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Original Research Article

# THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF SEROUS TUBAL INTRAEPITHELIAL CARCINOMA AND ITS ASSOCIATION WITH VARIOUS GYNECOLOGICAL MALIGNANCIES: AN INSTITUTIONAL STUDY

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## Abstract

**Background:** The prognosis for High-grade serous carcinomas (HGSC) remains dire, as they are often identified at late stages. Key to improving early detection is a deeper understanding of HGSC pathogenesis. The identification of Serous tubal intraepithelial carcinoma (STIC) as a primary precursor to HGSC has underscored the need for precise diagnostic measures to detect these early lesions. Objectives: The focus of this study is to appraise the utility of immunohistochemistry (IHC), specifically through p53 and Ki67 labeling indices, in confirming STIC diagnoses. It also aims to investigate the relationship between STIC and a range of gynecological malignancies. **Materials and Methods:** This study encompassed a review of 38 cases, including both ovarian and non-ovarian malignancies, collected over a span of three years. Fallopian tube samples were processed following the SEE-FIM protocol, and IHC was employed to assess the expressions of p53 and Ki67 markers. **Result:** From the total cases, 35 (92%) pertained to ovarian malignancies, while 3 (8%) were cases of endometrial serous carcinomas. Notably, the p53 signature was present in 8 cases (21%), and instances of STIL and STIC were detected in 3 cases each (8%). Observations of Secretory cell outgrowth (SCOUT) were made in 10 cases (26%). **Conclusion:** Accurate identification of HGSC precursor lesions in the ovary necessitates the integration of the SEEFIM protocol, rigorous morphological evaluation, and IHC analysis. This triad is crucial for the reliable detection of early lesions, potentially pivotal in the progression to HGSC, thereby playing a critical role in advancing diagnostic accuracy and early intervention strategies.

## INTRODUCTION

Gynecological cancers, particularly ovarian cancer, pose a significant threat to women's health in India and across the globe, with a high fatality rate that underscores the importance of early detection and management.[1,2] Understanding the pathogenesis of these cancers is crucial for early intervention. Although the ovarian surface epithelium was once considered the primary site of origin, it is now acknowledged that the fimbrial end of the fallopian tube plays a central role.[3,4,5,6] Shih and Kurman have identified two distinct pathways in the development of ovarian cancer[7], a classification first proposed in 2004 and later adopted by the WHO that same year.

The first pathway, Type I, includes low-grade serous and non-serous carcinomas such as mucinous, clear cell, and transitional subtypes. These cancers typically develop from benign or borderline precursors in the ovary, have a less aggressive nature, and generally present a favorable prognosis. The second pathway, Type II, encompasses the more aggressive forms such as high-grade serous carcinomas, undifferentiated carcinomas, and


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carcinosarcomas. These tend to proliferate quickly, spread extensively at the time of diagnosis, and are characterized by high mitotic rates (>12/10HPF) and necrosis. High-grade serous carcinomas are predominantly P53 mutants, which is evident through strong nuclear positivity in immunohistochemical staining.

The inception of these malignancies is often traced back to intraepithelial carcinomas at the fimbrial end of the fallopian tube, which includes a range of precursor lesions such as the p53 signature, STIL (serous tubal intraepithelial lesion), and STIC (serous tubal intraepithelial carcinoma).[8] The "sectioning and extensively examining fimbria" (SEE-FIM) protocol is particularly emphasized for a thorough examination, with a special focus on the fimbrial end of the fallopian tube.[9]

The morphological diagnosis of STIC presents its own set of challenges, including moderate reproducibility and observer variability. To bolster diagnostic accuracy, we integrate morphological assessment with immunohistochemical markers such as p53 and Ki67. This approach aims to clarify the incidence of precursor lesions among ovarian malignancies.

