Original Article

Risk factors predicting the occurrence of metachronous ovarian metastasis of gastric cancer

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Background: Ovarian metastasis following radical gastrectomy, also known as metachronous ovarian metastasis (MOM), pose a significant threat to the long-term survival of female gastric cancer (GC) patients. However, a mechanism to identify and characterize operated patients at high risk of developing MOM remains unknown. This retrospective study aimed to identify risk factors for the occurrence of MOM based on the profiling of clinicopathological parameters and expression of sex hormone receptors (SHR) of operated GC patients with and without ovarian relapse.

Methods: The clinicopathological data of 1,055 female GC patients from two medical centers who underwent surgery between January 2011 and December 2015 were reviewed. A total of 378 patients with and without the occurrence of MOM met the eligibility criteria, including the availability of medical records, adequacy of lymph node dissection, completeness of clinicopathological data, sufficient follow-up time, and no administration of neoadjuvant chemotherapy were selected for further analysis. Expressions of estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and progesterone receptor (PR) were detected by immunohistochemical staining on the surgical specimens of patients, and retrospective statistical analyses identified independent risk factors for the occurrence of MOM. A risk prediction model in the format of a polygenic hazard score (PHS) for the occurrence of MOM was established by introducing and modifying the previously validated polygenic risk score (PRS)/PHS.

Results: A Cox regression-based multivariate analysis identified premenopausal with an HR of 3.15 (95% CI, 1.66–5.98), more advanced pathological T stage with an HR of 3.79 (95% CI, 2.14–6.69), more advanced pathological N stage with an HR of 1.85 (95% CI, 1.35–2.54), and negative expression of ERβ with an HR of 0.33 (95% CI, 0.15–0.7) as independent risk factors for the occurrence of MOM ($P<0.01$). Accordingly, a PHS for the occurrence of MOM was established, with 1-, 2-, and 3-year ovarian relapse rates for the high-risk group estimated at 17.8%, 33.7%, and 46.2%, respectively.

Conclusions: Premenopausal status, depth of tumor invasion, number of positive lymph nodes, and negative expression of ERβ were independent factors for the occurrence of MOM. More frequent follow-up examinations are recommended to provide timely diagnosis and medical intervention.

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Keywords: Gastric cancer (GC); metachronous ovarian metastasis (MOM); risk factor; estrogen receptor beta (ERβ)

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Introduction

Gastric cancer (GC) is one of the most common and deadly malignancies worldwide. Although extensive efforts are made to improve the long-term survival rate, it remains the 3rd most lethal cancer, according to GLOBOCAN 2018 statistics (1). Distant metastasis occurs in approximately half of GC patients and significantly contributes to treatment failure and poor prognosis (2), and the ovary is a major target organ for the metastasis of multiple nongynecologic malignancies, especially gastric and colorectal cancer (3,4). While ovarian metastasis may be synchronously identified at the initial diagnosis of GC, a proportion of patients develop ovarian relapse after radical gastrectomy, which is termed as metachronous ovarian metastasis (MOM) (5). MOM occurs in 0.3–6.7% of GC patients undergoing radical surgery, and its incidence rate reaches up to 41% by autopsy (6). The prognosis of GC patients with MOM is reportedly poor, as its median survival time is less than 14 months and 3-year survival rate less than 10% (7). Although previous studies have characterized the clinical features of these patients and discussed potentially beneficial treatment strategies, risk factors for the occurrence of MOM after initial surgical procedures remain largely unknown. One study published approximately 20 years ago reported a significant association between the number of positive lymph nodes and patient age on the risk of post-gastrectomy ovarian relapse (8). Correlation between the expression status of sex hormone receptors (SHR) such as estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and progesterone receptor (PR) and GC progression and its ovarian metastasis have also been investigated (9-11).

Nevertheless, the predictive role of SHR expression in the recurrent ovarian metastasis of operated GC patients remains unclarified. Therefore, we performed this retrospective study to characterize the independent risk factors for the occurrence of MOM based on the systematic profiling of clinicopathological parameters and the expression status of ERα, Erβ, and PR in operated GC patients. Accordingly, we further established a risk prediction model for MOM development to identify operated GC patients at high risk of ovarian relapse so that tailored follow-up examination and treatment could be applied promptly. We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/atm-21-1419).

Methods

Patients

We initially evaluated the medical records of 1,055 female GC patients receiving D2 gastrectomy in the Fudan University Shanghai Cancer Center and Ruijin Hospital affiliated to the Shanghai Jiaotong University School of Medicine from January 2011 to December 2015. The eligibility criteria for the study included the availability of medical records, adequacy of lymph node dissection, completeness of clinicopathological data, sufficient follow-up time, and no administration of neoadjuvant chemotherapy, which led to a total of 378 patients meeting all criteria and selected for further analysis (*Figure 1*). Clinicopathological data including age, the status of menstruation, vessel and lymphatic thrombus, WHO pathological classification, tumor differentiation, depth of tumor invasion (pathological T stage, pT stage in short), number of positive lymph nodes (pathological N stage, pN stage in short), and the American Joint Committee on Cancer (AJCC) staging system were collected and are summarized in *Table 1*. Among all reviewed patients, 55 developed ovarian relapse after receiving radical D2 gastrectomy followed by chemotherapy, mainly comprised of fluorouracil, oxaliplatin, calcium folinate, and docetaxel. Most patients with ovarian metastasis recurrence (90.9%, 50/55) underwent exploratory laparotomy followed by either curative or palliative metastasectomy based on the distribution of metastatic foci remaining five patients did not undergo surgery and received palliative chemotherapy.

