be considered positive, admixture of non-tumor cells with tumor cells, the intensity of counterstaining with hematoxylin and sometimes small, non-representative tumorous area, to list only a few of these factors. Beside these issues, another important factor is the time taken by the evaluation. Although an international consensus recommends the examination of at least 500, but optimally 1,000 cells for deriving the Ki-67 LI
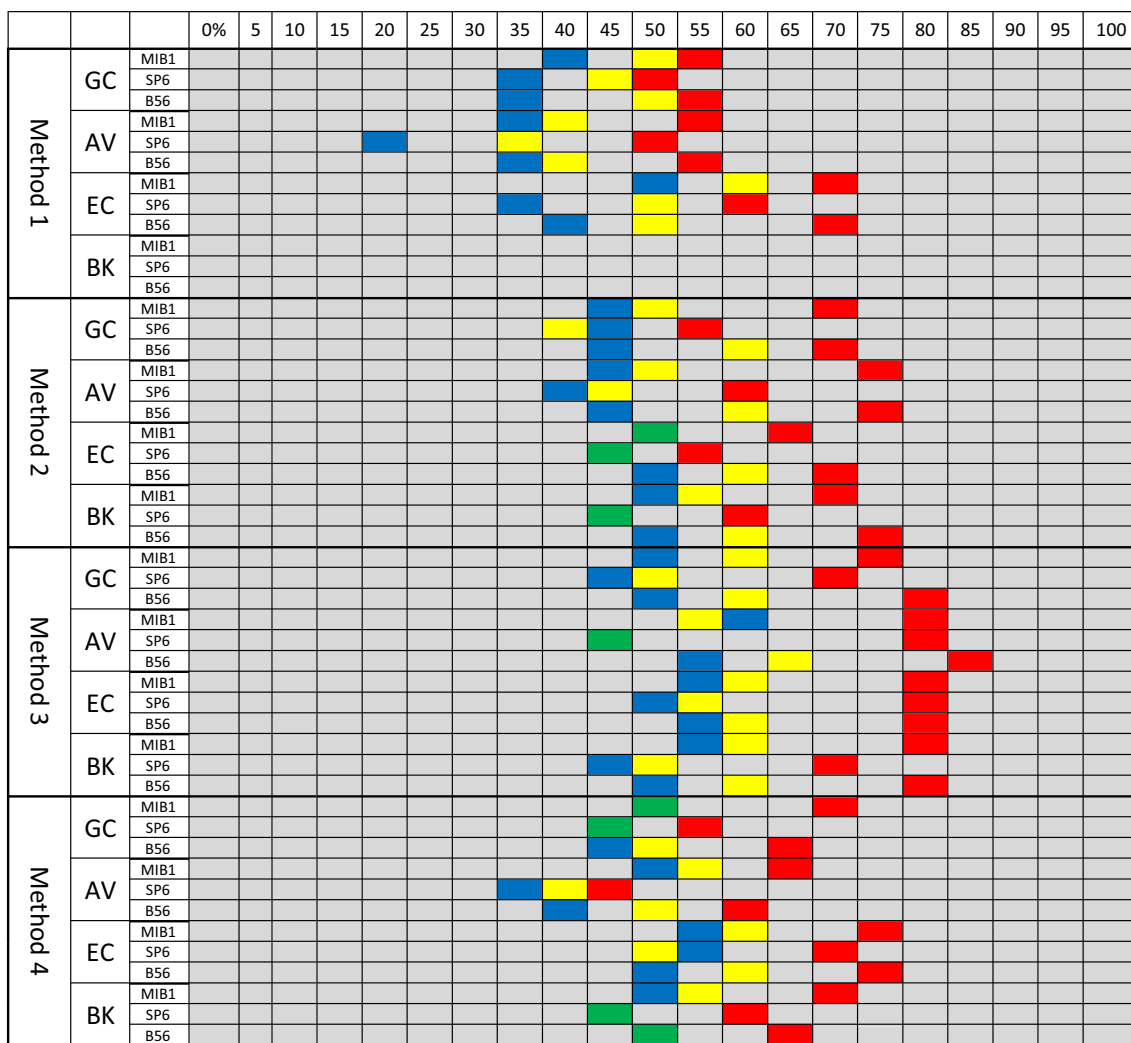
Fig. 2 Mean Ki-67 LIs gained by four different evaluation methods by 4 observers and 3 antibodies in the different categories of tumor regression after neoadjuvant chemotherapy. *Blue* fields represent values of tumors with no regression, the *yellow* ones are for tumors with partial regression and the red ones for those with pathological complete regression. The *green* color reflects the fusion of *blue* and *yellow* for instances when the mean values of partial regression and no regression fall in the same field. Each colored field represents the mean KI-67 LI value of ten cases in one of the three groups (no regression, partial regression and complete regression). GC, AV, EC, BK reflect the observers and MIB1, SP6, B56 the antibodies. The scale of the figure is by 5 %, i.e. each field represent a range of values with 5 integers from 1–5 to 96–100

