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Diagnosis of Serous Tubal Intraepithelial Carcinoma Based on Morphologic and Immunohistochemical Features: A Reproducibility Study

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Abstract

There is compelling evidence that serous tubal intraepithelial carcinoma (STIC) is a precursor of high-grade serous ovarian carcinoma. Large-scale studies are now required to determine its biological significance and clinical implication. Before conducting these studies, a reproducible classification for STIC is needed, and that is the goal of this study. This study involved 6 gynecologic pathologists from 4 academic institutions and 3 independent rounds of review. In round 1, sixty-seven lesions ranging from normal, atypical, to STICs were classified by 5 pathologists on the basis of predetermined morphologic criteria. Interobserver agreement for the diagnosis of STIC versus not STIC was fair [$\kappa=0.39$; 95% confidence interval (CI) 0.26, 0.52], and intraobserver reproducibility ranged from fair to moderate on the basis of percentage agreement and κ . Round 2 involved testing revised criteria that incorporated morphology and immunohistochemistry (IHC) for p53 protein expression and Ki-67 labeling in 10 sets by 3 of the pathologists. The result was an improvement in interobserver agreement for the classification of STIC ($\kappa=0.62$; 95% CI 0.18, 1.00). An algorithm was then created combining morphology and IHC for p53 and Ki-67, and reproducibility was assessed as part of round 3. In 37 lesions reviewed by 6 pathologists, substantial agreement for STIC versus no STIC was observed ($\kappa=0.73$; 95% CI 0.58, 0.86). In conclusion, we have developed reproducible criteria for the diagnosis of STIC that

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incorporate morphologic and IHC markers for p53 and Ki-67. The algorithm we propose is expected to help standardize the classification of STIC for future studies.

Keywords

serous tubular intraepithelial carcinoma (STIC); reproducibility; ovarian cancer

In the last few years, there have been impressive advances in our understanding of the molecular pathogenesis and origin of epithelial ovarian cancer, which have important clinical ramifications.⁵ The identification of a precursor lesion designated “serous tubal intraepithelial carcinoma” (STIC) in the fimbriated end of the fallopian tube that is morphologically and molecularly similar to high-grade serous carcinoma is a major advancement. This discovery has the potential to significantly alter the current approach to ovarian cancer risk reduction particularly among *BRCA1* and *BRCA2* mutation carriers who undergo prophylactic bilateral salpingo-oophorectomies at a young age. Molecular characterization of these lesions could enable the development of biology-based targeted prevention strategies for this deadly cancer. Accordingly, the establishment of uniform criteria for the diagnosis of STIC and other putative precursor lesions is critical: first, for translational scientists who are trying to further characterize the early events in serous carcinogenesis; second, for clinical investigators who will be placing patients on clinical trials on the basis of these diagnoses; and third for practicing pathologists who will be reviewing increasing numbers of fallopian tubes in high-risk women who have undergone risk-reducing salpingo-oophorectomies. In these women, an accurate diagnosis of STIC is likely to impact their subsequent clinical management.

The recognition of STIC has led investigators and pathologists in practice to more thoroughly evaluate the fallopian tubes using protocols such as Sectioning and Extensively Examining the Fimbria, which step sections the entire fallopian tube with particular attention to the fimbrial end of the tube.⁹ This has resulted in the “discovery” of a variety of lesions demonstrating degrees of proliferation and atypia that fall short of STIC.¹⁰ It has also resulted in a lesion designated “p53 signature,” which is characterized by a strip of normal-appearing fallopian tube epithelium, at least 12 cells in length, which strongly expresses p53. Some of these lesions have been reported to harbor *TP53* mutations.⁷ At present, it is not known whether these lesions are potential precursors of STIC overexpression of p53 in response to some form of cellular stress and not related to carcinogenesis. It is conceivable that there are subsets that are precursors and others that are not. As these lesions have only recently been recognized, their clinical significance is not known. Moreover, because the changes are relatively subtle, this presents new and challenging problems in diagnosis. A recently published report demonstrated that interobserver reproducibility for STIC even among expert gynecologic pathologists was moderate at best,² and in our initial investigations we encountered similar problems. Accordingly, this study was undertaken to determine whether we could develop more reproducible criteria for the diagnosis of STIC.

