



The role of SATB2, CDX2, CK7 and CK20 in differentiating primary versus metastatic ovarian carcinoma

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ABSTRACT

OBJECTIVES: To find out the association of primary ovarian carcinomas (POC) and metastatic gastrointestinal carcinoma (MGC) by utilizing a panel of antibodies including Cytokeratin (CK) 7, CK20, Caudal-type Homeobox Transcription Factor-2 (CDX2), and SATB2 with their clinicopathological parameters.

METHODS: This prospective cross-sectional study was conducted from Mar, 2023 to Dec 2023 at Dow University of Health Sciences, Karachi, Pakistan. Biopsy tissue specimens from female patients over the 40 years, diagnosed with primary and metastatic ovarian carcinomas (MOC), were included. Immunohistochemistry, employing a three-tier scoring system, assessed CK7, CK20, CDX2, and SATB2 expressions. Statistical analysis was performed, utilized SPSS version 26.0 by employing Chi-Square test.

RESULTS: Over a 9-month period, 100 biopsied tissue samples were collected from patients aged 42-71 years (mean age 53.24 ± 8.03 years). Among these, 79 were POC (51% serous carcinoma, 9% mucinous carcinoma, 18% endometrioid carcinoma, and 1% clear cell carcinoma), and 21 were MGC. CK7 showed diffuse staining predominantly in POC and was absent in MGC ($p < 0.001$). CK20 exhibited significant focal and diffuse staining in MGC and was largely absent in POC ($p = 0.005$). CDX2 was significantly absent in POC and showed diffuse and focal staining in MGC ($p < 0.001$). SATB2 was absent in POC and displayed focal or diffuse staining in MGC ($p < 0.001$).

CONCLUSION: The inclusion of SATB2 in immunohistochemical panels, alongside CK7, CK20, and CDX2, improves the accuracy of diagnosing primary and metastatic ovarian carcinomas. This approach provides support in the distinction between ovarian and gastrointestinal tumors.

KEYWORDS: Ovarian Neoplasms (MeSH); Primary and metastatic ovarian carcinoma (Non-MeSH); CK7 (Non-MeSH); CK20 (MeSH); CK20 protein, human (MeSH); CDX2 (MeSH); SATB2 (MeSH); Immunohistochemistry (MeSH).

THIS ARTICLE MAY BE CITED AS: Tahir S, Avesi L, Shamim SH, Danish F, Bukhari U, Ali FM. The role of SATB2, CDX2, CK7 and CK20 in differentiating primary versus metastatic ovarian carcinoma. *Khyber Med Univ J* 2024;16(3): 244-8. <https://doi.org/10.35845/kmu.2024.23594>

INTRODUCTION

Ovarian cancer (OC) presents a significant global health challenge, ranking seventh among malignant tumors in women and eighth in cancer-related mortality. Its high mortality rate makes it the third leading cause of cancer deaths among women, following cervical and uterine cancers.¹ Among the various histological subtypes, epithelial ovarian cancer is the most prominent, consisting of four main categories: serous, endometrioid, clear cell, and mucinous tumors. Each subtype exhibits distinct biological

characteristics and varying treatment responses. Additionally, there are rarer subtypes, including Brenner and seromucinous tumors.²

Ovarian cancer can be further categorized into two subtypes: Type I and Type II tumors. Type II tumors are generally more lethal, often linked to factors like continuous ovarian cycles, inflammation, and endometriosis. Type I tumors include low-grade serous, endometrioid, clear-cell, and mucinous carcinomas, with rarer forms such as seromucinous and Brenner tumors. These tumors typically present at an

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Date Submitted: January 28, 2024

Date Revised: September 30, 2024

Date Accepted: September 30, 2024

early stage, are predominantly low grade, and exhibit low proliferative activity, resulting in a more favorable prognosis. In contrast, Type II tumors are high-grade and usually diagnosed at an advanced stage. They are characterized by aggressive progression, heightened proliferative activity, significant chromosomal instability, and often involve p53 mutations.^{2,3}

