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Research Article

Evaluation of Ki-67 index in breast cancer cases with intratumor heterogeneity

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Summary

There are no specific recommendations for evaluating the Ki-67 index in heterogeneous breast carcinomas. This study aimed to evaluate the applicability of currently accepted recommendations for Ki-67 evaluation in breast cancer in the context of intratumor heterogeneity. Twelve cases of heterogeneous breast carcinomas obtained from 110 patients were retrospectively studied. Ki-67 staining was performed according to protocols provided by the reagent manufacturer. Results for Ki-67 of the separate components in each tumor were obtained, described, and analyzed statistically using a paired *t*-test. Values of $p < 0.05$ were considered as statistically significant. SPSS software was used for statistical analysis. Results from the comparison of the Ki-67 index evaluation in each heterogeneous component of the studied tumors demonstrated no statistically significant difference of mean values $t = 0.4802$, $p = 0.6405$. The anticipation of an average Ki-67 score in the evaluated cases would have changed the molecular subtype from Luminal B to Luminal A (due to the Ki-67 index below 14%) in two of the cases. Heterogeneous tumors had a different Ki-67 index in their separate components. Our observations suggest that Ki-67 in heterogeneous breast carcinoma is evaluated and reported separately for the distinguishable tumor components.

Key words: Evaluation, heterogeneous breast carcinoma, Ki-67 index

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Introduction

The role of Ki-67 in breast cancer as a prognostic and predictive marker regarding treatment response evaluation is still controversial (Mannel 2016; Davey et al. 2021).

The evaluation procedure for proliferative Ki-67 index in breast cancer is currently still subject to ongoing discussion and standardization (Nielsen et

al. 2021). Many factors can influence Ki-67 evaluation, including the type of biopsy, fixation and laboratory protocols, the used antibody, methods of scoring (global, average, or hot spot), interobserver variability, number of evaluated cells (500 set as absolute minimum) (Dowsett et al. 2011; Nielsen et al. 2021).

In breast cancer, heterogeneity refers to differences in the genomic, transcriptomic, epigenomic, and proteomic characteristics that result in different cancer properties, including proliferation. Heterogeneity is considered to be a dynamic tumor characteristic (Guo et al. 2023). According to the literature, heterogeneity of Ki-67 expression can be observed in carcinomas of different histologic grades and subtypes (Turashvili and Brogi 2017). The heterogeneity in Ki-67 levels in lymph node metastases is far less common as the proliferation index is generally high and relevant to the one observed in a hot spot of the particular tumor (Turashvili and Brogi 2017).

Currently, there are no specific widely accepted recommendations for Ki-67 evaluation in heterogeneous breast carcinomas.

We aimed to evaluate the applicability of currently accepted recommendations for Ki-67 assessment in breast cancer in the context of intratumor heterogeneity.

Patients and methods

Twelve cases of heterogeneous breast carcinomas obtained from 110 breast carcinomas were retrospectively studied for Ki-67 expression. Firstly, all 110 archival cases were revealed on H&E and immunostained slides. All tumors were initially routinely classified, graded, and sub-stratified into surrogate molecular subtypes and staged according to the latest edition of WHO (World Health Organization 2019). Results from core biopsies and resections were studied, and distinct tumor components were separately evaluated, if present, for the study. The selected twelve cases of heterogeneous tumors were further analyzed.

Ki-67 staining was performed according to protocols provided by the reagent manufacturer, using OPAI325T60 Ki-67 prediluted rabbit monoclonal antibody clone SP6 (Biocare USA) and visualization system M1U539G MACH 1 Univ. HRP kit (Biocare USA).

Ki-67 evaluation was conducted following currently accepted recommendations (Dowsett et al. 2011; Nielsen et al. 2021). Whenever possible, a minimum of 500 tumor cells were counted for each component. Tumor cells with nuclear staining were counted as positive, regardless of intensity. When present, “hot spots” and “cold spots” were included. If tumors contained more than two distinct morphologic components, the two most widely presented were analyzed. The results for Ki-67 of the separate components in each tumor were described and analyzed statistically using a paired *t*-test, and values of $p < 0.05$ were considered statistically significant. Statistics were computed using SPSS software.

Ethical approval for this study was obtained from the Medical University of Pleven Research Ethics Committee.

Results

The mean age of the studied population was 50.58 years, ranging between 31 and 73 years. Twelve of the studied 110 cases were found to represent heterogeneous breast carcinomas (10.91%). Six cases demonstrated morphological

and molecular heterogeneity, and 6 showed only morphological heterogeneity. Eight of the cases were unifocal, and four were multifocal (one of the multifocal cases was also bilateral, affecting both breasts). The most common morphological heterogeneity was the simultaneous presence of ductal NST and mucinous carcinoma, observed in four cases. Further details regarding the studied population are presented in Table 1.

