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Original Contribution

High-grade ovarian serous carcinomas: Significant correlation of histologic patterns with IMP3 and E-Cadherin predicting disease recurrence and survival

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ABSTRACT

Most high-grade serous carcinomas (HGSC) of the ovary are advanced stage tumors with early recurrences. However, some tumors do not recur and have a better survival. We identified such cases of HGSC and compared those with the cases that recurred and assessed the relationship between patterns of invasion (intracystic, IC; micropapillary, MP; nonpapillary, NP) with IMP3 and E-Cadherin expression, and evaluated their predictive role in recurrence and survival. The study comprised of seventeen tumors recurred within 18 months of follow-up and 14 cases that did not recur with a minimum follow-up of 49 months. 73% tumors with predominantly MP pattern recurred, while only 27% of non-recurrent tumors showed this pattern. In contrast, predominant NP and IC patterns were seen in 71% of the non-recurrent and in 35% of recurrent tumors. 67.7% tumors expressed IMP3 and all cases expressed E-Cadherin. The tumors with a higher percentage of destructive invasion showed higher IMP3 positivity and greater chances of recurrence, whereas tumors with higher percentage of pushing invasion showed lower IMP3 positivity and lesser chances of recurrence ($p = 0.02$). IMP3-negative tumors had lower odds of recurrence than IMP3-positive ones ($p = 0.01$). The patients with negative IMP3 staining had a significantly higher OS than those with IMP3 positive tumors ($p = 0.01$), regardless of the histologic patterns. Also, reduction in E-Cadherin staining in the metastatic site led to poor DFS ($p = 0.016$) and OS ($p = 0.006$). IMP3 may serve as a useful prognostic marker that can stratify patients of advanced stage, high-grade serous carcinomas into two distinct subsets: majority with early recurrence with an infiltrative pattern of invasion and IMP3 positivity particularly in the MP areas; and a smaller subset that do not show early recurrence having pushing borders and are IMP3 negative. Also, E-Cadherin showed significant decrease in expression in the metastatic site of the recurrent cases.

1. Introduction

Ovarian carcinomas account for the majority of cancer-related deaths in women among the cancers of the female reproductive tract [1]. Serous carcinoma is the most common type of ovarian cancer and usually presents at an advanced stage with overall 5-year and survival rates ranging from 19% to 63% (stage IIIA: 63%; stage IIIB: 53%; stage IIIC: 41%; stage IV: 19%) [2]. Patients with high-grade serous carcinoma (HGSC) often show fatal outcomes with a median interval of 1.7 years after diagnosis, whereas patients with low-grade tumors died at a median interval of 4.2 years after diagnosis [3]. Furthermore, many cases of HGSC recur within a short period of time after surgical resection and chemotherapy treatment; however, there are rare cases that do not show recurrence.

Various patterns of invasion have been studied in solid organ malignancies, including cervical carcinoma [4], squamous cell carcinomas

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[5], urothelial carcinoma of the bladder and the upper urinary tract [6,7], uterine endometrioid adenocarcinoma [8], and ovarian mucinous, high-grade serous (mainly in the BRCA-mutated cancers) [9-13], and endometrioid adenocarcinomas [14], serous borderline tumors [15], and low-grade serous tumors [16]; however, thus far, the invasive patterns in ovarian HGSC and their relationship with clinical outcome have rarely been described in the literature [11]. We recently identified cases of HGSC that did not have an early recurrence and compared those with cases that recurred early. We determined whether histologic patterns of invasion aid in distinguishing the two groups and their prognostic implications. IMP3 and E-Cadherin immunoreactivity has been described in a varieties of human malignancies, however, the correlation between IMP3 and E-Cadherin expression with various histomorphologic invasion phenotypes have not been described. Therefore, we sought to assess the differential expression pattern of epidermal growth factor receptor (EGFR)-signaling pathway molecule, IMP3 (insulin-like growth factor 2 mRNA-binding protein-3) and cell-adhesion molecule E-Cadherin among these two groups, and to assess their clinical (recurrence and survival) and histopathologic (invasion patterns) significance.

2. Materials and methods

2.1. Patient demographics and case selection criteria

An Institutional Review Board approval was obtained at Cedars-Sinai Medical Center, Los Angeles, CA. Using a computerized database from the Department of Gynecologic Oncology, cases of stage III, pure HGSC of the ovary were searched for over an 18 year period (from January 1990 to December 2008). Exclusion criteria consisted of tumors where the main tumor mass was present either in the fallopian tube or peritoneum and primary ovarian tumors with predominant surface involvement. Thus, a total of 216 cases of HGSC were identified. Clinical and follow-up information including date of initial staging surgery, chemotherapy regimen, amount of residual disease, recurrence status, date of recurrence, date of last follow-up, date of death disease-free (DFS), and overall survival (OS) were recorded. The cases satisfying the following inclusion criteria were included in this study: (1) at least six cycles of treatment with carboplatin and taxol/cytoxan, (2) optimal clearance of disease (no gross residual disease after adequate surgical resection), (3) FIGO stage III tumors, (4) a minimum follow-up of 49 months for cases where no recurrence was observed, (5) a minimum follow-up recurrence time of 18 months for cases which recurred, and (6) where hematoxylin and eosin-stained (H&E) slides were available. The case number was low due to low incidence of cases of pure serous histology with recurrence. Using our exclusion and inclusion criteria, 31 cases (14 cases that did not recur and 17 cases that recurred) were included in the study. The Anatomic Pathology database was searched for each selected case and the slides were reviewed.

Recurrence of disease was detected clinically at follow-up appointments and confirmed by imaging and/or pathology. DFS was calculated as the time in months between the date of surgery and date of first recurrence. If the patient did not show recurrence of tumor, the date of death or date of last follow-up was used as the endpoint. OS was similarly calculated to be the time in months between the date of surgery and date of death (or date of last follow-up for living patients).