Histologically, the fallopian tube epithelium is composed of secretory, ciliated, and intercalated cells—with ciliated cells being predominant, especially at the fimbrial end. It is from the secretory epithelium that the p53 signature, STIL, and STIC originate, all supported by a foundation of fibroconnective tissue and smooth muscle.

STIC is recognized by its morphological deviation from normal epithelium, manifesting as darker under low magnification, potential multifocality, and a predilection for the fimbrial end. Its epithelial cells may present a flat or stratified appearance, sometimes with detached cells within the tubal lumen. Under higher magnification, STIC is characterized by enlarged nuclei, altered nuclear-cytoplasmic ratios, irregular chromatin patterns, pronounced nucleoli, a loss of cellular polarity, and heightened mitotic figures—yet without invading the stroma. Notably, STIC lacks ciliated cells.[10]

In most cases, STIC exhibits either a strong, diffuse expression of mutated p53 or a complete absence thereof (in cases of null mutations), coupled with an elevated Ki67 index over 10%. Conversely, STIL shows cytological atypia with limited proliferation and insufficient criteria for a STIC diagnosis. Here, either p53 or Ki67 will exceed 10%, but not both.

The p53 signature represents an entity identified by overexpression of p53 in otherwise normal-looking tubal epithelium. This immunohistochemical finding, rather than a morphological one, is typified by intense nuclear expression across 12 contiguous secretory cells, often seen in the distal fallopian tube and sometimes appearing multifocally. While its clinical significance remains uncertain, it is considered the earliest precursor to cancer.

An even earlier histopathological change, SCOUT (Secretory cell outgrowth), marks a shift toward a secretory phenotype in the fallopian tube epithelium, characterized by an absence of ciliated cells and a continuous stretch of secretory cells.

While a definitive association between STIC and all high-grade serous carcinomas (HGSC) is not established, literature suggests that thorough sampling reveals STIC in only 40-50% of HGSC cases, though its identification jumps to 59% in women with peritoneal and high-grade serous carcinoma.[11,12] It is suggested that many Type II lesions may originate from tubal intraepithelial carcinomas and then progress to involve the ovary and other extragonadal sites.

The aims of this study are twofold: firstly, to investigate the effectiveness of immunohistochemical markers, particularly p53 and Ki67 labeling indices, in diagnosing STIC; and secondly, to explore the relationship between STIC and various gynecological malignancies.

## MATERIALS AND METHODS

**Study Design and Setting**: This retrospective and prospective hospital-based study was conducted at the Department of Pathology, Guntur Medical College, Guntur, from January 2020 to December 2022. The Institutional Ethics Committee approved the research protocol, and informed consent was obtained from all participating subjects.

**Study Population**: The study included fallopian tubes excised from patients with ovarian carcinomas and serous carcinoma of the endometrium. Exclusion criteria were other malignancies, non-neoplastic lesions, benign ovarian tumors, and metastatic malignancies.

**Specimen Processing**: Specimens were procured from the Gynecology and Surgical Oncology departments of the Government General Hospital. For the prospective component, specimens were freshly grossed, and for the retrospective component, previously obtained specimens were re-grossed. The SEE-FIM protocol, as recommended by the College of American Pathologists (CAP), guided the sectioning process. The fimbriated ends of the fallopian tubes were longitudinally sectioned to maximize the surface area for histopathological examination (HPE). The remaining tube portions were serially sectioned transversely. Standard paraffin embedding procedures were followed, and sections were stained using Hematoxylin and Eosin (H&E). In total, 38 cases were analyzed during the study period.

**Histopathological and Immunohistochemical Analysis**: Initial H&E stained sections were independently reviewed by 3 to 4 pathologists, classifying them as Negative for STIC, Suspicious for STIC, or Unequivocal for STIC. For immunohistochemistry (IHC), 1 to 2 representative slides per case were selected, and sections were taken on poly-L-lysine coated slides from selected blocks. After deparaffinization and rehydration, antigen


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retrieval was performed using the pressure cooker method, followed by peroxidase blocking. The slides were incubated with a monoclonal primary mouse anti-p53 antibody for 30 minutes, washed in buffer, and treated with polymer Rb HRP for 20 minutes. Diaminobenzidine (DAB) was applied for 10 minutes, and counterstaining was completed with hematoxylin. The slides were then dehydrated and mounted.

