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%

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).

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

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Digital Total Count</th>
<th>IKWG Unweighted</th>
<th>Hotspot Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-100</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
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The Digital Total Count and IKWG Unweighted methods altered Ki67 categories in 3 cases each. The Digital Total Count:

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

IKWG Unweighted:

- Moderate (20%, 17%) became Low (16.25%, 16%)
- 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.

REFERENCES