Follow-up

Follow-up was performed during outpatient visits at

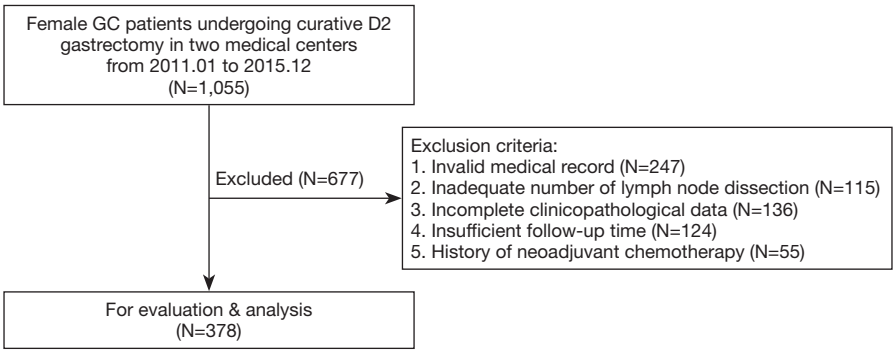

Figure 1 Schematic diagram of eligibility/exclusion criteria.

1 month after the operation and then at 3–6 months intervals in the first year and twice per year after that. Regular examinations included physical examination, tumor marker testing, and abdominal and pelvic ultrasonography. Abdominal and pelvic computed tomography (CT) with or without intravenous contrast was either performed every 6 months or when other regular examinations suggested pelvic occupation, and other auxiliary examinations such as positron emission tomography-computed tomography (PET-CT) and single-photon emission computed tomography (SPECT) were applied when distant metastasis was suspected. Confirmation of MOM was based on either pathological inspection of surgically removed metastatic foci or imaging diagnosis, including pelvic CT/PET-CT scanning. For all 55 patients with the occurrence of MOM, metastatic foci-related information, including the laterality of ovarian foci, the presence/absence of ascites, distribution of metastatic foci, and tumor marker tests 3–6 months before the diagnosis of MOM were collected and are summarized in *Table 2*. Information on the status and outcome of patients were obtained via admission records, outpatient clinic, follow-up telephone call, and E-mail. Follow-up was terminated when MOM was clinically and pathologically diagnosed, and the median follow-up time was 41 months.

Pathology and immunohistochemistry

Upon completion of the initial curative gastrectomy and secondary metastasectomy, fresh tumor tissues were fixed in 10% neutralized formalin, embedded in paraffin, and processed for hematoxylin and eosin (H&E) staining, which were further used for pathological diagnosis and staging. Paraffin blocks were stored at room temperature within two weeks and at 4 degrees C for long-term use. The 8th edition of The American Joint Committee on Cancer (AJCC) was applied to determine the pathological TNM stage of primary GC based on the depth of tumor invasion (pT stage), number of tumor-invaded lymph nodes (pN stage), and absence/presence of distant tumor metastasis (pM stage). Ovarian metastasis of GC was diagnosed according to (I) the history of primary gastric adenocarcinoma; (II) the histological correspondence between primary tumor and ovarian metastasis; and (III) the representative morphology by histological inspection such as poorly cohesive/signet-ring cells (12,13).

Fixed and embedded paraffin blocks were processed into tissue microarray (TMA) to determine the expression status of ERα, ERβ, and PR on primary tumor specimens, and the construction and storage of TMA and immunohistochemical staining of these markers were performed according to the methodology published by Ryu *et al.* (9). Briefly, the specimens underwent sequential processes, including deparaffinization by xylene, rehydration by graded ethanol, and heat-induced epitope retrieval. Sections were then subjected to incubation with H_2O_2, phosphate-buffered saline (PBS) washing, and blocking with 3% hydrogen peroxide followed by pre-immune goat serum. Following this, the sections were incubated with primary antibodies against ERα, Erβ, and PR, followed by incubation with secondary biotinylated anti-mouse/rabbit antibodies. Processed sections were visualized after incubation with red chromogen at 40°C, and further counterstained using the Mayer hematoxylin method. The products of primary antibodies included anti-ERα (ab37438, dilution 1:200; Abcam, Cambridge, UK), anti-ERβ (ab288, dilution 1:100; Abcam), and anti-PR (ab16661, dilution 1:100; Abcam). The definition of positivity was based on the scoring system

Table 1 Clinicopathological parameters of 378 female GC patients with or without MOM