2.2. Histopathologic assessment

Three H&E-stained slides each with the maximum amount of invasive tumor from the ovary and peritoneum (metastatic site) was selected for each case. Using a blinded method, two pathologists-in-training reviewed the slides independently. The cases were further evaluated by a senior pathologist with a long experience and expertise in Gynecologic Pathology. They confirmed the histologic diagnosis and evaluated two distinct patterns of invasion in the ovary and at the metastatic site. The categories of invasion were well-demarcated/pushing and destructive types. Well-demarcated/pushing pattern of invasion was defined as large group(s) of neoplastic cells arranged in the form of clusters and/or solid sheets, abutting the adjacent stroma with a sharply demarcated border between the neoplastic cells and the stroma. The destructive pattern of invasion was defined as irregular, angulated clusters, cords, or single dispersed tumor cells in a desmoplastic stroma or variably sized groups and clusters of tumor cells within non-epithelial/endothelial-lined spaces (NELS) surrounded by desmoplastic stroma. In addition, we evaluated the various architectural attributes within NELS, initially described by Silva et al. [17] as follows: (1) Nesting or Nidi pattern (nests of tumor composed of approximately 2 to 50 cells each, with or without cribriform pattern), (2) micropapillae (papillae lacking a fibrovascular core and with a length that is at least 5 times that of the width), (3) macropapillae with true fibrovascular cores (with or without a hierarchical order from primary to secondary to tertiary papillae), (4) papillae with branching architecture and lacking fibrovascular cores, (5) single cells, (6) single/scant cells with associated dystrophic calcifications, and (7) solid areas (incorporating > 50 tumor cells). The percentage of tumor showing destructive and pushing invasion were estimated in each slide in a semi-quantitative fashion. The individual mean as a percentage for primary and metastatic sites were then calculated for each case. Finally, we have summarized and further categorized the invasion phenotype into three major categories, which included invasive infiltrating micropapillary (MP), intracystic (IC), and nonpapillary (NP; solid, microcystic, nesting, glandular, transitional cell carcinoma-like morphology, and well-demarcated pushing patterns) subtypes.

2.3. Immunohistochemistry

Immunostains were performed on four-micrometer thick formalin-fixed and paraffin embedded sections. Immunostaining was performed in automated immunostainers. The antibody clones, commercial vendors, dilutions, antigen retrieval methods, incubation time for the primary antibodies, localization techniques, and appropriate positive controls are summarized in Table 1.

2.4. Interpretation of stains

The status of IMP3, EGFR, and E-Cadherin were assessed by researchers (SM, CS, and FD) without knowledge of the clinical and pathologic features of the cases and the clinical outcome. Positive staining of IMP3 was defined as dark brown cytoplasm staining pattern in the tumor epithelial cells, which can be easily observed at low power magnification ($<40\times$). Scant fine granular background staining of epithelial cells, which cannot be seen at low power magnification ($\leq 40\times$), or no staining at all was considered negative. Positive staining for E-Cadherin and EGFR was defined as membranous staining pattern in the tumor epithelial cells, which can be easily observed at low power magnification ($<40\times$). For each case, immunoreactivity for IMP3, EGFR, and E-Cadherin were interpreted as follows: negative $\leq$ 5% tumor cell staining; positive = $\geq$5% tumor cell staining. The percentage of immunoreactivity for these markers were evaluated in a semi-quantitative fashion as follows: 0 = up to 5% tumor cell staining; 1+ = 5%–10% tumor cell staining; 2+ = 11%–50% tumor cell staining; 3+ = 51%–70% tumor cells staining; 4+ $\geq$ 70% tumor cell staining. Further, the intensity of immunoreactivity for IMP3 and EGFR was graded as weak, moderate and strong. For E-Cadherin intensity of staining was assessed as follows: 1+ = weak and incomplete/complete membranous or moderate and incomplete membranous staining; 2+ = moderate and complete membranous or strong and incomplete membranous; 3+ = strong and complete membranous staining. The staining of individual immunostains was assessed in the micropapillary, intracystic, and nonpapillary areas and the predominant staining area in a particular tumor is evaluated.

Table 1
Summarizes the details of antibodies used and the staining protocols.

IHC markers	Antibody clone	Vendor	Dilution	Antigen retrieval method	Incubation time and temperature for the antibody	Instrument	Detection kit	Positive controls
IMP3	69.1	Dako, Carpinteria, CA	1:50	HIER-Hi pH (8 to 8.4)	32 min 37 °C	Ventana Benchmark Ultra	Optiview DAB Det. Kit	Placenta (syncitiotrophoblast, cytotrophoblast, and mesenchymal cells)
EGFR	3C6	Ventana, Tucson, AZ	Pre-diluted	Protease 1	20 min 37 °C	Ventana Benchmark Ultra	UltraView DAB Det. Kit	Normal skin
E-cadherin	36B5	Leica, Buffalo Grove, IL	Pre-diluted	HIER-Hi pH (8 to 8.4)	15 min 37 °C	Leica Bond 3	Bond Polymer Refine Det. Kit	Normal skin and normal breast tissue

Abbreviations used: HIER: Heat-induced Epitope Retrieval; DAB: 3,3′-diaminobenzidine.

2.5. Statistical analysis

Logistical regression analysis (SAS Statistical Software) was utilized to compare recurrence status and mean percentages of well-demarcated/pushing and destructive patterns of invasion in the ovary and peritoneum for each case. A linear regression analysis (SAS Statistical Software) was used to compare disease-free survival and overall survival with mean percentage of pushing and destructive patterns of invasion in the ovary and peritoneum for each case. p value of < 0.05 was defined to be statistically significant. Univariate cross tabulation was performed among type of invasion, recurrence status, IHC staining for IMP3, E-cadherin, and EGFR; Chi-square analysis was done. Survival analysis (Gehan-Breslow-Wilcoxon) was done to decipher the significance of recurrence status, invasion type, and IHC marker status using SPSS 22.0 software.

3. Results

3.1. Clinical characteristics

The age ranged from 43 to 85 years (median = 57.5 years; mean = 57.6 years). All 31 patients had stage III disease (left ovarian primary, n = 14; right ovarian primary, n = 17). The primary tumors ranged from 1.9 to 14.0 cm in maximum dimension. Three of the 31 cases were positive for BRCA1 germline mutation. All the cases within the recurrence group showed disease recurrence within 18 months of initial resection (range of DFS = 4.8 to 17.7 months, median = 5.9 months), whereas none of the cases from the non-recurrence group recurred after a follow-up of 49 months (range of DFS/OS = 49 to 249.2 months, median = 123.2 months). The OS for the recurrence group ranged from 11.5 to 100.7 months (median = 30.9 months).