MATERIALS AND METHODS

Case Selection

There were 3 rounds of review over a 12-month period. For each review we were able to identify a unique set of slides that included normal fallopian tube epithelium and a variety of tubal lesions that were obtained from cancer-free women who underwent bilateral salpingo-oophorectomy. In round 1, hematoxylin and eosin (H&E) slides for 67 lesions from 48 cases were collected from 3 academic institutions (Toronto University Health Network, Johns Hopkins, and Memorial Sloan-Kettering). The lesions were identified and marked on the slides by 1 pathologist (P.S.). They were then randomly ordered by the epidemiologist (K.V.) and scored in the same order by the other 5 pathologists (R.J.K., I.S., R.V., V.P., and R.S.) who were blinded to any clinical information. These 67 lesions were then relabeled and redistributed by the epidemiologist for rescoring by the same 5 pathologists. In round 2, ten lesions were reviewed by 3 pathologists (R.J.K., R.V., and I.S.) on the basis of revised criteria outlined below. For each lesion, 1 H&E slide and immunohistochemical (IHC) stains for p53 and Ki-67 were evaluated on the basis of 1 slide each. Pathologists were given all the slides at the same time, but they reported first on morphology and then on both p53 and Ki-67 expression before rendering a final diagnosis. The results of this study led to the development of an algorithm incorporating morphologic and IHC features. In round 3, all 6 pathologists evaluated 37 new lesions from 29 cases using the algorithm. As in round 2, for each lesion an H&E slide and IHC stains for p53 and Ki-67 were reviewed.

IHC Analysis

Both p53 antibody and Ki-67 antibody were obtained from Ventana, Tucson, Arizona, prediluted 1:1. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections as described previously.¹² In brief, antigen retrieval was performed by steaming the sections in citrate buffer (pH 6.0) for 20 minutes. After incubation with the primary antibodies at room temperature for 2 hours, a positive reaction in tissue sections was detected by the EnVision+System (DAKO, Carpinteria, CA) and developed with 3, 3'-diaminobenzidine. The percentage of intensely immunoreactive nuclei was determined.

Statistical Analysis

For each round, overall agreement and category-specific agreement were determined between participating pathologists on the basis of the Cohen κ statistics and corresponding 95% confidence intervals (CIs).⁶ In addition, agreement was also assessed for specific criteria within a diagnostic category in round 1. If all the pathologists agreed on a diagnosis, this was coded as complete agreement. Intraobserver agreement was also tested for each pathologist in round 1 using similar methods. Statistical analyses were performed using STATA software package (version 11.1; StataCorp LP).

RESULTS

Figure 1 is a flow chart showing the process that was undertaken to develop reproducible criteria for STIC.

First Round

The initial classification consisted of 3 categories: normal/reactive, atypical, and STIC. Predefined morphologic characteristics for each category are shown in Table 1 and 2. A total of 67 lesions were evaluated. The variation in classification among all 5 pathologists is shown in Figure 2A. The interobserver agreement based on all 3 categories and the interobserver agreement comparing a diagnosis of STIC with not STIC were poor to fair ($\kappa=0.27$, 95% CI 0.18, 0.38; $\kappa=0.39$, 95% CI 0.26, 0.52). The category that the pathologists least agreed upon was atypical ($\kappa=0.11$; 95% CI 0.0, 0.25). A similar interobserver agreement was obtained when repeated a second time (see Fig. 2A and Table 3).

The κ value for each of the specific morphologic criteria used was as follows: 0.64 for at least 1 mitotic figure, 0.39 for epithelial stratification, 0.38 for apoptotic bodies, 0.18 for nuclear enlargement and/or nuclear rounding, 0.18 for marked pleomorphism, 0.16 for abnormal chromatin, and 0.08 for nuclear molding. The κ values were very similar in the second round. The intraobserver agreement for STIC versus not STIC was better, as shown in Table 4 and 5. Percentage agreements ranged from 72.6% to 84.1%. Kappa values ranged from 0.41 (95% CI 0.18, 0.65) to 0.68 (95% CI 0.50, 0.86).