Variables	Patient without MOM (%)	Patient with MOM (%)	P value*
Age (years)			
>50	217 (67.2)	15 (27.3)	<0.001
≤50	106 (32.8)	40 (72.7)	
Status of menstruation			
Non-pausal	221 (68.4)	13 (23.6)	<0.001
Menopausal	102 (31.6)	42 (76.4)	
Vessel/lymphatic thrombus			
Negative	182 (56.3)	3 (5.5)	<0.001
Positive	141 (43.7)	52 (94.5)	
WHO histologic classification			
Tubular	15 (4.6)	2 (3.6)	0.881
Papillary	5 (1.5)	1 (1.8)	
Mucinous	12 (3.7)	2 (3.6)	
Signet-ring cell	106 (32.8)	22 (40.0)	
Mixed subtype	185 (57.3)	28 (50.9)	
Differentiation			
High	21 (6.5)	0 (0.0)	<0.001
Medium	70 (21.7)	1 (1.8)	
Low	232 (71.8)	54 (98.2)	
Depth of tumor invasion (= pathological T stage)			
T1	180 (55.7)	1 (1.8)	<0.001
T2	27 (8.4)	0 (0.0)	
T3	34 (10.5)	4 (7.3)	
T4	82 (25.4)	50 (90.9)	
Number of positive lymph nodes (= pathological N stage)			
N0	189 (58.5)	3 (5.5)	<0.001
N1	47 (14.6)	3 (5.5)	
N2	32 (9.9)	13 (23.6)	
N3	55 (17.0)	36 (65.5)	
AJCC stage			
I–II	241 (74.6)	5 (9.1)	<0.001
III–IV	82 (25.4)	50 (90.9)	

*, Chi-square test. GC, gastric cancer; WHO, World Health Organization; MOM, metachronous ovarian metastasis; AJCC, the American Joint Committee on Cancer.

Table 2 Clinicopathological variables of 55 female GC patients with MOM

Variables	Number	Percentage
Laterality of ovarian metastasis		
Unilateral	14	25.5
Bilateral	41	74.5
Ascites		
Presence	34	61.8
Absence	17	30.9
Undetermined	4	7.3
WHO pathological classification		
Signet-ring cell	19	40.4
Mixed subtype	28	59.6
Distribution of metastatic foci		
Confined to the ovary	31	56.4
Disseminated in pelvic peritoneum	7	12.7
Disseminated in abdominal peritoneum	17	30.9
Tumor marker*		
Normal	13	35.1
Elevated CA125	3	8.1
Elevated CA199	2	5.4
Elevated CEA	3	8.1
Elevated CA724	10	27.0
Elevated CA724 + CA199	3	8.1
Elevated CA199 + CA125	1	2.7
Elevated CA199 + CA242	2	5.4

*, tumor markers were regularly tested for 37 patients with MOM. GC, gastric cancer; WHO, World Health Organization; MOM, metachronous ovarian metastasis.

introduced by Gan *et al.* (14), and the inspection and scoring were performed by two independent pathologists who were unaware of the clinical outcomes. The expression status of ERα, Erβ, and PR are presented in *Table 3*.

Statistical analysis

Clinicopathological parameters, tumor marker test results, and the expression status of SHRs of surgical GC patients were included for statistical analyses. Spearman chi-squared test was used to compare the clinicopathological parameters and expression status of SHRs between surgical GC patients with and without MOM. The same statistical methodology was applied to compare tumor marker testing results between patients with ovarian relapse with confined and disseminated metastatic foci. Univariate and multivariate analyses were sequentially conducted using a Cox proportional hazards regression model to determine independent risk factors for the occurrence of MOM. As four independent risk factors, including menstrual status, depth of tumor invasion (pT stage), the number of positive lymph node (pN stage), and expression status of ERβ were determined, a polygenic hazard score (PHS) tool was introduced to establish the risk prediction model as previously described (15). The best cutoff value between high-risk and low-risk groups was defined and manifested via the Survminer R package (https://CRAN.R-project.org/package=survminer). All statistical analyses were performed two-sided at a significance level of P=0.05 with the application of R package version 3.5.1.

Statement of ethics and consent

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the local ethics committee of the Fudan University Shanghai Cancer Center (Number: 050432-4-1911D) and Ruijin hospital affiliated to Shanghai Jiaotong University School of Medicine [Number: (2018) Linlunshen No. 151]. Informed consent was obtained from all patients

Results

Clinicopathological features of female GC patients with and without MOM

Between January 2011 to December 2015, 1,055 female GC patients administered in the Fudan University Shanghai Cancer Center and Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine for radical D2 gastrectomy were reviewed, and a total number of 378 patients meeting all eligibility criteria (described in the section of Methods) were further selected for this retrospective study (illustrated in *Figure 1*). The general clinicopathological parameters of all 378 patients are summarized in *Table 1*. The median follow-up period was 41 months (range, 3–93 months), and 55 patients developed MOM during the post-operative follow-up phase.

Table 3 Expression status of SHRs of female GC patient with or without MOM

Variables	Patients without the occurrence of MOM (%)	Patients with the occurrence of MOM (%)	P value*
ERα expression			
Negative	297 (92.0)	51 (92.7)	1.000
Positive	26 (8.0)	4 (7.3)	
ERβ expression			
Negative	181 (56.0)	47 (85.5)	<0.001
Positive	142 (44.0)	8 (14.5)	
PR expression			
Negative	227 (70.3)	41 (74.5)	0.629
Positive	96 (29.7)	14 (25.5)	

*, Chi-square test. SHR, sex hormone receptor; GC, gastric cancer; MOM, metachronous ovarian metastasis; ERα, estrogen receptor α; ERβ, estrogen receptorβ; PR, progesterone receptor.

