Ki-67 Labeling Index in Pulmonary Carcinoid Tumors

Comparison Between Small Biopsy and Resection Using Tumor Tracing and Hot Spot Methods

Jennifer M. Boland, MD; Trynda N. Kroneman, CT(ASCP), SCT(ASCP); Sarah M. Jenkins, MS; Simone B.S.P. Terra, MD; Hao Xie, MD, PhD; Julian Molina, MD, PhD; Taofic Mounajjed, MD; Anja C. Roden, MD

Context.—Pulmonary carcinoids are classified as typical or atypical by assessing necrosis and mitoses, which usually cannot be adequately assessed on small biopsies. Ki-67 is not currently used to grade pulmonary carcinoids, but it may be helpful to determine preliminary grade in biopsies. However, the rate at which Ki-67 could underestimate or overestimate grade on small biopsies has not been well studied.

Objective.—To compare Ki-67 labeling obtained on small biopsies to subsequent resection.

Design.—Ki-67 was performed on paired biopsy and resection specimens from 55 patients. Slides were scanned using Aperio ScanScope. Labeling index was determined using automated hot spot and tumor tracing methods.

Results.—The study included 41 typical and 14 atypical carcinoids. Atypical carcinoids were larger and had more distant metastases. Death from disease occurred in 3 patients (all had atypical carcinoids). Median hot spot Ki-67 labeling index was greater in resection compared with biopsy by 0.7% (P = .02). Median tumor tracing Ki-67 was lower in resection compared with biopsy by 0.5% (P < .001). Receiver-operating characteristic analysis showed similar hot spot Ki-67 cutoffs to predict atypical histology (3.5% for biopsy, 3.6% for resection; area under the curve [AUC], 0.75 and 0.74, respectively). Different optimal cutoffs were needed for tracing method based on biopsy (2.1%; AUC, 0.75) compared with resection (1.0%; AUC, 0.67).

Conclusions.—Hot spot Ki-67 tends to underestimate grade on small biopsies, whereas grade is overestimated by tumor tracing. Hot spot Ki-67 cutoff of 3.5% predicted atypical histology for both biopsy and resection. Different biopsy and resection cutoffs were necessary for tumor tracing, which would make clinical implementation more difficult.

Arch Pathol Lab Med. 2020;144:982–990; doi: 10.5858/arpa.2019-0374-0A
67 may be assessed by concentrating on areas of highest activity, the “hot spot” method, or by counting all available tumor cells on the stain, the “tumor tracing method.” The hot spot method has the advantage of identifying the most proliferative area, but it could underestimate Ki-67 on a small biopsy because a “hotter spot” could be missed because of sampling. Tumor tracing has the advantage of providing an “average” Ki-67 throughout the tumor, and is therefore a very stable measure, but it could overestimate Ki-67 on a small biopsy if an area of relatively high proliferation is sampled. As might be expected, values established by hot spot counting are routinely higher than tumor tracing values, and thus any proposed cutoffs would be expected to be quite different depending on the method used.

Despite these shortcomings, Ki-67 has particular appeal in the evaluation of small biopsies of pulmonary carcinoids, where it may be helpful to determine preliminary grade, or to estimate grade when surgical excision is not clinically feasible (high stage, comorbidities, etc). The rate at which Ki-67 could underestimate or overestimate grade on small biopsies is not fully known. One study using manual hot spot Ki-67 index showed good concordance between biopsies and resections. The goal of this study is to compare automated Ki-67 index of preoperative biopsies to subsequent resection specimens, in order to determine how often they are different. Additionally, we sought to determine whether hot spot or tumor tracing method might provide the best preliminary grading information.

**MATERIALS AND METHODS**

Institutional pathology archives (1997–2017) were searched for patients with biopsy showing pulmonary carcinoid and subsequent resection. Diagnoses were confirmed and agreed upon by 2 pulmonary pathologists, with classification using 2015 WHO criteria applied to the resected tumor. Ki-67 immunostaining (clone MIB1, Dako, Carpinteria, California) was performed on formalin-fixed, paraffin-embedded tissue sections from both biopsy/cell block and resection specimens (1 representative whole tissue section was stained for resection specimens to include any areas of increased mitotic activity, if applicable). Slides were scanned at ×20 magnification on the Aperio ScanScope AT Turbo brightfield instrument (Leica Biosystems, Buffalo Grove, Illinois) at a resolution of 0.50 microns per pixel. The images were 24-bit contiguous standard pyramid tiled TIFFs compressed via JPEG with a quality setting of 70. Cases that had insufficient tumor on the Ki-67 stain (fewer than 300 cells) or complete lack of Ki-67 staining (lack of internal positive control) were excluded. The biopsies from the final study group included 38 transbronchial biopsies, 11 needle core biopsies, and 6 cytology cell blocks.