3.2. Histopathologic features

Each tumor showed significant cytologic atypia, high mitotic index (> 12/10 high power fields) and variable degrees of necrosis, confirming the diagnosis of HGSC. Well-demarcated/pushing pattern of invasion was seen as large groups of neoplastic cells, with sharp, pushing borders in a broad-front in relationship to the non-neoplastic ovarian stroma (Fig. 1A) or the stroma in the metastatic sites (Fig. 1B). Various morphologic attributes as previously described, including irregular angulated clusters, cords, and single dispersed cells in the stroma, were observed and documented as the destructive pattern of invasion in the ovary (Fig. 1C) and metastasis (Fig. 1D). In addition, areas of destructive pattern of invasion in the form of non-epithelial/endothelial-lined spaces (Fig. 1E and F) were present in the majority of cases in variable proportion. Within the non-epithelial/endothelial-lined spaces, several morphologic patterns were observed, which were listed below according to the frequency.

Nesting or Nidi pattern was present in 94% tumors in the recurrence group and 87% tumors in the non-recurrence group; m*icropapillary* pattern was present in 82% tumors in the recurrence group and 53% tumors the non-recurrence group; *single cell pattern* was present in 29% tumors in the recurrence group and 40% tumors in the non-recurrence group; b*ranching papillae with no fibrovascular core* pattern was present in 35% tumors in the recurrence group and 13% tumors in the non-recurrence group; *scant cells with dystrophic calcifications* pattern was present in 24% tumors in the recurrence group and 13% tumors in the non-recurrence group; m*acropapillae with true fibrovascular core* pattern was present in 18% tumors in the recurrence group and 7% tumors in the non-recurrence group. No areas of solid tumor were seen in the non-epithelial/endothelial-lined spaces in any of the cases.

Finally, we summarized and further categorized the invasion phenotype into three major categories, which included invasive infiltrating micropapillary, intracystic, and nonpapillary (solid, microcystic,

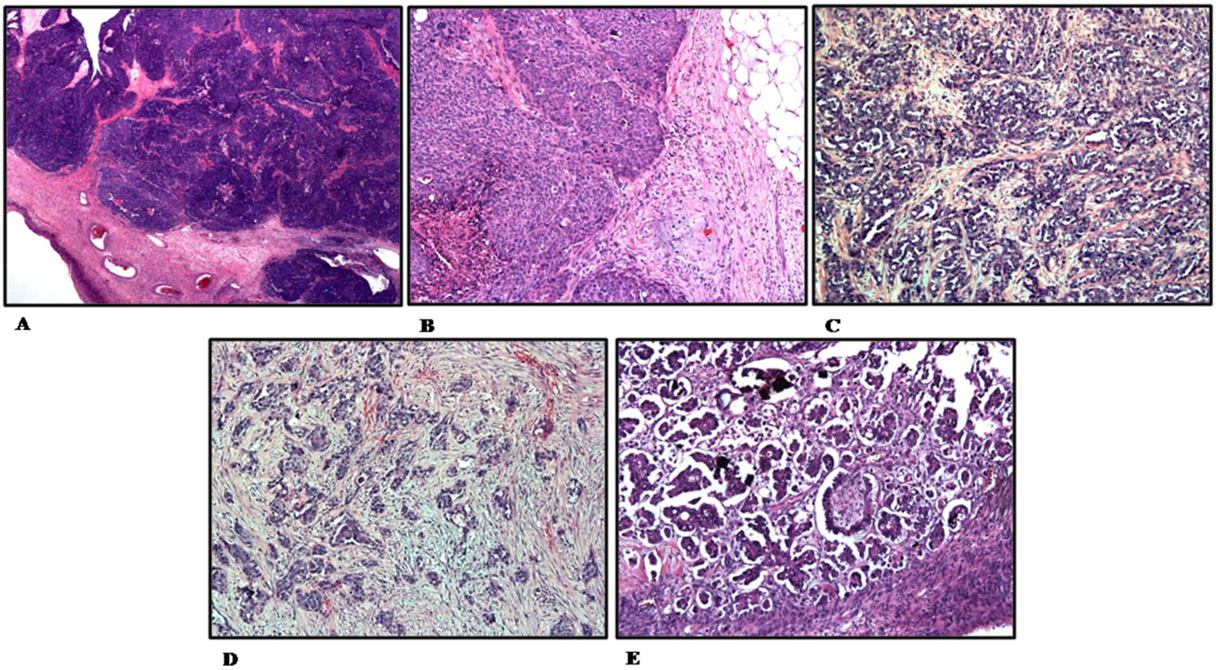

Fig. 1. (A) Representative image of pushing borders in a case of high-grade serous carcinoma of the ovary (Hematoxylin and Eosin magnification ×4); (B) representative image of pushing borders in the metastatic site (peritoneum) in case of a high-grade serous carcinoma of the ovary (Hematoxylin and Eosin magnification ×10); (C) image representing destructive invasion pattern in the ovary in high-grade serous carcinoma (Hematoxylin and Eosin magnification ×10); (D) image illustrates destructive invasion pattern in the peritoneum in high-grade serous carcinoma (Hematoxylin and Eosin magnification ×10); (E) this figure highlights non-epithelial/endothelial-lined spaces with true and pseudopapillae in the areas of destructive invasion in the ovary of high-grade serous carcinoma (Hematoxylin and Eosin, original magnification ×10).

Table 2
Distribution of patterns in the ovary and peritoneum of the cases of high-grade ovarian serous carcinomas.

Stage	MP (O)	IC (O)	NP (O)	MP (P)	IC (P)	NP (P)
III R (n = 17)	8 (47%)	5 (29%)	4 (24%)	11* (65%)	2** (11%)	4** (24%)
III NR (n = 14)	2 (14%)	9 (64%)	3 (21%)	4* (28%)	6** (44%)	4** (28%)

Abbreviations used: MP: Micropapillary; IC: Intracystic; NP: Nonpapillary; R: recurrence group; NR: non-recurrence group; O: ovary; P: peritoneum.
* p = 0.02.
** p = 0.002.

nesting, glandular, transitional cell carcinoma-like morphology, and well-demarcated pushing patterns) subtypes. The distribution of the aforementioned patterns in the primary and peritoneal tumors was illustrated in Table 2.