Clinical features of the 55 patients with MOM were further characterized, revealing the interval between the initial diagnosis of GC and post-surgical ovarian relapse was on average 20 months (ranging from 3 to 72 months), with up to 40 patients developing MOM within the first two years of gastrectomy. Among all 55 patients, 72.7% (40/55) were under the age of 50, 76.4% (42/55) were premenopausal, and the mean age at initial GC diagnosis was 43 years. Upon the discovery of a mass in the pelvic and/or lower abdominal cavity leading to the suspicion of ovarian relapse, 85.5% of patients (47/55) underwent secondary explorative laparotomy followed by either radical (R0) or palliative (R1) metastasectomy based on the distribution of metastatic foci. Of the 47 patients undergoing laparotomy, unilateral and bilateral ovarian metastasis were found in 13 and 34 patients, respectively. Pathological inspection of the primary gastric lesions showed that all were low differentiation adenocarcinoma with either signet-ring cell carcinoma (19/47) or mixed subtype (28/47) of histology, and the same findings were witnessed on their matching metastatic ovarian lesions, indicating the consistency of pathological types between paired primary and metastatic tumors. Remarkably, we also found that metastatic foci were either confined to the ovary in 25 operated patients and disseminated in the pelvic and/or abdominal peritoneum in the other 22. For the other eight patients receiving only palliative chemotherapy, radiographic/imaging examinations indicated that the ovarian tumors were unilateral/bilateral in 1/7 cases and metastatic foci were confined/disseminated in 6/2 cases, respectively. Additionally, the presence/absence of ascites was confirmed in 51 cases, with its presence in four cases was unclear due to technical limitations. All results are summarized in *Table 2*.

Serum tumor markers were routinely tested in 37 patients undergoing metastasectomy. Revision of the tumor markers tested 3–6 months before the diagnosis of MOM revealed that 13 patients (35.1%) were negative for all markers, 3 (8.1%) were positive for CA125, 2 (5.4%) were positive for CA199, 3 (8.1%) were positive for CEA, 10 (27.0%) were positive for CA724, and 6 patients (16.2%) were positive for multiple markers (summarized in *Table 2*). Notably, the positive rate of CA724 in patients with metastatic foci disseminated in the pelvic and/or abdominal peritoneum was borderline significantly higher than patients with metastatic foci confined to the ovary (P=0.05). This indicated that the metastatic foci of female GC patients with positive CA724 were prone to disseminate in the pelvic and/or abdominal cavity rather than remaining confined to the ovaries

Expression status of SHRs of female GC patients with and without MOM

As previous studies have indicated a significant association between the expression status of SHR and prognosis of patients both with and without ovarian metastasis (9,11,14), we investigated whether SHR expression was correlated with MOM. Firstly, we profiled the expression status of SHRs including ERα, Erβ, and PR on the primary

