Prognostic Significance of Intra-tumoral Budding in Serous Ovarian Carcinomas

Toru Hachisuga (thachisu@med.uoeh-u.ac.jp)
University of Occupational and Environmental Health Japan
Midori Murakami
University of Occupational and Environmental Health Japan
Hiroshi Harada
University of Occupational and Environmental Health Japan
Taeko Ueda
University of Occupational and Environmental Health Japan
Tomoko Kurita
University of Occupational and Environmental Health Japan
Seiji Kagami
University of Occupational and Environmental Health Japan
Kiyoshi Yoshino
University of Occupational and Environmental Health Japan
Ryosuke Tajiri
University of Occupational and Environmental Health Japan
Masanori Hisaoka
University of Occupational and Environmental Health Japan

Research Article

Keywords: Ovarian carcinoma, Serous ovarian carcinoma, Tumor budding, Epithelial-mesenchymal transition, Prognosis, progression

Posted Date: June 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-595373/v1

Abstract

Intra-tumoral budding (ITB) has been well demonstrated to be an independent risk factor for adverse outcomes in colorectal carcinoma. This study investigated the prognostic significance of ITB in serous ovarian carcinomas (SOCs). The medical records and slides of 84 SOCs, including 13 with neoadjuvant chemotherapy (NAC), were retrospectively reviewed. The following clinicopathological parameters were noted: age at the diagnosis, CA125 level, 2014 FIGO stage, architectural grade, nuclear grade, presence of ITB, lymphovascular space invasion (LVSI), mitotic index and lymph node metastasis. ITB was found in 65 (77.4%) of the 84 SOCs. The presence of ITB was significantly correlated with a higher level of CA125, an advanced 2014 FIGO stage, a worse architectural grade, and the presence of LVSI. The median progression-free survival (PFS) was 20 months in women with SOC with ITB and 38 months in women with SOC without ITB (P<0.01), and their median overall survival (OS) was 52 months and 72 months (P=0.05). The multivariate analysis revealed that ITB was not an independent prognostic factor. ITB is a cost-effective prognostic indicator for women with SOC and ITB in ovarian tumor tissue is considered a useful histological biomarker of the progression of SOCs.

Introduction

Many authors have examined the prognostic significance of histopathologic features for invasive ovarian carcinomas. Although Shimizu and Silverberg[1] proposed a three-tier grading system, which was based on the dominant architectural pattern, degree of nuclear pleomorphism, and mitotic index, for all ovarian epithelial malignancies, the international collaboration on cancer reporting (ICCR) [2] recommends that different grading systems should be used for different morphological subtypes.

Ovarian high- and low-grade serous carcinoma, was defined by Malpica et al.[3] in 2004 and subsequently adopted in the 2014 WHO Classification of Tumours of Female Reproductive Organs [4]. Using this two-tier grading system for serous ovarian carcinomas (SOCs) [3], cases assigned to the low-grade category were characterized by the presence of mild to moderate nuclear atypia. As a secondary feature, they tended to show up to 12 mitoses/10 high-power fields (HPFs), whereas those in the high-grade category had marked nuclear atypia and as a secondary feature more than 12 mitoses/10 HPFs. Recent molecular genetic studies have shown that low-grade serous carcinomas demonstrate mutations in KRAS or BRAF and few chromosomal abnormalities. In contrast, high-grade serous carcinomas harbor mutations in TP53 and are, chromosomally, highly unstable[5]. Immunohistochemical algorithms and prediction models have been proposed for the five major histologic types of epithelial ovarian carcinomas[6]. Based on a dualistic model of epithelial ovarian carcinogenesis, the importance of the molecular classification and origin of ovarian carcinomas was reported[5].

Tumor budding (TB) and its association with disease progression in patients with various solid cancers was first described by Imai in the 1950s[7]. TB is a morphological phenotype representing a destructive stromal invasion and was included as an additional prognostic factor for colorectal carcinoma in the

2019 WHO Classification of Digestive System Tumours [8]. The term intra-tumoral budding (ITB), which is TB found within the main tumor body, was introduced to distinguish this form of budding from the classic peritumoral budding[9]. The reported interobserver variability for assessing TB has ranged from moderate to very good, depending on the study [10].