Despite advancements in understanding ovarian cancer subtypes, distinguishing primary ovarian mucinous neoplasms from metastatic tumors, especially those from the lower gastrointestinal tract, remains challenging. Immunohistochemical markers, including cytokeratin 7 (CK7), cytokeratin 20 (CK20), and Caudal-type Homeobox Transcription Factor 2 (CDX2), are valuable tools for this differentiation. CK7 is often positive in primary mucinous ovarian carcinomas, but it can also be found in colon and rectal carcinomas, complicating its diagnostic use. CK20 typically shows diffuse positivity in metastatic colorectal carcinomas, yet it may also appear focal or diffuse in primary ovarian mucinous neoplasms. CDX2, a nuclear transcription factor involved in intestinal differentiation, is expressed in both colon carcinoma and mucinous ovarian neoplasms, further obscuring the distinction between primary and metastatic tumors.^{4,6}

This study addresses the clinical challenges in differentiating mucinous ovarian neoplasms, especially in distinguishing primary tumors from

metastatic ones originating in the lower gastrointestinal tract, such as colorectal adenocarcinomas and appendiceal neoplasms. The challenge arises from overlapping immunohistochemical profiles that complicate accurate diagnosis. Accurate diagnosis is crucial, as it significantly impacts prognosis and treatment strategies. With the rise of targeted therapies, understanding the biological profiles of these tumors could open new therapeutic pathways for future clinical trials.

The primary aim of this study was to investigate the association between the immunohistochemical profiles of primary ovarian carcinoma (POC) and metastatic gastrointestinal carcinoma (MGC) using a panel of antibodies. This included traditional markers like CK7, CK20, and CDX2, as well as novel markers such as SATB2.

METHODS

This prospective cross-sectional study was conducted from March to December 2023, at the Histopathology Department of the Dow Diagnostic, Research and Reference Laboratory at Dow University of Health Sciences (DUHS) in Karachi, Pakistan. Convenience sampling was used, applying strict inclusion criteria that involved tissue specimens from female patients over 40 years old, including both primary and secondary ovarian carcinomas, provided they consented to participate. Exclusion criteria ruled out poorly fixed tissues, improperly sectioned or stained slides, requisition forms lacking adequate clinical details, benign ovarian lesions identified via light microscopy, patients under 40, and those who had received chemotherapy or radiation therapy. The study's dependent variables included patients diagnosed with ovarian and gastrointestinal malignancies, while independent variables comprised SATB2 expression, risk factors, socioeconomic status, ethnicity, and tumor grading and staging.

Tissue specimens suspected of ovarian malignancy were collected and processed according to internationally recognized grossing protocols in the histopathology department. Initial diagnoses were made using light

microscopy on Hematoxylin and Eosin-stained slides, and only specimens confirmed as carcinomas were included in the study after rigorous review by consultant pathologists.

Tissue processing for Immunohistochemistry: Immunohistochemistry was conducted on formalin-fixed, paraffin-embedded (FFPE) tissue sections, which were deparaffinized, rehydrated, and treated for epitope retrieval and endogenous peroxidase inactivation. After primary antibody incubation using ready-to-use DAKO Mouse, secondary antibody incubation (DAKO, HRP [Code K4065]) followed for 30 minutes.

Results were interpreted by evaluating

immunohistochemical staining of tumor cells: CK7 and CK20 positivity in epithelial cells, and nuclear staining for CDX2 and SATB2. Marker expression was classified using a three-tier system: "Absent" for no detectable staining, "Focal" for 1–50% of tumor cells, and "Diffuse" for over 50%, considering specific subcellular locations during evaluation.⁷

Ethical approval: Ethical considerations were crucial in this study, with approval obtained from the Institutional Review Board (IRB) of DUHS, Karachi (REF #IRB-2905/DUHS/Approval/2023/110). Patient confidentiality was rigorously upheld, and no harm was inflicted on

