Refining Ki-67 Index Reporting with AI-Based Open-Source Software (KoShiPath) Using Routine Microscope Camera and Computer

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Abstract

Background: The grading of neuroendocrine tumors (NET) based on Ki-67 index is critical. In this study, we evaluated artificial intelligence (AI) based, open-source software on Windows: KoShiPath (engineered recently by RK under guidance of VS), to overcome problems with the current routine approach in calculating Ki-67 index.

Material and Methods: Ki-67 indices were calculated using A) Eyeballing-guesstimation, B) manual counting on printed images, and C) KoShiPath software. Archived images without identifiers from 18 NETs were analyzed by three observers (MK, AK, MC). Each lesion had at least 3 images from hotspots with approximately >1000 tumor nuclei. Each observer counted at least 1000 tumor nuclei on each lesion.

Results: The average time required was 27 minutes for B and 15 minutes for C, while Method A was relatively quick. Cohen's kappa coefficient was 0.366 with A, 0.2995 with B, and 0.71 with C (Table 1). Paired t-test showed a significant difference between A and C (p=0.006), A and B (p=0.004), but not between B and C (p=0.238) (Table 2).

Conclusion: KoShiPath demonstrated high interobserver reproducibility and improved efficiency. Methods A and B had low interobserver reproducibility without a significant difference. Although B and C did not show a significant difference, interobserver reproducibility with B was less. Method C was fastest with improved efficiency after using the software on multiple lesions. However, an experienced interpreter is required for corrections in the Auto-counting mode to refine the final results.

Table 1: Cohen's kappa coefficient for inter-observer agreement

Method	Kappa coefficient	Agreement
A	0.366	Fair
B	0.2995	Fair
C	0.71	Substantial

Table 2: Paired t-test results for comparison of counting methods

Comparison	t-statistic	p-value	Significant difference
A vs. C	3.158	0.006	Yes
B vs. C	-1.229	0.238	No
A vs. B	3.348	0.004	Yes

JASC 12(5), Sep-Oct 2023, S75, Abstract #PST120.
https://doi.org/10.1016/j.jasc.2023.07.138

Background

Correction with the help of two-color assistance for weeding out brown nuclear Ki-67 immunoreactive lymphocytes with red cytoplasmic LCA immunostaining in neuroendocrine tumors (NET) is reported previously. Even after that, manual counting for Ki-67 index has problems compromising the pattern of outcome based on grading.

Material and Methods

Figure 1

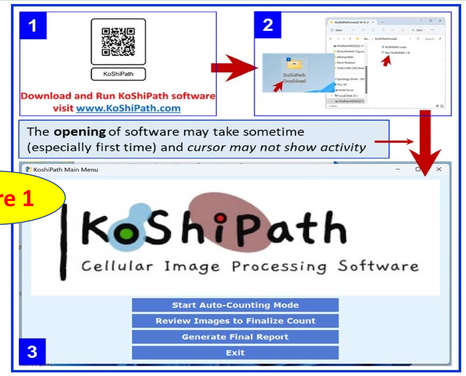

Figure 2

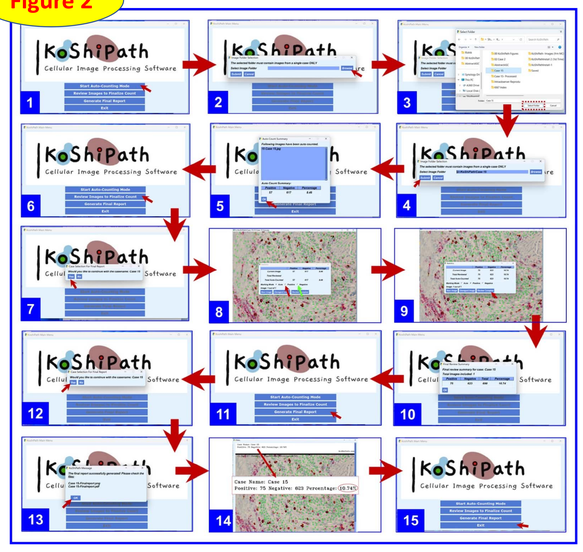

Figure 1.

Downloading KoShiPath software.

1. Visit www.KoShiPath.com and click on 'Download KoShiPath'. (It can be done by scanning the QR code shared here).
2. Open the 'Downloaded folder' and then click on 'Run KoShiPath 1.0'.
3. Open KoShiPath software (as mentioned, this may take some time to open) to use it for analyzing and calculating Ki-67 index.

