

# Tumor-stroma Ratio as a New Prognosticator for Pseudomyxoma Peritonei: a Comprehensive Clinicopathological and Immunohistochemical Study

**Ru Ma**

Capital Medical University Affiliated Beijing Shijitan Hospital

**Yu-Lin Lin**

Capital Medical University Affiliated Beijing Shijitan Hospital

**Xin-Bao Li**

Capital Medical University Affiliated Beijing Shijitan Hospital

**Feng-Cai Yan**

Capital Medical University Affiliated Beijing Shijitan Hospital

**Hong-Bin Xu**

Aerospace Central Hospital: Central Hospital of China Aerospace Corporation

**Zheng Peng**

Chinese PLA General Hospital

**Yan Li** (✉ [liyansd2@mail.ccmu.edu.cn](mailto:liyansd2@mail.ccmu.edu.cn))

Capital Medical University Affiliated Beijing Shijitan Hospital <https://orcid.org/0000-0001-6018-6538>

---

## Research

**Keywords:** Pseudomyxoma peritonei, Tumor-stroma ratio, Histopathology, Immunohistochemistry, Prognosis

**Posted Date:** April 19th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-412210/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Diagnostic Pathology on December 1st, 2021. See the published version at <https://doi.org/10.1186/s13000-021-01177-1>.

# Abstract

**Background:** As a rare clinical tumor syndrome, pseudomyxoma peritonei (PMP) is usually diagnosed at an advanced stage. In-depth pathological analysis is essential to assessing tumor biological behaviors, assisting treatment decision, and predicting clinical prognosis of PMP. Tumor-stroma ratio (TSR) is a promising prognostic parameter based on tumor and stroma. This study was to explore the relationship between TSR with pathological characteristics and prognosis of PMP.

**Methods:** PMP patients with complete data who underwent last cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy were collected. The TSR of postoperative pathological images was quantitatively analyzed by Image-Pro Plus. Then the relationship between TSR with clinicopathological characteristics, immunohistochemical characteristics and prognosis of PMP was analyzed.

**Results:** Among the 50 PMP patients included, there were 27 males (54.0%) and 23 females (46.0%), with a median age of 55 (31 - 76) years. The patients with histopathological types of disseminated peritoneal adenomucinosis (DPAM) and peritoneal mucinous carcinomatosis (PMCA) were both 25 (50.0%), 4 cases (8.0%) with vascular tumor emboli, 3 cases (6.0%) with nerve invasion, and 5 cases (10.0%) with lymph node metastasis. Immunohistochemical results showed that Ki67 label index was  $< 25\%$  in 18 cases (36.0%) and  $\geq 25\%$  in 32 cases (64.0%). The range of TSR was 2% - 24% (median: 8%). The cutoff value of TSR was 10% based on receiver operating characteristic (ROC) curve. There were 31 cases with TSR  $< 10\%$  while 19 cases with TSR  $\geq 10\%$ . It showed that TSR was closely related to histopathological types ( $P < 0.001$ ) and Ki67 label index ( $P < 0.001$ ). Univariate analysis showed that preoperative carcinoembryonic antigen (CEA), preoperative carbohydrate antigen (CA) 19-9, pathological types, vascular tumor emboli and TSR influenced prognosis of PMP patients ( $P < 0.05$ ). Multivariate analysis showed preoperative CEA, vascular tumor emboli and TSR were independent prognostic factors.

**Conclusion:** TSR could be a new independent prognosticator for PMP.

## Introduction

Pseudomyxoma peritonei (PMP) is a malignant clinical syndrome characterized by the accumulation and redistribution of copious mucus produced by mucinous tumor cells in the peritoneal cavity, with typical clinical manifestations including mucinous ascites, peritoneal implantation, omental cake, and ovarian involvement [1]. Currently, cytoreductive surgery (CRS) plus hyperthermic intraperitoneal chemotherapy (HIPEC) is the standard treatment, which can significantly prolong the survival [2]. As a rare clinical tumor syndrome, early diagnosis of PMP is difficult, and there is a widespread misunderstanding of the ambiguous clinical signs and symptoms among the medical field in general, easily causing missed diagnosis and misdiagnosis, and thus delaying the best treatment opportunity. Improving the accuracy of PMP diagnosis contributes to better assess the disease progression and prognosis to provide more effective treatment options for patients.

The histopathological classification and grade of PMP are of vital importance for disease assessment. The pathologists of Peritoneal Surface Oncology Group International (PSOGI) agreed that based mainly on the number of tumor cells and morphology of tumor nests, cells atypia, mitotic figures, and the form of surrounding invasion, PMP was divided into acellular mucin, disseminated peritoneal adenomucinosis (DPAM), peritoneal mucinous carcinomatosis (PMCA) and PMCA with signet ring cells [3], hence in the qualitative stage based on experience. Therefore, the accuracy of precise pathological diagnosis is susceptible to subjectivity mood and fatigue of readers.

Over the last decade, more and more attention has been paid to the interaction between tumor cells and microenvironment. Tumor microenvironment, also known as the tumor-associated matrix, is composed of immune cells, fibroblasts, pericytes, and endothelial cells in the extracellular matrix. Tumor invasion is a multifactorial process, which is significantly affected by tumor microenvironment, and the synergistic interaction between tumor cells and stromal components is the main driving force of tumor progression and metastasis [4]. Tumor-stroma ratio (TSR) is a promising prognostic parameter based on tumor and stroma, which reflects the area of tumor and stromal cells and is determined by histopathological sections stained with hematoxylin and eosin (HE) [5]. Currently, TSR has been proved to impact the prognosis of esophageal squamous carcinoma, lung cancer, breast cancer, gastric cancer, and other malignant solid tumors [6, 7]. The purpose of this study was to analyze the relationship between TSR and histopathological and immunohistochemical characteristics of PMP, and to investigate the impact of TSR on PMP prognosis.

## Materials And Methods

### Patients' selection

We selected 50 patients with PMP who received CRS + HIPEC at our center from June 2015 to May 2020 and had complete clinical data. The data included clinicopathological characteristics, immunohistochemical characteristics and follow-up data. Selected criteria were as follows: (1) DPAM and PMCA were diagnosed according to histopathological classification criteria of PSOGI on PMP; (2) Neoadjuvant therapy was not performed before CRS + HIPEC; (3) Follow-up time was longer than 3 months; (4) Clinical data were complete. The study was approved by the Ethics Committee of Beijing Shijitan Hospital, and all patients have signed the informed consent.

### Methods

#### *The workflow of quantitative analysis of pathological images (Fig. 1)*

The whole specific process is as follows:

(1) Section preparation: The surgical specimens of PMP patients undergoing CRS + HIPEC at our center were collected and made into 5  $\mu$ m sections for routine HE staining (Dako Hematoxylin, Dako Eosin and Dako Bluing Buffer, catalog number CS701; Dako CoverStainer, Agilent Technologies Inc., USA) (Fig. 1a1).