Ki-67 labeling index was determined via digital image analysis by an experienced technologist, using both hot spot and tumor tracing methods. Aperio ImageScope Software (Leica Biosystems) was used. Tumor areas were circled by a pathologist on corresponding hematoxylin-eosin slides for reference, to ensure only tumor areas were scored. For the tumor tracing method, using the reference hematoxylin-eosin, a minimum 85% of tumor was traced with a digital pen tool to indicate the region of analysis. Care was taken, either in the tracing process or by using a negative pen tool, to eliminate tissue folds and avoid staining artifacts. For hot spot method, a second annotation layer was added to the image and 10 fixed-size boxes were placed on hottest-staining region. Each box was 107 × 106 μm, for a total analyzed hot spot area of 0.11 mm². A nuclear algorithm was used to analyze the selected tissue. Manual assessment of Ki-67 was performed in 10% of cases by a pathologist as a quality control measure, which included verification of placement of hot spot boxes, confirmation of excluded nontumor areas/cells, and counting of at least 5 high-power fields for a manual estimate of Ki-67 both in hot spot areas and at random; no discrepancies were identified from the automated reads.

Follow-up information was obtained from clinical records. Descriptive statistics were employed to summarize the data (frequencies and percentages, means and SDs, or medians and ranges, as appropriate). Patient characteristics were compared between those with TCT versus ACT with Fisher exact tests (categoric variables) or Wilcoxon rank sum tests (ordinal or continuous variables). Within each of the 2 methods (hot spot, tracing), Ki-67 was compared between paired biopsy and resection samples with Wilcoxon signed rank tests, and the median differences were reported. Additionally, the Pearson correlation coefficient was used to further characterize the association between the methods. For each method and specimen type, receiver-operating characteristic (ROC) analysis was performed to identify a Ki-67 cutoff to jointly optimize the sensitivity and specificity for predicting ACT, and the area under the curve (AUC) was reported. P values less than .05 were considered statistically significant. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina) and R.

**RESULTS**

Demographic, clinical, and pathologic information is summarized in Table 1. Based on WHO criteria, 41 tumors were TCT (74.5%; Figure 1, A) and 14 were ACT (25.5%; Figure 1, B and C). Sex and age were similar between groups. The ACTs were significantly larger than TCTs (mean, 4.0 versus 2.6 cm; P = .03). The rate of lymph node metastasis at the time of surgery was not significantly different (8 of 41 [19.5%] for TCT; 4 of 14 [28.6%] for ACT; P = .48). Three patients had distant metastases at the time of diagnosis, all of whom had ACT metastatic to the liver and had concomitant mediastinal lymph node metastases. The rate of distant metastasis at the time of diagnosis was significantly higher in ACT (3 of 14 [21.4%] for ACT versus 0 of 41 for TCT; P = .01). Of 55 patients, 49 were treated with surgery alone (89.1%). Adjuvant treatment regimens included radiotherapy and chemotherapy (cisplatin, etoposide) in 1 patient with ACT; salvage cisplatin and etoposide in 1 patient with ACT; and salvage sandostatin in 2 patients with ACT and 2 patients with TCT (1 with metastatic disease, 1 with diffuse idiopathic pulmonary neuroendocrine cell hyperplasia). Median follow-up was 48.8 months (range, 11 days to 191 months). Recurrence/metastases occurred after surgery in 5 of 15 ACT patients (2 of whom also had liver metastases at diagnosis), and 1 of 40 TCT patients. An additional 3 patients with TCT had either multifocal carcinoid tumor (1 patient with 2 endobronchial TCTs in separate lobes) or multiple carcinoid tumors in the setting of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. Sites of late metastasis included liver (4), bone (2), pericardium/heart (1), lung (1), eye (choroid) and orbit (1), lymph nodes (1), spleen (1) and soft tissue (1). Three patients died of disease (17, 73, and 75 months after diagnosis), all of whom had ACT.

