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Comparing Aperio and QuPath in the Quantitative Analysis of p53 Immunohistochemistry in Primary and Metastatic Sarcomas

D.J. Bastaic*, G.E. Sandusky, D. Rushing and l. Baldridge

Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, in, USA.

Corresponding Author: Bastaic, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, in, USA.

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Abstract

Soft tissue sarcomas are rare, aggressive malignancies arising from mesenchymal tissues and account for a significant proportion of pediatric cancers. The tumor suppressor protein p53 plays a central role in maintaining genomic stability by regulating cell cycle arrest, DNA repair, and apoptosis. Mutations in TP53 are common in sarcomas and are associated with tumor progression and adverse outcomes. This study evaluated p53 expression in 50 clinical sarcoma specimens, including 28 primary tumors and 22 metastatic lung tumors, using immunohistochemistry and compared quantitative results generated by two digital pathology platforms, Aperio and QuPath. Slides were stained using the DAKO FLEX system and scanned at 20× magnification. Aperio analysis utilized the Positive Pixel Count (V9) algorithm, while QuPath employed a machine learning–based cellular segmentation classifier. Aperio reported p53 positivity rates of 4.83% in primary tumors and 4.65% in metastatic tumors, whereas QuPath detected higher rates of 16.70% and 15.64%, respectively. These findings suggest that segmentation methodology significantly influences quantitative biomarker detection and highlight the importance of platform selection in digital pathology research.

Keywords: Therapy, Heterogenous, Pathology and Pediatric

1. Introduction

Sarcomas are a heterogeneous group of malignant tumors originating from connective tissues such as bone, muscle, adipose tissue, and fibrous structures. Although relatively rare in adults, they represent a notable percentage of pediatric malignancies. Histologic subtypes include liposarcoma, leiomyosarcoma, fibrosarcoma, synovial sarcoma, osteosarcoma, and Ewing sarcoma, among others. The TP53 gene encodes the p53 tumor suppressor protein, which serves as a key regulator of genomic integrity. Upon detection of DNA damage or oncogenic stress, p53 mediates cell cycle arrest, facilitates DNA repair, or induces apoptosis when cellular damage is irreparable. Loss of p53 function through mutation disrupts these protective pathways and contributes to tumor initiation and progression [1].

Immunohistochemical detection of p53 overexpression is commonly used as a surrogate indicator of TP53 mutation. However, the increasing adoption of digital pathology platforms has introduced variability in quantitative interpretation. Aperio and QuPath are widely used whole slide image analysis systems, yet they differ fundamentally in algorithm design. Aperio primarily relies on pixel-based color thresholding, whereas QuPath integrates cellular segmentation with machine learning–based classification. Understanding how these methodological differences impact biomarker quantification is critical for ensuring reproducibility in oncologic research.

2. Materials and Methods

Fifty sarcoma tissue specimens were selected under Institutional Review Board–approved protocols with informed consent and HIPAA compliance. The cohort consisted of 28 primary tumors and 22 metastatic lung tumors. Most patients had received Adriamycin and Etoposide therapy prior to tissue evaluation. Specimens were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histologic confirmation. Immunohistochemical staining for p53 was performed using the DAKO FLEX automated immunostainer. All incubations were conducted at room temperature with Tris-buffered saline used for washing steps. The primary antibody against p53 was obtained from DAKO. Slides were scanned at 20× magnification using the Aperio ScanScope CS system to generate whole slide images. Digital image analysis was conducted using both Aperio and QuPath. Aperio quantification utilized the Positive Pixel Count (V9) algorithm, which classifies staining intensity based

on predefined hue, saturation, and intensity thresholds. In contrast, QuPath implemented a machine learning–based classifier to distinguish tumor cells from background tissue following nuclear segmentation. For metastatic cases, three lung sections per case were analyzed. Positivity rates were calculated as the percentage of positively stained tumor cells relative to the total number of tumor cells identified by each platform [2-4].

3. Results

Quantitative analysis revealed notable differences between the two digital pathology platforms as shown in Figure 1.