The combination of more aggressive and more indolent components resulted in significant difficulties in selecting sites for appropriate tumor evaluation. Different components resulted in different results. A good illustration of this observation may be a case of NST (ductal carcinoma) and micropapillary carcinoma coexistent in one lesion (Fig. 1A–E). The NST component demonstrated relatively low proliferative activity with a Ki-67 index of 12% (Fig. 1C) in contrast to 35% for the micropapillary component (Fig. 1E).

Another problem was encountered in the presence of a combination of mucinous and ductal carcinoma on core needle biopsy samples. In these cases, the low-in-cellularity mucinous component rarely contained enough cells for appropriate evaluation.

Results from the comparison of the Ki-67 index evaluation in each heterogeneous component of the studied tumors demonstrated no statistically significant difference of mean values $t = 0.4802$, $p = 0.6405$.

At the same time, the anticipation of the average Ki-67 score in the evaluated cases would have changed the molecular subtype from Luminal B to Luminal A (due to a Ki-67 index below 14%) in two of the cases.

Discussion

Although it is rather a rare phenomenon, morphologic tumor heterogeneity may be challenging and may affect breast cancer treatment and its consecutive effect (Turashvili and Brogi 2017). We observed such heterogeneity in 10.91% of the breast cancer cases in our study.

Table 1. Details, regarding Ki-67 index evaluation and tumor heterogeneity.

Age	Morphologic variant	Focality	Ki-67 index in predominant component	Ki-67 index in lesser component	Surrogate molecular subtypes before and after neoadjuvant therapy
68	Morphological NST (ductal), lobular	Unifocal	22% Lobular	39% NST (ductal)	Lum B/Lum B
68	Morphological NST (ductal), with noticeable variation in morphology and grade	Multifocal	14% NST (ductal)	16% NST (ductal)	Lum B/Lum B
37	Morphological NST (ductal), mucinous	Multifocal	30% NST (ductal)	8% Mucinous	Lum B (HER2)/Lum A
73	Morphological NST (ductal), micropapillary	Unifocal	12% NST (ductal)	35% Micro papillar	Lum A/Lum B (HER2)
67	Morphological NST (ductal), metaplastic	Unifocal	20% NST (ductal)	40% Metaplastic	Lum B/Triple negative
72	Morphological NST (ductal), lobular	Unifocal	70% NST (ductal)	20% Lobular	Lum B/Lum B
41	Morphological NST (ductal), mucinous	Unifocal	35% Mucinous	75% NST (ductal)	Lum B(HER2)/ Lum B(HER2)
37	Morphological NST (ductal), with noticeable variation in morphology	Multifocal	25% NST (ductal)	10% NST (ductal)	Lum B (HER2)/Lum A
40	Morphological NST (ductal), lobular	Multifocal	10% Lobular	15% NST (ductal)	Lum A/Lum B
32	Morphological NST (ductal), metaplastic	Unifocal	90% Metaplastic	20% NST (ductal)	Triple negative/Triple negative
31	Morphological NST (ductal), mucinous	Unifocal	12% Mucinous	14% NST (ductal)	Lum A/Lum B
41	Morphological NST (ductal), mucinous	Unifocal	22% NST (ductal)	18% Mucinous	Lum B/Lum B