(2) Pathological images acquisition: 5 sections at the most aggressive tumor site were selected for multi-site and multi-filed image acquisition (Axio Scope.A1 biological microscope, Carl Zeiss AG, Germany; MS60 microscope camera, Mshot Technology Co., Ltd, China). For each section slide, the whole slide was first observed under low-power field ( $\times 50$ ) for overall image quality evaluation, and 3 fields with the most obvious tumor infiltration were randomly selected under high-power fields ( $\times 100$ ) for image acquisition, thus producing 3 non-overlapping JPG images with a resolution of  $2048 \times 2072$  pixels. Therefore, a total of 15 images were acquired from each specimen for quantitative analysis (Fig. 1a2, a3).

(3) Image preprocessing: the above obtained images were first preprocessed to create unified images for subsequent quantitative evaluation, using Image-Pro Plus (IPP) 6.0 software (Media Cybernetics Inc., USA). Major techniques for image preprocessing included contrast enhancement, color normalization and denoising (Fig. 1b).

(4) Tumor-stroma segmentation and area measurement: The standardized images were segmented using IPP, to separate the tumor area and the stroma area (Fig. 1c). The percentage of tumor area in each pathological image was measured, and the remaining area was the percentage of stroma. Thus, the ratio of tumor and stroma was calculated (Fig. 1d).

### ***Detection and determination of immunohistochemistry (IHC)***

IHC were performed on IntelliPATH FLX (BIOCARE MEDICAL LLC, USA) with Polymer Immunohistochemical Detection System (Wuxi OriGene Technologies Inc., China). We analyzed the protein expression by IHC of Ki67 (clone UMAB107, OriGene, catalog number ZM-0166), p53 (clone DO7, OriGene, catalog number ZM-0408), MUC1 (clone MRQ-17, OriGene, catalog number ZM-0391), MUC2 (clone MRQ-18, OriGene, atalog number ZM-0392), MUC5AC (clone MRQ-19, OriGene, catalog number ZM-0395), MUC6 (clone MRQ-20, OriGene, catalog number ZM-0396), MLH1 (clone ES05, OriGene, catalog number G10090), MSH2 (clone 25D12, OriGene, catalog number G07855), MSH6 (clone EP49, OriGene, catalog number G07841), PMS2 (clone EP51, OriGene, catalog number G14519), CDX2 (clone EP25, OriGene, catalog number ZA-0520), CK7 (clone EP16, OriGene, catalog number ZM-0071), and CK20 (clone EP23, OriGene, catalog number ZA-0574). All antibodies were ready-to-use. Positive control was set up according to the instructions, and phosphate buffer solution was used as blank control instead of primary antibody.

IHC images were interpreted as the following. The positive expression of Ki67, p53, mismatch repair (MMR) gene-related proteins (MLH1, MSH2, MSH6, and PMS2), CDX2 were in cell nucleus, while mucin (MUC1, MUC2, MUC5AC, MUC6) was in cytoplasm, and CK7 and CK20 were in cytoplasm and cytomembrane. Among them, the wild type of p53 showed positive nuclear staining with different strength and uneven distribution, while the mutant type showed strong positive nuclear staining with dense and uniform intensity. Any loss of MLH1, MSH2, MSH6, and PMS2 was a loss of MMR gene-related protein. The presence of brown-yellow or tan granules in tumor cells was defined as positive.

### ***Study parameters***

The clinicopathological characteristics were gender, age, previous surgical history, body mass index (BMI), Karnofsky (KPS) score, preoperative serum tumor markers, histopathological type, peritoneal cancer index (PCI), completeness of cytoreduction (CC), vascular tumor emboli, nerve invasion, and lymph node metastasis.

Immunohistochemical characteristics were Ki67, p53, MUC1, MUC2, MUC5AC, MUC6, MMR gene-related proteins (MLH1, MSH2, MSH6 and PMS2), CDX2, CK7 and CK20.

Survival data: survival status, overall survival (OS) from last surgery.

### ***Follow-up***

Follow-up records mainly included general conditions and survival status. The last unified telephone follow-up was on Feb. 21, 2021, with a follow-up rate of 100%.

## **Statistical analysis**

All statistical analyses were performed using SPSS V24.0 statistical software (IBM SPSS Inc., USA). The age, BMI, KPS, and PCI score were expressed as median (range), while the rest features were expressed as rate or percentage. The relationship between TSR and pathological features was analyzed with Chi-square test. Kaplan-Meier method and log-rank test were used for survival analysis. The cutoff values were obtained by receiver operating characteristic (ROC) curve, which were used as the grouping critical values of age, PCI score, TSR and Ki67.  $P < 0.05$  was considered as statistically significant.

## **Results**

### **Demographic and clinical features of 50 patients in this study**

A total of 50 patients were selected in the study, including 27 males (54.0%) and 23 females (46.0%), with a median age of 55 (31–76) years and a median KPS score of 90 (60–100). 43 patients (86.0%) had previous surgery, 23 patients (46.0%) had intravenous chemotherapy and 23 patients (46.0%) had intraperitoneal chemotherapy, respectively. The patients with histopathological type of DPAM and PMCA were both 25 (50.0%). The median PCI score was 36 (3–39); CC score was 0–1 in 18 cases (36.0%) and 2–3 in 32 cases (64.0%) (Table 1).

Table 1  
Major clinicopathological features of 50 PMP patients

Items	Value
Gender, n (%)	
Male	27 (54.0)
Female	23 (46.0)
Age (years), median (range)	55 (31–76)
BMI (kg/m <sup>2</sup> ), median (range)	22.0 (15.9–27.8)
KPS score, median (range)	90 (60–100)
Operation History, n (%)	
No	7 (14.0)
Yes	43 (86.0)
History of intravenous chemotherapy, n (%)	
No	27 (54.0)
Yes	23 (46.0)
History of intraperitoneal chemotherapy, n (%)	
No	27 (54.0)
Yes	23 (46.0)
Preoperative CEA, n (%)	
Normal	9 (18.0)
Increased	41 (82.0)
Preoperative CA19-9, n (%)	
Normal	22 (44.0)
Increased	28 (56.0)
Preoperative CA125, n (%)	
Normal	22 (44.0)
Increased	28 (56.0)
Histopathological type, n (%)	
DPAM	25 (50.0)

Items	Value
PMCA	25 (50.0)
PCI score, median (range)	36 (3–39)
CC score	
0–1	18 (36.0)
2–3	32 (64.0)
PMP: Pseudomyxoma peritonei; BMI: Body mass index; KPS: Karnofsky performance status; DPAM: Disseminated peritoneal adenomucinosis; PMCA: Peritoneal mucinous carcinomatosis; PCI: Peritoneal cancer index; CC: Completeness of cytoreduction; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19 – 9; CA125: Carbohydrate antigen 125.	

## Routine histopathological and immunohistochemical characteristics of 50 patients in this study

Histopathological results showed that among the 50 patients in this study, there were 25 cases each in DPAM (Fig. 2a) (50.0%) and PMCA (50.0%) (Fig. 2b) groups. Other prominent histological features were 4 cases (8.0%) with vascular tumor emboli (Fig. 2c), 3 cases (6.0%) with nerve invasion, and 5 cases (10.0%) with lymph node metastasis.

IHC showed that Ki67 (Fig. 2d) label index was < 25% in 18 cases (36.0%) and  $\geq$  25% in 32 cases (64.0%). The mutant rate of p53 was 62.0%. The expression rates of MUC1, MUC2, MUC5AC and MUC6 were 74.3%, 89.5%, 100.0% and 22.9%, respectively. The loss rate of MMR gene-related protein expression was 6.7%. The expression rate of CDX2, CK7 and CK20 were 83.3%, 55.6% and 95.7%, respectively.