The prognostic significance of destructive invasive implants in extra-ovarian tissues has been well studied in serous borderline tumors (SBTs) of the ovary [11]. Such destructive invasion was suggested to be associated with a poor prognosis in patients with stage I endometrioid and mucinous ovarian carcinomas [12]. TB is an independent, unfavorable, prognostic factor for patients with early-stage cervical cancer following radical surgery[13]. However, ITB has not been well described in SOCs.

The present study evaluated the prognostic significance of ITB in SOCs, describing the correlation between ITB and other conventional histologic parameters.

Results

Clinicopathologic features

The clinicopathologic data of 84 SOCs are shown in Table 1. ITB was found in 65 (77.4%) of 84 SOCs (Fig. 1). The values of CA125 level ($P = 0.04$), 2014 FIGO stage ($P < 0.01$), architectural grade ($P = 0.01$) and presence of LVSI ($P < 0.01$) in women with SOC with ITB were significantly higher than those in women with SOC without ITB (Fig. 2). The differences in nuclear grade and lymph node metastasis between these two groups did not reach statistical significance ($p = 0.06$). The levels of CA125 before initial treatment and after 3cycles of cisplatin-based chemotherapy were measured in 84 and 75 women with SOC, respectively. The CA125 level after 3 cycles of chemotherapy was shown to be normal in all 16 women with SOC without ITB and 45 (76.2%) of 59 women with SOC with ITB. All 13 women with SOC who had undergone NAC showed ITB. Nine of these 13 SOCs showed a mitotic index ≤ 12 mitoses/10HPFs (SOCs with NAC vs. without NAC, $P < 0.01$). Poly (ADP-ribose) polymerase (PARP) inhibitor and bevacizumab were administered to three and three women with SOC without ITB, and five and eight women with SOC with ITB, respectively.

SOCs with mild to moderate nuclear atypia are shown in Table 2. Three SOCs without ITB and an SOC with ITB (5.6%) showed histologic features of mild to moderate nuclear atypia and ≤ 12 mitoses/10 HPFs among 71 SOCs without NAC.

The prognosis

Figure 3 shows the differential Kaplan-Meier PFS and OS curves of women with SOC for the presence or absence of ITB. The median PFS was 20 months in 61 women with SOC with ITB (range, 2 to 156 months) and 38 months in 17 women with SOC without ITB (range, 12 to 148 months), and their median OS was 52 months (range, 6 to 189 months) and 72 months (range, 14 to 148 months).

A univariate analysis showed that the 2014 FIGO stage (I and II vs. III and IV), the CA125 level before initial treatment (median, 680 U/ml ; ≤680.0 U/ml vs. > 680.0 U/ml), LVSI (presence vs. absence) and ITB (presence vs. absence) were significantly associated with the PFS (hazard ratio for progression or death, 6.56; 95% IC, 2.36 to 18.2; P < 0.01, 2.04; 95% IC, 1.20 to 3.47; P = 0.01, 2.09; 95% IC, 1.21 to 3.61; P = 0.01 and 1.42; 95% IC, 1.13 to 1.82; P < 0.01, respectively) and that the 2014 FIGO stage and the CA125 level before initial treatment were significantly associated with the OS (hazard ratio for death, 4.72; 95% IC, 1.13 to 19.74; P = 0.03 and 2.19; 95% IC, 1.06 to 4.50; P = 0.03, respectively). LVSI and ITB were associated with the OS (hazard ratio for death, 1.50; 95% IC, 0.75 to 3.00; P = 0.26 and 1.41; 95% IC, 0.96 to 2.00; P = 0.05, respectively). In a multivariate analysis, the significance of the 2014 FIGO stage was preserved in the PFS of patients (hazard ratio for progression or death, 5.22; 95% IC, 1.69 to 16.2; P < 0.01), whereas the significance of the CA125 level before initial treatment, LVSI and ITB disappeared. No independent variable was associated with OS.

Among the 13 patients treated with NAC, the prognostic statuses were dead of disease in 12 patients and alive with disease in 1 with median follow-up of 46 (range, 12 to 63) months.