[22], this practice is rarely followed. In everyday practice, pathologists are reluctant to spend the required time for counting hundreds of tumor cells on each case. It is also obvious from personal discussions, that many pathologists believe that counting the Ki-67 LI on the basis of 1,000 cells is a waste of time. Our first study highlighted the limitations of daily practice based tumor cell counting, as all three participating pathologists counted only a hundred cells, and the effort resulted in poor reproducibility of the Ki-67 LI [23]. That study showed that the Ki-67 LI values were significantly influenced by the person of the investigator. Not only the inter-observer, but also the intra-observer agreement was found to be poor to moderate. A later study of ours suggested that by choosing a limited area of the tumor representing the highest proliferation of the core biopsy sample and fitting into

a single digital image, and by helping to choose which cells to count with a grid, the reproducibility of the Ki-67 LI can be improved [24].

As mentioned previously, the Ki-67 LI has prognostic value in breast cancer and may indicate the need for additional chemotherapy in special cases. Cytotoxic therapy acts on proliferating cells, therefore it would be very logical to hypothesize that tumors with a higher baseline proliferation may better benefit from chemotherapy than those with a low baseline proliferation. In keeping with this theoretical approach, a better response to neoadjuvant chemotherapy was reported in tumors with high baseline Ki-67 LI [9, 27–29]. In contrast, tumors with a high Ki-67 LI showed similar response rate (77 %) to chemoendocrine therapy than those with a low Ki-67 LI (81 %) in a retrospective series from the Royal Marsden
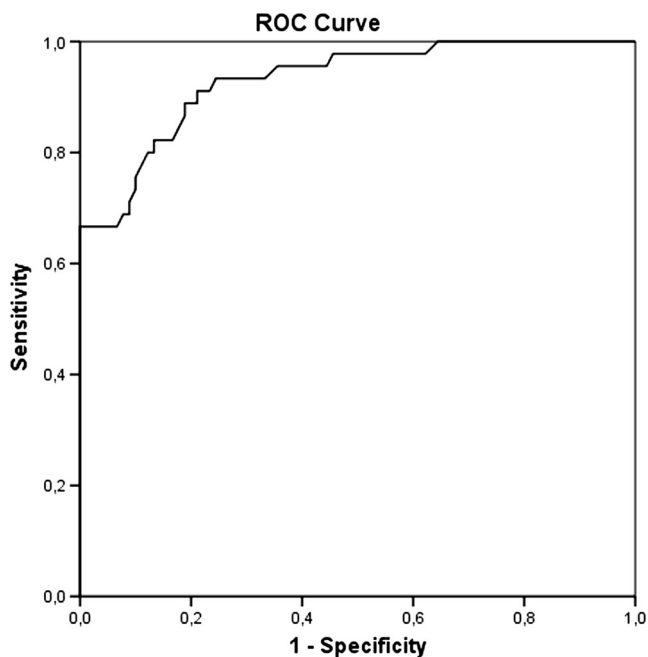
Fig. 3 ROC curve derived from all average Ki-67 values (all methods, observers and antibodies) as predictors of the group with pCR and those without it (partial response and no response together) AUC: 0.93 (sensitivity: 0.89; specificity: 0.81)