**IHC Interpretation**: The p53 staining was categorized as mutant (strong, diffuse nuclear positivity), null type (complete absence of staining), and wild type (cytoplasmic positivity). The Ki67 labeling index was quantitatively assessed, with a cut-off value of 10% delineating low (<10%) from high (>10%) proliferation rates[5,13].

**Final Classification**: After IHC analysis and reassessment of H&E slides, the cases were reclassified into one of the following categories: Normal/reactive, p53 signature, SCOUT, STIL, or STIC, following the diagnostic algorithm proposed by Russell Vang.[13]

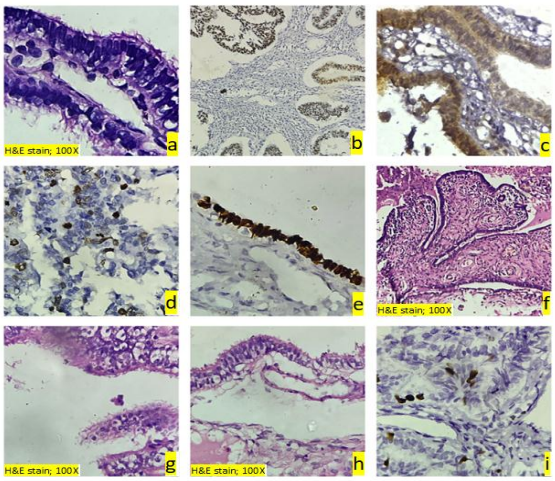


**Fig a) STIC: H&E - on high power(400X) displaying hyperchromatic nuclei,few with prominent nucleoli Fig b),c),d)STIC: IHC – p53 expression mutant type(100X) , c) p53 on (400X) d) Ki67 >10% (100X) Fig e),f) p53 signature : p53 signature (100X,IHC),f) p53 signature(H&E,100X) Fig g) SCOUT – showing cells with loss of cilia(H&E,400X) Fig h),i) STIL – showing normal morphology with mild atypia (H&E,400X),i) Ki67 < 10%, (IHC,400X).**

## RESULTS

The study encompassed 38 cases, with 35 (92%) being ovarian malignancies and 3 (8%) endometrial serous carcinomas. Of the ovarian cases, 24 (63%) presented unilaterally, while 11 (29%) were bilateral. The most prevalent ovarian tumors were serous carcinomas, totaling 25 cases (66%); within this subgroup, 19 (50%) were high-grade serous carcinomas and 6 (16%) were low-grade. Bilateral presentation occurred in 14 serous carcinoma cases, and 11 were unilateral.

Mucinous carcinomas were the second most frequent, representing 7 cases (18%), all unilateral. Additionally, there was a single case (3%) each of endometrioid carcinoma, clear cell carcinoma, and carcinosarcoma, respectively (Table 1).

Patient ages mainly fell within the 5th decade with 15 cases, then the 6th decade with 11 cases, and 6 cases in the 4th decade. The 7th decade featured 4 cases, with one case each in the 8th and 9th decades, ranging from 35 to 85 years (Table 2).

p53 signatures (Figures e, f) appeared in 8 cases (21%), comprising 5 high-grade serous ovarian carcinomas, 2 serous endometrial carcinomas, and 1 carcinosarcoma. Serous Tubal Intraepithelial Lesions (STIL, Figures h, i) were diagnosed in 3 cases (8%), including 2 high-grade serous carcinomas and 1 carcinosarcoma. Secretory cell outgrowths (SCOUT, Figure g) were identified in 10 cases (26%), with 9 being serous carcinomas and 1 a clear cell ovarian carcinoma. Serous Tubal Intraepithelial Carcinoma (STIC, Figures a, b, c) was noted in 3 cases (8%) of high-grade serous carcinoma. Ki67 indexes were notably elevated, ranging from 15% to 75%. The remaining 14 cases (37%) tested negative for these lesions, deemed as non-specific/normal (Table 3).