Discussion

When we examined the presence of ITB as a maker of destructive stromal invasion of SOCs, it was found to be a significant poor prognostic indicator for the PFS of women with SOC, but did not reach statistical significance for the OS of women with SOC in the univariate analysis. In a multivariate analysis, ITB was not an independent prognostic indicator. The prognostic significance of ITB was considered to be strongly affected by the 2014 FIGO stage. The large size studies stratified by the 2014 FIGO stage may be recommended.

It is well-known that TBs are part of the tumor microenvironment and are associated with epithelial-mesenchymal transition (EMT) [7]. EMT is characterized by cytoskeletal rearrangements, cell motility and invasion, increased cell-associated proteolytic activity and reprogramming of the gene expression[14]. Although the association between ITB and lymph node metastasis did not reach statistical significance, ITB was significantly associated with the incidence of LVSI in the present study. Increasing evidence has highlighted a close relationship between EMT and chemoresistance in epithelial ovarian carcinoma (EOC) [15]. In one study, the SKOV-3 EOC cells were shown to trigger both EMT and chemoresistance after treatment by carboplatin [16]. ITB was found in all SOCs after NAC, except for in two complete regression cases, and was associated with a low mitotic index of tumors and poor prognosis of patients.

The stromal-epithelial patterns of invasion in SBTs of the ovary have been subclassified as destructive and nondestructive. By definition, well-differentiated serous tumors featuring destructive stromal invasion are classified as low-grade serous carcinomas whereas those with either no stromal invasion or stromal microinvasion are classified as SBTs of the ovary [17]. In a review, low-grade serous carcinoma was defined as a serous neoplasm showing destructive invasion, mild to moderate cytologic atypia, and relatively low proliferative activity. Low-grade serous carcinoma accounts for 4.7% of SOCs and has an excellent

prognosis, but long-term follow-up indicates that the prognosis for patients with stage III-IV disease is poor [18]. In the present study, 3 SOCs without ITB and 1 SOC with ITB (5.6%) showed both histologic features of mild to moderate nuclear atypia and ≤ 12 mitoses/10 HPFs in 71 SOCs without NAC.

Analyses of gene expression microarray data from The Cancer Genome Atlas (TCGA) project revealed that high-grade SOC could be classified as one of four gene expression subtypes: mesenchymal, immunoreactive, proliferative, or differentiated [19]. Tumors with the mesenchymal phenotype had poor prognoses, whereas those with the immunoreactive type had favorable prognoses. Murakami et al. [20] said that a feature of spindle and isolated cells with destructive stromal reaction was referred to as the mesenchymal phenotype. Tothill et al. [21] reported that a novel subtype of high-grade serous cancers reflected a mesenchymal cell type, characterized by the overexpression of N-cadherin and P-cadherin and low expression of differentiation markers, including CA125 and MUC1. A poor prognosis subtype was defined by a reactive stroma gene expression signature, correlating with extensive desmoplasia in such samples.

In conclusion, recent studies concerning the origin and molecular pathogenesis of SOCs support the performance of prophylactic salpingo-oophorectomy in high-risk women and new molecular target therapy in women with high-grade SOC. ITB was shown to be a cost-effective prognostic indicator for women with SOC and a histopathologic biomarker of the progression of SOC. We may need to pay closer attention to the progression of SOCs

Materials And Methods

Case selection

After a review of the 318 malignant ovarian tumors found in the database of the Department of Pathology at the University of Occupational and Environmental Health Hospital between 2000 and 2017, 84 SOCs were selected. Twenty peritoneal carcinomas, defined according to the Gynecological Oncology Group inclusionary criteria[22], were not included in the present study. The ovarian specimens were taken from 71 patients at primary surgery and 13 after neoadjuvant chemotherapy (NAC). All cases with available histopathologic slides were included in the present study, except for two women with complete regression of SOC after NAC. Hematoxylin and Eosin (H&E)-stained slides of the SOCs were cut from a median of 6 (range, 2–18) tissue blocks per case.

Ethical approval for this study was granted by the Review Board of the University Hospital of Occupational and Environmental Health on Ethical Issues (H27-185). All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants.