Table I: Characteristics of ovarian cancers

Characteristics		SC	MC	EC	CCC	MGC	Total
Size	< 10cm	21	3	3	0	14	41
	> 10cm	30	6	15	1	7	59
Tumor Laterality	Unilateral	49	7	17	1	4	78
	Bilateral	2	2	1	0	17	22
Nodal Involvement	Involved	1	0	2	0	13	16
	Not Involved	50	9	16	1	8	84

SC: serous carcinomas; MC: mucinous carcinoma; EC: Endometrioid carcinomas; CCC: clear cell carcinoma; MGC: metastatic gastrointestinal carcinomas

Table II: Immunohistochemical results of ovarian cancers

Variables		SC	MC	EC	CCC	MGC	p-value
CK 7	Absent	3	0	2	0	17	<0.001
	Focal	30	2	13	1	4	
	Diffuse	18	7	3	0	0	
CK 20	Absent	36	6	13	0	4	0.005
	Focal	14	3	4	0	13	
	Diffuse	1	0	1	0	4	
CD X2	Absent	43	8	11	0	4	<0.001
	Focal	8	1	7	1	10	
	Diffuse	0	0	0	0	7	
SAT B2	Absent	47	7	15	0	2	<0.001
	Focal	4	2	3	1	9	
	Diffuse	0	0	0	0	10	

SC: serous carcinomas; MC: mucinous carcinoma; EC: Endometrioid carcinomas; CCC: clear cell carcinoma; MGC: metastatic gastrointestinal carcinomas

participants throughout the research.

Statistical analysis: Data were entered and analyzed using IBM SPSS version 26.0. Descriptive statistics and Chi-square tests assessed the association between histopathological patterns and clinical hysterectomy diagnoses, with statistical significance set at a p-value of <0.05.

RESULTS

Over a 9-month period, 100 samples were collected from patients aged 42 to 71 years (mean age 53.24 ± 8.03 years). Among these, 79 tumors (79%) were classified as primary ovarian cancers:

(serous carcinoma accounted for 51%, mucinous carcinoma represented 9%, endometrioid carcinoma (EC) comprised 18%, and clear cell carcinoma constituted for only 1%). The remaining 21 tumors were identified as metastatic gastrointestinal carcinomas.

Analysis of tumor size showed that most primary ovarian tumors, particularly serous carcinomas (SC), were larger than 10 cm, while mucinous carcinomas (MC) and endometrioid carcinomas (EC) varied in size. Unilateral presentation was common across all histological types, especially in SC, whereas bilateral cases were mainly associated with metastatic gastrointestinal tumors. Nodal involvement was limited, primarily occurring in metastatic gastrointestinal carcinomas (MGC) [Table I].

Immunohistochemical

Analysis: SC exhibited a distinct immunohistochemical profile, with predominantly focal and diffuse staining for CK7, absent CK20, and consistently absent CDX2 and SATB2. Mucinous carcinoma (MC) showed primarily focal CK7 staining, CK20 was mostly absent, while CDX2 and SATB2 presented with focal or diffuse staining. EC displayed varied staining patterns, and clear cell carcinoma (CCC) generally showed absent staining for SATB2 and CK20, though focal positivity for CK7 and CDX2 was noted (Figure 2). In metastatic gastrointestinal carcinoma (MGC), CK7, CK20, CDX2, and SATB2 predominantly exhibited diffuse staining (Figure 3). Statistically significant

associations ($p < 0.001$) were found between the expression of these markers in POC and MGC (Table II).