neoadjuvant therapy) may better predict the response and the outcome of disease [27, 28, 31], but multiple biopsies are not always easy to obtain. Our results displayed in Fig. 2 show that breast cancers of the cohort selected for the study showing pCR consistently had a higher mean Ki-67 LI than those not responding or showing only partial pathological response whatever the method of evaluation or the antibody used were or whoever the observer was. Although this difference was seen among the small groups showing regression, looking into the details demonstrates that the regression of individual cases of breast carcinoma cannot be precisely predicted (Fig. 4), and prediction on the basis of high versus low Ki-67 LI does not work for the individual cases. The only thing that can be stated is that tumors with higher Ki-67 LIs are more likely to regress completely, independently of the method of evaluation.

The methods compared in this study had different reproducibility [23, 24]. Although a good to excellent correlation was found between the results gained by different methods, antibodies and observers, the interobserver and intraobserver reproducibility of the everyday practice based counting on glass slides (Method 1) was only fair to moderate. Reproducibility of the Ki-67 LI has improved when the field to be assessed was limited to a digital image, chosen to represent the highest proliferation in the core biopsy sample (Method 2) [23, 24]. Interestingly, reproducibility was not worse when the proportion of

Hospital [30]. It is also likely that Ki-67 applied as a dynamic marker (i.e. taken as a baseline value and also during
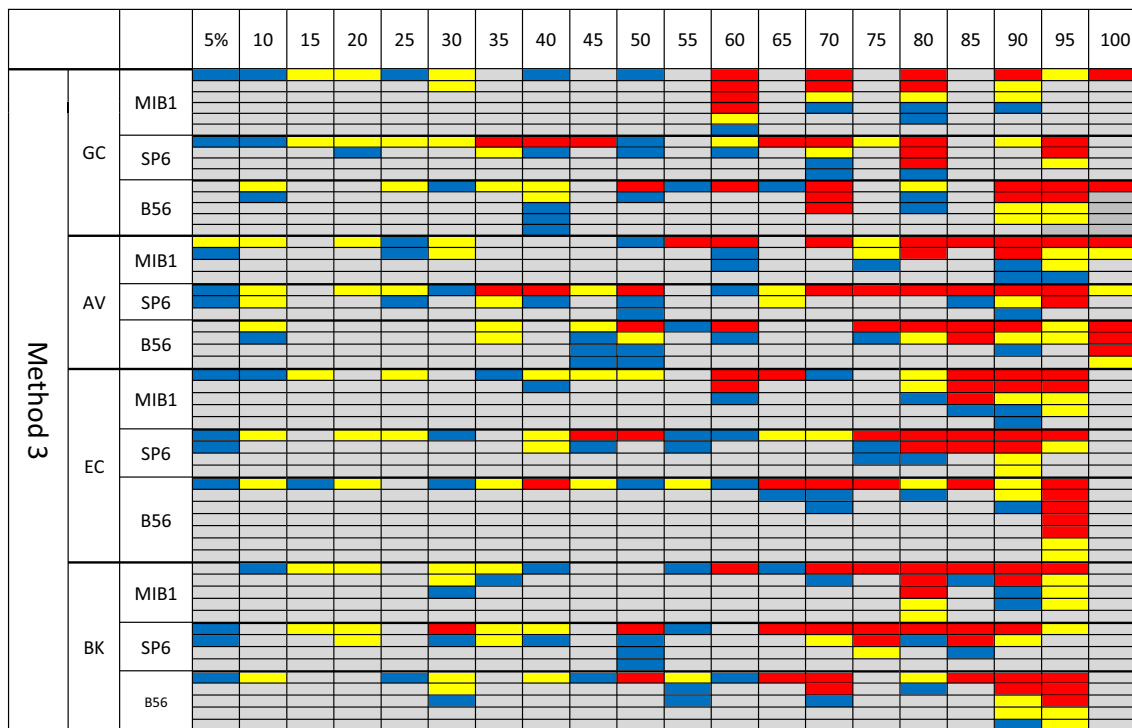