### TSR evaluation

After image segmentation and target-object calculation of 15 images for each patient specimen, the TSR was produced. For the 50 patients, the median TSR was 8% (range: 2% – 24%) (Fig. 3a, b). Based on ROC curve (Fig. 3c), at the cutoff value of 10%, TSR reached 0.500 sensitivity and 0.929 specificity in predicting prognosis. Therefore, TSR was divided into < 10% and  $\geq$  10% for the subsequence analysis.

### The relationship between TSR and pathological features

TSR was divided into < 10% and  $\geq$  10% according to the cutoff value of ROC curve, and then the correlation analysis was conducted between TSR and the above histopathological characteristics. The results showed that TSR was correlated with histopathological types ( $P < 0.001$ ) and Ki67 ( $P < 0.001$ ), but not correlated with the other histopathological and immunohistochemical characteristics ( $P > 0.05$ ) (Table 2).

Table 2  
The relationship between TSR and pathological characteristics of PMP

Items	n (%)	TSR		<i>P</i>
		< 10%	≥ 10%	
Histopathological type				
DPAM	25 (50.0)	23	2	<b>&lt; 0.001</b>
PMCA	25 (50.0)	8	17	
Vascular tumor emboli				
Yes	4 (8.0)	1	3	0.293
No	46 (92.0)	30	16	
Nerve invasion				
Yes	3 (6.0)	0	3	0.095
No	47 (94.0)	31	16	
Lymph node metastasis				
Yes	5 (10.0)	2	3	0.560
No	45 (90.0)	29	16	
Ki67 label index				
< 25%	18 (36.0)	17	1	<b>&lt; 0.001</b>
≥ 25%	32 (64.0)	14	18	
p53				
Wild type	19 (38.0)	15	4	0.053
Mutant type	31 (62.0)	16	15	
MUC1				
+	26 (74.3)	16	10	1.000
-	9 (25.7)	5	4	
MUC2				
+	17 (89.5)	12	5	0.964
-	2 (10.5)	2	0	
MUC5AC				
+	18 (100.0)	13	5	-

Items	n (%)	TSR		<i>P</i>
		< 10%	≥ 10%	
-	0 (0.0)	0	0	
MUC6				
+	8 (22.9)	3	5	0.285
-	27 (77.1)	18	9	
MMR protein expression				
Normal	28 (93.3)	15	13	0.464
Loss	2 (6.7)	0	2	
CDX2				
+	35 (83.3)	21	14	0.676
-	7 (16.7)	3	4	
CK7				
+	25 (55.6)	14	11	0.787
-	20 (44.4)	12	8	
CK20				
+	44 (95.7)	26	18	1.000
-	2(4.3)	1	1	
TSR: Tumor-stroma ratio; DPAM: Disseminated peritoneal adenomucinosis; PMCA: Peritoneal mucinous carcinomatosis; MMR: Mismatch repair				

## Survival analysis

### Survival curve analysis

At the median follow-up time of 42.0 months (95%CI: 9.38–75.02 months), 36 patients (72.0%) died, 14 patients (28.0%) survived, and the median OS from last surgery was 16.7 months (95%CI: 2.95–30.47 months) from last CRS + HIPEC. The 1-, 2- and 3-year survival rates were 65.4%, 47.0% and 29.8%, respectively (Fig. 4a).

### Univariate analysis

Univariate analysis demonstrated the following factors with statistically significant impact on OS: preoperative CEA ( $P= 0.039$ ), preoperative CA19-9 ( $P= 0.032$ ), pathological types ( $P= 0.022$ ), vascular tumor emboli ( $P= 0.007$ ), and TSR ( $P= 0.012$ ).

## Multivariate analysis

Factors with  $P < 0.05$  in univariate survival analysis was included in Cox regression model for multivariate analysis, which showed that preoperative CEA, vascular tumor emboli and TSR were independent prognostic factors (Fig. 4b-d). The mortality risk for patients with elevated preoperative CEA was 4.091 times that of those with normal preoperative CEA ( $P= 0.008$ , 95%CI: 1.441–11.616); The mortality risk for patients with vascular tumor emboli was 5.377 times that of patients without it ( $P= 0.004$ , 95%CI: 1.706–16.944); The mortality risk for patients with  $TSR \geq 10\%$  was 2.550 times that of patients with  $TSR < 10\%$  ( $P= 0.007$ , 95%CI: 1.286–5.058) (Table 3).

Table 3  
Multivariate survival analysis of 50 PMP patients

Variable	Wald	HR	95%CI	<i>P</i>
Preoperative CEA (Normal vs. Increased)	7.003	4.091	1.441–11.616	0.008
Vascular tumor emboli (Yes vs. No)	8.250	5.377	1.706–16.944	0.004
TSR (< 10% vs. $\geq 10\%$ )	7.179	2.550	1.286–5.058	0.007
PMP: Pseudomyxoma peritonei; CEA: Carcinoembryonic antigen; TSR: Tumor-stroma ratio				

## Examples of correlation between OS with TSR

This study showed that TSR was an independent prognostic factor in PMP patient. Figure 3 shows two examples that TSR was negatively correlated with OS. In patient A, the TSR was 2% and the OS was 36.5 months. However, in patient B, the TRS was 24% and the OS was only 2.3 months (Fig. 5).

## Discussion

In this study, TSR was obtained by quantitative analyze on HE histopathological images of PMP. We retrospectively analyzed the relationship between TSR and histopathological characteristics, immunohistochemical characteristics, and prognosis in 50 patients with complete clinicopathological information. Our results showed that TSR was closely related to histopathological types and Ki67 ( $P < 0.05$ ). Multivariate survival analysis showed that preoperative CEA, vascular tumor emboli and TSR were independent prognostic factors.

As a rare clinical malignant tumor syndrome, the incidence of PMP is about 2–4 per million, and early diagnosis is difficult. Surgical resection is the main treatment. CRS + HIPEC has been recommended as the standard treatment of PMP internationally, which significantly improves the prognosis of patients [8].

However, about 1/3 of PMP patients treated with CRS + HIPEC will relapse even if complete CRS is achieved [9]. Therefore, accurate disease assessment is particularly important for treatment decision and response evaluation. Nowadays the internationally recognized pathologic classification of PMP is mainly based on the subjective qualitative evaluation of PMP pathological images by pathologists. The results are easily affected by the experience level of pathologists, the complexity of images and the visual search process, which leads to inaccurate pathological diagnosis. It showed that pathological type is an independent prognostic factor in PMP patients [10]. However, Mhamed et al. [11] found that although most DPAM patients had a relatively better prognosis, some DPAM patients showed highly aggressive disease progression, with a 5-year survival rate much lower than those patients with worse tumor differentiation. In addition, Baratti et al. [12] used PSOGI pathological classification to analyze the prognosis of 265 patients with PMP, and the results showed that acellular mucinous and PMCA-S were identified as subgroups with good prognosis and poor prognosis, respectively, but pathological classification was not an independent prognostic factor of PMP patients. These studies indicated that current pathological classification is not enough to accurately predict the prognosis of patients, and more in-depth and objective indicators need to be explored to improve the precision and clarity of pathological diagnosis. Few studies have been reported on quantitative analysis of pathological images of PMP. Nevertheless, due to the rarity of PMP and the late start of research, pathological images related research is still at the semi-quantitative level, with the relatively rough research and simple calculation method [13, 14].