Median and range of Ki-67 results are summarized in Table 2, including comparison between biopsy and resection specimens (Figure 1, D through F), and comparison between hot spot and tumor tracing methods. As expected, based on the method of Ki-67 evaluation, the median value of the hot spot method was greater than the tumor tracing method for biopsy and resection specimens. The differences observed between biopsy and resection values by tumor tracing (Figure 2, A) and hot spot (Figure 2, B) methods are summarized in Table 3. Tumor tracing Ki-67 was lower on the resection compared with biopsy by a median of 0.5%,
which was significant ($P < .001$), and this significance was maintained when TCT (median, 0.4%; $P < .001$) and ACT (median, 0.7%; $P = .009$) were considered separately. The difference between biopsy and resection for tumor tracing Ki-67 was more pronounced in ACT (0.7%) versus TCT (0.4%), but this was not significant ($P = .25$). The hot spot Ki-67 index was greater in the resection compared with biopsy by a median of 0.7%, which was statistically significant ($P = .02$), although the difference between biopsy and resection did not reach significance when TCT ($P = .11$) and ACT ($P = .10$) were considered separately. The median difference was greater in ACT (1.3%) compared with TCT (0.7%), but this difference was not significant ($P = .28$). The correlation between Ki-67 values determined by hot spot and tumor tracing methods was stronger in biopsy compared with resection (Pearson correlation coefficient 0.88 for biopsies, 0.70 for resections). The Pearson correlation coefficient between biopsy and resection Ki-67

| Table 1. Summary of Clinical and Pathologic Characteristics of the Study Group |
|---------------------------------|---------------------------------|---------------------------------|------------------|
|                                 | Typical Carcinoid  | Atypical Carcinoid  | Total (n = 55)   |
|                                 | (n = 41)           | (n = 14)            | $P$ Value       |
| Age, y                          | 53.4 (17.9)        | 60.8 (14.0)         | 55.3 (17.1)     | .11               |
| Mean (SD)                       | 52.6 (14.8–87.1)   | 62.8 (25.1–83.2)    | 58.5 (14.8–87.1)|                |
| Median (range)                  |                    |                    |                 |
| Sex, No. (%)                    | Female 23 (56.1)   | 7 (50.0)            | 30 (54.5)       | .76               |
| Male 18 (43.9)                  | 7 (50.0)           | 25 (45.5)           |                 |
| Surgical procedure, No. (%)     | Lobectomy 27 (65.9)| 9 (64.3)            | 36 (65.5)       | .03               |
| Wedge resection                 | 4 (9.8)            | 1 (7.1)             | 5 (9.1)         |
| Segmentectomy                   | 6 (14.6)           | 0 (0.0)             | 6 (10.9)        |
| Sleeve resection                | 3 (7.3)            | 2 (14.3)            | 5 (9.1)         |
| Bilobectomy                     | 0 (0.0)            | 1 (7.1)             | 1 (1.8)         |
| Pneumonectomy                   | 0 (0.0)            | 1 (7.1)             | 1 (1.8)         |
| Lobectomy and wedge resection   | 1 (2.4)            | 0 (0.0)             | 1 (1.8)         |
| Tumor size, cm                  | Mean (SD) 2.6 (1.2)| 4.0 (2.3)           | 2.9 (1.7)       | .03               |
| Median (range)                  | 2.3 (0.8–5.5)      | 3.4 (1.7–8.3)       | 2.5 (0.8–8.3)   |
| Mitoses per 2 mm²               | Mean (SD) 0.3 (0.5)| 3.6 (2.8)           | 1.2 (2.0)       | .03               |
| Median (range)                  | 0.0 (0.0–1.0)      | 3.0 (0.0–9.0)       | 0.0 (0.0–9.0)   |
| Necrosis, No. (%)               | 0                 | 2 (14.3)            | 2 (3.6)         | .48               |
| LN metastases at time of        | Mean (SD) 8 (19.5) | 4 (28.6)            | 12 (21.8)       | .48               |
| resection, No. (%)              | Median (range) 3.4 (1.7–8.3) | 4.0 (2.3) | 2.9 (1.7)       | .03               |
| Distant metastases at time of   | 0                 | 3 (21.4)            | 3 (5.5)         | .01               |
| resection, No. (%)              |                    |                    |                 |
| Pathologic tumor stage, 8th AJCC, No. (%) | 3 (7.3) | 0 (0.0) | 3 (5.5) | .16               |
| IA1                             | 13 (31.7)          | 5 (35.7)            | 18 (32.7)       |
| IA2                             | 9 (22.0)           | 1 (7.1)             | 10 (18.2)       |
| IA3                             | 6 (14.6)           | 1 (7.1)             | 7 (12.7)        |
| IB                              | 1 (2.4)            | 1 (7.1)             | 2 (3.6)         |
| IIa                             | 5 (12.2)           | 2 (14.3)            | 7 (12.7)        |
| IIb                             | 4 (9.8)            | 1 (7.1)             | 5 (9.1)         |
| IVB                             | 0 (0.0)            | 3 (21.4)            | 3 (5.5)         |
| Follow-up interval, median (range), mo | 48.8 (11 d to 191.1 mo) | 43.5 (17 d to 184.6 mo) | 48.8 (11 d to 191.1 mo) | .16               |
| Metastasis after surgery, No.   | 1                 | 5 (2 had liver metastasis at diagnosis) | 6 |
| Follow-up status, No.          | Alive without disease 33 | 8 | 41 |
| Alive with disease              | 3 (1 with distant metastases, 2 with DIPNECH) | 1 | 4 |
| Death from disease              | 0                 | 3                  | 3               |
| Death from other cause          | 4                 | 1                  | 5               |
| Death from unknown cause        | 1                 | 1                  | 2               |