Second Round

Revisions were made to the morphologic classification to focus primarily on STICs by 3 of the pathologists (R.J.K., R.V., and I.S.), as it became apparent on the basis of the results of round 1 that defining atypical versus normal and reactive at this stage was challenging and that STIC encompassed a broad number of morphologic criteria. The revised morphologic classification was again of 3 categories, but it was simplified further: not STIC, equivocal for STIC, and STIC. There was no predetermined number of features required for a diagnosis. IHC staining for p53 and Ki-67 was also incorporated. P53 was considered compatible with a *TP53* mutation when it was strongly expressed in >75% of lesional cells or if completely negative (ie, no staining). Our studies have demonstrated that these 2 different staining patterns correlate with the presence of a *TP53* mutation in nearly 95% of cases.¹⁶ The Ki-67 labeling index was classified into 2 categories, <10% and 10%, on the basis of our studies of the Ki-67 labeling index in normal fallopian tube epithelium and STICs (unpublished data). Ten sets were evaluated by 3 of the 5 study pathologists (R.J.K., R.V., and I.S.) (Table 1 and 2 and Fig. 2B). The κ value for morphology alone was in fact lower than that of round 1 and reflects a difference in how 1 of the 3 pathologists defined STIC. This led to further clarification of the categories. However, the evaluation of sets that incorporated morphology and IHC (Ki-67 and p53) into the diagnosis of STIC improved agreement for STIC compared with morphology alone in round 1. The κ value for STIC versus not STIC based on morphology and IHC was 0.62 (95% CI 0.18, 1.00), as shown in Table 3. The broad CIs reflect the small sample size. This approach also appeared to significantly increase the agreement of the other categories; however, the data are less robust. These results led to further refinement of the classification and to the development of an algorithm described in Figure 3.

Third Round

The classification used in round 2 was further refined on the basis of the results of rounds 1 and 2, and an algorithm was created (Fig. 3). All 6 pathologists used the algorithm to evaluate 37 lesions from 25 cases in round 3. Morphology was further refined as follows: not suspicious for STIC, suspicious for STIC, and unequivocal for STIC. The results of round 3 are shown in Figure 2C. Use of the morphologic and IHC-based algorithm in this large sample set resulted in substantial agreement among the 6 pathologists in classifying STICs versus not STICs ($\kappa=0.73$; 95% CI 0.58, 0.86), as shown in Table 3.

Figures 4 to 8 present examples of STIC, serous tubal intraepithelial lesion (STIL), normal/reactive, and P53 signature that were agreed upon by all 6 pathologists.

DISCUSSION

We have developed reproducible criteria for the diagnosis of STIC versus not STIC using an algorithm that incorporates morphology coupled with IHC for p53 expression and Ki-67 labeling. Using only morphologic criteria for a diagnosis of STIC in round 1 did not yield sufficient reproducibility. Despite good intraobserver agreement, the reproducibility of STIC versus not STIC based on morphology alone was fair and did not improve over time. In fact, we observed poor-to-fair agreement in round 1 among the 5 pathologists for the majority of STIC-associated diagnostic criteria listed in, Table 1 and 2 irrespective of whether or not the lesion was a STIC. The most commonly agreed upon criterion was the presence of at least 1 mitotic figure, followed by epithelial stratification. Other criteria such as apoptotic bodies, nuclear enlargement and/or nuclear rounding, marked pleomorphism, abnormal chromatin, and nuclear molding were poorly agreed upon among the pathologists. It is noteworthy that we did not specifically investigate polarity, which has been mentioned in association with STICs previously; however, it was only commented upon by 1 of the 5 pathologists. Given the lack of agreement for these criteria, it is difficult to assess which of them are more commonly seen in STICs. Further, distinguishing between normal/reactive and atypical/STILs (referred to as TILTS by others) based on morphology alone was challenging. However, simplifying the classification into 3 broad categories (not suspicious, suspicious, and unequivocal), in combination with group training and discussions, did improve reproducibility in round 2.