3.3. Results of immunohistochemistry

3.3.1. IMP3 expression

88% recurrent and 43% non-recurrent ovarian tumors expressed IMP3 (p = 0.007). The staining was more pronounced in the micropapillary regions in 11 recurrent and 2 non-recurrent tumor. 71% recurrent and 64% of non-recurrent tumors exhibited IMP3 staining in the peritoneal metastasis (p = 0.70) (Table 3). Predominant IMP3 staining was confined to the micropapillary areas in the peritoneal metastasis of recurrent tumors, in all cases (Fig. 2A to D).

3.3.2. E-Cadherin expression

All ovarian and peritoneal tumors showed E-Cadherin positivity. Among both the groups either a 3+ or 4+ staining intensity and extent of positivity was observed, except for one case in non-recurrent

Table 3
Illustrates IMP3 expression in our cohort of high-grade ovarian serous carcinomas.

IMP3 staining	Stage IIIR (O)	Stage IIIR (P)	Stage IIINR (O)	Stage IIINR (P)
IMP3 (+)	15* (88%)	12 (71%)	6* (43%)	9 (64%)
IMP3 (−)	2** (12%)	5 (29%)	8** (57%)	5 (36%)

Abbreviations used: R: recurrence group; NR: non-recurrence group; O: ovary; P: peritoneum.
* p = 0.01.
** p = 0.001.

category, which depicted 2+ staining intensity. Extent of positive E-Cadherin staining among the peritoneal tumors did not show significant result (Table 4; p = 0.31). Interestingly, 76% peritoneal tumors in the recurrence category showed 1+ staining pattern, while only 21% tumors in the non-recurrence group exhibited similar pattern of staining. Similarly, 79% tumors in the non-recurrence group showed 2+ or 3+ staining pattern, whereas only 24% cases revealed similar staining pattern in the tumors which recurred (p = 0.018) (Table 4 and Fig. 2E to H).

3.3.3. EGFR expression

EGFR expression was illustrated in Table 5.

3.4. Statistical comparison

Patterns of invasion dependent survival analysis were performed for ovary and peritoneum. The percentage of tumor showing well-demarcated/pushing and destructive patterns of invasion in the ovary was recorded. The individual means as a percentage were then calculated for each case and are summarized in the supplements 1 to 2. In the ovary, more destructive than pushing type of invasion was seen in 1 of 15 (6.67%) cases in the non- recurrence group as compared to 4 of 17

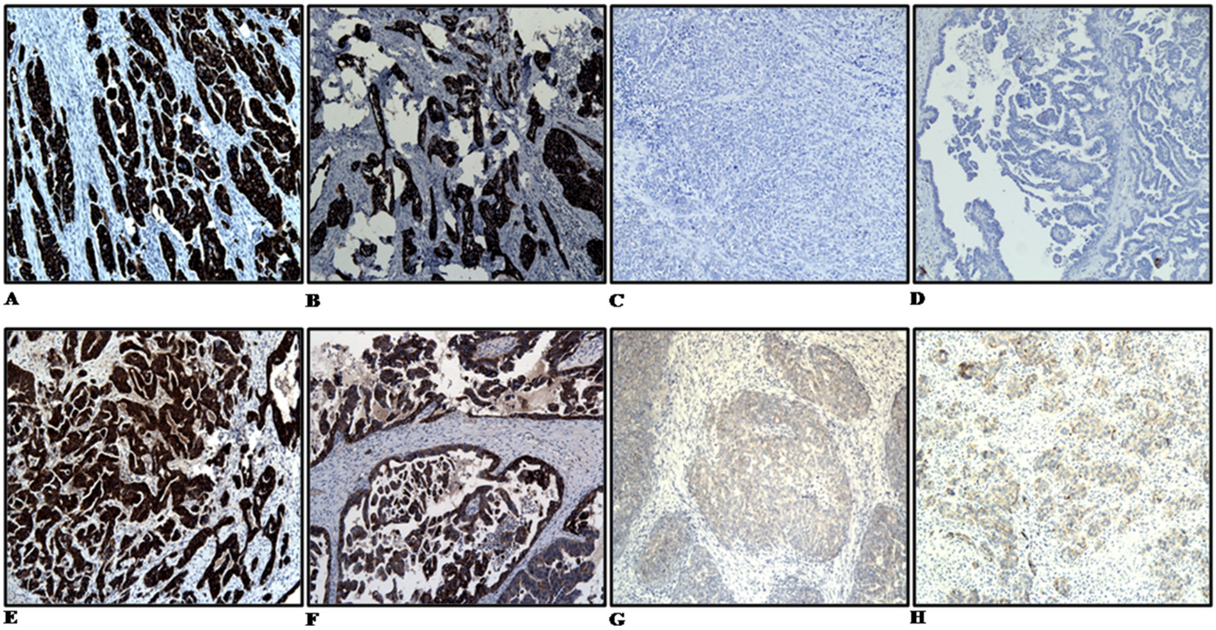

Fig. 2. (A) Representative image of strong IMP3 staining in the micropapillary areas in an ovarian high-grade serous carcinoma (magnification ×10); (B) image illustrates IMP3 staining within the micropapillary areas in the peritoneum of a high-grade ovarian serous carcinoma (magnification ×10); (C) negative IMP3 staining in the nonpapillary area in high-grade serous carcinoma of the ovary (magnification ×10); (D) image depicts absence of IMP3 immunoreactivity in the intracystic area in high-grade serous carcinoma of the ovary (magnification ×10); (E) representative image showing 4 + E-Cadherin staining intensity in the ovary of a high-grade serous carcinoma that recurred (magnification ×10); (F) representative image showing 4 + E-Cadherin staining intensity in tumor tissue of a high-grade serous carcinoma of ovary that did not recur (magnification ×10); (G) image depicts 1 + E-Cadherin staining intensity in the tumor at the metastatic site (peritoneum) of a high-grade serous carcinoma of the ovary that recurred (magnification ×10); (H) image depicts 1 + E-Cadherin staining intensity in peritoneal metastasis of a high-grade serous carcinoma that did not recur (magnification ×10).