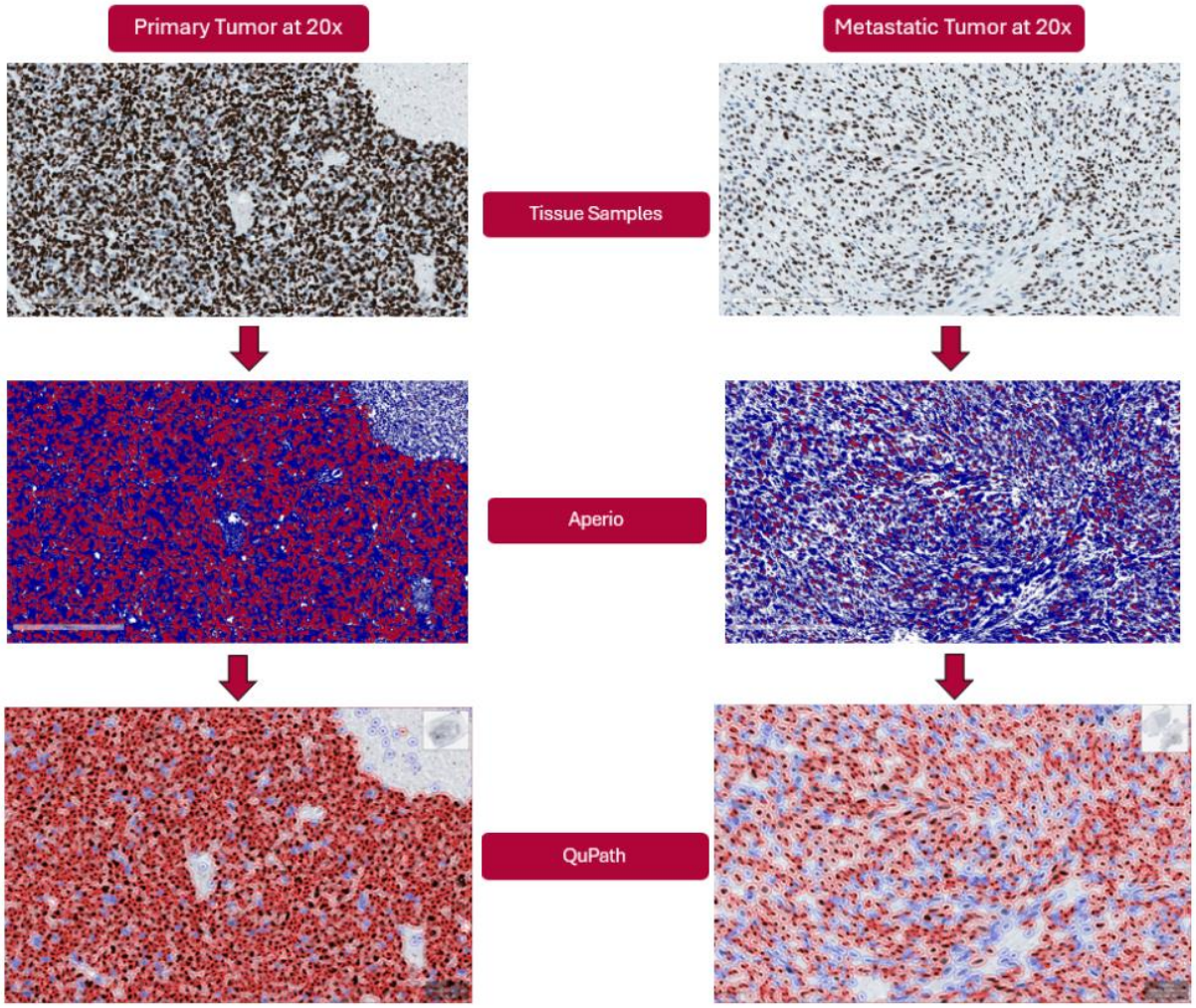

Figure 1: Aperio VS QU Path Comparison

Representative 20× whole-slide images demonstrating p53 immunohistochemical staining in primary sarcoma and metastatic lung tumor specimens. Brown nuclear staining indicates p53 positivity. Representative digital analysis overlays showing tumor cell detection and nuclear segmentation using Aperio's Positive Pixel Count algorithm and QuPath's machine learning–based classifier. Differences in cellular delineation and positivity detection are illustrated. In primary tumors, Aperio reported a p53 positivity rate of 4.83%, while QuPath identified a substantially higher rate of 16.70%.

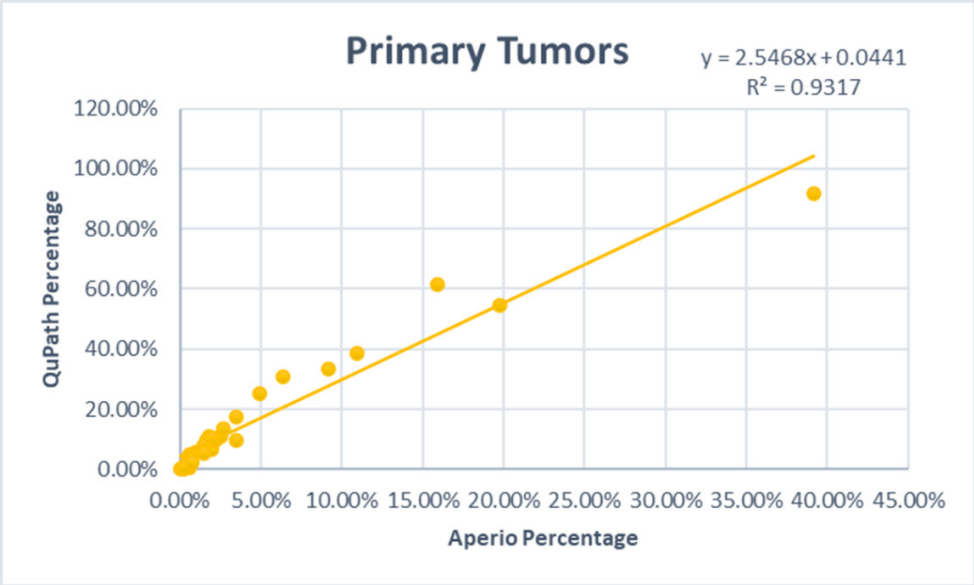

Figure 2: Primary Tumors Aperio and Qu Path Comparison

Scatter plot comparing percentage of p53-positive tumor cells quantified by Aperio (x-axis) and QuPath (y-axis) in primary sarcoma specimens. The linear regression line (y = 2.5468x + 0.0441) demonstrates a strong positive correlation (R^2 = 0.9317), with QuPath consistently identifying higher positivity rates. A similar trend was observed in metastatic tumors, with Aperio detecting 4.65% positivity compared to 15.64% using QuPath. Across both tumor groups, QuPath consistently identified approximately threefold higher positivity rates than Aperio [5].

