Automated Ki-67 assessment: From invasive tumor component detection to Ki-67 quantification in hot spots

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INTRODUCTION

In standard clinical practice the assessment of the nuclear proliferation biomarker Ki-67 is seen as having potential as both a prognostic and predictive marker in breast cancer and other cancers [1]. One of the main obstacles with regards to Ki-67 immunohistochemistry (IHC) assessment in the clinic is the lack of standardization in scoring methods. Traditionally, the scoring has been performed manually using a global method that attempts to derive an average score across all tissue available for assessment. More recent approaches utilize a hot spot method, where the area that appears to be the most active in cell division is identified and used as basis for scoring. However, both methods are often found to be subjective and prone to both intra- and inter-observer variability [2].

A more objective approach to Ki-67 assessment is the use of digital image analysis (DIA). Here we present a newly developed DIA workflow for automated and objective Ki-67 assessment that implements the hot spot scoring method, and is able to discriminate between invasive and non-invasive tumor components. We compare this DIA workflow to a more conventional DIA workflow using the global assessment method and with no discrimination between invasive and non-invasive tumor components.

MATERIALS

The data consisted of 84 formalin-fixed paraffin-embedded (FFPE) breast cancer resection specimens obtained from the archives of the Institute of Pathology, Aarhus University Hospital. For each resection specimen two serial sections were cut 3 μm apart and IHC staining was performed. The first section was stained with the proliferation marker Ki-67 (clone 30-9, Ventana Medical Systems, USA) and the other section was manually double stained with the myoepithelial nuclei marker p63 (clone 44A, Ventana Medical Systems, USA) and the cytokeratin (CK) cocktail of CK7 (clone OV-TL12/30, Dako, Denmark) and CK19 (A53-B/A2-26, AH-Diagnostics, Denmark) for epithelial visualization. Whole slide images were captured using a NanoZoomer HT-2.0 scanner (Hamamatsu Photonics K. K., Japan) at 20X scan mode.

METHODS

The aligned serial sections were analyzed using two different DIA workflows for Ki-67 assessment. Both workflows are developed by Visiopharm. The first DIA workflow uses the conventional approach to Ki-67 assessment. It automatically identifies the tumor on the CK19/19 slide, performs a Ki-67 quantification within the tumor regions and reports the Ki-67 proliferation index [%] for the whole slide. The second DIA workflow uses a more advanced and fully automated approach to Ki-67 assessment: It automatically identifies the tumor and separates it in invasive and non-invasive regions based on the joint p63 and CK/19 staining. It then performs a Ki-67 quantification within the invasive tumor components, creates a heatmap based on the density of the positive nuclei, identifies a hot spot, and finally outputs the Ki-67 proliferation index [%] for the hot spot. We defined the hot spot as the area consisting of 200 cells for which the density of positive cells was the highest.

RESULTS

The Ki-67 proliferation index for Workflow 1 and for Workflow 2 were compared to one another, and a $R^2 = 0.77$ was obtained (ct Figure 2). A paired t-test showed that there was a significant difference ($p<0.01$) in the proliferation index for Workflow 1 ($μ=21.66, σ=18.64$) and Workflow 2 ($μ=5.15, σ=23.57$). On average Workflow 2 is found to give a proliferation index that is roughly 20 units higher than Workflow 1. In addition, with Workflow 2 it was identified that 62% of the cases contained at least 10% non-invasive tumor.

CONCLUSION

The presented newly introduced DIA workflow increases the level of automation for Ki-67 assessment and reduces the need for manual interaction, since it can automatically discard non-invasive tumor components such as ductal carcinoma in situ. As the assessment of Ki-67 in hot spots, rather than as an average of the entire tumor area, is currently being discussed and implemented into some scoring guidelines we have here presented a method for conducting this type of analysis in an objective way.