Abbreviations: AJCC, American Joint Committee on Cancer; DIPNECH, diffuse idiopathic neuroendocrine cell hyperplasia; LN, lymph node.
Figure 1.  Typical carcinoid tumor (A), showing classic trabecular growth and rosette formation, with finely stippled chromatin. No necrosis or increased mitotic activity is observed. Atypical carcinoid tumor, showing increased mitotic activity (B, arrows) and focal necrosis (C). Representative levels of Ki-67 labeling index are pictured, including <1% (D), 2% (E), and >10% (F) (hematoxylin-eosin, original magnification ×200 [A through C]; original magnification ×200 [D through F]).
for the hot spot method was 0.65, whereas it was 0.58 for the tumor tracing method.

The ROC analysis was performed to determine what Ki-67 cutoff best discriminated between histologic classification of TCT versus ACT (Figure 3). For the hot spot method, ROC analysis of both biopsy and resection showed a similar cutoff to optimize sensitivity and specificity (3.5% for biopsy [Figure 3, C] and 3.6% for resection [Figure 3, D]; AUCs of 0.75 and 0.74, respectively). However, the ROC analysis of biopsy versus resection gave quite different optimal cutoffs for the tracing method, with a proposed cutoff of 2.1% based on the biopsy data (AUC, 0.75; Figure 3, A), but a lower cutoff of 1.03% generated based on resection data (AUC, 0.67, Figure 3, B). The distribution of Ki-67 labeling index for TCT and ACT around the cutoffs determined by ROC analysis is illustrated in Figure 4. Ki-67 labeling index determined on resection specimens of tumors near the mitotic rate cutoff between TCT and ACT is summarized in Table 4, which includes TCT cases with 1 mitotic figure per 2 mm² (n = 14) and ACT with 2 to 3 mitoses per 2 mm² (n = 9); although the median Ki-67 was higher in ACT versus TCT for both methods, considerable overlap was observed in Ki-67 values for these cases.

Of the 7 patients who experienced distant metastasis, the range of hot spot Ki-67 on the biopsy was 2.7% to 7.2%, with only 1 patient below 3.5%. The range of tumor tracing Ki-67 in the biopsies of these patients was 1.1% to 3.7%, with only 1 patient below 2.1%. On subsequent resection specimens, the range of hot spot Ki-67 was 0.9% to 12.6%, with 1 patient below 3.6%. The range of tumor tracing Ki-67 on the resections was 0.2% to 5.0%, with 3 tumors showing values 1.0% or less. Three patients with ACT had hot spot Ki-67 less than 3.5% on resection; 1 of these patients (hot spot Ki-67 on resection 0.9%; hot spot Ki-67 on biopsy 3.9%) experienced distant metastases and died of disease, whereas the other 2 patients did not experience recurrence or distant metastases. Six patients with ACT had tumor tracing Ki-67 less than 1.03%; 1 had distant metastases and died of disease, 1 had distant metastases with unknown outcome, 1 had lymph node metastases with no other distant disease, and 3 had no recurrence or metastases.

**DISCUSSION**

Ki-67 labeling index is a marker of cell proliferative activity, similar to mitotic count. However, it is more sensitive than mitotic count because it detects all cells in the G1, G2, and S phases of the cell cycle as well as cells in mitosis.²⁷ Ki-67 labeling index has been incorporated into routine practice for grading of gastrointestinal neuroendocrine tumors.⁸ Although manual Ki-67 reading can be tedious because of the high cell count required for accurate assessment, automated Ki-67 labeling index has been widely adopted in the grading of gastrointestinal neuroendocrine tumors, where it has shown to correlate very well with manual counting.⁸,²⁸ Automated counting has also been shown to have good to excellent concordance with