In summary, ovarian high-grade serous carcinomas exhibited a higher frequency of lesions.

**Table 1: Classification of Serous and Non-Serous Carcinomas (Total Cases: 38)**

| Malignancies | No. of cases | Percentage |
|---|---|---|
| **Serous carcinoma** | | |
| Serous carcinoma endometrium | 3 | 8% |
| High grade serous carcinoma of ovary | 19 | **50%** |
| Low grade serous carcinoma of ovary | 6 | 16% |
| **Non Serous carcinoma** | | |
| Endometroid carcinoma | 1 | 3% |
| Mucinous carcinoma | 7 | 17% |
| Clear cell carcinoma | 1 | 3% |
| Carcinosarcoma | 1 | 3% |
| **Total** | 38 | 100% |

**Table 2: Age Distribution Among Patients with Serous and Non-Serous Carcinomas (N=38)**

| Malignancies | No of cases | Mean age |
|---|---|---|
| **Serous carcinoma** | | |
| Serous carcinoma endometrium | 3 | 58.66 |
| High grade serous carcinoma of ovary | 19 | 52.57 |
| Low grade serous carcinoma of ovary | 6 | 43.5 |
| **Non Serous carcinoma** | | |


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| | | |
|---|---|---|
| Endometroid carcinoma | 1 | 45 |
| Mucinous carcinoma | 7 | 45.71 |
| Clear cell carcinoma | 1 | 38 |
| Carcinosarcoma | 1 | 57 |
| **Total** | 38 | |

**Table 3: Correlation of SCOUT, p53 Signature, STIL, and STIC with Serous and Non-Serous Carcinomas (Total Cases: 38)**

| Lesion type | Serous carcinomas | | Non serous carcinomas |
|---|---|---|---|
| | Ovary | Endometrium | |
| SCOUT | 9(24%) | - | 1(3%) |
| P53 sig | 5(13%) | 2(5%) | 1(3%) |
| STIL | 2(5%) | - | 1(3%) |
| STIC | 3(8%) | - | - |

## DISCUSSION

In India, ovarian carcinoma is a prominent concern among gynecological malignancies. The prevalence of tubal intraepithelial carcinoma (TIC) in many ovarian serous carcinomas has catalyzed the adoption of the SEEFIM protocol. This protocol is significant in that it involves a detailed sectioning of the entire fimbrial end longitudinally to maximize tubal surface exposure, and transversely for the remainder of the tube.[14] This approach is particularly relevant given that the fimbria is a common site for STIC lesions. The primary objective of the current study was to assess the effectiveness of p53 and Ki67 in diagnosing STIC and its precursor lesions and to determine the incidence of STIC across various gynecological malignancies.

In our study, STIC was morphologically detected in 6 cases, with 2 being unequivocally identified as STIC and 4 deemed suspicious for STIC. Subsequent immunohistochemistry revealed that 3 of these cases were intensely positive for p53, and the Ki67 index was elevated, exceeding 10% as per the criteria established by Vang et al.[13] Examining the 38 cases in total, we found 35 ovarian malignancies comprising 19 high-grade serous ovarian carcinomas (HGSOC), 6 low-grade serous ovarian carcinomas (LGSOC), and 10 non-serous carcinomas, along with 3 cases of endometrial serous carcinoma. Among the 19 HGSOC cases, STIC was identified in 3, translating to an incidence rate of 15.7%. This is in stark contrast to the findings of Aswathi et al[15], who reported a much higher incidence of 66.6% in HGSOC. However, our results are somewhat aligned with those of Tang et al[16], who observed an incidence rate of 18.8% (6 out of 32 cases). The comparatively lower incidence in our study could be attributed to the smaller number of cases examined. Notably, no STIC instances were found in LGSOC.

