Ki67 Quantitation in Breast Cancer: A Comparative Analysis of Four Counting Methodologies

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INTRODUCTION

Ki67 is an important tumor marker in breast cancer that is associated with cell proliferation. Ki67 has been suggested to be of clinical value in determining whether adjuvant chemotherapy is necessary in ER positive and HER2 negative breast cancer patients. While immunohistochemical stains are commonly used to assess Ki67, quantitation is limited by lack of standard scoring methods

Most commonly, the total percentage of Ki67 in the entire tumor is visually evaluated. More recently, the International Ki67 Working Group (IKWG) proposed counting 100 cells across four areas of varying Ki67 density and taking the average of the counts. Another method is selecting representative areas of "hotspots," areas with the highest Ki67 nuclear labeling. At this time however, no single method has gained universal acceptance and a reliable and reproducible method for Ki67 quantitation remains to be identified.

The goal of this study was to compare and evaluate four different methods of estimating Ki67 nuclear staining by Visual Total Count, Digital Total Count, IKWG Unweighted and Hotspot Count.

MATERIALS

Selection of Cases

100 cases were chosen from a database of 657 ER positive and HER2 negative invasive breast carcinomas. A consensus review of the Ki67 by a team of pathologists was estimated using the Visual Total Count method. The 100 cases were classified into the following Ki67 groups: 49 Low, 29 Moderate, and 22 High cases.

Immunohistochemistry

Paraffin-embedded formalin-fixed slides were incubated with MIB-1/Ki67 (DAKO) antibodies.

Analysis of Immunohistochemical Staining

Stained slides were digitized using Aperio® AT2 (Leica Biosystems) apparatus. Ki67 immunohistochemical staining were classified as follows:

- Low positive expression, <17%
- Moderate positive expression, 17%-35%
- High positive expression, >35%

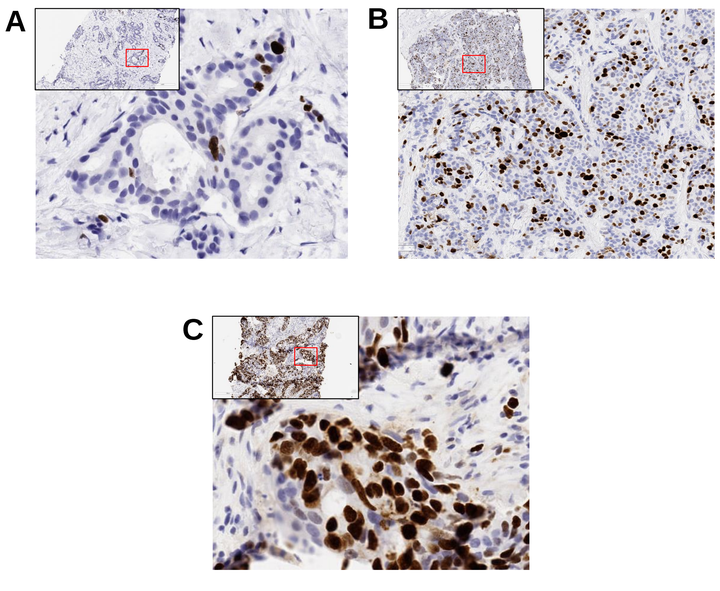
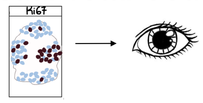


Fig. 1: Examples of Ki67 staining. IHC stain showing low positive **(A)**, moderate positive **(B)** and high positive **(C)** Ki67 staining.

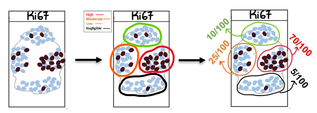
METHODS

1) Visual Total Count



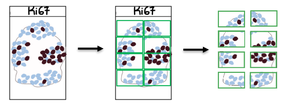
Total percentage of Ki67 nuclear staining within the entire invasive tumor was visually estimated. This method was used as the "gold-standard" method to which all others were compared.

3) IKWG Unweighted



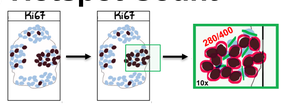
The pathologists selected areas with high, moderate, low, and negligible Ki67 staining within each sample. A digital image was obtained and Ki67 of 100 cells in each area were counted using the manual cell counter function in Aperio®.

2) Digital Total Count



A digital image of the slide was obtained. Screenshots of the entire tumor were taken at 10x magnification. The Ki67 staining of the entire tumor was quantified using digital analysis (Image J).

4) Hotspot Count



A screenshot of the most prominent Ki67 labeling was taken at 10x magnification, a color photo was printed and then used to count Ki67 labeling in 400 cells.