---

**Table 2. Median and Range of Ki-67 Labeling Indices Observed on Biopsy and Resection, Using Hot Spot and Tumor Tracing Methods**

| Diagnosis            | Biopsy, % | Resection, % |
|----------------------|-----------|--------------|
|                       | Median Ki-67 | Range Ki-67  | Median Ki-67 | Range Ki-67  |
| Hot spot method      |            |              |              |              |
| Typical carcinoid (n = 41) | 1.6        | 0–10.7       | 2.9          | 0.2–7.4      |
| Atypical carcinoid (n = 14) | 4.3        | 1.2–12.2     | 4.9          | 0.5–14.2     |
| Tumor tracing method |            |              |              |              |
| Typical carcinoid (n = 41) | 1.4        | 0.09–5.0     | 0.6          | 0–4.1        |
| Atypical carcinoid (n = 14) | 2.6        | 0.5–4.4      | 1.1          | 0.1–5.0      |

---

**Figure 2.** Correlation between Ki-67 labeling index values on biopsy and resection, using tumor tracing (A) and hot spot (B) methods.
manual counts in lung neuroendocrine tumors.\textsuperscript{16,19,29,30} Ki-67 may have the additional advantage of higher interobserver agreement than mitotic count.\textsuperscript{29} However, data on the value of adding Ki-67 labeling index to evaluation of lung carcinoid tumors have been mixed. Some data support that it does not add additional prognostic value in addition to the WHO grading system,\textsuperscript{18} or that Ki-67 is of borderline significance, with stage and histologic typing providing more clearly significant information.\textsuperscript{13,18–20} Conversely, other studies have found that Ki-67 is an independent predictor of outcome, whereas mitotic count and in some instances even stage and histologic type are not; some of these studies have proposed that Ki-67 may be a better predictor of true tumor biologic potential.\textsuperscript{2,4,5,11,24,31} The best grading system may incorporate both WHO classification and Ki-67. In 1 large study, incorporation of Ki-67, necrosis, and mitotic count provided very good separation of survival in a 3-tiered grading system.\textsuperscript{16} However, another study showed that this

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Receiver-operating characteristic analysis of Ki-67 labeling index cutoffs for predicting atypical carcinoid tumor histology, based on biopsy using tumor tracing (A), resection using tumor tracing (B), biopsy using hot spot method (C), and resection using hot spot method (D).}
\end{figure}
system did not outperform the current WHO classification.\textsuperscript{19} Unfortunately, we had too few events in our cohort to perform a robust statistical evaluation of the prognostic value of Ki-67 values independent of WHO classification. Our follow-up data are somewhat limited in length as well, which limits our ability to perform robust survival analysis, because pulmonary carcinoid tumors have an indolent course that requires very long follow-up for optimal assessment of survival.

Increased Ki-67 labeling index has been associated with other markers of aggressive behavior, including increased apoptotic index, aneuploidy, BCL2 expression, and mutant p53 expression.\textsuperscript{9,10,21} Ki-67 may be an indicator of adverse prognosis in TCT when greater than 5%, where it is associated with higher stage.\textsuperscript{18,32} Atypical carcinoid tumors with Ki-67 values higher than 10% or mitotic rate of 6 or more per 10 high-power fields also appear to have a particularly dismal prognosis.\textsuperscript{3,5,20,33} Interestingly, metastatic carcinoid tumors tend to show significantly higher Ki-67 values compared with the primary tumor.\textsuperscript{18}

Ki-67 index is generally very dichotomous between low/intermediate-grade and high-grade pulmonary neuroendo-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.pdf}
\caption{Distribution of Ki-67 labeling values in typical and atypical pulmonary carcinoid tumors. Cutoff values determined by receiver-operating characteristic analysis are illustrated with red lines. Pictured data include biopsy samples using tumor tracing (A), resection specimens using tumor tracing (B), biopsy samples using hot spot method (C), and resection specimens using hot spot method (D).}
\end{figure}
Ki-67 in Pulmonary Carcinoids—Boland et al

Abbreviations: ACT, atypical carcinoid tumor; TCT, typical carcinoid tumor.

crine neoplasms. It can be useful to avoid the diagnostic pitfall of overcalling small cell carcinoma in the setting of a crushed carcinoid tumor, where confident morphologic distinction may be impossible.34,35 High Ki-67 index above 20% has also been proposed as an indicator of high-grade neuroendocrine carcinoma on small biopsies.25 However, the discrimination between TCT and ACT is unfortunately much more difficult. Sensitivity and specificity are not particularly robust and discriminatory capacity is only moderate for Ki-67 in predicting TCT versus ACT.16,25 This is also true of mitotic count, where the distinction between low/intermediate and high grade is often clear, whereas the distinction between low and intermediate grade requires more rigorous evaluation and still does not provide the same discriminatory capacity.16 Therefore, it is not surprising that there is substantial interobserver disagreement in the application of the WHO classification of pulmonary neuroendocrine tumors, especially for the diagnosis of ACT.36 Ki-67 has been suggested as a way to increase diagnostic agreement.36