Table 4
Illustrates E-cadherin expression in our cohort of high-grade ovarian serous carcinomas.

Anatomic location	Stage IIIR (n = 17)	Stage III NR (n = 14)
Ovary (% positivity)	3+ (n = 1); 4+ (n = 16)	3+ (n = 2); 4+ (n = 12)
Ovary (intensity and type)	3+ (n = 17)	2+ (n = 1); 3+ (n = 13)
Peritoneum (% positivity)	1+ (n = 10); 2+ (n = 3); 3+ (n = 1); 4+ (n = 3)	1+ (n = 3); 2+ (n = 4); 3+ (n = 3); 4+ (n = 4)
Peritoneum (intensity and type)	1+ (n = 13); 2+ (n = 3); 3+ (n = 1)	1+ (n = 3); 2+ (n = 8); 3+ (n = 3)

Abbreviations used: R: recurrence group; NR: non-recurrence group.

Table 5
Illustrates EGFR expression in our cohort of high-grade ovarian serous carcinomas.

EGFR staining	Stage IIIR (O)	Stage IIIR (P)	Stage IIINR (O)	Stage IIINR (P)
EGFR (+)	10 (59%)	7 (41%)	6 (43%)	4 (29%)
EGFR (−)	7 (41%)	10 (59%)	8 (57%)	10 (71%)

Abbreviations used: R: recurrence group; NR: non-recurrence group; O: ovary; P: peritoneum.

(23.53%) cases in the recurrence group (p = 0.3382) (Supplement 3).

Of the three cases showing BRCA1 germline mutation, two showed recurrence. One of the recurrent cases and the BRCA1 mutated non-recurrent tumor showed more pushing type than destructive type invasion; the other BRCA1 mutated recurrent tumor showed greater destructive pattern than pushing pattern of invasion.

3.4.1. Patterns of invasion and recurrence status

A logistic regression analysis showed that cases with greater pushing type of invasion in the ovary had lower recurrence rates (Odds Ratio, OR = 0.956; 95% CI 0.920–0.956; p = 0.0202) as compared to cases with greater destructive pattern of invasion in the ovary (OR = 1.046; 95% CI 1.07–1.087; p = 0.0202). Micropapillary pattern in the peritoneal tumor was observed to be associated with recurrence (p = 0.045), but not in the ovary (p = 0.052). Similarly, intracystic pattern was predominantly observed in non-recurrent cases, in the perironeal metastasis (p = 0.049), however, not in the ovary (p = 0.052). Nonpapillary pattern has no significant association with the recurrence status of the primary (p = 0.889) and metastatic tumors (p = 0.750).

3.4.2. Patterns of invasion and disease-free survival

The pushing pattern of invasion in the ovary was compared with DFS, which indicated that the more extensive the pushing pattern of invasion, the higher the DFS (parameter estimate =1.095, p = 0.0284, linear regression). In contrast, the destructive pattern of invasion including non-epithelial/endothelial-lined spaces in the ovary, when compared to DFS, showed an inverse relationship (parameter estimate =1.095, p = 0.0284, linear regression).

3.4.3. Patterns of invasion and overall survival

The ovarian cases with higher percentage of pushing type of invasion showed a significantly longer OS (parameter estimate =1.068, p = 0.0116, linear regression); however, cases with higher percentage of destructive invasion including non-epithelial/endothelial-lined spaces in the ovary showed a significantly decreased OS (parameter estimate =1.068, p = 0.0116, linear regression).

3.4.4. Patterns of invasion and results of the immunostains

3.4.4.1. Ovarian destructive and pushing invasion % vs. IMP3 status. Higher the % of destructive invasion in the ovary, higher the chances of IMP3 positivity. This difference was, however, not statistically significant (OR: 1.006; 95% CI: 0.977–1.035; p = 0.7076).

Higher the % of pushing invasion in the ovary, lower the chances of IMP3 positivity. This difference was, however, not statistically significant (OR: 0.995; 95% CI: 0.966–1.024; p = 0.7076).

3.4.4.2. Relationship among immunostains and micropapillary, intracystic, and nonpapillary patterns. Greater than 50% IMP3 staining in ovary was positively associated with micropapillary pattern (p = 0.007) and negatively associated with intracystic pattern independent of the recurrence status (p = 0.008). IMP3 expression had no significant association with other patterns in the ovary as well as in the peritoneum. E-Cadherin and EGFR staining did not show any significant association among the various patterns of invasion.

3.4.5. Recurrence and immunostains

IMP3 expression in the ovary was significantly associated with tumor recurrence, with IMP3 negative staining in the ovary had lower odds of recurrence than those with IMP3 positive staining (OR: 0.1; 95% CI: 0.016–0.615; p = 0.0129, Logistic regression analysis). However IMP3 staining in the peritoneum was not significantly associated with recurrence status (p = 0.709). Association of E-Cadherin immunostain status was found to be non-significant in case of ovary (p = 0.313), while low E-Cadherin expression was observed in the peritoneal metastasis of cases which showed recurrence (p = 0.018). EGFR staining pattern in both ovary and peritoneum had no significant association with recurrence in patient.

3.4.6. Immunostain and survival

Kaplan-Meier plots showed that patients with negative IMP3 staining had a significantly higher OS than those with IMP3 positive tumors (p = 0.017), however patients with positive IMP3 staining did not significantly differ from those with IMP3 negative staining in terms of DFS (p = 0.425). The tumors with higher percentage of destructive invasion showed higher IMP3 positivity (OR: 1.006; 95% CI: 0.977–1.035; p = 0.7076), and similarly, the tumors with higher percentage of pushing invasion showed lower IMP3 positivity (OR: 0.995; 95% CI: 0.966–1.024; p = 0.7076) (Fig. 3A and B). However, these differences were not statistically significant. Lower E-Cadherin staining score (1+ in intensity and type) in the peritoneum had a significantly low DFS (median DFS = 6.9 months) as compared to the cases with high (2+ or 3+) E-Cadherin staining (median DFS = 108 months) (Hazard ratio, HR = 0.52, p = 0.016). Similarly, OS in reduced (1+) E-Cadherin expression in the peritoneum showed poor median survival period of 33.9 month, compared to strong (2+ or 3+) E-Cadherin expression, median survival of 107.8 months (HR = 0.49; p = 0.0057) (Fig. 3C and D). Further we performed a correlation curve analysis between IMP3 staining in the ovary and E-Cadherin staining in the peritoneum. We observed significant correlation between IMP3 in the ovary and E-Cadherin in the peritoneum (Pearson correlation co-efficient r = −0.362; p = 0.045) (Fig. 4). Patients with positive EGFR staining did not significantly differ from those with EGFR negative staining in terms of OS and DFS (p = 0.4273 and p = 0.0170).