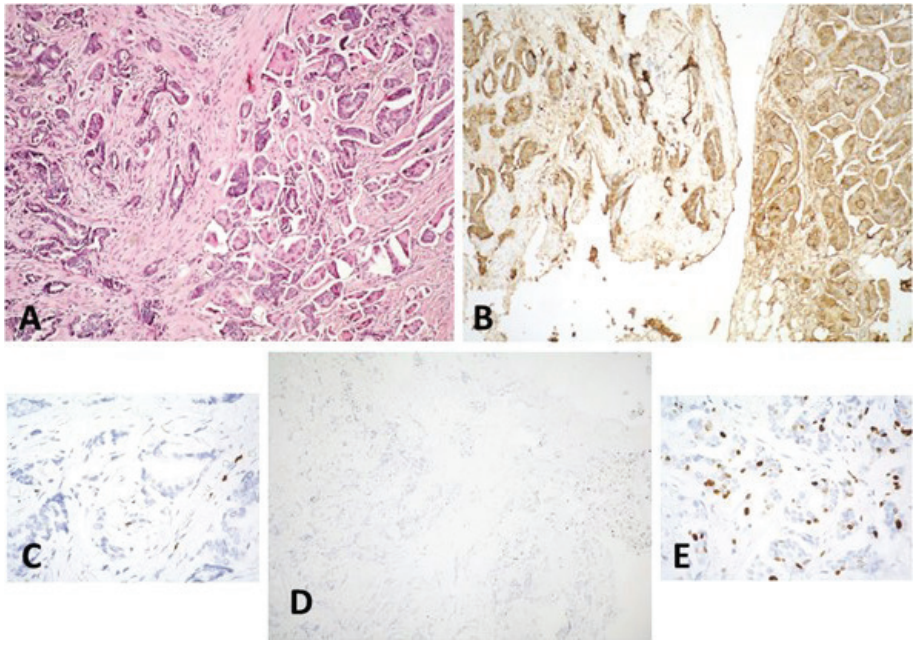

Figure 1. Representative case of NST (ductal) carcinoma and micropapillary carcinoma HE100× – **A**; **B** NST (ductal) carcinoma (luminal EMA stain) and micropapillary carcinoma demonstrating reverse polarity of EMA expression EMA, 100×; **C** NST component Ki-67, 400×; **D** Comparison of the immunostaining of the two components for Ki-67, Ki-67 40×; **E** Micropapillary component Ki-67, 400×. Abbreviations: NST – no special type; HE - hematoxylin and eosin; EMA - epithelial membrane antigen.

According to the WHO, tumors with distinguishable morphological patterns are more easily noticed as heterogeneous and are referred to as mixed or containing a particular component (World Health Organization 2019).

Other cases demonstrating no obvious morphologic heterogeneity may show molecular heterogeneity (Martelotto et al. 2014). For example, HER2 expression heterogeneity can be divided into genetic- (clustered, mosaic, and scattered types) and non-genetic (Hou et al. 2023). In such cases, HER2 diagnostic guidelines suggest additional evaluation for the separate components (Wolff et al. 2013) and eventual retesting metastasis in primary tumors with heterogeneity and positive components less than 10% (Wolff et al. 2018). It is quite likely that Ki-67 follows the pattern of HER2 distribution in such heterogeneous cases. In agreement with this statement, Kim KI et al. reported that values of Ki-67 equal to or above 25% were predictive for therapy response to neoadjuvant chemotherapy in ER-negative, HER2-positive breast carcinomas (Kim et al. 2014). Currently, no recommendations exist concerning Ki-67 evaluation on mixed breast tumors.

Tumors containing two morphologically distinct components represent a significant challenge in Ki-67 examination for various reasons. One of the described challenges is related to the heterogeneous cellularity of the tumor mass. The combination of mucinous and NST (ductal) carcinoma demonstrates variable Ki-67 expression and cellularity. The option to focus on the NST (ductal) carcinoma that is more cellular and likely to contain hot spots is tempting. However, it will result in estimated proliferation neglecting a low proliferative component, which is unlikely to respond to chemotherapy (Mannell 2016). On the contrary, the average Ki-67 will be descriptive for neither of the components. We believe tumor heterogeneity requires special attention in further Ki-67 guidelines and recommendations.

It may not be easy to assess the Ki-67 index adequately according to the accepted recommendations on some biopsies, particularly core biopsies. They contain hypocellular components of heterogeneous carcinomas or small fractions of one of the components (Dowsett et al. 2011). In such cases, it might be suggested that both components are reported (as accurately as possible, with a remark of low cell counts). Once more, Ki-67 count evaluation and clinical meaning should be studied in a wide population of heterogeneous breast carcinomas.

Heterogeneous breast carcinomas may present with one of its components in diagnostic biopsies and with the other component - in post-neoadjuvant resections. The comparison between the proliferation index, evaluated on diagnostic biopsy on one component (with a high proliferation index that will undergo complete pathologic response) and the other component (with a low proliferation index that will remain in the specimen due to the lack of therapeutic response) (Mannell 2016) may lead to poor understanding of the treatment-related changes in Ki-67 positivity in heterogeneous breast carcinomas. Some studies suggest different therapeutic approaches based on Ki-67 values, demonstrating different effectiveness of the applied treatment and predictive value when used to monitor response to neoadjuvant therapy (Zhang et al. 2021). However, the reviewed studies did not mention the heterogeneity of the primary tumors, so the use and role of Ki-67 should be discussed in the absence of morphological and biological heterogeneity in breast cancer.

To the best of our knowledge, this is the first study to evaluate the applicability of currently accepted recommendations for Ki-67 assessment in cases of heterogeneous breast carcinomas. Further studies on a larger population may be needed to completely understand the specifics of this problem and its relevance to clinical practice.

Conclusion

Based on our observations, we suggest that Ki-67 in heterogeneous breast carcinoma is evaluated and reported separately for the distinguishable tumor components. This evaluation would provide clinicians with detailed information concerning the tumor components and further assessment of tumor heterogeneity and Ki-67 evaluations in a clinical setting.

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