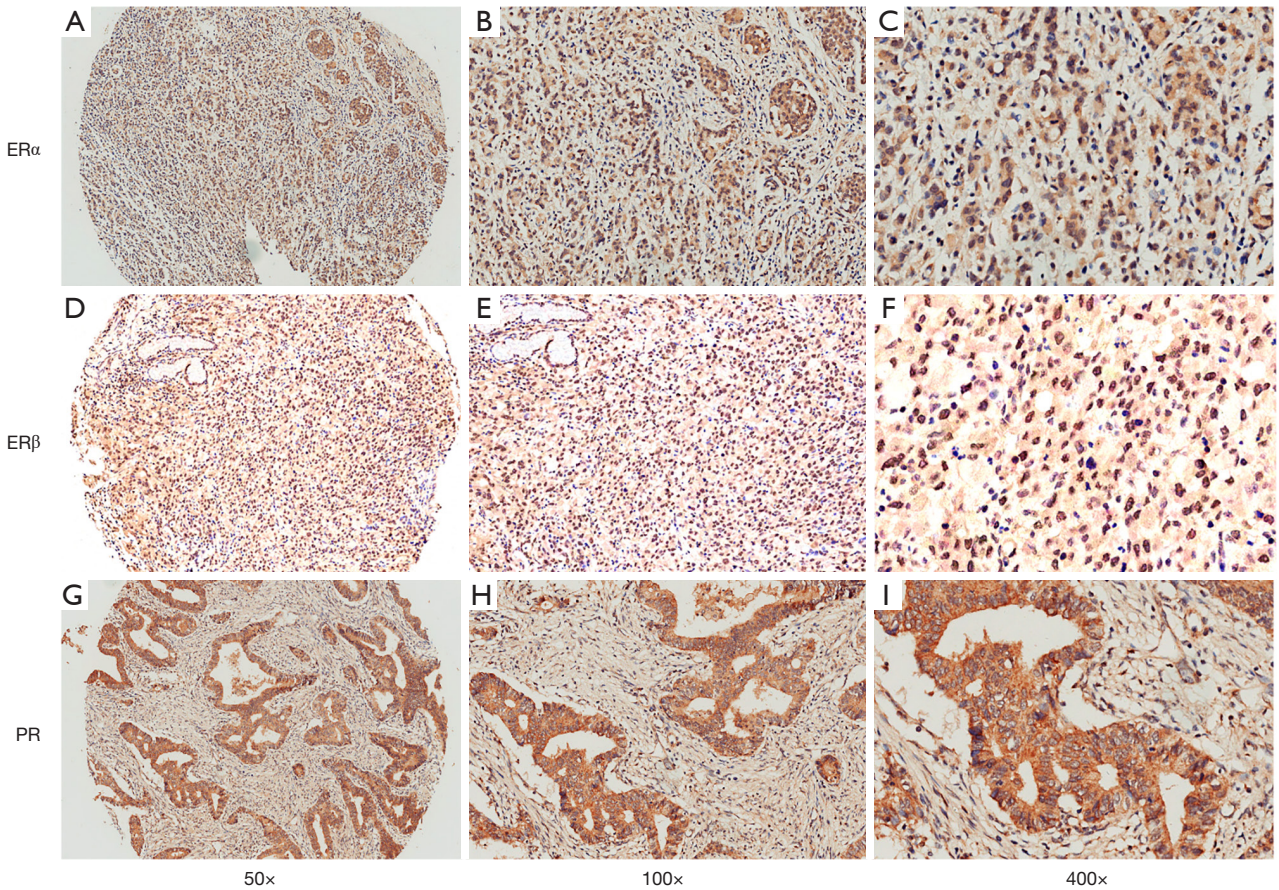

Figure 2 Representative image of immunohistochemical-based staining of ERα, ERβ, and PR in primary gastric cancer tissues. (A,B,C) Positive expression of ERα (A. ×50; B. ×100; C. ×400). (D,E,F) Positive expression of ERβ (D. ×50; E. ×100; F. ×400). (G,H,I) Positive expression of PR (G. ×50; H. ×100; I. ×400). ERα, estrogen receptor α; ERβ, estrogen receptor β; PR, progesterone receptor.

GC specimens of all 378 patients by performing TMA -based immunohistochemical staining of these markers (demonstrated in *Figure 2*). A specific scoring system (described in Methods section) was applied to determine the positivity of ERα/ERβ/PR and showed that for the 323 patients without ovarian relapse after primary radical gastrectomy, the positive expression rate of ERα, ERβ, and PR was 8.0%, 44.0%, and 29.7%, respectively. However, in the 55 patients with MOM, the positive expression rate of ERα, ERβ, and PR was 7.3%, 14.5%, and 25.5%, respectively (summarized in *Table 3*), and the difference between the two groups was significant (P<0.001). On this basis, we further performed immunohistochemical staining of ERβ on the resected ovarian tumors of 47 GC-MOM patients undergoing secondary laparotomy and oophorectomy to evaluate the consistency of ERβ expression between paired primary and metastatic lesions. This revealed that among eight ERβ (+) gastric tumors, seven had matching ovarian tumors with positive ERβ expression, but ERβ expression was not detected in the matching ovarian tumors of the other 39 ERβ (–) gastric tumors (illustrated in Figure S1).

Identification of independent risk factors and establishment of a risk prediction model for MOM

To determine independent risk factors for MOM, we firstly conducted a univariate analysis by taking clinicopathological parameters and SHR expression status into account. This showed that age less than 50 years, premenopausal status, the presence of vessel and/or lymphatic thrombus, low differentiation, more advanced pT stage (depth of tumor invasion), more advanced pN stage (number of the positive lymph node), more advanced AJCC pathological stage, and

Table 4 Univariate Cox regression analysis of clinicopathological variables of female GC patients with MOM

Variables	HR	95% CI	P value
Age (<50 years)	4.74	2.62–8.58	<0.001
Menstruation status (premenopausal)	6.02	3.23–11.21	<0.001
Vessel/lymphatic thrombus (positive thrombus)	22.19	6.91–71.29	<0.001
Differentiation (low differentiation)	16.36	2.35–114.07	0.005
Depth of tumor invasion (more advanced pT stage)	5.76	3.33–9.94	<0.001
Number of positive lymph nodes (more advanced pN stage)	3.01	2.27–4.00	<0.001
AJCC TNM stage (more advanced stage)	35.68	13.69–93.01	<0.001
Expression status of ERα (positive ERα expression)	0.94	0.34–2.61	0.910
Expression status of ERβ (positive ERβ expression)	0.24	0.12–0.52	<0.001
Positive expression of PR (positive PR expression)	0.84	0.46–1.54	0.577

GC, gastric cancer; MOM, metachronous ovarian metastasis; AJCC, American Joint Committee on Cancer; TNM, tumor (T), nodes (N), and metastases (M); HR, hazard ratio; CI, confidence interval; ERα, estrogen receptor α; ERβ, estrogen receptor β; PR, progesterone receptor.

Table 5 Stepwise Cox regression model-based multivariate analysis of clinicopathological variables of female GC patients with MOM

Variables	HR	95% CI	P value
Menstruation status (premenopausal)	3.15	1.66–5.98	<0.001
Depth of tumor invasion (more advanced pT stage)	3.79	2.14–6.69	<0.001
Number of positive lymph nodes (more advanced pN stage)	1.85	1.35–2.54	<0.001
Expression status of ERβ (positive ERβ expression)	0.33	0.15–0.7	0.004

GC, Gastric cancer; MOM, Metachronous ovarian metastasis; HR, hazard ratio; CI, confidence interval; ERβ, estrogen receptor β.

negative expression of ERβ were all significantly associated with an increased risk of MOM (P<0.05). In contrast, the expression status of ERα and PR were statistically irrelevant for ovarian relapse after initial gastrectomy (*Table 4*).