Fig. 4 Individual Ki-67 LI values for Method 3 (estimation on representative digital images). *Blue* fields represent values of tumors with no regression, the *yellow* ones are for tumors with partial regression and the *red* ones for those with pathological complete regression. Each colored field represents a case with a given Ki-67 LI. GC, AV, EC, BK reflect the observers and MIB1, SP6, B56 the antibodies with 30 cases falling in each of the assessments. The scale of the figure is by 5 %, as the estimation was done in steps of 5 % precision

stained cells was only estimated (Method 3) rather than counted (Methods 1 and 2). This is in keeping with the results of a very carefully designed study by Varga et al, which showed that the reproducibility achieved by estimation was not worse than that reached by meticulous counting [32]. This suggests that the simple approach of estimating by eyeballing has also diagnostic value, and may be better received by the pathological society as a simple and fast method, taking 4 to 12 times less time than counting [23, 24]. The daily use of estimating rather than counting the Ki-67 LI by at least some pathologists was also highlighted in a series evaluating the distribution of Ki-67 LI values in different pathology departments [33].

Although all of our study cases received neoadjuvant chemotherapy on the basis of locally advanced stage according to general practice and recommendations, biological markers, including those reflecting a high proliferation rate might be more suitable to select patients with potential benefit from this treatment. Pathological complete regression is associated with better prognosis [34, 35], and tumors achieving pCR have a higher mean Ki-67 LI than those which do not achieve pCR, in keeping with earlier works [9, 27–29]. However, proliferation alone is not sufficient to predict the response to neoadjuvant chemotherapy [29], as demonstrated by Fig. 4, and it is also a matter of debate what cut-off value should separate tumors with high and low KI-67 LIs. In this respect, Fig. 2 seems useless, and suggests that depending on the methods, antibodies and observers, different cut-offs could be used for this purpose. Fasching et al used a >13 % cut-off [9], in keeping with the value suggested for the immunohistochemistry based separation of luminal A and luminal B carcinomas [18, 36]. A recent analysis by members of the European Working Group for Breast Screening Pathology found that the distribution pattern of 1709 Ki-67 LI values has peaks at values ending with 0 or 5, and therefore an inclusive or non-inclusive cut-off ending with these values would seem more realistic [33], and independently, the latest St Gallen recommendations have happily adopted a 20 % cut-off in keeping with the cited results. Figure 2 would suggest that with most of the methods included, a cut-off of 50 % would delineate high proliferation, and the ROC curve analysis also supports a similar value (i.e. 55 %), but the analysis of the distribution pattern by the members of the European Working Group for Breast Screening Pathology suggests that only a minority of breast cancers would fall into this category, as the median Ki-67 LI of the large cohort was 17 % whereas the estrogen receptor-positive cases had a median of only 14 % [33]. Therefore, the small subset of locally advanced breast cancers analyzed in the present work would fall into the higher end of the Ki-67 LI values of a general breast cancer cohort, and might not be representative enough. This would also counteract the determination of a cut-off value. Individual cases with high proliferation (e.g. Ki-67 LIs above 50 %) would still have overlapping Ki-67 LI values with non-regressing tumors (Fig. 4).