Tumor invasion is a multi-factory-driven process in which the synergistic interaction between tumor cells and stromal components is the driving force for tumor progression and metastasis. Tumor microenvironment is involved in tumor genesis, progression, invasion, and metastasis by inducing stem cell-like characteristics and epithelial-mesenchymal transformation of tumor cells [15]. Therefore, the ratio of tumor and stroma can more accurately evaluate the biological behavior of tumor to a certain extent. TSR is the ratio of tumor and stroma area, which can be obtained from pathological sections of routine HE staining of postoperative specimens without additional cost, so it is simple to operate and easy to promote. In recent years, TSR has been proved to have prognostic value in many tumors. Vangangelt et al. [16] evaluated HE sections of 1794 patients of breast cancer and found that TSR was not affected by clinically relevant factors such as age, tumor size and histology, and patients with low TSR had a relatively poor prognosis, which could be used as a potential prognostic indicator. Wu et al. [17] carried out Meta-analysis of 4238 cases of solid tumors including cervical cancer, non-small cell lung cancer, esophageal cancer, ovarian cancer, hepatocellular carcinoma, colorectal cancer, and nasopharyngeal carcinoma patients. It showed that low TSR was significantly associated with severe clinical stage, advanced depth of invasion, and positive lymph node metastasis. Patients with high TSR were related with good clinical prognosis, and TSR may be an independent prognostic factor for solid tumors [18].

Our study investigated the correlations between TSR and histopathological and immunohistochemical indicators of PMP. It showed that the TSR level of DPAM was significantly lower than that of PMCA group. TSR was positively correlated with Ki67, and patients with high Ki67 levels had higher TSR.

Previous studies have shown that pathological types of PMP is an independent prognostic factor. High-grade pathological type indicates the biological behavior of malignant and aggressive tumors, which is in vital importance for the selection of treatment regimens and prognosis evaluation of patients. Ki67 is a biological indicator reflecting the state of cell proliferation, and its expression changes with the cell cycle. The expression of Ki67 increases from G1 and decreases rapidly after mitosis. Ki67 protein was present in the nucleus during G1, S, G2 and mitotic phases, but not in the quiescent phase G0. Therefore, the higher the value of Ki67, the more active the cell proliferation is, which has been proved to be a prognostic marker of tumors [19].

In recent years, the relationship between the pathological characteristics of PMP and survival prognosis has been gradually studied. Multiple studies have shown that histopathological type is an independent prognostic factor in PMP patients. Zhou et al. [10] searched studies of PMP patients underwent debulking surgery from PubMed, Wiley Online Library and Cochrane Library before Jan. 2020. 766 patients were included for Meta-analysis of pathological indicators and survival, and it showed that the 5-year survival rate of PMP patients with different pathological types after surgery was significantly different, and the low-grade biological behavior of PMP had a better survival comparing with high-grade PMP. Yan et al. [20] performed HE staining and immunohistochemical analysis on the postoperative specimens of 155 patients of PMP from the appendiceal origin. Histopathological features such as primary tumor site, histological type, lymph node metastasis, as well as immunohistochemical indicators such as Ki67 label index, p53, mucin expression and prognosis were carried out with univariate and multivariate analyses. It showed that pathological type was an independent prognostic factor. Choudry et al. [21] retrospectively analyzed 310 PMP patients who underwent CRS + HIPEC and included tumor cell density in the study. It showed that the higher the cell density, the shorter the progressive-free survival, which may indicate the higher degree of malignant.

Three categories of indicators were included in this study, including gender, age, KPS score, preoperative serum tumor markers and other systemic indicators, pathological type, vascular tumor emboli, lymph node metastasis, Ki67, p53 and other traditional histopathological and immunohistochemical indicators, as well as TSR. The results showed that preoperative CEA, vascular tumor emboli and TSR were independent prognostic factors, which were derived from systemic indicator, traditional pathological indicator and subvisual pathological features describing tumor and its microenvironment. CEA is one of the most widely used serum tumor markers in clinical practice, which can assist in judging the degree of tumor invasion, and has important clinical value in the diagnosis disease detection and efficacy evaluation of gastrointestinal cancer and other malignant tumors. Carmignani et al. [22] analyzed the preoperative serum tumor markers of 532 patients with PMP, and it showed that the elevated serum CEA at the time of recurrence indicated a poor prognosis, which could provide information related to disease progression. With the increase of CEA and CA19-9 in PMP patient after second operation, the prognosis became worse. Vascular tumor emboli are the result of a series of pathological changes in the lymphatic and hematologic system during tumor invasion and metastasis. It is closely associated with poor prognosis of various malignant tumor, including gastric cancer, colorectal cancer, and lung cancer [23]. Previous studies have shown that patients with high cell density had a high risk of relapse, while our

study shows TSR is an independent prognostic factor. The survival of patients with high TSR is significantly shorter than that of patients with low TSR, that is, the tumor area proportion is increased, and the prognosis of patients is worse, which is similar to previous studies. However, our study took stromal components into account, and analyzed the effects of both tumor and stroma on tumor biological behavior of PMP. Large amount of mucus aggregation in stroma leading to intestinal obstruction is the main cause of death in PMP patients, and the study of stroma is also of great significance for the prognosis of PMP patients [24].

Our study showed that patients with low TSR had better prognosis, which is contrary to other solid tumors. Several factors may account for these differences. First, the recurrence risk of PMP with high cell density is relatively higher, indicating that the large the tumor area, the stronger the invasion and metastasis of PMP [14]. Secondly, as a special clinical malignancy syndrome, PMP is characterized by vigorous mucus secretion, and a large amount of mucus accumulates in the stroma and envelops the tumor, thus significantly increasing the stroma area of PMP [25]. Finally, due to the lack of quantitative pathological studies on PMP and the relatively small sample size in this study, the result may be limited to some extent, which is also the weakness of this study. Future studies with large sample size and multiple centers are warranted to verify the findings from this study.

## Conclusion

This study found that TSR was closely related to histopathological types and Ki67, two features of tumor aggression and proliferation, indicating that TSR could help evaluate the biological behavior of PMP. Moreover, TSR has significant impact on PMP prognosis, suggesting it may be a promising new prognostic indicator for PMP.

## Abbreviations

PMP: Pseudomyxoma peritonei; TSR: Tumor-stroma ratio; DPAM: Disseminated peritoneal adenomucinosis; PMCA: Peritoneal mucinous carcinomatosis; ROC: Receiver operating characteristic; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9; CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; PSOGI: Peritoneal Surface Oncology Group International; HE: Hematoxylin and eosin; IPP: Image-Pro Plus; IHC: Immunohistochemistry; MMR: Mismatch repair; BMI: Body mass index; KPS: Karnofsky; PCI: Peritoneal cancer index; CC: Completeness of cytoreduction; OS: Overall survival; CA125: Carbohydrate antigen 125.