4. Discussion

Patterns of invasion have been delineated in various tumor types. Spiro et al. [5] showed that an infiltrative pattern of small groups or single cells at the deepest point of invasion in oral cancers from the tongue was associated with lymph nodal and distant metastases, and a lower cumulative survival. Likewise, a spray-like (infiltrative) pattern in cervical carcinoma has been associated with higher stage, pelvic lymph node metastasis, and reduced recurrence-free survival [4]. Infiltrative tumor, as compared to expansile or mixed tumor patterns, was related to a lower cumulative survival in endometrioid adenocarcinoma of the endometrium [8]. Denzinger et al. [6], and Langner et al. [7] confirmed the value of infiltrative tumor pattern in predicting outcome in urothelial carcinomas of the bladder and upper urinary tract, respectively. Interestingly, in gastric and breast cancers, quantum dot technology has been used to highlight four different patterns of invasion of cancer cells in relationship to the extracellular matrix barrier [18], providing new areas of research in the tumor-stromal interaction. Several patterns of stromal invasion have also been described in serous borderline tumor of the ovary [15]. Micropapillary serous carcinoma of the ovary, which was described as a low-grade serous carcinoma [19], shows areas of stromal invasion consisting of micropapillae and nests of cells surrounded by spaces with adjacent fibroblastic stroma [20]. Various architectural patterns of tumor within these spaces (non-epithelial/endothelial-lined spaces) were further characterized by Silva et al. [17]. A similar histologic pattern has been described as micropapillary carcinoma of the bladder, lung, salivary duct, colon, and breast [21-25]. In these carcinomas, micropapillae or clusters of cells predominantly lacking fibrovascular cores lie within lacunar spaces which can often be confused with lymphovascular spaces. Amin et al. [21] demonstrated that the cleft-like spaces in micropapillary carcinoma of the bladder are indeed void of endothelial lining. Interestingly, the commonality between the above-mentioned micropapillary carcinomas is an aggressive course with lymphotrophic nature [26–28]. The similarity of micropapillary carcinomas and tumor within non-epithelial/endothelial-lined spaces in HGSC is striking and may be explained by stromal-epithelial interactions, which facilitate the creation of cleft-like spaces.

We determined whether histologic patterns of invasion aid in distinguishing the two groups and their prognostic implications. Our study showed that destructive pattern of invasion in HGSC of the ovary is associated with tumor recurrence, decreased disease-free survival, and decreased overall survival. Conversely, a well-demarcated/pushing pattern of invasion correlates to increased survival. Also, we found that a destructive pattern within metastatic sites, which has not been evaluated in the previously mentioned studies, is predictive of decreased survival and may be more important than the pattern in the ovary in determining recurrence. This characteristic destructive pattern of invasion may be linked to poor prognosis due to an aggressive biologic behavior which allows tumor cells to break down the surrounding stroma.

In our cohort of HGSC, tumor within non-epithelial/endothelial-lined spaces was included within the destructive (infiltrative) invasion category, and we found the predominant pattern of tumor within non-epithelial/endothelial-lined spaces to be a nesting or nidi pattern. Furthermore, our study showed that destructive (infiltrative) pattern of invasion in the form of non-epithelial/endothelial-lined spaces in HGSC of the ovary is associated with tumor recurrence, decreased disease-free survival, and decreased overall survival. In order to analyze the invasion patterns and develop a predictive model, we categorized the invasion phenotype into three major invasion phenotypes, which included invasive infiltrating micropapillary, intracystic, and nonpapillary (solid, microcystic, nesting, glandular, transitional cell carcinoma-like morphology, and well-demarcated pushing patterns) subtypes.

Based on our observations, we propose that infiltrative (destructive) invasion in HGSC, which includes tumor within non-epithelial/endothelial-lined spaces, is associated with aggressive tumor behavior and poor patient outcome. Furthermore, analysis of the pattern of invasion in metastatic sites, which may reflect resectability, is important in determining prognosis. It is unusual to find cases of high stage HGSC which do not recur; however, this study shows a clear difference in the