Confident pathologic grading of pulmonary carcinoid tumors using the current WHO system usually requires surgical excision, because necrosis is patchy and can be missed because of sampling, and mitotic activity can show “hot spots” of increased activity that may not be represented in a biopsy sample.25 Therefore, the increased sensitivity for proliferative activity detected by Ki-67 is particularly attractive as a potential tool to predict primary grade in pulmonary carcinoid tumors, but its utility in this setting has not been well studied. One study of Ki-67 on tissue microarrays, which mimic the scenario of a small biopsy, did show Ki-67 index higher than 2.5% was associated with worse prognosis.4 Another study has looked at manual hot spot Ki-67 correlation between biopsy and resection specimens and has found good correlation.25 In our study, we found a small but statistically significant difference in Ki-67 between biopsy and resection, when using both hot spot and tumor tracing methods. Hot spot Ki-67 was lower in the biopsy, which could underestimate preliminary grade, and the Ki-67 determined by tumor tracing was higher in the biopsy compared with resection, which could overestimate preliminary grade. These limitations of determining Ki-67 labeling index on biopsy specimens largely make sense when considering each method. Determination of hot spot Ki-67 is limited in small samples, because “hotter” areas of the tumor may exist in unsampled regions, hence the tendency to underestimation of grade. It is possible that the increased hot spot values in the resections could be at least partially due to the biopsy procedure itself, with induction of a hot spot due to tissue damage and regeneration (i.e., perhaps the biopsy actually provides better assessment, because the resection may overestimate grade because of “artificial” hot spots); however, it is also logical that true hot spots would be better detected when a whole tumor section is available for analysis. The number of cells counted by tumor tracing in a resection specimen is generally very large, which leads to a stable estimate of overall tumor proliferative activity. However, this method would be predicted to be susceptible to sampling variability in proliferative activity when only a small area of the tumor is sampled via biopsy. The reason for the propensity to have a higher tumor tracing Ki-67 in biopsy is not entirely clear; one could imagine that the surface of a tumor accessible by bronchoscopy could have reactive changes that would lead to relatively higher proliferative activity, but it seems there must also be other factors at play.

The cutoff value of Ki-67 that might be useful to divide TCT from ACT is not standardized. Proposed cutoffs have varied widely in different studies, which is not surprising given the wide variety of methods that have been employed to determine this index (number of cells counted, manual versus automated methods, differing definitions of hot spots, hot spot counting versus random counting, different statistical methods to determine the “best” cutoff, etc). Although 1 study found that there was no overlap between the Ki-67 index observed between TCT and ACT,31 we found significant overlap in our study, which has been observed in other studies as well.18,19,20 Proposed Ki-67 cutoffs to divide TCT from ACT in the literature include 1%, 2%, 3%, 4%, 5%, 7%, and 7.5%.17 Although it would be ideal to use the same Ki-67 cutoff as the gastrointestinal tract to increase uniformity and reduce confusion, there is at least some evidence that this provides suboptimal discrimination of pulmonary TCT versus ACT, with less separation of the survival curves than the WHO system.26 Although disappointing, it is not

| Table 3. Difference Between Biopsy and Resection Values for Ki-67 Labeling Index, Using Hot Spot and Tumor Tracing Methods |
|---|
| Ki-67 hot spot method: biopsy value | TCT, % (n = 41) | ACT, % (n = 14) | Total, % (n = 55) |
| Ki-67 tumor tracing method: biopsy value | | | |
| − resection value (difference), median (range) |
| − resection value (difference), median (range) |
| P Value for Difference Between Biopsy and Resection, All Patients |

| Table 4. Ki-67 Labeling Index Determined on Resection Specimens for Cases With a Mitotic Count Near the Cutoff Between Typical and Atypical Carcinoid, Including Typical Carcinoids With 1 Mitotic Figure per 2 mm² and Atypical Carcinoids With 2 to 3 Mitoses per 2 mm² |
|---|
| Diagnosis | Ki-67, %, Median (Range) |
| Hot spot | |
| Typical carcinoid (n = 14) | 3.2 (0.2–7.4) |
| Atypical carcinoid (n = 9) | 4.8 (0.9–14.2) |
| Tumor tracing | |
| Typical carcinoid (n = 14) | 0.4 (0–2.3) |
| Atypical carcinoid (n = 9) | 0.6 (0.2–5.0) |