Round 2 demonstrated the benefit of incorporating both IHC markers for p53 expression and Ki-67 as part of the diagnosis of STIC. Published literature and our own experience indicate that Ki-67 is elevated in STIC and that p53 is more frequently increased or absent.^{4,8,11–13,15} The κ value for STIC compared with not STIC improved from 0.39 (95% CI 0.26, 0.52) to 0.62 (95% CI 0.18, 1.00). It was also clear from the results of round 2 that defined cutoffs were necessary. A cutoff of less than 10% Ki-67 labeling showed very good agreement among all 6 pathologists ($\kappa=0.73$; 95% CI 0.61, 0.84); however, there was only moderate agreement for p53 “positivity” ($\kappa=0.51$; 95% CI 0.37, 0.68) defined by diffuse moderate-to-strong expression of p53 in >75% of at least 12 epithelial cells (with or without intervening ciliated cells) or complete absence of staining has been shown to be compatible with a TP53 mutation either missense or nonsense p53 MUT versus p53WT pattern in algorithm. It is

possible that normal-appearing cells that are intermingled (scattered) within an STIC compromise a precise assessment of p53-positive cells.

The results of rounds 1 and 2 led to the development of an algorithm that was evaluated in round 3. An important aspect of our algorithm is that, regardless of whether the same focus of concern is interpreted as “unequivocal for STIC” by 1 pathologist and “suspicious for STIC” by another on the basis of morphology alone, abnormal coordinate expression of both p53 and Ki-67 which is necessary for making a final diagnosis of STIC (ie, p53 >75% positive or negative/high Ki-67 with either of those 2 morphologic designations). This redundancy of the IHC arms of the algorithm helps to compensate for differences in morphologic diagnosis by individual gynecologic pathologists. In addition, by incorporating 2 IHC markers in the algorithm we increased the rigor necessary for a diagnosis of STIC and are able to distinguish different tubal lesions (eg, STIC, STIL, p53 signature, and normal/reactive) on the basis of the various p53/Ki-67 immunoprofiles. The reproducibility of STIC versus not STIC using this algorithm was very good with a κ value of 0.73 (95% CI 0.58, 0.86).

One study has evaluated the reproducibility of STIC versus not STIC in 30 cases on the basis of morphology alone using high-quality digital images (14 that had been determined to be STIC and 16 benign lesions by 2 gynecologic pathologists on the basis of morphologic criteria and IHC staining for p53 and Ki-67).² Nine of the 14 lesions designated as STIC were classified correctly, and 2 of 16 lesions were designated as benign by 6 experienced gynecologic pathologists. The κ value was 0.453 for the experienced gynecologists. Interestingly, the κ value was very similar to what we reported with morphology alone in round 1 ($\kappa=0.40$; 95% CI 0.29, 0.52). They did not evaluate atypical lesions and did not present reproducibility data on the combination of both morphology and IHC or the potential for improvement in diagnosis of STIC over time. In our study, we did not include other proteins that have been shown to be present in STICs, nor did we include etiologically important genes and proteins associated with high-grade serous carcinoma, such as p21, p16 cyclin E1, Rsf-1, fatty acid synthase, and laminin C1protein.^{1,3,12,14} If they are validated in larger data sets, they may improve the reproducibility of STIC even further.

The strengths of our study include the evaluation of over 100 lesions including approximately 40 STICs, atypical lesions, and variants of normal tubal epithelium in cancer-free women by at least 5 and sometimes 6 experienced gynecologic pathologists from 4 different institutions over multiple time points. This has enabled us to generate robust estimates to compare the classification of STICs of STICs to mimickers of STIC. The weakness is that the sample size of the subcategories is not large, making the κ values for those lesions less robust, particularly when there are imbalances between the categories being assessed. Larger studies will be required to assess the reproducibility of these lesions. In conclusion, we have developed reproducible criteria for the diagnosis of STIC that incorporate morphologic and IHC markers for p53 and Ki-67. Validation of this algorithm in larger data sets and among pathologists with a variety of experiences is now required.

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FIGURE 1.

A flow chart describing the process that was undertaken to assess reproducibility of STIC.