We then conducted a Cox proportional hazards regression model-based multivariate analysis and determined that premenopausal status [hazardous ratio (HR) 3.15; 95% CI, 1.66–5.98], more advanced pT stage (HR 3.79, 95% CI, 2.14–6.69), more advanced pN stage (HR 1.85, 95% CI, 1.35–2.54), and positive expression of ERβ (HR 0.33, 95% CI, 0.15–0.7) were four independent factors predicting the occurrence of MOM (P<0.01) (*Table 5*).

Furthermore, to facilitate the timely detection of operated patients at high risk of developing MOM, we established a risk prediction model in PHS format by adopting and modifying the polygenic risk score (PRS) and PHS as previously described (15,16). The calculation of PHS was based on the four independent factors and their corresponding HR values (see equation below).

$$Polyfactorial\ Hazard\ Score\ (PHS)=\sum_{i}^{n} HR_i \times independent\ factor_i \quad [1]$$

As the risk of ovarian relapse is proportional to PHS, we then determined the best cutoff value to discriminate between patients at high and low risk of ovarian relapse, and the probability is manifest in *Figure 3* (P<0.0001). Further, the 1-, 2-, and 3-year occurrence rates of MOM in high-risk patients were estimated at 17.8%, 33.7%, and 46.2%, respectively.

Discussion

Ovarian metastasis from GC, also termed as Krukenberg tumor, was first reported by the German physician Friedrich Ernst Krukenberg in 1896. Metastatic ovarian tumors arise from multiple primary sites, including the gastrointestinal tract, biliary tract, breast, and even

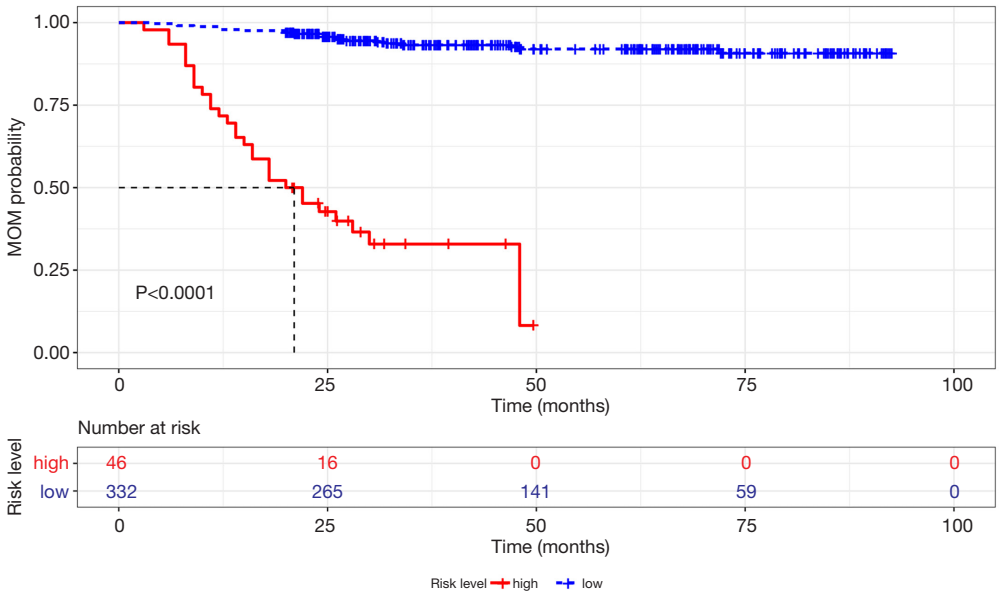

Figure 3 Kaplan-Meier ovarian relapse-free curve demonstrates the rate of MOM of high and low-risk groups partitioned by the best cutoff value. MOM, metachronous ovarian metastasis.

urinary bladder, although the stomach remains the most frequent site of primary tumors (17). Approximately 7% of female GC patients undergoing curative operation develop ovarian reoccurrence, which is termed MOM, and the clinicopathological parameters of these patients have been systematically reviewed in several studies. The most extensive study to date, conducted by Feng *et al.*, reviewed 63 cases of ovarian metastasis after gastrectomy and showed that the mean age of initial diagnosis of GC was 45 years, 65.1% of patients were premenopausal, and the mean interval between ovarian metastasis and primary GC was 16 months. Most primary tumors were reportedly at the T4 pathological stage (87.3%) and N2–3 (68.3%), 85.7% of metastatic ovary foci were bilateral, and 73% of cases were positive for peritoneal membrane seeding, leading to an R0 resection rate as low as 31.7%. Similar findings were demonstrated by other study groups (5,8,18), and data from our investigation generally conforms with these results (see Results section).

