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Impact Of Tissue Sampling On Accuracy Of Ki67 Immunohistochemistry Evaluation In Breast Cancer

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Introduction/ Background

Gene expression studies have identified molecular subtypes of breast cancer with implications to chemotherapy recommendations. For distinction of these types a combination of hormone receptors and proliferative activity of tumor cells, estimated by Ki67 labeling index from immunohistochemistry (IHC) is used. Clinical studies are frequently based on IHC performed on tissue microarrays (TMA) with variable tissue sampling. This raises the need for evidence-based sampling criteria for individual studies. We present a novel tissue sampling simulation model and demonstrate its application on Ki67 assessment in breast cancer tissue taking into account its intra-tumoral heterogeneity.

Aims

The aim is, using a novel method for easy virtual TMA simulation, to determine the optimal tissue sampling requirements in the context of variable intra-tissue heterogeneity level of Ki67 immunohistochemistry in breast cancer.

Methods

Whole slide images (WSI) of 297 primary breast tumors immunohistochemically stained for Ki67 were subjected to digital image analysis (DIA). Percentage of invasive tumor cells stained for Ki67 were computed for hexagonal tiles superimposed on the WSI. From this, intra-tumoral Ki67 heterogeneity indicators (Haralick's entropy values) were extracted and used to dichotomize the tumors into homogeneous and heterogeneous populations. Simulations with random selection of hexagons, equivalent to 0.75 mm circular diameter, were performed. The tissue sampling requirements were investigated in relation to tumor heterogeneity using linear regression and extended error analysis.

Results

The sampling requirements were dependent on the heterogeneity of the biomarker expression. To achieve a coefficient error of 10%, 5-6 cores were needed for homogeneous cases, while 11-12 cores for heterogeneous cases. In mixed tumor population, 8 TMA cores were required. Similarly, to achieve the same accuracy, approximately 4,000 nuclei must be counted when the intra-tumor heterogeneity is mixed/unknown. Tumors at the lower scale of proliferative activity would require larger sampling (10-12 TMA cores, or 5,000 nuclei) to achieve the same error measurement results as for highly proliferative tumors. Our data show that optimal tissue sampling for IHC biomarker evaluation is dependent on the heterogeneity of the tissue under study and needs to be determined on a per-use basis. We propose a method that can be applied to determine the TMA sampling

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strategy for specific biomarkers, tissues and study targets. In addition, our findings highlight the importance of high-capacity computer-based IHC measurement techniques to improve accuracy of the testing.