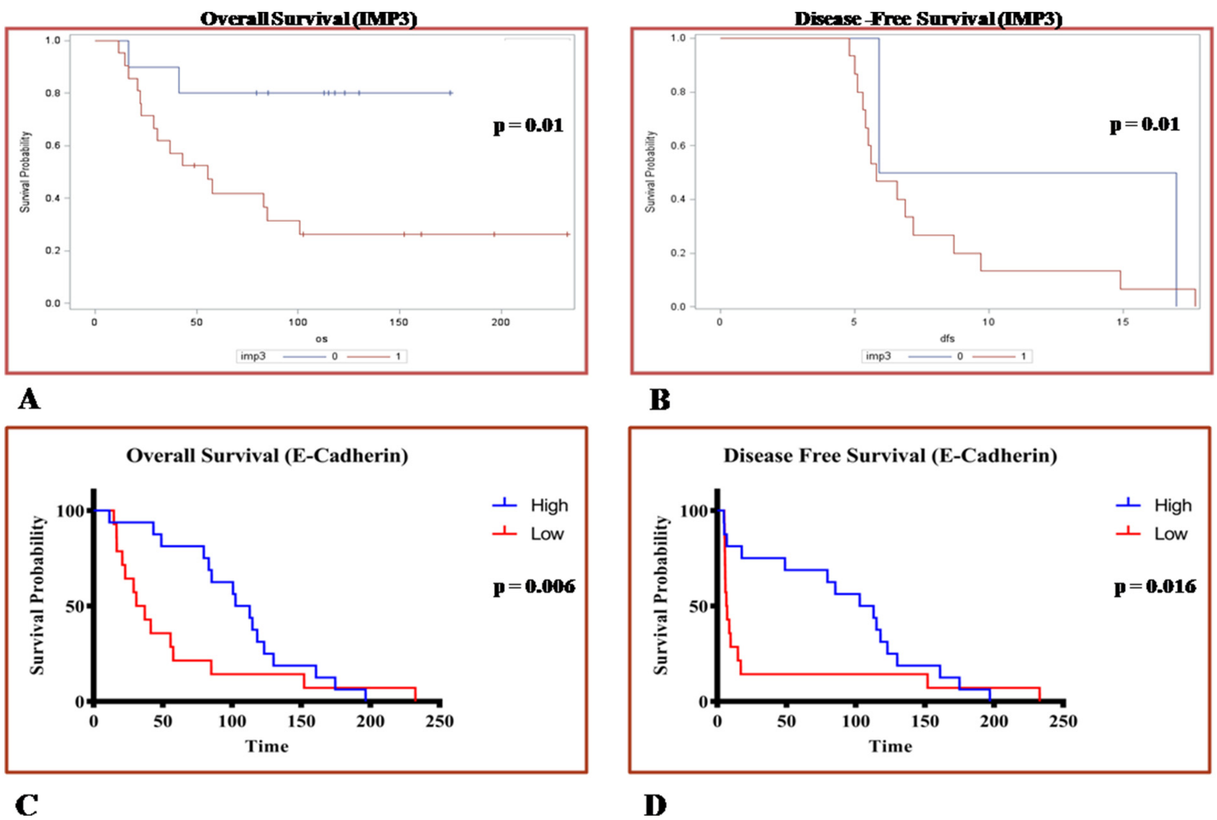

Fig. 3. (A) Kaplan-meier plot showing correlation between the overall survival of with IMP3 positivity in the ovary in patients of high-grade serous carcinoma; (B) Kaplan-meier plot showing correlation between disease-free survival with IMP3 expression in the ovary in cases of high-grade serous carcinoma; (C) Kaplan-meier plot showing correlation between overall survival in high-grade serous carcinoma with low vs. high E-Cadherin expression in the peritoneum (D) Kaplan-meier plot showing correlation between disease-free survival in high-grade serous carcinoma with low vs. high E-Cadherin expression in the peritoneum.

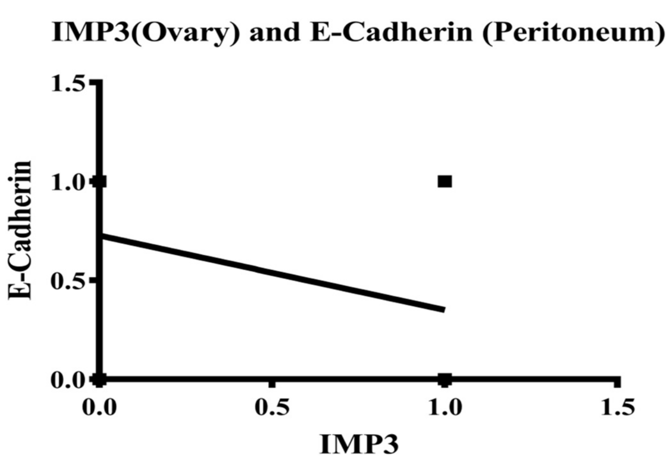

Fig. 4. A correlation curve analysis shows a significant correlation between IMP3 in the ovary and E-Cadherin in the peritoneum (Pearson correlation coefficient $r = -0.362$; $p = 0.045$) in high-grade serous carcinoma, regardless of the recurrence status of the primary tumor.

pattern of invasion between these cases and the usual recurrent cases. Furthermore, we attempted to correlate the morphologic invasion phenotypes with the differential expression pattern of EGFR-signaling pathway molecule, IMP3, and cell-adhesion molecule E-Cadherin among our tumor cohort in order to assess their histopathologic (invasion patterns/phenotypes) correlation and clinical (recurrence and survival) implications.

Insulin-like growth factor 2 (IGF-2) mRNA-binding protein (IMP) family members play an important role in RNA trafficking and stabilization, cell growth, and cell migration during the early stages of embryogenesis [29,30]. IMP family consists of IMP1, IMP2 and IMP3 [30]. IMP3 is identical to the KH domain containing protein that is over expressed in cancer (KOC). It was originally cloned from a pancreatic tumor cDNA screen [31]. IMP3 is expressed in developing epithelium, muscle, and placenta during early stages of human and mouse embryogenesis, but it is expressed at low or undetectable levels in adult tissues [31]. The expression of IMP3 is also found in malignant tumors including pancreas, lung, endocervical, endometrial, renal cell, stomach, urothelial, neuroendocrine (small cell), breast, and colon cancers, leukemias, and soft tissue sarcomas but it is not detected in adjacent benign tissues [32-42]. Moreover, recent studies have demonstrated that IMP3 is involved in cell growth, adhesion, invasion, and migration, suggesting this to be an oncofetal protein. Recently, IMP3 was found to be a prognostic biomarker for patients with renal cell carcinoma and meningioma [32,43]. IMP3 level has shown correlation with chemoresistance and poor prognosis in epithelial ovarian tumors [44]. Findings from Kobel et al. reflected that IMP3 positivity leads to unfavorable prognosis in ovarian clear cell carcinoma but serous and endometrioid ovarian carcinoma showed no significant prognostic association with IMP3 levels [45].

E-Cadherin, a transmembrane protein considered to be a tumor suppressor and its loss has been reported in various tumors, enhancing cell invasion and metastasis [46-48]. Loss of E-Cadherin has been often reported to have poor prognostic value among cancer patients. One of the unique aspects of E-Cadherin expression in case of ovarian cancer is that at early stages of ovarian cancer E-Cadherin level is increased which is reduced at the latter stages of tumor [49]. Various studies have presented a conflicting approach towards the prognostic significance of E-Cadherin in ovarian cancer, but it could be concluded that with increase FIGO stage E-Cadherin levels tend to reduce in a subtype independent manner, with lowest level of E-Cadherin levels were