## Declarations

### Acknowledgments

Not applicable.

### Authors' contributions

Ru Ma contributed to the design of the study, data collection and analysis, writing of the manuscript; Yu-Lin Lin participated in data collection and manuscript writing; Xin-Bao Li participated in data collection and analysis; Feng-Cai Yan contributed to the collection of pathological images of PMP; Hong-Bin Xu and Zheng Peng revised the manuscript; and Yan Li designed the content of the manuscript, directed and revised the writing.

## **Funding**

This manuscript is supported by the General Program of National Natural Science Foundation of China, NO. 82073376; Beijing Municipal Administration of Hospitals' Ascent Plan, NO. DFL20180701; Beijing Municipal Grant for Medical Talents Group on Peritoneal Surface Oncology, NO. 2017400003235J007.

## **Availability of data and materials**

All data generated or analysed during this study are included in this published article.

## **Ethics approval and consent to participate**

This retrospective study was approved by the Ethics Committee of Beijing Shijitan Hospital, Capital Medical University [2019 Research Ethics Review No. (8)].

## **Consent for publication**

Not applicable.

## **Competing interests**

The authors declare that they have no competing interests.

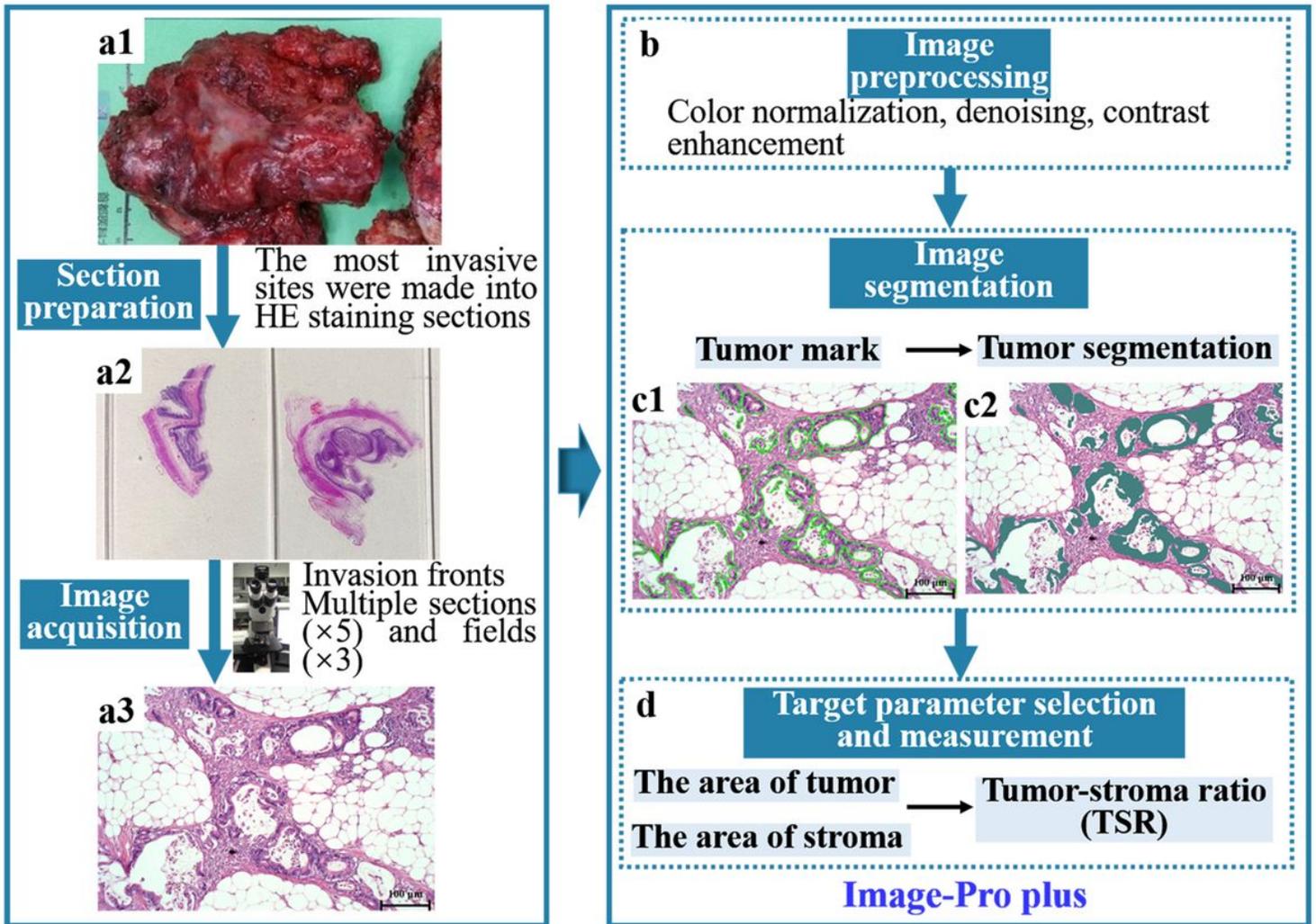
## **References**

1. Rizvi SA, Syed W, Shergill R. Approach to pseudomyxoma peritonei. *World J Gastrointest Surg.* 2018; 10(5): 49-56.
2. Li XB, Ma R, Ji ZH, Lin YL, Zhang J, Yang ZR, et al. Perioperative safety after cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy for pseudomyxoma peritonei from appendiceal origin: Experience on 254 patients from a single center. *Eur J Surg Oncol.* 2020; 46(4 Pt A): 600-6.
3. Carr NJ, Cecil TD, Mohamed F, Sobin LH, Sugarbaker PH, Gonzalez-Moreno S, et al. A consensus for classification and pathologic reporting of pseudomyxoma peritonei and associated appendiceal neoplasia: The results of the peritoneal surface oncology group international (PSOGI) modified delphi process. *Am J Surg Pathol.* 2016; 40(1): 14-26.
4. Vangangelt KMH, Green AR, Heemskerk IMF, Cohen D, van Pelt GW, Sobral-Leite M, et al. The prognostic value of the tumor-stroma ratio is most discriminative in patients with grade III or triple-negative breast cancer. *Int J Cancer.* 2020; 146(8): 2296-304.