FIGURE 2.

Pathologic classification of fallopian tube lesions for rounds 1 to 3. A, Morphologic classification of fallopian tube lesions by each pathologist at 2 time points in round 1. B, Morphologic and immunohistochemical classification of fallopian tube lesions by each pathologist at 1 time point in round 2. C, Morphologic and immunohistochemical classification of fallopian tube lesions by each pathologist at 1 time point in round 3 based on the algorithm described in Figure 3. STIL indicates serous tubal intraepithelial lesion, STIC for serous tubal intraepithelial carcinoma and N/R indicates for normal/reactive. p53 signature is characterized by a strip of normal-appearing fallopian tube epithelium, at least 12 cells in length, which strongly expresses p53.

FIGURE 3.

Algorithm for the diagnosis of STIC. Foci showing a Ki-67 labeling index >10% are considered Ki-67 high, whereas a Ki-67 labeling index of <10% is considered low. Diffuse moderate-to-strong expression of p53 in >75% of at least 12 epithelial cells (with or without intervening ciliated cells) or complete absence of staining has been shown to be compatible with a TP53 mutation either missense or nonsense (p53 MUT versus p53WT pattern in algorithm).

FIGURE 4.

Final diagnosis of STIC by all 6 observers. Normal epithelium for comparison is present in the upper left corner. A, Morphologic designation of unequivocal STIC by all 6 observers. The epithelium is slightly stratified with loss of polarity and nuclear molding. A longitudinal cleft within the epithelium is present at the center of the photograph. The nuclei are enlarged with prominent nucleoli, and the nuclearto-cytoplasmic ratios are increased. B, The p53 immunostain was interpreted as “compatible with a TP53 mutation” by all 6 observers. C, High Ki-67 labeling index by all 6 observers.

FIGURE 5.

Final diagnosis of STIL by all 6 observers. Normal epithelium for comparison is present in the lower left corner. A, Morphologic designation of suspicious for STIC by all 6 observers. The epithelium shows a suggestion of nuclear molding. Some nuclei are enlarged, rounded, and hyperchromatic; however, the overall morphologic features were not interpreted as sufficient for a morphologic designation of unequivocal STIC. B, The p53 immunostain was interpreted as “compatible with a TP53 mutation” by all 6 observers. C, Low-Ki-67 labeling index by all 6 observers.

FIGURE 6.

Final diagnosis of normal/reactive by all 6 observers. Normal epithelium for comparison is present in the lower right corner. A, Morphologic designations of suspicious for STIC by 2 observers and not suspicious for STIC by 4 observers. The epithelium is slightly stratified, and the nuclei are questionably hyperchromatic. B, The p53 immunostain was interpreted as “NOT compatible with a TP53 mutation” by all 6 observers. C, Low-Ki-67 labeling index by all 6 observers.

FIGURE 7.

Final diagnosis of p53 signature by all 6 observers. A, Morphologic designation of not suspicious for STIC by all 6 observers. Although no obviously ciliated cells are evident within the epithelium, no appreciable atypia is identified. B, The p53 immunostain was interpreted as “compatible with a TP53 mutation” by all 6 observers. C, Low-Ki-67 labeling index within the same segment showing a positive p53 pattern in (B) by all 6 observers.

FIGURE 8.

Final diagnoses of STIC by 4 observers and STIL by 2 observers. A, Morphologic designations of unequivocal for STIC by 3 observers and suspicious for STIC by 3 observers. The epithelium is slightly stratified, and the nuclei are questionably enlarged and hyperchromatic. B, The p53 immunostain was interpreted as “compatible with a TP53 mutation” by all 6 observers. C, High Ki-67 labeling index by 4 observers and low-Ki-67 labeling index by 2 observers.