As to the best method, statistics do not allow to suggest that any one of these methods is better than the other, they are just different from each other with regard to the Ki-67 LIs. It seems obvious that Method 3, the estimation made on the digital image taken from the area thought to represent the highest proliferation in the core biopsy sample had substantial reproducibility [24], is fast and simple, and seems to separate responders from non-responders better than the other methods. However, individual cases would fail to follow the prediction even on the basis of this small sample. (The number of data evaluated in this study did not allow an ROC curve analysis of sufficient value to estimate the best cut-off value for this individual method.) Response to therapy is obviously a phenomenon depending on multiple factors of which the number of cells in the cycle is only one. For example, the predictive role of the HR status has also been widely investigated [9–15]. A recent meta-analysis by Houssami also concluded that ER-negative tumors are more likely to achieve pCR [37]. The data of this small series are in keeping with this, since ER-negative tumors were more common in the pCR group than in the rest of the patients.

Conclusion

Tumors of patients selected for this study achieving pCR after neoadjuvant chemotherapy had a higher mean Ki-67 LI than those who achieved either partial pathological response or did not show regression. This statement is true for all the 3 antibodies tested, all the 4 observers and all the methods evaluated. Therefore, the simplest method of evaluating the Ki-67 LI by eyeballing rather than time-consuming counting could be a good alternative to assess the proliferation rate. The estimation of a cut-off value would require a larger series of cases and should probably be different for each evaluating method. Despite the association of a higher Ki-67 LI with pCR, a high Ki-67 LI alone is not sufficient to predict response to neoadjuvant chemotherapy, since other factors (including the HR status of the tumor) also influence the response to treatment.

References

1. Stuart-Harris R, Caldas C, Pinder SE, Pharoah P (2009) Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 17(4):323–334
2. de Azambuja E, Cardoso F, de Castro G, Jr CM, Mano DV et al (2007) Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 96:1504–1513

3. Urruticoechea A, Smith IE, Dowsett M (2005) Proliferation marker Ki-67 in early breast cancer. J Clin Oncol 23:7212–7220

4. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA (2010) Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 11:174–183

5. Boom HJ, Richardson WW (1957) Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer 11(3):359–377

6. Beresford MJ, Wilson GD, Makris A (2006) Measuring proliferation in breast cancer: practicalities and applications. Breast Cancer Res 8:216

7. Gerdes J, Li L, Schlueter C, Duchrow M, Wohlenberg C, Gerlach C et al (1991) Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 138:867–873

8. Gerdes J, Schwab U, Lemke H, Stein H (1983) Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20

9. Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM et al (2011) Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 11:486

10. Bozzetti C, Musolino A, Camisa R, Bisagni G, Flora M, Bassano C et al (2006) Evaluation of HER-2/neu amplification and other biological markers as predictors of response to neoadjuvant anthracycline-based chemotherapy in primary breast cancer: the role of anthracycline dose intensity. Am J Clin Oncol 29(2):171–177

11. MacGrogan G, Mauriac L, Durand M, Bonichon F, Trojani M, de Mascarel I, Coindre JM (1996) Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. Br J Cancer 74(9):1458–1465

12. Faneyte IF, Schrama JG, Peterse JL, Remijnse PL, Rodenhuis S, van de Vijver MJ (2003) Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 88(3):406–412

13. Penault-Llorca F, Abrial C, Raoelfils I, Chollet P, Cayre A, Mouret-Reynier MA et al (2008) Changes and predictive and prognostic value of the mitotic index, Ki-67, cyclin D1, and cyclo-oxygenase-2 in 710 operable breast cancer patients treated with neoadjuvant chemotherapy. Oncologist 13(12):1235–1245

14. Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C et al (2004) Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 40(2):205–211

15. Pohl G, Rudas M, Taucher S, Stranzl T, Steger GG, Jakesz R et al (2003) Expression of cell cycle regulatory proteins in breast carcinomas before and after preoperative chemotherapy. Breast Cancer Res Treat 78(1):97–103

16. Luporsi E, André F, Spyratos F, Martin PM, Jacquemier J, Penault-Llorca F et al (2012) Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Res Treat 132:895–915

17. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann B, Senn HJ (2009) Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol 20:1319–1329

18. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ (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 2011. Ann Oncol 22:1736–1747

19. Gnant M, Harbeck N, Thomssen C (2011) St. Gallen, 2011: summary of the consensus discussion. Breast Care (Basel) 6:136–141