The pathogenesis of ovarian metastasis from GC remains undetermined. The classic model of direct tumor seeding across the peritoneal and pelvic cavity has been gradually replaced by recognizing that multiple factors contribute to ovarian metastasis (19). Notably, retrograde lymphatic spread is increasingly recognized as the major route of ovarian metastasis considering the richness of lymphatic tissues in the stomach-ovary axis (12). Malignant cells in the lymphatic plexus of the gastric mucosa and submucosa spread through retroperitoneal lymph nodes and converge with ovarian reticular lymphatic reflux in the waist lymph nodes so that the interlinked lymphatic vessels facilitate the paradoxical metastasis of malignant cells to the ovary (13,20). This is in line with our finding that the occurrence of ovarian metastasis is significantly correlated with the number of positive lymph nodes and is also supported by the previously reported observation that most metastatic foci in the ovary occur in areas of rich lymphatic tissue such as the hilum and cortex, rather than on the surface with where there is little lymphatic content (17). Hematogenous metastasis is also recognized as a significant route of ovarian metastasis as it frequently occurs in premenopausal patients whose ovaries are in a status of greater vascularity (21). Additionally, the observation that metastasis overwhelmingly occurs in bilateral ovaries instead of unilaterally also supports the pattern that cancer cells are spread via a vascular route (5,7).

The prognosis of GC patients developing MOM is remarkably poor, with the median overall survival reportedly ranging from 11 to 21.7 months (5,7,11,18). To address this challenge, the early detection of ovarian reoccurrence is urgently needed to provide timely medical intervention (22,23). Unfortunately, this remains a major

challenge, as most patients with ovarian reoccurrence are either asymptomatic or demonstrate non-specific symptoms such as lower abdominal discomfort, irregular bleeding, and weight loss (19). While it is essential to identify independent risk factors for the occurrence of MOM and establish a risk prediction model accordingly, most previous studies have focused on determining prognostic factors for patients with ovarian reoccurrence rather than identifying specific factors in patients who have undergone surgery and are at high risk of ovarian relapse. However, one study by Kim *et al.* in 1999 reported on independent risk factors for ovarian reoccurrence based on a systematic revision of the clinicopathological parameters of 690 female GC patients undergoing curative gastrectomy (8). In that study, factors including age, menopausal status, histologic classification, Lauren classification, greatest tumor dimension, tumor size, depth of invasion, number of positive lymph node, and AJCC staging were compared between 32 patients with MOM and 658 patients without, and the number of positive lymph nodes and age were reported as two independent risk factors for the occurrence of ovarian relapse. Furthermore, a risk prediction model which partitioned patients into high-risk (positive lymph nodes >6 and age <50 years), intermediate-risk (positive lymph nodes >6 and age ≥50 years) and low-risk groups (positive lymph nodes ≤6 and age </≥50 years) showed an estimated 3-year ovarian relapse rate of 39.5% (95% CI, 23.8–55.7%), 10.7% (95% CI, 0.2–21.1%), and 2.1% (95% CI, 0.5–3.8%), respectively. However, in our analysis, premenopausal patients and those with more advanced pathological T stage and N stages were significantly associated with an increased risk of ovarian relapse. It is worth noting that menstrual status was not identified as an independent risk factor in the study by Kim *et al.* as their record of menstrual status was incomplete, despite their compensation by using the average Korean women menopausal age (50 years) as a cutoff value. By contrast, our complete record of menstrual status enabled us to incorporate this factor into multivariate analysis in which premenopause outcompeted age as an independent risk factor.

Another independent risk factor for ovarian recurrence identified in our study was the negative expression of ERβ. This finding highlighted the significance of estrogen and its receptors in GC progression and its ovarian metastasis, especially considering the impact of elevated sex hormone activity on the reproductive organs, including the ovaries, in young females (24,25). Estrogen exerts a broad influence on multiple aspects of malignant cells, the process of which is mainly mediated by its receptors such as ERα and ERβ (26). Once activated by estrogen, ER translocates into the nucleus and function as nuclear transcription factors that influence the transcription activity of target oncogenic or tumor-suppressing genes directly and/or indirectly binding to their regulation domains (27). ERs can induce transcription by binding to a cognate DNA binding element called estrogen response elements (ERE) within the promoters and/or enhancers of target genes with a significant impact on cell growth and differentiation in breast and prostate cancer (28,29), and it is increasingly recognized that ERβ mainly functions as a tumor suppressor which could potentially serve as a treatment target in cancer therapy (30). The role of ERβ in GC progression has been broadly investigated in previous studies, including Ryu *et al.*, who profiled its expression status in 148 GC patients and found ERβ was more highly expressed in the older age group, pT1/2 stage tumor group, and Lauren's intestinal type group than in the younger age, T3/4 stage tumor and Lauren's diffuse type group. More importantly, those authors found that the negative expression of ERβ was significantly associated with a higher rate of GC recurrence and a worse 3-year overall survival rate (9). Their conclusions were also in line with other studies, which suggested the negative expression of ERβ was correlated with an unfavorable prognosis in GC patients (31-33). On the other hand, the significance of SHR expression in ovarian metastasis of patients with GC was also investigated. Yan *et al.* reviewed the clinicopathological data and expression status of ERα, Erβ, and PR in 103 GC patients with synchronous ovarian metastasis (SOM) undergoing treatment in a single cancer center. They found that the negative expression of ERβ and PR were both favorable prognostic factors for overall survival (34). They further conducted a similar study by reviewing the same parameters in 152 patients (93 patients with SOM and 59 patients with MOM), and the results also indicated that the negative expression of ERβ (HR 0.404; 95% CI, 0.251–0.648; P<0.001) and PR (HR 0.496; 95% CI, 0.301–0.817; P<0.001) unfavorably predicted the long-term survival of GC patients with ovarian metastasis (11). Unfortunately, although numerous studies have been conducted to investigate the prognostic value of SHR expression in GC patients with ovarian metastasis, the potential predictive value of SHR expression in the occurrence of MOM has not yet been specifically addressed, and our investigation is so far the only known study addressing this issue. We compared the clinicopathological features and SHR