RESULTS

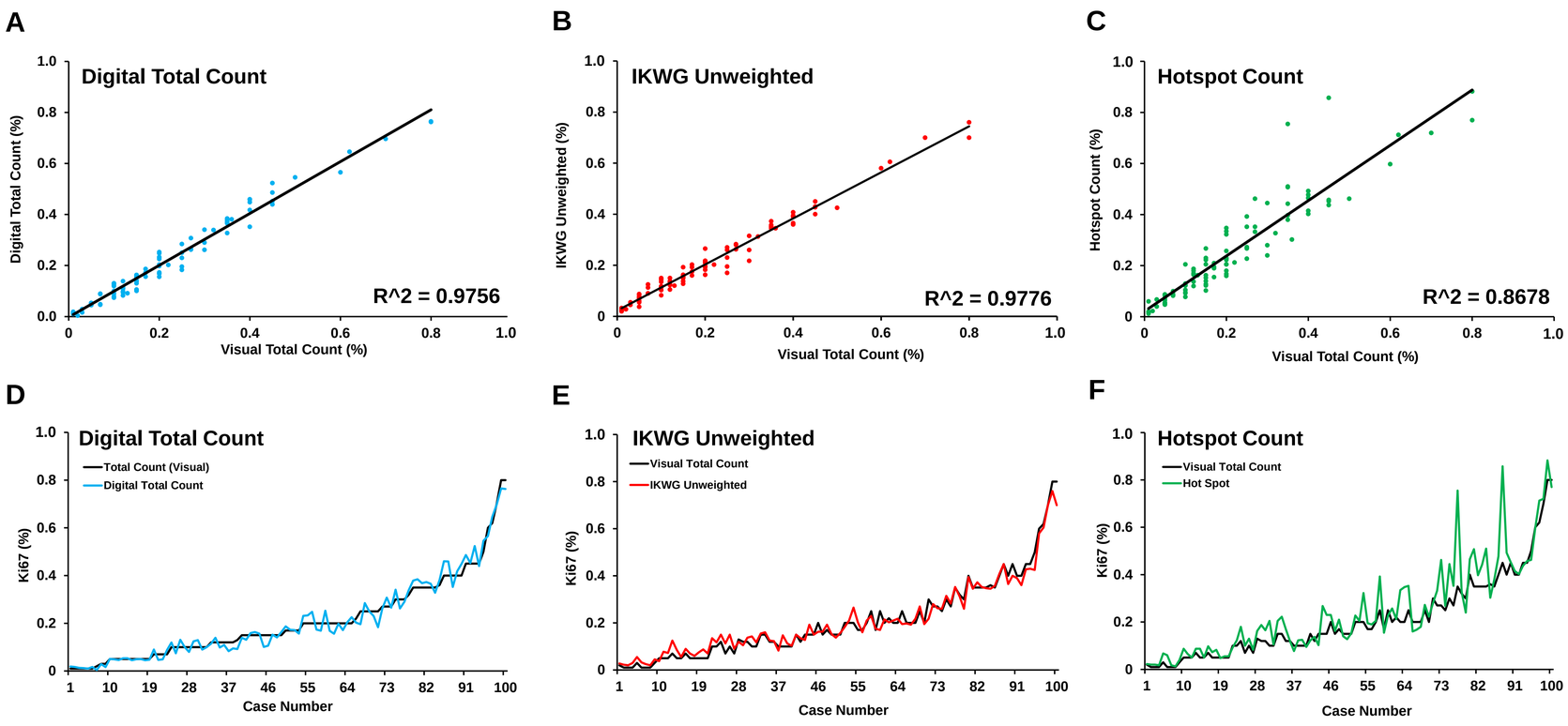


Fig. 2: Correlation of Ki67 Scoring Between Visual Total Count and the 3 Methods. Scatterplot matrix plots for correlation between Visual Total Count and Digital Total Count **(A)**, IKWG Unweighted **(B)**, and Hotspot Count **(C)**. Line graphs showing relationship between Visual Total Count and Digital Total Count **(D)**, IKWG Unweighted **(E)**, and Hotspot Count **(F)**.

- Digital Total Count and IKWG Unweighted yield comparable results to pathologist estimations (Visual Total Count). However, careful selections of intact images for digitized analysis are optimal.
- Hotspot Count had a low correlation with Visual Total Count. The error was likely caused by variation in staining throughout samples, making the hotspot a poor representation of the overall Ki67 percentage.

Table 1: Comparison of Ki67 Visual Total Count to the three different quantitation methods: Digital Total Count, IKWG Unweighted, and Hotspot Count.

		Visual Total Count		
		Ki67 Low (n = 49)	Ki67 Moderate (n = 29)	Ki67 High (n = 22)
Digital Total Count	Low (n = 51)	49	2	0
	Moderate (n = 28)	0	27	1
	High (n = 21)	0	0	21
IKWG Unweighted	Low (n =50)	48	2	0
	Moderate (n = 28)	1	27	0
	High (n = 22)	0	0	22
Hotspot Count	Low (n = 42)	40	2	0
	Moderate (n = 31)	9	21	1
	High (n = 27)	0	6	21

The Digital Total Count and IKWG Unweighted methods altered Ki67 categories in 3 cases each.

Digital Total Count:

- 2 Moderate (20%, 17%) became Low (15.6%,15.7%)
- 1 High (35%) became Moderate (32.8%)

IKWG Unweighted:

- 2 Moderate (20%, 17%) became Low (16.25%, 16%)
- 1 Low (15%) became Moderate (19.25%)

The Hotspot Count method altered Ki67 categories in 16 cases.

CONCLUSIONS

Our study successfully demonstrated that Visual Total Count, Digital Total Count and IKWG Unweighted are reliable and reproducible methods for Ki67 evaluation. In contrast, the Hotspot Count had a lower correlation with Visual Total Count, and its use in clinical practice may be limited. Further studies to establish the clinical significance of these Ki67 categories and their impact on clinical management are necessary.

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