5. Aurello P, Berardi G, Giulitti D, Palumbo A, Tierno SM, Nigri G, et al. Tumor-stroma ratio is an independent predictor for overall survival and disease free survival in gastric cancer patients. *Surgeon*. 2017; 15(6): 329-35.
6. van Pelt GW, Krol JA, Lips IM, Peters FP, van Klaveren D, Boonstra JJ, et al. The value of tumor-stroma ratio as predictor of pathologic response after neoadjuvant chemoradiotherapy in esophageal cancer. *Clin Transl Radiat Oncol*. 2020; 20: 39-44.
7. Smit M, van Pelt G, Roodvoets A, Meershoek-Klein Kranenbarg E, Putter H, Tollenaar R, et al. Uniform noting for international application of the tumor-stroma ratio as an easy diagnostic tool: Protocol for a multicenter prospective cohort study. *JMIR Res Protoc*. 2019; 8(6): e13464.
8. Merrell DS, McAvoy TJ, King MC, Sittig M, Millar EV, Nieroda C, et al. Pre- and post-operative antibiotics in conjunction with cytoreductive surgery and heated intraperitoneal chemotherapy (HIPEC) should be considered for pseudomyxoma peritonei (PMP) treatment. *Eur J Surg Oncol*. 2019; 45(9):1723-6.
9. Stearns AT, O'Dwyer ST. Aso author reflections: Quality of life after hipec for pseudomyxoma peritonei. *Ann Surg Oncol*. 2018; 25(Suppl 3): 739-40.
10. Zhou S, Zhao H, He X. The prognostic impact of pathology on patients with pseudomyxoma peritonei undergoing debulking surgery: A systematic review and meta-analysis of retrospective studies. *Front Surg*. 2020; 7: 554910.
11. Mohamed F, Gething S, Haiba M, Brun EA, Sugarbaker PH. Clinically aggressive pseudomyxoma peritonei: A variant of a histologically indolent process. *J Surg Oncol*. 2004; 86(1): 10-5.
12. Baratti D, Kusamura S, Milione M, Bruno F, Guaglio M, Deraco M. Validation of the recent psogi pathological classification of pseudomyxoma peritonei in a single-center series of 265 patients treated by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Ann Surg Oncol*. 2018; 25(2): 404-13.
13. Horvath P, Yurttas C, Birk P, Struller F, Konigsrainer A. Cellularity in low-grade pseudomyxoma peritonei impacts recurrence-free survival following cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *Langenbecks Arch Surg*. 2018; 403(8): 985-90.
14. Bhatt A, Mishra S, Prabhu R, Ramaswamy V, George A, Bhandare S, et al. Can low grade PMP be divided into prognostically distinct subgroups based on histological features? A retrospective study and the importance of using the appropriate classification. *Eur J Surg Oncol*. 2018; 44(7): 1105-11.
15. Calabrò ML, Lazzari N, Rigotto G, Tonello M, Sommariva A. Role of epithelial-mesenchymal plasticity in pseudomyxoma peritonei: Implications for locoregional treatments. *Int J Mol Sci*. 2020; 21(23): 9120.
16. Vangangelt KMH, van Pelt GW, Engels CC, Putter H, Liefers GJ, Smit V, et al. Prognostic value of tumor-stroma ratio combined with the immune status of tumors in invasive breast carcinoma. *Breast Cancer Res Treat*. 2018; 168(3): 601-12.
17. Wu JY, Liang CX, Chen MY, Su WM. Association between tumor-stroma ratio and prognosis in solid tumor patients: A systematic review and meta-analysis. *Oncotarget*. 2016; 7(42): 68954-65.

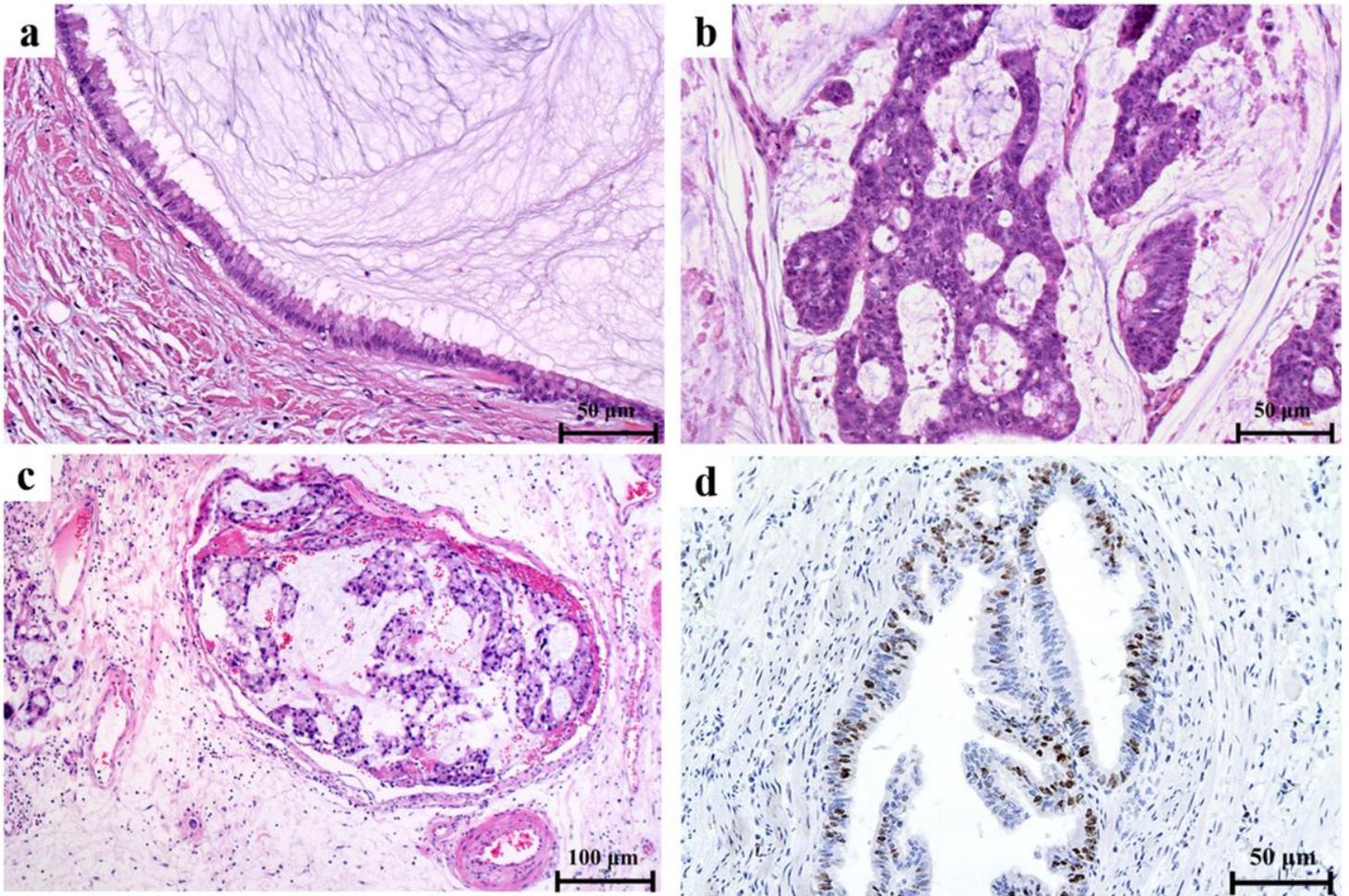
18. Sandberg TP, Stuart M, Oosting J, Tollenaar R, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. *BMC Cancer*. 2019; 19(1): 284.
19. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer. *Mol Med Rep*. 2015; 11(3): 1566-72.
20. Yan F, Lin Y, Zhou Q, Chang H, Li Y. Pathological prognostic factors of pseudomyxoma peritonei: Comprehensive clinicopathological analysis of 155 cases. *Hum Pathol*. 2020; 97: 9-18.
21. Choudry HA, Pai RK, Shuai Y, Ramalingam L, Jones HL, Pingpank JF, et al. Impact of cellularity on oncologic outcomes following cytoreductive surgery and hyperthermic intraperitoneal chemoperfusion for pseudomyxoma peritonei. *Ann Surg Oncol*. 2018; 25(1): 76-82.
22. Carmignani CP, Hampton R, Sugarbaker CE, Chang D, Sugarbaker PH. Utility of CEA and CA 19-9 tumor markers in diagnosis and prognostic assessment of mucinous epithelial cancers of the appendix. *J Surg Oncol*. 2004; 87(4): 162-6.
23. Nummela P, Leinonen H, Jarvinen P, Thiel A, Jarvinen H, Lepisto A. Expression of CEA, CA19-9, CA125, and EpCAM in pseudomyxoma peritonei. *Hum Pathol*. 2016; 54: 47-54.
24. Choudry HA, O'Malley ME, Guo ZS, Zeh HJ, Bartlett DL. Mucin as a therapeutic target in pseudomyxoma peritonei. *Journal of Surgical Oncology*. 2012; 106(7): 911-7.
25. Valasek MA, Pai RK. An update on the diagnosis, grading, and staging of appendiceal mucinous neoplasms. *Adv Anat Pathol*. 2018; 25(1): 38-60.