Figure 2.

Analysis of the images (Figure 3a) of Two-color immunohistochemistry for Ki-67 nuclear brown and Red LCA with Hematoxylin counterstain for Ki-67 index calculation using KoShiPath software.

1. Open the KoShiPath software. Click on 'Start Auto-counting Mode'.
2. Click on 'Browse'.
3. *Select the folder* with images of Ki-67 immunostained slides from hot spots (NOT the image file itself).
4. Then click on 'Submit'.
5. This will generate auto-counted numbers for the 'first' image in the folder. Click on 'OK'.
6. Click on 'Review Images to Finalize Count'.
7. Click on 'Yes' to continue with case (in this example 'Case 15').
8. This will open a new window to show the image with auto-counted marks (negative nuclei- green, positive nuclei- Red) with a box showing the counting status.
The interpreter has to review all the nuclei (Figure 3b&c). Adjust each Positive or Negative nucleus after selecting Positive or Negative radio button. CLICK (has to be precise) would unmark the existing auto-mark if needed to cancel that nucleus not to be counted. Clicking the unmarked nucleus would mark that nucleus as Positive (red) or Negative (green) depending on the mode selected.
Unmark all auto-marked non-tumor nuclei depending on interpreter's decision (un-mark all lymphocytes: negative and positive nuclei in cells with LCA cytoplasmic red immunostaining) (**Figure 2: 8 & 9**).
9. If the total nuclei counted so far are less than 1000 nuclei, click on 'Next image' (the folder should have enough number of images with various representative hotspots from the case) to count preferably more than 1000 nuclei).
If one has to end counting, click on 'Review Complete' (in the example used here, we had only one image in the folder, so we completed the counting after one image only).
10. This will lead to the window with 'Final Review Summary'. Click 'OK'.
11. This will lead to the main software window. Click on 'Generate Final Report'.
12. This will open "Case Selection For Final Report". Click 'Yes'.
13. Next window will mention "The final report successfully generated". Click 'OK'.
14. This will lead to the *Final Report* as shown (Figure 3d).
15. At this stage, the interpreter can proceed to other case(s) in other folder(s).
Or close the program by clicking 'Exit'.

Method C: KoShiPath, AI based image analysis software was established using Python by RK under guidance of VS for cytomorphological variables in the images of immunostained sections taken with routine microscope camera (**Figure 3**). The final software KoShiPath 1.0 is now available as FREE tool from KoShiPath.com **Figure 1** with instructions how to use **Figure 2**. It was evaluated by comparing the Ki-67 results with current commonly available suboptimal alternatives A and B.

Figure 3

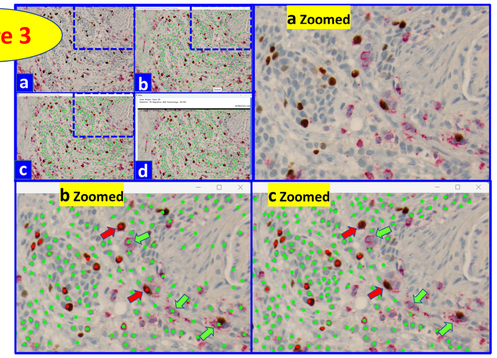

Figure 3.

Images under analysis (different stages: see legends for Figure 2).

Results

Figure 4

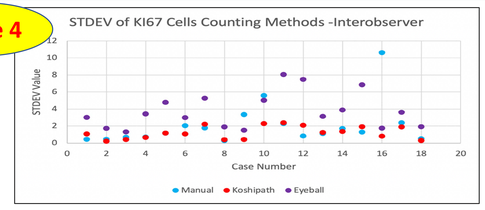

Figure 4.

Ki-67 indices by the 3 observers: Standard deviations (SD) for KoShiPath (Red dots) in 18 NETs were lower than other two methods, highlighting least variation, which was statistically significant (**Tables 1 and 2**).

Conclusions

1. Interobserver reproducibility is improved with efficiency with **KoShiPath 1.0** AI based imaging software.
2. Correction is required by pathologist for interpretation of tumor and non-tumor nuclei after Auto-counting mode.

Acknowledgements: We thank Deepti Jain, MD for participating in image analysis and Ayushi Gupta for statistical analysis.