DISCUSSION

The present study highlights the key finding that metastatic ovarian tumors

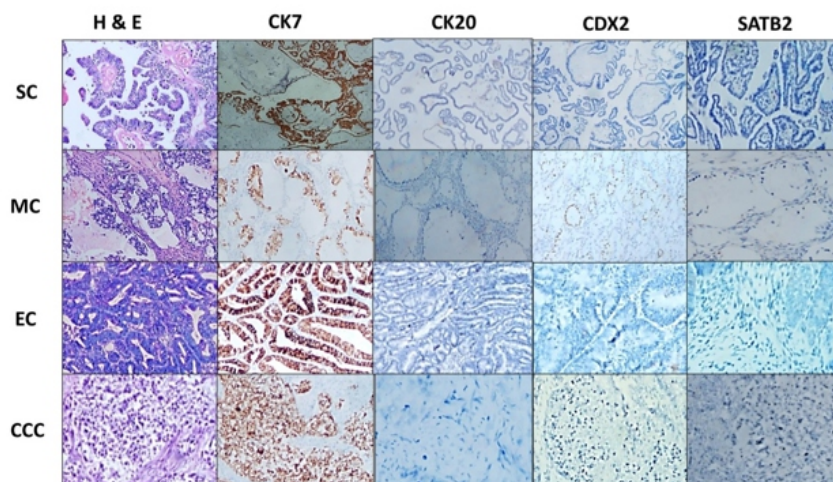


Figure 1: Various Types of Primary Ovarian Cancers (Haematoxylin and Eosin and Immunohistochemical Stains, 40x Magnification; Scale Bar: 500 μ m)

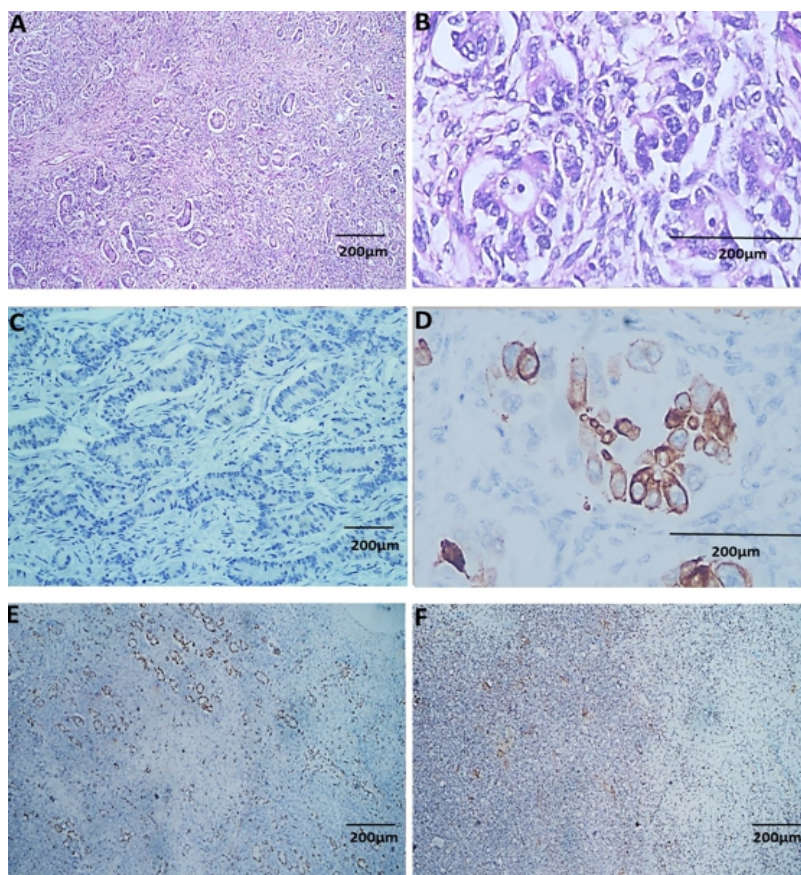


Figure 2: Metastatic Gastrointestinal Carcinoma. **A & B:** H & E, **C:** Diffuse Positive expression of CK7, **D:** Diffuse Positive expression of CK20, **E:** Diffuse Positive expression of CD X2, **F:** Diffuse Positive expression of SAT B2 (Magnification A, C, E & F: 40x; B & D: 400x) [Scale bar: 200 μ m]

exhibit distinct immunohistochemical profiles compared to primary ovarian mucinous carcinomas. Specifically, metastatic carcinomas were positive for CK7, CK20, CDX2, and SATB2, while primary ovarian mucinous carcinomas showed positivity for CK7 and CK20, but negativity for CDX2 and SATB2. Notably, CDX2 was expressed in both primary ovarian mucinous carcinomas and metastatic gastrointestinal carcinomas, whereas SATB2 exhibited diffuse positivity exclusively in metastatic gastrointestinal tumors. This differentiation is critical, as metastatic tumors often share histological and immunohistochemical features with well-differentiated primary ovarian tumors, complicating the diagnostic process despite advanced tools such as macroscopic, microscopic, and histochemical evaluations. Therefore, an accurate diagnosis requires a comprehensive medical approach that takes into account these overlapping characteristics to distinguish between primary and metastatic ovarian tumors.^{8,10}