Beyond STIC, our study also identified various lesions in the fallopian tubes considered precursors to STIC, namely P53 signature, STIL, and SCOUT. The P53 signature was observed in 8 cases, accounting for 21% of our study population. This included 5 instances in HGSOC (13%), 2 in serous carcinoma of the endometrium (5%), and 1 in carcinosarcoma (3%). These figures are considerably lower compared to the 50% occurrence of P53 signature in HGSOC reported by R. Aswathi and P S Jayalakshmi et al.[15] Neha Mittal and Radhika Srinivasan et al[17] noted 7 cases of multifocal P53 signature, whereas our study found only unifocal occurrences.

Moreover, in our research, 2 cases of serous carcinoma of the endometrium (5%) and 1 case of carcinosarcoma exhibited the P53 signature. Tang Shangguo et al[16] reported 4 cases of STIC in endometrial serous carcinoma, contrasting with our findings where we identified P53 signatures rather than STIC in these cases.

Regarding STIL, it was present in 2 cases of HGSOC and 1 case of carcinosarcoma in our study, with the carcinosarcoma case also displaying a P53 signature. This finding highlights the spectrum of these two lesions within a single case and their association with carcinosarcoma, categorized as a type II lesion.

SCOUT was detected in 10 cases in our study, encompassing 9 HGSOC and 1 clear cell carcinoma, which is classified as a type I lesion. SCOUT was observed across all categories, indicating its non-specific nature. Aswathi et al[15] reported SCOUT in 42.6% of benign, 50% of borderline, 90.9% of low-grade, and 83.3% of HGSOC cases.

In total, our study identified 3 cases of STIC, 2 of STIL, and 5 of P53 signature, cumulatively accounting for 10 precursor lesion cases exclusively associated with HGSOC. This constitutes 52.6% of the total, underscoring a strong connection between these precursor lesions at the fimbrial end of the tube and the type II tumorigenesis of HGSOC.

All studies conducted on STIC to date emphasize their utility, especially for women with BRCA1 and BRCA2 mutations undergoing prophylactic salpingo-oophorectomy. Implementing the SEEFIM protocol in conjunction with immunohistochemistry (IHC) for p53 and Ki67 allows for earlier detection and diagnosis of precursor lesions like STIC, STIL, P53 signature, and SCOUT.

**Limitations**

The study's limitations include a small sample size, which may limit the generalizability of the findings. Being a single-institution study, the results may not be applicable elsewhere. The retrospective nature raises the potential for selection and information bias. Variability in immunohistochemistry interpretation could affect diagnostic consistency, and the absence of genetic profiling omits a significant aspect of


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cancer risk assessment. Additionally, the study lacks longitudinal follow-up to confirm the progression from precursor lesions to invasive cancer, and findings require external validation to establish broader clinical relevance.

## CONCLUSION

The study reinforces that precursor lesions like the P53 signature, STIL, and STIC, classified with the aid of p53 and Ki67 markers, have a strong association with type II tumorigenesis, particularly HGSOC. The SEE-FIM protocol, combined with meticulous morphological analysis and the judicious application of IHC, is critical for diagnosing these precursor lesions, which could ultimately refine the prognosis of HGSC of the ovary. While lesions resembling STIL could be classified as epithelial atypia in clinical reports, it's important to note their insufficiency for a definitive STIC diagnosis. Meanwhile, the P53 signature, while not routinely reported, serves an important academic function. The prevalence of precursor lesions in our cohort suggests a notable link with type 2 carcinomas, especially serous types. These findings underline the significance of early detection and the potential for improved management in high-risk groups, such as BRCA1 and BRCA2 mutation carriers undergoing prophylactic salpingo-oophorectomy.

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