The following clinicopathological parameters were evaluated: age at the diagnosis, CA125 level (before initial treatment and after three cycles of chemotherapy), 2014 FIGO stage, architectural grade, nuclear

grade, ITB, lymphovascular space invasion (LVSI), mitotic index and lymph node metastasis while referring to previous studies[1,3,23]. The postsurgical assessment of the interval debulking surgery was available for the 2014 FIGO stage of patients who received platinum-based NAC. Adjuvant platinum-based chemotherapy was not performed in one patient with stage IA tumor without ITB, and six patients with severe medical complication and/or advanced age, (including two with SOC without ITB and four with SOC with ITB,). These latter six patients were not included in the analysis of the patient prognosis.

According to the statements of the international tumor budding consensus conference (ITBCC) [10], ITB was defined as a single tumor cell or a cell cluster of up to four tumor cells at the invasive tumor front. ITB was evaluated in the surgical specimens of the ovarian tumors to be clinically the site of the greatest tumor volume/size.

While we initially attempted to use a three-tier grading system of ITB (none, focal and diffuse) according to the recommendation of the ITBCC, there was no significant difference in the cumulative survival between women with SOC with focal and diffuse ITB. The SOCs were therefore ultimately divided into two groups (: those with and without ITB).

Statistical analyses

Statistical analyses were carried out using the IBM SPSS Statistics, version 27 (IBM SPSS Statistics for Windows, IBM Corporation, Armonk, NY, USA). Data for the clinicopathological factors were evaluated using the Chi-square test or Mann-Whitney *U* test. The progression-free survival (PFS) was defined as the time from the date of initial treatment to the date of objective disease progression or last follow-up. The overall survival (OS) was defined as the time from the date of initial treatment to the date of death or last follow-up. The PFS and OS curves were estimated using the Kaplan-Meier method and compared using the log rank test. Univariate and multivariate Cox proportional hazards models were used to determine the association between potential risk factors and progression as well as death from disease. Statistical significance was considered to exist at a value of $P < 0.05$.

Declarations

Funding: This research did not receive any specific grant from any funding agency.

Conflicts of interest/competing interests: All authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material: not applicable

Code availability: not applicable

Authors' contributions: Hachisuga, Yoshino and Hisaoka contributed to conception and design. Material preparation, data collection and analysis were performed by Hachisuga, Murakami, Harada, Ueda, Kurita, Kagami and Tajiri. Murakami, Kagami and Harada contributed the statistical analysis. The first draft of

the manuscript was written by Hachisuga, Murakami, Kagami and Tajiri. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Ethics approval: Ethical approval for this study was granted by the Review Board of the University Hospital of Occupational and Environmental Health on Ethical Issues (H27-185).