Abbreviations: ACT, atypical carcinoid tumor; TCT, typical carcinoid tumor.
necessarily surprising that site-specific factors may impact the Ki-67 index, which could include changes of prior biopsy, exposure to the environment within the bronchus, and potential traumatization due to cough, etc. In our study, a Ki-67 hot spot cutoff value of 3.5% provided optimal sensitivity and specificity for distinguishing TCT from ACT, which was applicable to both biopsy and resection. Tumor tracing method required different cutoff values in biopsy (2.1%) versus resection (1.03%), which is a limitation of this method. The AUC was also slightly higher for the hot spot method when resected tumors were considered. Therefore, the hot spot method may be better suited to distinguish pulmonary TCT from ACT.

In summary, Ki-67 labeling index in pulmonary carcinoid tumors determined on biopsy versus subsequent resection shows a small but statistically significant difference using automated hot spot and tumor tracing methods. Hot spot method has a tendency toward undergrading based on biopsy, whereas tumor tracing has the opposite tendency toward overgrading. Tumor tracing has the drawback of requiring different cutoff values for biopsy versus resection. These potential shortcomings should be kept in mind when deciding when and how to use Ki-67 labeling index in clinical practice.

References

1. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, eds. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. 4th ed. Lyon, France: IARC Press; 2015. WHO Classification of Tumours; vol 7.
2. Grimaldi F, Muser D, Beltrami CA, et al. Partitioning of bronchopulmonary carcinoids in two different prognostic categories by ki-67 score. Front Endocrinol (Lausanne). 2011;2:20.
3. Beasley MB, Thunnessen FB, Brambilla E, et al. Pulmonary atypical carcinoid: predictors of survival in 106 cases. Hum Pathol. 2000;31(10):1255–1265.
4. Vesterinen T, Mononen S, Salmenkivi K, et al. Clinopathological indicators of survival among patients with pulmonary carcinoid tumor. Acta Oncol. 2018;57(8):1109–1116.
5. Ramirez RA, Beyer DT, Diebold AE, et al. Prognostic factors in typical and atypical pulmonary carcinoids. Ochsner J. 2017;17(4):335–340.
6. Cusumano G, Fournel L, Strano S, et al. Surgical resection for pulmonary carcinoid: long-term results of multicentric study—the importance of pathological N status, more than we thought. Lung. 2017;195(6):789–798.
7. Kornrump LS, Dam G, Gronbaek H. Survival and predictors of death for patients with bronchopulmonary carcinoid at a Danish tertiary NET centre. In Vivo. 2017;31(3):397–402.
8. Bosman FT, Carneiro F, Hruban RH, Weiss CL, eds. WHO Classification of Tumours of the Digestive System. 4th ed. Lyon, France: IARC Press; 2010: 417.
9. Beasley MB, Thunnessen FB, Brambilla E, et al. Pulmonary atypical carcinoid: predictors of survival in 106 cases. Hum Pathol. 2000;31(10):1255–1265.
10. Vesterinen T, Mononen S, Salmenkivi K, et al. Clinopathological indicators of survival among patients with pulmonary carcinoid tumor. Acta Oncol. 2018;57(8):1109–1116.
11. Papaxoinis G, Lamarca A, Quinn AM, Mansoor W, Nonaka D. Clinical and-pathological characteristics of carcinoid tumors in central and peripheral locations. Endocr Pathol. 2010;21(3):259–269.
12. Neubauer E, Wirtz RM, Kaemmerer D, et al. Comparative evaluation of three proliferation markers, Ki-67, TOP2A, and RacGAP1, in bronchopulmonary neuroendocrine neoplasms: issues and prospects. Oncotarget. 2016;7(27): 41959–41973.
13. Skov BG, Holm B, Ereboe A, Skov T, Mellengaard A. ERCC1 and Ki67 in small cell lung carcinoma and other neuroendocrine tumors of the lung: distribution and impact on survival. J Thorac Oncol. 2010;5(4):453–459.
14. Clay V, Papaxoinis G, Sanderson B, et al. Evaluation of diagnostic and prognostic significance of Ki-67 index in pulmonary carcinoid tumors. Clin Transl Oncol. 2017;19(5):579–586.
15. Arbour ZK, Arbour JL, Cohen G, Cal AA. Neuroendocrine lung tumors: grade correlates with proliferation but not angiogenesis. Mod Pathol. 2001;14(12):1195–1199.