20. Curigliano G, Criscitiello C, Andrè F, Colleoni M, Di Leo A (2013) Highlights from the 13th St Gallen International Breast Cancer Conference 2013. Access to innovation for patients with breast cancer: how to speed it up? Ecancermedicalscience 7:299

21. Fasanella S, Leonardi E, Cantaloni C, Eccher C, Bazzanella I, Aldovini D et al (2011) Proliferative activity in human breast cancer: Ki-67 automated evaluation and the influence of different Ki-67 equivalent antibodies. Diagn Pathol 6

22. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J et al (2011) Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 103:1–9

23. Vörös A, Csörgő E, Nyári T, Cserni G (2012) An intra- and interobserver reproducibility analysis of the Ki-67 proliferation marker assessment on core biopsies of breast cancer patients and its potential clinical implications. Pathobiology 80:111–118

24. Vörös A, Csörgő E, Kővári B, Lázár P, Kelemen G, Cserni G. (2013) The Use of Digital Images Improves Reproducibility of the Ki-67 Labeling Index as a Proliferation Marker in Breast Cancer. Pathol Oncol Res. 2013 Nov 8

25. Nikolényi A, Sükösd F, Kaizer L, Csörgo E, Vörös A, Uhercsák G et al (2011) Tumor topoisomerase II alpha status and response to anthracycline-based neoadjuvant chemotherapy in breast cancer. Oncology 80(3–4):269–277

26. Wells CA, Amendoeira I, Bellocq JP, Bianchi S, Boecker W, Borisch B et al (2012) S2: Pathology update. Quality assurance guidelines for pathology. In: Perry N, Broeders M, de Wolf C, Törnberg S, Holland R, von Karsa L (eds) European guidelines for quality assurance in breast cancer screening and diagnosis. Fourth edition, Supplements. European Commission, Office for Official Publications of the European Union, Luxembourg, pp 73–120

27. Chang J, Ormerod M, Powles TJ, Allred DC, Ashley SE, Dowsett M (2000) Apoptosis and proliferation as predictors of chemotherapy response in patients with breast carcinoma. Cancer 89:2145–2152

28. Faneyte IF, Schrama JG, Peterse JL, Remijnse PL, Rodenhuis S, van de Vijver MJ (2003) Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 88:406–412

29. Keam B, Im SA, Park S, Nam BH, Han SW, Oh DY et al (2011) Nomogram predicting clinical outcomes in breast cancer patients treated with neoadjuvant chemotherapy. J Cancer Res Clin Oncol 137:1301–1308

30. Makris A, Powles TJ, Dowsett M, Osborne CK, Trott PA, Fernando IN et al (1997) Prediction of response to neoadjuvant chemoendocrine therapy in primary breast carcinomas. Clin Cancer Res 3:593–600

31. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R et al (2007) Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst 99(2):167–170

32. Zs V, Diebold J, Dommann-Scherrer C, Frick H, Kaup D, Noske A et al (2012) How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. PLoS ONE 7:e37379

33. Cserni G, Vörös A, Liepniece-Karele I, Bianchi S, Vezzosi V, Grabau D et al (2014) Distribution pattern of the Ki67 labelling index in breast cancer and its implications for choosing cut-off values. Breast 2014 Mar 6

34. Valero V, Buzdar AU, McNeese M, Singletary E, Hortobagyi GN (2002) Primary chemotherapy in the treatment of breast cancer: the University of Texas M. D. Anderson Cancer Center experience. Clin Breast Cancer 3(Suppl 2):S63–S68

35. Montagna E, Bagnardi V, Rotmensz N, Viale G, Pruneri G, Veronesi P et al (2010) Pathological complete response after preoperative systemic therapy and outcome: relevance of clinical and biologic baseline features. Breast Cancer Res Treat 124(3):689–699

36. Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J et al (2009) Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 101:736–750

37. Houssami N, Macaskill P, von Minckwitz G, Marinovich ML, Mamounas E (2012) Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer 48:3342–3354