References

1. Shimizu, Y., Kamoi, S., Amada, S., Akiyama, F. & Silverberg, S. G. Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. *Cancer* **82**, 893-901, doi:10.1002/(sici)1097-0142(19980301)82:5<893::aid-cncr14>3.0.co;2-w (1998).
2. McCluggage, W. G. *et al.* Data set for reporting of ovary, fallopian tube and primary peritoneal carcinoma: recommendations from the International Collaboration on Cancer Reporting (ICCR). *Mod. Pathol.* **28**, 1101-1122, doi:10.1038/modpathol.2015.77 (2015).
3. Malpica, A. *et al.* Grading ovarian serous carcinoma using a two-tier system. *Am. J. Surg. Pathol.* **28**, 496-504, doi:10.1097/00000478-200404000-00009 (2004).
4. Kurman, R. J., Carcangiu, M. L., Herrington, S. & Young, R. H. *WHO Classification of Tumours of Female Reproductive Organs*. Vol. 6 (International Agency for Research on Cancer, 2014).
5. Kurman, R. J. & Shih Ie, M. The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded. *Am. J. Pathol.* **186**, 733-747, doi:10.1016/j.ajpath.2015.11.011 (2016).
6. Kobel, M. *et al.* An Immunohistochemical Algorithm for Ovarian Carcinoma Typing. *Int. J. Gynecol. Pathol.* **35**, 430-441, doi:10.1097/PGP.0000000000000274 (2016).
7. Lugli, A., Zlobec, I., Berger, M. D., Kirsch, R. & Nagtegaal, I. D. Tumour budding in solid cancers. *Nat. Rev. Clin. Oncol.* **18**, 101-115, doi:10.1038/s41571-020-0422-y (2021).
8. Board, W. C. o. T. E. *WHO Classification of Digestive System Tumours*. Vol. 1 (World Health Organization, 2019).
9. Lugli, A. *et al.* Intratumoral budding as a potential parameter of tumor progression in mismatch repair-proficient and mismatch repair-deficient colorectal cancer patients. *Hum. Pathol.* **42**, 1833-1840, doi:10.1016/j.humpath.2011.02.010 (2011).
10. Cho, S. J. & Kakar, S. Tumor Budding in Colorectal Carcinoma: Translating a Morphologic Score Into Clinically Meaningful Results. *Arch. Pathol. Lab. Med.* **142**, 952-957, doi:10.5858/arpa.2018-0082-RA (2018).
11. Vang, R. *et al.* Long-term Behavior of Serous Borderline Tumors Subdivided Into Atypical Proliferative Tumors and Noninvasive Low-grade Carcinomas: A Population-based Clinicopathologic Study of 942 Cases. *Am. J. Surg. Pathol.* **41**, 725-737, doi:10.1097/PAS.0000000000000824 (2017).

12. Chen, S., Leitao, M. M., Tornos, C. & Soslow, R. A. Invasion patterns in stage I endometrioid and mucinous ovarian carcinomas: a clinicopathologic analysis emphasizing favorable outcomes in carcinomas without destructive stromal invasion and the occasional malignant course of carcinomas with limited destructive stromal invasion. *Mod. Pathol.* **18**, 903-911, doi:10.1038/modpathol.3800366 (2005).
13. Huang, B., Cai, J., Xu, X., Guo, S. & Wang, Z. High-Grade Tumor Budding Stratifies Early-Stage Cervical Cancer with Recurrence Risk. *PLoS One* **11**, e0166311, doi:10.1371/journal.pone.0166311 (2016).
14. Derynck, R. & Weinberg, R. A. EMT and Cancer: More Than Meets the Eye. *Dev. Cell* **49**, 313-316, doi:10.1016/j.devcel.2019.04.026 (2019).
15. Deng, J. *et al.* Targeting epithelial-mesenchymal transition and cancer stem cells for chemoresistant ovarian cancer. *Oncotarget* **7**, 55771-55788, doi:10.18632/oncotarget.9908 (2016).
16. Rohnalter, V. *et al.* A multi-stage process including transient polyploidization and EMT precedes the emergence of chemoresistent ovarian carcinoma cells with a dedifferentiated and pro-inflammatory secretory phenotype. *Oncotarget* **6**, 40005-40025, doi:10.18632/oncotarget.5552 (2015).
17. McKenney, J. K., Balzer, B. L. & Longacre, T. A. Patterns of stromal invasion in ovarian serous tumors of low malignant potential (borderline tumors): a reevaluation of the concept of stromal microinvasion. *Am. J. Surg. Pathol.* **30**, 1209-1221, doi:10.1097/01.pas.0000213299.11649.fa (2006).
18. Slomovitz, B. *et al.* Low-grade serous ovarian cancer: State of the science. *Gynecol. Oncol.* **156**, 715-725, doi:10.1016/j.ygyno.2019.12.033 (2020).
19. Network, C. G. A. R. Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609-615, doi:10.1038/nature10166 (2011).
20. Murakami, R. *et al.* Establishment of a Novel Histopathological Classification of High-Grade Serous Ovarian Carcinoma Correlated with Prognostically Distinct Gene Expression Subtypes. *Am. J. Pathol.* **186**, 1103-1113, doi:10.1016/j.ajpath.2015.12.029 (2016).
21. Tothill, R. W. *et al.* Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. *Clin. Cancer Res.* **14**, 5198-5208, doi:10.1158/1078-0432.CCR-08-0196 (2008).
22. Schorge, J. O. *et al.* BRCA1-related papillary serous carcinoma of the peritoneum has a unique molecular pathogenesis. *Cancer Res.* **60**, 1361-1364 (2000).
23. Matsuo, K. *et al.* Estrogen receptor expression and increased risk of lymphovascular space invasion in high-grade serous ovarian carcinoma. *Gynecol. Oncol.* **133**, 473-479, doi:10.1016/j.ygyno.2014.03.563 (2014).