16. Rindi G, Klersy C, Inzani F, et al. Grading the neuroendocrine tumors of the lung: an evidence-based proposal. Endocr Relat Cancer. 2014;21(1):1–16.
17. Garg R, Bal A, Das A, Singh N, Singh H. Proliferation marker (Ki67) in subcategories of neuroendocrine tumours of the lung. Turk Patoloj Derg. 2019; 35(1):15–21.
18. Waltz AE, Ines D, Marchevsky AM. Limited role of Ki-67 proliferative index in predicting overall short-term survival in patients with typical and atypical pulmonary carcinoid tumors. Mod Pathol. 2012;25(9):1258–1264.
19. Swarts DR, Ruedelius M, Claessen SM, et al. Limited additive value of the Ki-67 proliferative index on patient survival in World Health Organization-classified pulmonary carcinoids. Histopathology. 2017;70(3):412–422.
20. Marchio C, Gatti G, Massa F, et al. Distinctive pathological and clinical features of lung carcinoids with high proliferation index. Virchows Arch. 2017; 471(6):713–720.
21. Santinelli A, Ranaldi R, Baccarin M, Mannello B, Beerzi I. Ploidy, proliferative activity, p53 and bcl-2 expression in bronchopulmonary carcinoids: relationship with prognosis. Pathol Res Pract. 1999;195(7):467–474.
22. Bohm J, Koch S, Gais P, et al. Prognostic value of MiB-1 in neuroendocrine tumours of the lung. J Pathol. 1996;187(4):402–409.
23. Barbareschi M, Girlando S, Mauri FA, et al. Tumour suppressor gene products, proliferation, and differentiation markers in lung neuroendocrine neoplasms. J Pathol. 1992;166(4):335–350.
24. Costes V, Marty-Ané C, Picot MC, et al. Typical and atypical bronchopulmonary carcinoid tumors: a clinicopathologic and Ki-67-labeling study. Hum Pathol. 1995;26(7):740–745.
25. Fabbi R, Cossa M, Sonzogni A, et al. Ki-67 labeling index of neuroendocrine tumors of the lung has a high level of correspondence between biopsy samples and surgical specimens when strict counting guidelines are applied. Virchows Arch. 2017;470(2):153–164.
26. Team RC; R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2017. https://www.R-project.org/.
27. Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol. 1984;133(4):1710–1715.
28. Tang LH, Gonen M, Heddav C, Modlin IM, Klimstra DS. Objective quantification of the Ki-67 proliferative index in neuroendocrine tumors of the gastroenteropancreatic system: a comparison of digital image analysis with manual methods. Am J Surg Pathol. 2012;36(12):1761–1770.
29. Warth A, Fink L, Fisseler-Eckhoff A, et al. Interobserver agreement of proliferation index (Ki-67) outperforms mitotic count in pulmonary carcinoids. Virchows Arch. 2013;462(5):507–513.
30. Joseph MG, Shihani A, Panwar N, et al. Usefulness of Ki-67, mitoses, and tumor size for predicting metastasis in carcinoid tumors of the lung: a study of 48 cases at a tertiary care centre in Canada. Lung Cancer Int. 2015;2015:545601.
31. Liu SZ, Staats PN, Goicochea L, et al. Automated quantification of Ki-67 proliferative index of excess neuroendocrine tumors of the lung. Diag Pathol. 2014;9:174.
32. de Vilhena AF, de Neves Pereira JC, Parra ER, et al. Histomorphometric evaluation of the Ki-67 proliferation rate and CD34 microvascular and D2-40 lymphovascular densities drives the pulmonary typical carcinoid outcome. Hum Pathol. 2018;81:201–210.
33. Pericelous M, Karpathakis A, Tounapakis C, et al. Well-differentiated bronchial neuroendocrine tumors: clinical management and outcomes in 105 patients. Clin Respir J. 2018;12(13):904–914.
34. Aslan DL, Gulbahce HE, Parmuccian SE, Manivel JC, Jessurun J. Ki-67 immunoreactivity in the differential diagnosis of pulmonary neuroendocrine neoplasms in specimens with extensive crush artifact. Am J Clin Pathol. 2005; 123(6):874–878.
35. Pelos G, Rodriguez J, Viale G, Rosai J. Typical and atypical pulmonary carcinoid tumor overdiagnosed as small-cell carcinoma on biopsy specimens: a major pitfall in the management of lung cancer patients. Am J Surg Pathol. 2005; 29(2):179–187.
36. Swarts DR, van Sayjen RJ, den Bakker MA, et al. Interobserver variability for the WHO classification of pulmonary carcinoids. Am J Surg Pathol. 2014;38(10): 1429–1436.
37. Amin MB. AJCC Staging Manual. 8th ed. New York, NY: Springer; 2018.