## Figures



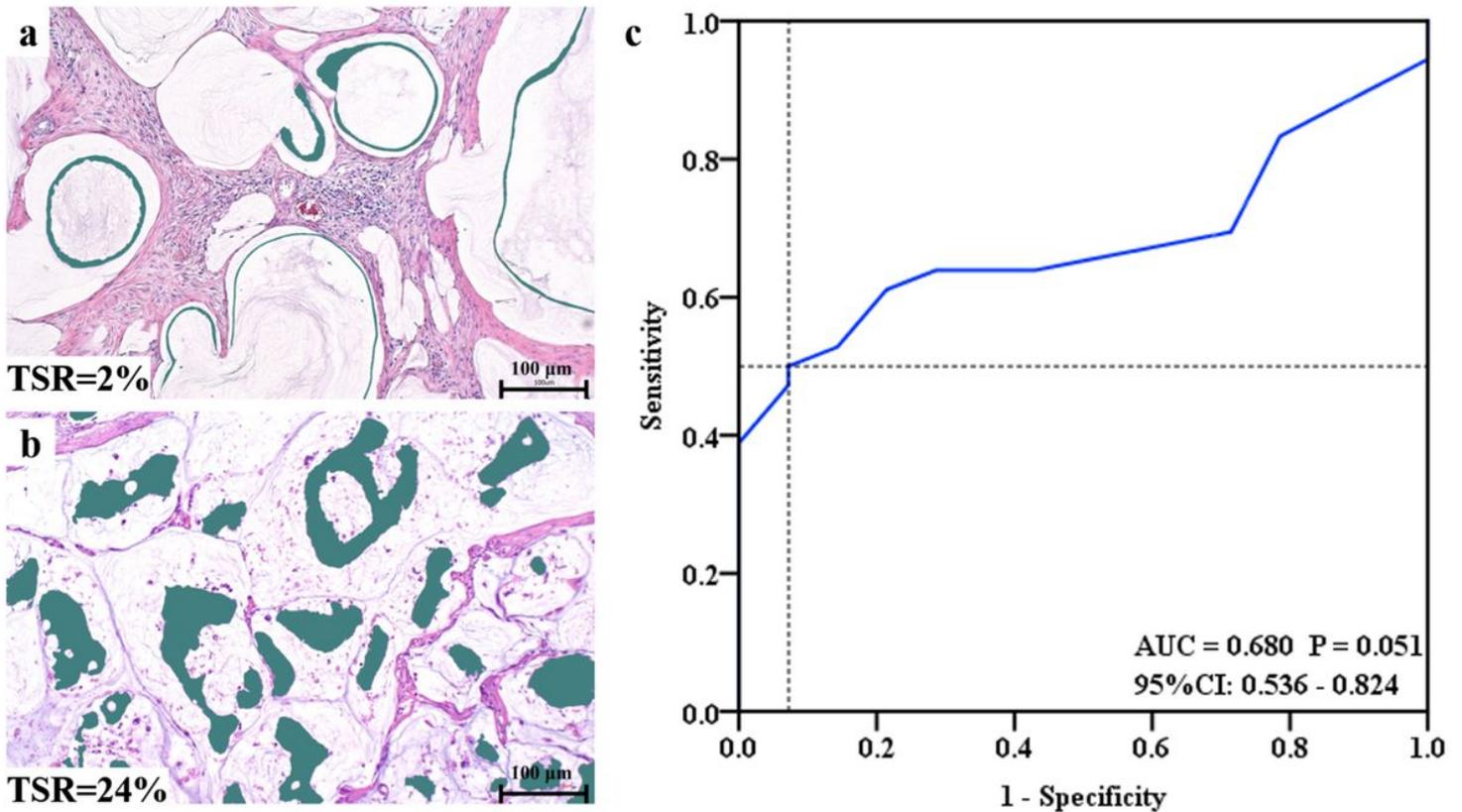
**Figure 1**

The workflow of histopathological quantitative analysis. a1 The most invasive sites of PMP; a2 5 μm HE stained sections; a3 Pathological image of invasion front (HE staining, ×100); b1-2 Tumor and stroma were segmented by Image-Pro Plus (HE staining, ×100).