Tables

Due to technical limitations, table 1 and 2 PDFs are only available as a download in the Supplemental Files section.

Figures

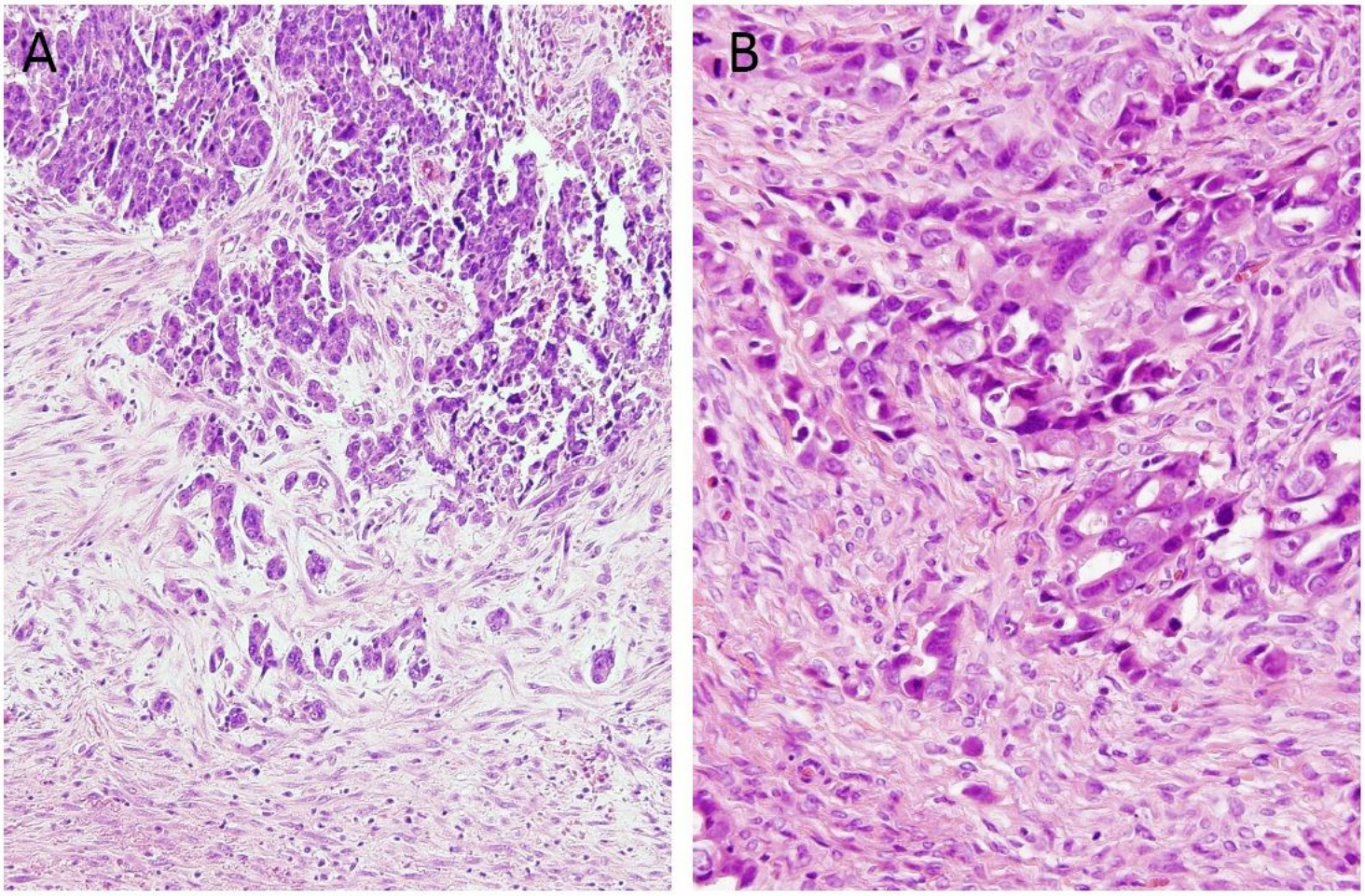

Figure 1

Serous ovarian carcinomas with intra-tumoral budding in a 48-year-old woman (A) and 51-year-old woman (B). Single tumor cells and clusters of small numbers of tumor cells were noted at the invasive edge.