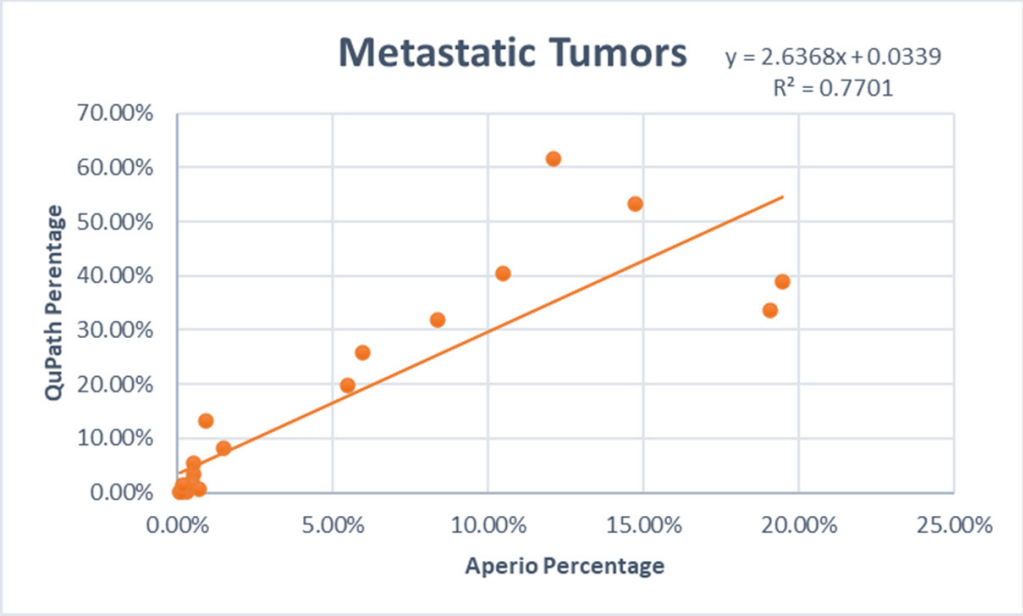

Figure 3: Metastatic Tumors Aperio And Qu Path Comparison

Scatter plot comparing percentage of p53-positive tumor cells quantified by Aperio (x-axis) and QuPath (y-axis) in metastatic lung sarcoma specimens. The linear regression line (y = 2.6368x + 0.0339) indicates a positive correlation (R^2 = 0.7701), with QuPath detecting higher overall positivity compared to Aperio. Visual inspection of segmentation outputs suggested that QuPath more precisely delineated individual tumor nuclei and more effectively excluded stromal and non-tumor regions from analysis. In contrast, Aperio's pixel-based thresholding approach appeared more susceptible to underestimating nuclear staining, particularly in cases with weak or heterogeneous expression. Despite inter-platform variability, there were no major differences in p53 positivity between primary and metastatic tumors within each individual platfor [6].

4. Discussion

The findings of this study demonstrate that digital pathology

platform selection can significantly influence quantitative biomarker results. The discrepancy observed between Aperio and QuPath likely reflects fundamental differences in algorithmic design. Aperio's Positive Pixel Count algorithm evaluates color intensity at the pixel level without explicit cellular segmentation, which may limit its sensitivity in detecting nuclear-localized biomarkers such as p53. Weakly stained nuclei or heterogeneous tumor regions may therefore be underrepresented. In contrast, QuPath's implementation of nuclear detection and machine learning–based classification enables more precise identification of tumor cells and exclusion of background tissue. This cell-based segmentation approach likely contributes to the higher positivity rates observed. The approximately threefold difference in detected p53 expression underscores the importance of understanding algorithmic behavior when interpreting quantitative digital pathology data. As digital pathology becomes increasingly integrated into both research and clinical workflows, standardization of analytic methods will be essential. Variability introduced by segmentation models, threshold parameters, and classifier training can meaningfully affect reported biomarker expression levels. Future studies should incorporate statistical concordance testing, validation against pathologist manual scoring, and correlation with clinical outcomes to further clarify platform performance.

5. Conclusion

QuPath demonstrated higher sensitivity in detecting p53 positivity compared to Aperio in both primary and metastatic sarcoma specimens. These results highlight the impact of algorithmic methodology on quantitative immunohistochemical analysis and emphasize the need for careful platform selection in biomarker studies. Rather than viewing these systems as competing tools, their complementary strengths may be leveraged to enhance robustness and reproducibility in digital pathology research.

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