Similar findings were observed in four distinct studies, which also investigated the immunohistochemical markers CK7, CK20, CDX2, and SATB2.^{11,15} These studies revealed a pattern consistent with the outcomes of our research. CK7 was predominantly expressed in POC, closely aligning with the trend identified in our study. In contrast, CK20, CDX2, and SATB2 were more commonly expressed in metastatic ovarian carcinomas (MOC). Moreover, these patterns were accompanied by statistically significant associations (p -value < 0.005), further corroborating our findings.^{11,12}

Our study investigated the immunohistochemical profiles of POC and MOC using the markers CK7, CK20, CDX2, and SATB2. We observed that a significant proportion of POC measured larger than 10 cm, whereas the majority of MOC were smaller, typically less than 10 cm in size. These findings are consistent with Strickland S, et al.,¹¹ who reported similar results, and Mostafa et al.,¹² whose study showed that a significant number of both primary and metastatic tumors exceeded 10 cm. Furthermore, in agreement with previous literature,^{11,12} our study found that most POC cases were unilateral, while MOC cases were predominantly bilateral. However, Montiel DP, et al.,¹³

reported a contrasting outcome, indicating that both primary and metastatic tumors frequently exhibited bilateral involvement.

In the study by Meagher NS, et al.,⁷ a strong positive expression of CK7 was observed in 97% of primary ovarian carcinomas (POC), while its presence was notably lacking in metastatic ovarian carcinomas (MOC). Their findings align with our results, as CDX2 and SATB2 were predominantly expressed in MOC and were largely absent in POC. However, a discrepancy emerged regarding CK20; Meagher NS, et al.⁷ reported its presence in both POC and MOC, whereas our study found a significant absence of CK20 in most POC cases compared to MOC.

Differentiating between POC and MOC is essential for patient prognosis and treatment planning. Early-stage POC has a high 5-year survival rate, while MOC often presents at a more advanced stage and is associated with a poorer prognosis.^{16,17} Our study highlights the critical role of immunohistochemical markers in accurately determining tumor origin, thereby facilitating appropriate therapeutic strategies and improving patient outcomes.

A primary limitation of our study was the relatively small sample size, which may affect the generalizability of our findings. Additionally, the study was conducted in a single center, potentially limiting the representation of the broader spectrum of ovarian tumor characteristics. This confinement could introduce selection bias, thereby affecting the external validity of the results. Another notable limitation was the exclusion of molecular or genetic analyses. Relying solely on immunohistochemical markers, without incorporating molecular profiling, may have restricted the depth and precision of our diagnostic approach.

To overcome these limitations, future research should focus on involving a larger and more diverse sample population to validate our findings. Multi-center studies across different geographic regions would allow for a broader representation of ovarian tumor characteristics and increase the generalizability of the results. Additionally, integrating molecular and genetic analyses alongside immunohistochemical markers could offer a more comprehensive

understanding of tumor origins, potentially enhancing diagnostic accuracy and improving patient care strategies.

CONCLUSION

The inclusion of SATB2 in immunohistochemical panels, alongside CK7, CK20, and CDX2, improves the accuracy of diagnosing primary and metastatic ovarian carcinomas. This approach provides support in the distinction between ovarian and gastrointestinal tumors.

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AUTHORS' CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

ST: Study design, acquisition of data, drafting the manuscript, approval of the final version to be published

LA: Acquisition of data, drafting the manuscript, approval of the final version to be published

SHS: Analysis and interpretation of data, critical review, approval of the final version to be published

FD, UB & FMA: Concept and study design, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Author declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Author declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request



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