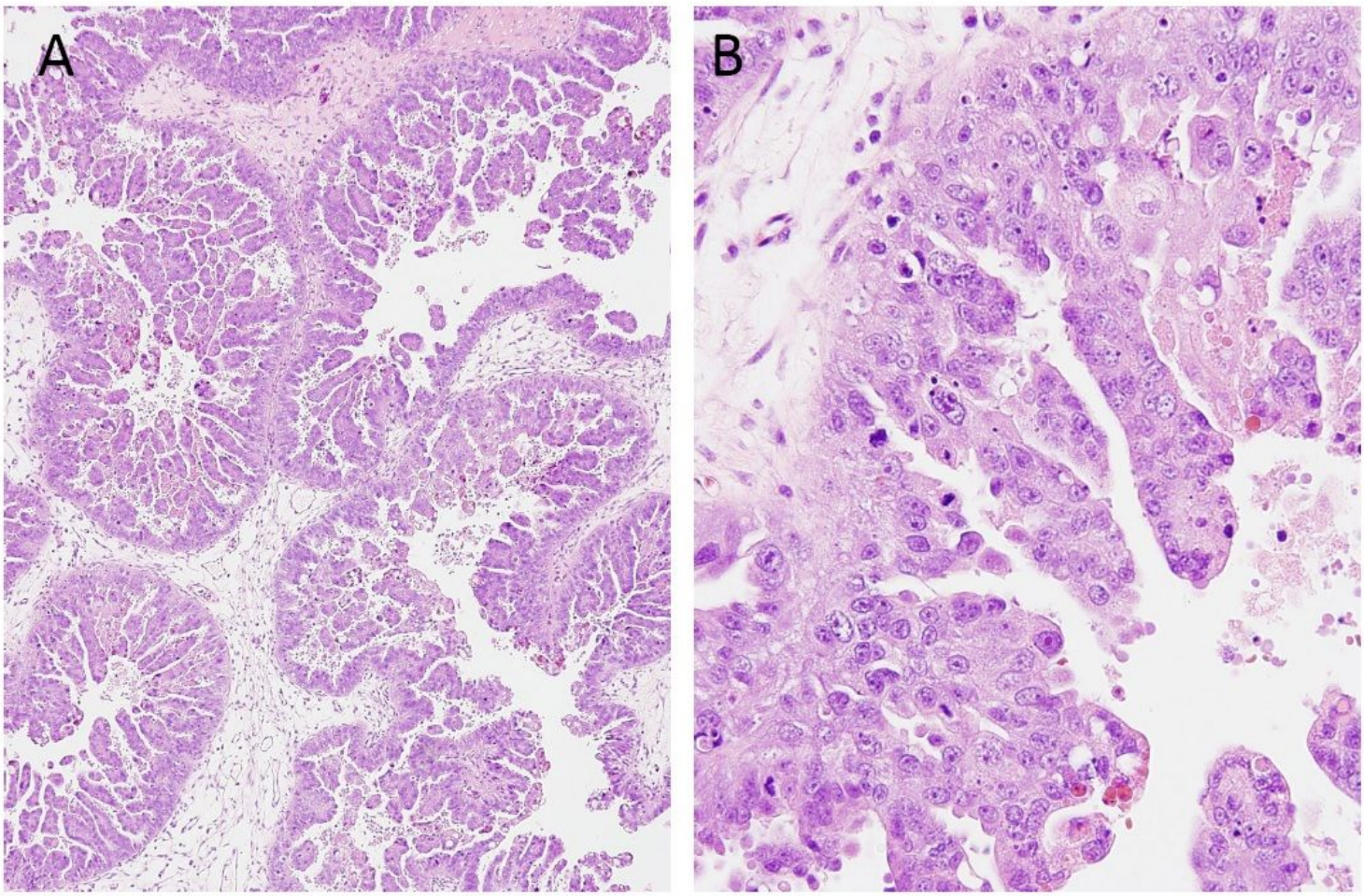

Figure 2

Serous ovarian carcinoma in a 49-year-old woman. The stage IC1 tumor showed no intra-tumoral budding, architectural grade 1 (A), nuclear grade 3 and a high mitotic index (B).