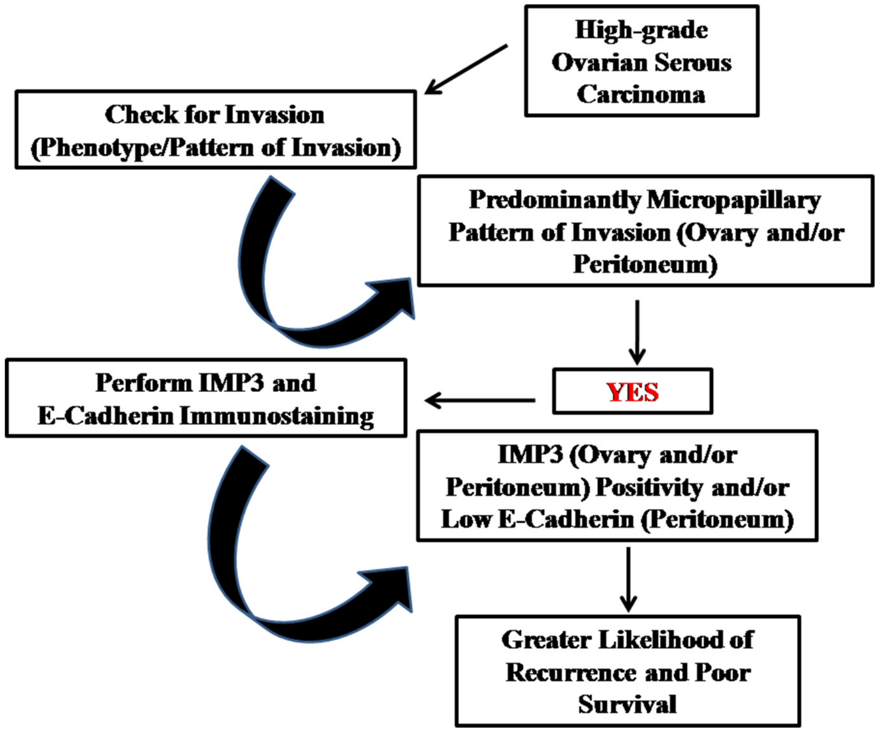

Fig. 5. Model representing an algorithmic approach that aid in determination of survival and recurrence in cases of high-grade serous carcinoma of the ovary.

observed in serous subtype compared to other type of ovarian cancer [50].

EGFR is trans-membrane protein which belongs to Erb family of tyrosine kinase receptors. EGFR over-expression has been reported in various cancers ranging from lung, oral cavity, anal lesions, to glioblastoma [51-53]. Enhanced EGFR expression and protein levels were observed in ovarian serous carcinoma. The enhanced expression corelated with high-grade tumor and poor patient outcome [54].

Although IMP3 and E-Cadherin immunoreactivity has been described in a varieties of human malignancies, as far as the correlation between IMP3 and E-Cadherin expression pattern with various invasion phenotypes have not been described. In our cohort of stage III carcinomas, the histologic pattern is important as 73% of the tumors with MP pattern recurred, while only 27% of non-recurrent tumors showed this pattern. In contrast, NP and IC patterns were seen in 71% of the non-recurrent and 35% of recurrent tumors. 67.7% and 29% of the tumors expressed IMP3 and EGFR, respectively, while all cases expressed E-Cadherin. The EGFR negative tumors showed slightly lower recurrence rate as compared to EGFR positive tumors (48% vs. 52% in the ovary; 65% vs. 35% in the peritoneum). The tumors with a higher percentage of destructive invasion showed higher IMP3 positivity and higher chances of recurrence, whereas tumors with higher percentage of pushing invasion showed lower IMP3 positivity and lower chances of recurrence ($p = 0.02$). IMP3-negative tumors had lower odds of recurrence than IMP3-positive ones, and this difference was statistically significant. The patients with negative IMP3 staining had a significantly higher OS than those with IMP3 positive tumors, regardless of the histologic patterns. Lower E-Cadherin staining score (1+ in intensity and type) in the peritoneum had a significantly low DFS (median DFS = 6.9 months) as compared to the cases with high (2+ or 3+) E-Cadherin staining (median DFS = 108 months). Similarly, OS in reduced (1+) E-Cadherin expression in peritoneum showed poor median survival period of 33.95 month, compared to strong (2+ or 3+) E-Cadherin expression, median survival of 107.8 months. Further we performed a correlation curve analysis between IMP3 staining in the ovary and E-Cadherin staining in the peritoneum. We observed a significant correlation between IMP3 in the ovary and E-Cadherin in the peritoneum (Fig. 4).

From the results of our study, we propose that infiltrative (destructive) micropapillary pattern of invasion in HGSC, with IMP3 positivity in the primary and/or peritoneal tumor and lower expression of E-Cadherin in the peritoneum, is associated with an aggressive tumor behavior, higher likelihood of recurrence, and poor survival (Fig. 5). Also, analysis of the pattern of invasion in metastatic sites, which may reflect resectability, is important in determining prognosis. It is unusual to find cases of high stage HGSC which do not recur; however, this study shows a clear difference in the pattern of invasion between these cases and the usual recurrent cases, and expression of immunostains.

In conclusions, IMP3 may serve as a useful prognostic marker that can stratify patients of advanced stage, high grade serous carcinomas into two distinct subsets: majority with early recurrence (< 24 months) with an infiltrative pattern of invasion and IMP3 positive; and a smaller subset that do not show disease recurrence for at least 49 months follow-up with pushing borders and IMP3 negative. IMP3-positive cases showed a significantly lower overall survival rate as compared to IMP3-negative tumors. Also, E-Cadherin showed significant decrease in expression in the invasive component, particularly in the peritoneal metastases of the cases which recurred and had significant correlation with increase IMP3 levels in ovary.

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None.

Conflict of interest

The authors declare there are no relevant conflicts of interest.

Author's contributions

Conception and Design: S. Mohanty, F. Dadmanesh, and C. Walsh.
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Acquisition of Data: S. Mohanty, F. Dadmanesh, and C. Walsh.

Analysis of Data: S. Mohanty, A. Tiwari, C. Singh, and K. Singh.

Interpretation of Data: S. Mohanty, A. Tiwari, F. Dadmanesh, and C. Walsh.

Writing and review/revision of the manuscript: S. Mohanty, A. Tiwari, C. Singh, F. Dadmanesh, and C. Walsh.

Technical Support: E. Kim and F. Chuang.

Study Supervision: F. Dadmanesh and C. Walsh.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anndiagpath.2019.02.013.

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