TABLE 1**Diagnostic Criteria Used in Round 1**

Morphologic classification used in round 1
Normal/reactive: <2 diagnostic features* in any length of nonciliated cells
Atypical:
–2 diagnostic features* in 10 consecutive nonciliated cells
2 diagnostic features* in <10 consecutive nonciliated cell
STIC†: >2 diagnostic features* in 10 consecutive nonciliated cells
*Diagnostic features
Nuclear enlargement (>2X nuclear area compared with nonciliated cells within the focus of interest or in adjacent normal mucosa) and/or nuclear rounding
Marked pleomorphism
Abnormal chromatin (hyperchromasia and/or vesicular nuclei with prominent nucleoli)
1 mitotic figure (either normal or abnormal)
Epithelial stratification (>2 cell layers)
Nuclear molding
Apoptotic bodies

TABLE 2**Morphologic Classification Used in Round 2**

Not STIC
Equivocal
STIC
The pathologists could comment on the morphologic features they observed
Immunohistochemical stains used in round 2
p53 positive:
Diffuse intense staining in >75% of all cells in the focus of interest
Heterogenous in the focus of interest
Completely negative in the focus of interest
Ki-67% (including all cells in the focus of interest)
<10%
10%
Final diagnosis (based on morphology and IHC staining)
Normal/reactive
Not STIC
STIC

TABLE 3

Interobserver Agreement Between Pathologists for Rounds 1, 2, and 3

Round 1 (n = 67)	Category	κ_1 (95% CI)	κ_2 (95% CI)*
Morphology alone	Normal/reactive	0.26 (0.15–0.42)	0.30 (0.17–0.45)
	Atypical	0.11 (0.00–0.25)	0.10 (0.01–0.22)
	STIC	0.39 (0.26–0.52)	0.40 (0.29–0.52)
	Overall	0.27 (0.18–0.38)	0.27 (0.20–0.37)
Round 2 (n = 10)	Category	κ (95% CI)	
Morphology alone	not STIC	0.57 (0.06–1.00)	
	equivocal for STIC	0.03 (0.18–0.52)	
	STIC	0.09 (0.12–0.47)	
	Overall	0.23 (0.06–0.57)	
p53 alone	heterogenous (wild type) vs. positive or flat negative	0.39 (0.00–0.88)	
Ki-67 alone	<10% vs. 10% staining	0.88 (0.73–1.00)	
Morphology+IHC	Normal	1.00 (–)	
	STIL	0.51 (0.15–1.00)	
	STIC	0.62 (0.18–1.00)	
	Overall	0.72 (0.47–1.00)	
Round 3 (n = 37)	Category	κ (95% CI)	
Morphology alone	Not suspicious for STIC	0.38 (0.26–0.57)	
	Suspicious for STIC	0.21 (0.08–0.37)	
	Unequivocal for STIC	0.60 (0.44–0.76)	
	Overall	0.39 (0.27–0.52)	
p53 alone	heterogenous (wild type) vs. positive or flat negative	0.50 (0.35–0.66)	
Ki-67 alone	<10% vs. 10% staining	0.71 (0.58–0.85)	
Morphology+IHC	Normal	0.41 (0.22–0.62)	
	p53 signature	0.40 (0.17–0.66)	
	STIL	0.32 (0.15–0.52)	
	STIC	0.73 (0.58–0.86)	
	Overall	0.48 (0.37–0.61)	

STIL, serous tubal intraepithelial lesions. P53 signature is defined as morphologically normal-appearing tubal epithelium that strongly expresses p53 in at least 12 consecutive nonciliated cells.

* κ_2 repeat agreement for the same set of slides in round 1.

TABLE 4

Intraobserver Agreement Comparing STIC and not STIC Among 5 Pathologists

Pathologist	Agreement (%)	κ (95% CI)
Round 1		
1	72.6	0.45 (0.23–0.67)
2	84.1	0.68 (0.50–0.86)
3	73.0	0.41 (0.18–0.65)
4	83.9	0.64 (0.44–0.84)
5	77.8	0.53 (0.33–0.74)

TABLE 5

Intraobserver Agreement for the various lesions among 5 pathologists

Pathologist	Agreement (%)	κ (95% CI)
Round 1		
1	53.2	0.34 (0.17–0.50)
2	61.9	0.47 (0.29–0.63)
3	61.9	0.43 (0.23–0.59)
4	69.4	0.57 (0.40–0.72)
5	54.0	0.38 (0.21–0.54)