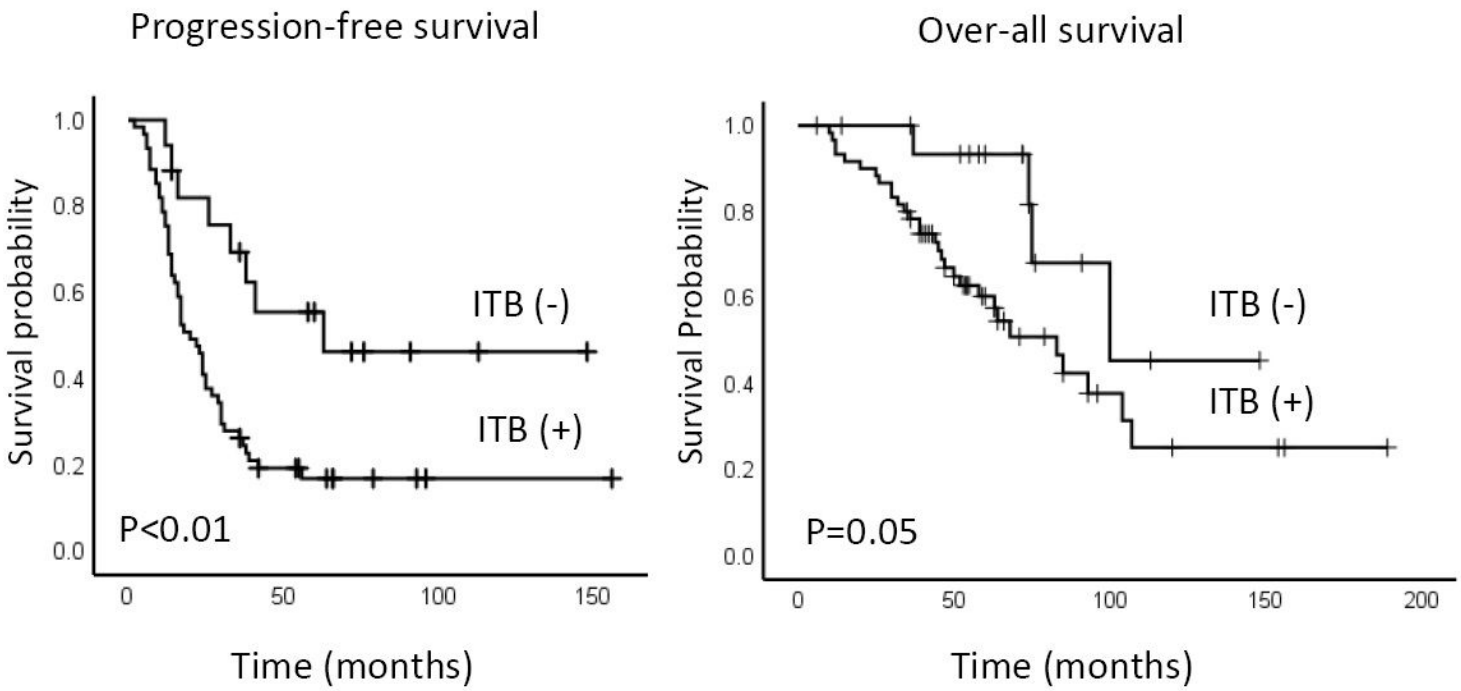

Figure 3

Kaplan-Meier survival analyses of the progression-free and over-all survivals in women with serous ovarian carcinomas with and without intra-tumoral budding (ITB).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1..pdf
- Table2..pdf