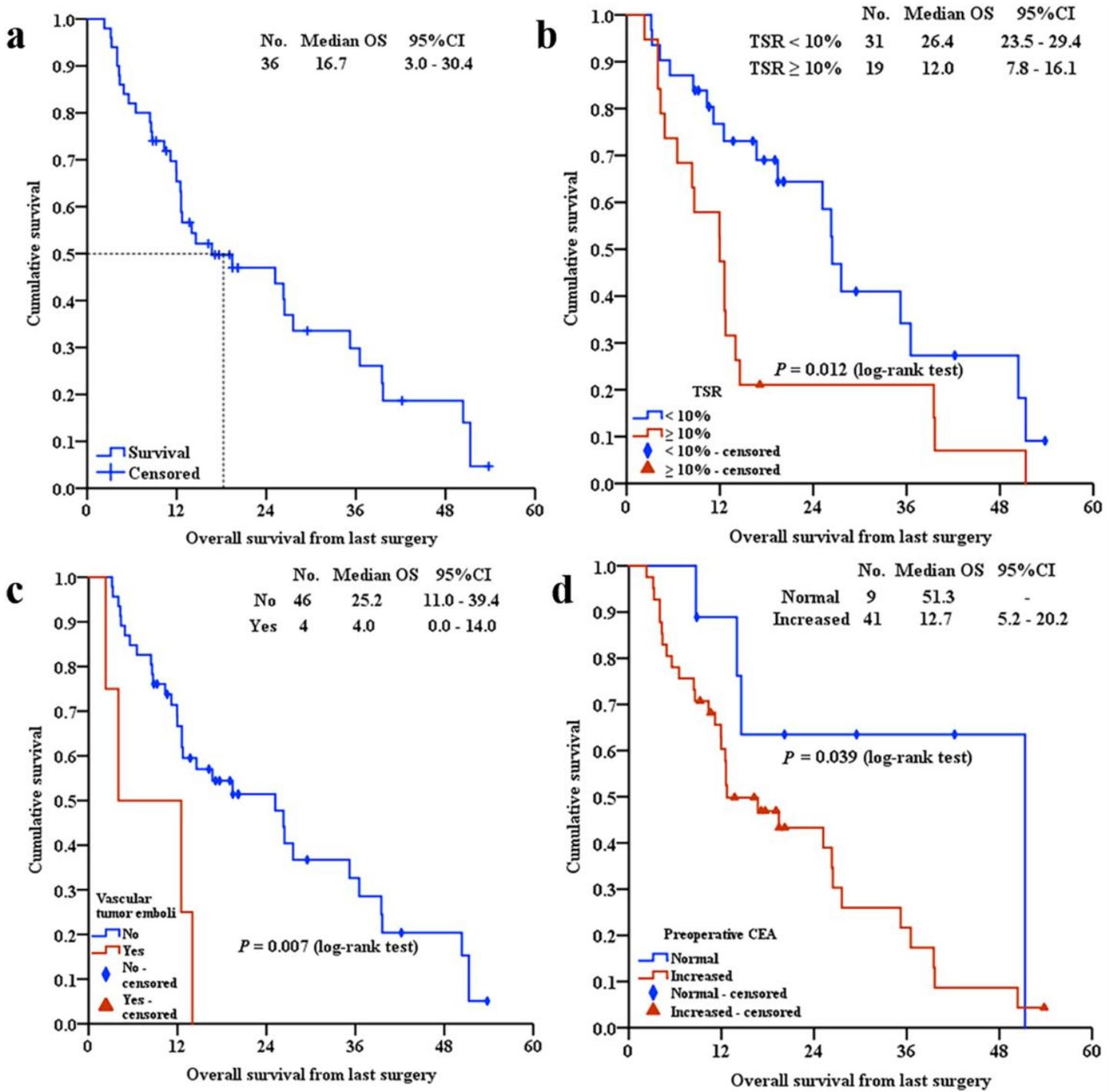
**Figure 2**

Histopathological and immunohistochemical characteristics of PMP. a DPAM: There were few tumor epithelial cells in the abundant mucus. Tumor cells were single-layer banding, with minimal atypia, small and regular nuclei, and few mitoses (HE staining,  $\times 200$ ); b PMCA: There were many epithelial cells in the mucus, which were arranged in the shape of cribriform or island. The tumor cells were highly atypical, with large and prominent nucleoli, and numerous mitosis (HE staining,  $\times 200$ ); c PMP patients with vascular tumor emboli (HE staining,  $\times 100$ ); d Ki67 showed partially positive in the nucleus (IHC,  $\times 200$ ). PMP: Pseudomyxoma peritonei; DPAM: Disseminated peritoneal adenomucinosis; PMCA: Peritoneal mucinous carcinomatosis; HE: Hematoxylin and eosin; IHC: Immunohistochemistry.



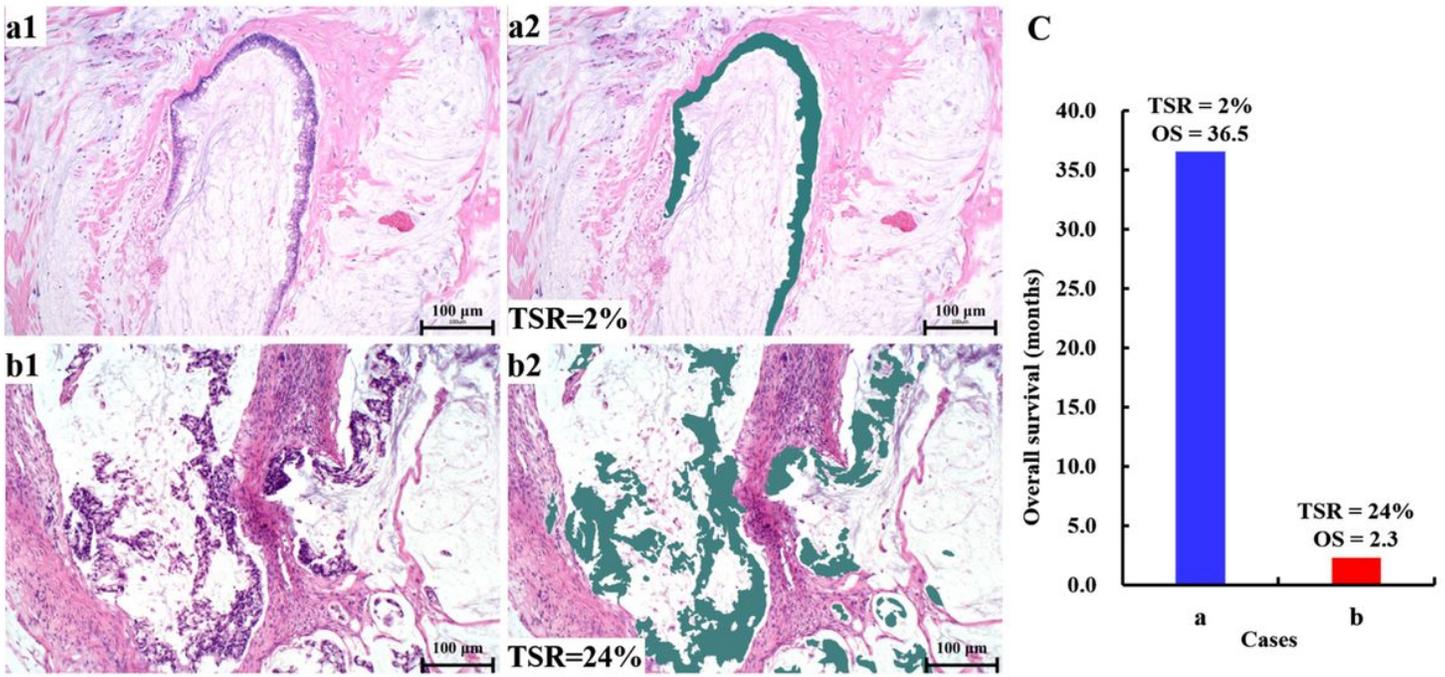
**Figure 3**

The description of TSR. a The pathological image of patient with minimum TSR of 2%; b The pathological image of patient with maximum TSR of 24% (A, B: HE staining,  $\times 100$ ); c ROC curve of TSR on overall survival of 50 patients. The dotted line indicated that TSR of 10% was the best cutoff value with high sensitivity and specificity. TSR: Tumor-stroma ratio; HE: Hematoxylin and eosin; ROC: Receiver operating characteristics.



**Figure 4**

Survival curve and univariate analysis of 50 PMP patients. a Survival curve; b TSR; c Vascular tumor emboli; d Preoperative CEA. PMP: Pseudomyxoma peritonei; TSR: Tumor-stroma ratio; OS: Overall survival.



**Figure 5**

Examples of survival associated TSR extracted from histopathological images of PMP. a1-a2 Histopathological images of patient with the minimum TSR (2%) (HE staining,  $\times 100$ ); b1-b2 Histopathological images of patient with the maximum TSR (24%) (HE staining,  $\times 100$ ). The second column images are segmentation results of histopathological images. c The corresponding overall survival of cases respectively. TSR: Tumor-stroma ratio; PMP: Pseudomyxoma peritonei; OS: Overall survival.