

Enhancing Ki-67 Quantification in Canine Mammary Carcinoma: A Digital Pathology Approach Using QuPath

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Objectives: Accurate Ki-67 quantification is crucial for canine mammary carcinoma molecular (MAC) classification. Traditional manual quantification (MQ) suffers from variability and inconsistencies. This study evaluated QuPath, a free digital tool, to standardize Ki-67 assessment and improve reproducibility.

Materials and Methods: Ki-67 immunohistochemistry was performed on 25 MAC cases. Three different approaches were used and compared with MQ obtained from five observer-selected hotspots (~1000 cells) from Targeted High-Power Field (HPF) images: 1 HPF Analysis: Automated quantification (AQ) of the same fields as the MQ (n=25) 2 Area-Based Analysis: Manual outlining of whole-slide tumor areas (~1000 cells), excluding non-tumor regions (n=21) 3 Algorithmic Hotspot Detection (AHS): Whole-slide algorithmic detection of the most proliferative areas (n=21)

Results:

Targeted HPF Analysis

MQ and AQ showed a strong correlation ($r=0,92$, $p<0,0001$) but the results were statistically different ($p=0,01$). MQ yielded higher Ki-67 ratios in 72% of cases, with an average difference of 3,71%. AQ identified more negative cells (80% of cases), while detecting more positive cells in only 36% of cases. The higher quantification of negative cells is due to the inability of Qupath to distinguish neoplastic negative cells from non-neoplastic negative cells.

Area-Based Analysis

MQ still showed higher ratios in 57% of cases compared to area-based AQ but differences were not statistically different ($p=0,7081$). The ability to avoid non neoplastic regions with this method improved accuracy.

Algorithmic Hotspot Analysis (AHS)

AHS revealed higher Ki-67 ratios in 76% of cases with the results being statistically different compared to MQ ($p= 0,0077$). QuPath's systematic, unbiased whole-slide scanning likely contributed to detecting hotspots with higher ratios.

Conclusion: AHD seems to be the most effective method for identifying hotspots. While AQ enhances Ki-67 quantification, it remains susceptible to artifacts and requires optimal staining. Further standardization is crucial for improving accessibility and reliability in veterinary pathology.

Keywords: Ki-67; QuPath; Mammary Anaplastic Carcinoma, dog