expression between operated GC patients with and without ovarian relapse. We conducted a Cox regression model-based univariate and multivariate analysis, which identified negative expression of ERβ as an independent risk factor for the reoccurrence of ovarian metastasis. Our study confirmed the correlation between ERβ expression and the risk of ovarian relapse for GC patients undergoing curative surgery for the first time.

To further facilitate the early detection of ovarian relapse, we established a risk prediction model based on four independent risk factors to identify patients at high risk of developing MOM. Here, we referred to the PRS model, which initially predicted the genetic predisposition for disease based on associated variants such as single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) (15). This model essentially aggregates the impact of genetic variants into a specific value that proportionally assesses the risk for a given disease such as cancer (16,35). In our risk prediction model, we proposed the PHS by adopting the essence of PRS with modification. We replaced genetic variants with four independent risk factors, which were given weightings according to their hazardous ratio (HR) calculated by multivariate analysis. With the application of PHS, we were able to estimate the 1-, 2-, and 3-year occurrence rate of MOM for operated GC patients. As PHS partially enables the quantification of the risk of ovarian reoccurrence, we could better determine which group of operated patients are prone to develop MOM.

Caution must be exercised when interpreting the independent risk factors and risk prediction model as certain limitations should be considered. Since the incidence of operated GC patients developing MOM remains low, the retrospective nature and relatively low patient numbers in this study could compromise our analyses' quality (36). Furthermore, a small proportion of patients (10.9%, 6/55) developed MOM over 41 months (median follow-up period in our study) after gastrectomy. Nevertheless, given that the median time to ovarian relapse was 20 months after the initial diagnosis of GC, our median follow-up time is conceivably sufficient to perform statistical analysis to identify risk factors for the reoccurrence of ovarian metastasis. Additionally, although the expression status of SHR was included in our analysis, the total number of candidate risk factors for ovarian relapse is still minimal, especially considering that our risk prediction model originated from PRS, which is based on a large number of independent risk variants by remarkably informative GWAS (37).

It is noted in our study that metastatic foci either remained confined to the ovary (56.4%, 31/55) or disseminated into the pelvis (12.7%, 7/55) and abdominal peritoneum (30.9%, 17/55). Previous studies demonstrated that both the treatment strategy and prognosis essentially varied between patients with localized and disseminated metastatic foci. Feng *et al.* reported the highest incidence of peritoneal seeding as 73.0% (46/63) in their systematic revision of patients with MOM and claimed that treatment targeting peritoneal seeding-induced metastasis could improve the prognosis (7). Cheong *et al.* reviewed 34 cases of post-surgical ovarian metastasis and reported that metastatic tumors were either limited to the ovary (26.5%, 9/34) and pelvic cavity (23.5%, 8/34) or beyond (50.0%, 17/34). There was also a significant difference in both median overall survival and progression-free survival between 17 patients receiving total metastasectomy (R0) and 17 patients receiving palliative metastasectomy (R1) (18). Similar results were reported by other groups claiming that patients with confined metastatic foci (ranging from 50.0% to 74.6%) undergoing R0 resection were rendered a significantly prolonged survival benefit compared with patients undergoing palliative operations (5,11). In our study, the number of elevated CA724 of patients with disseminated foci was borderline significantly higher than patients in the confined foci group (P=0.05), indicating that operated patients with ovarian reoccurrence showing abnormally elevated CA724 are more likely to have disseminated metastatic foci, which further indicates R0 resection may not be achievable and a poor prognosis is likely.

In summary, our retrospective study determined premenopausal status, depth of tumor invasion (pT stage), number of positive lymph nodes (pN stage), and negative expression of ERβ as four independent risk factors for MOM in female GC patients undergoing curative gastrectomy. Based on these findings, we further established a risk prediction model which could be applied for the early detection of post-surgical ovarian relapse so that medical intervention could be provided promptly. Future investigations should introduce high throughput technology-based methods (such as genome and/or transcriptome sequencing) to identify more specific factors essentially involved in MOM's process to optimize the risk prediction model in the light of precision completeness. Such sequencing-based technologies could also identify candidate molecular signatures distinguishing patients

with confined and disseminated metastatic foci who will be treated with distinctive strategies.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the local ethics committee of the Fudan University Shanghai Cancer Center (Number: 050432-4-1911D) and Ruijin hospital affiliated to Shanghai Jiaotong University School of Medicine [Number: (2018) Linlunshen No. 151]. Informed consent was obtained from all patients.

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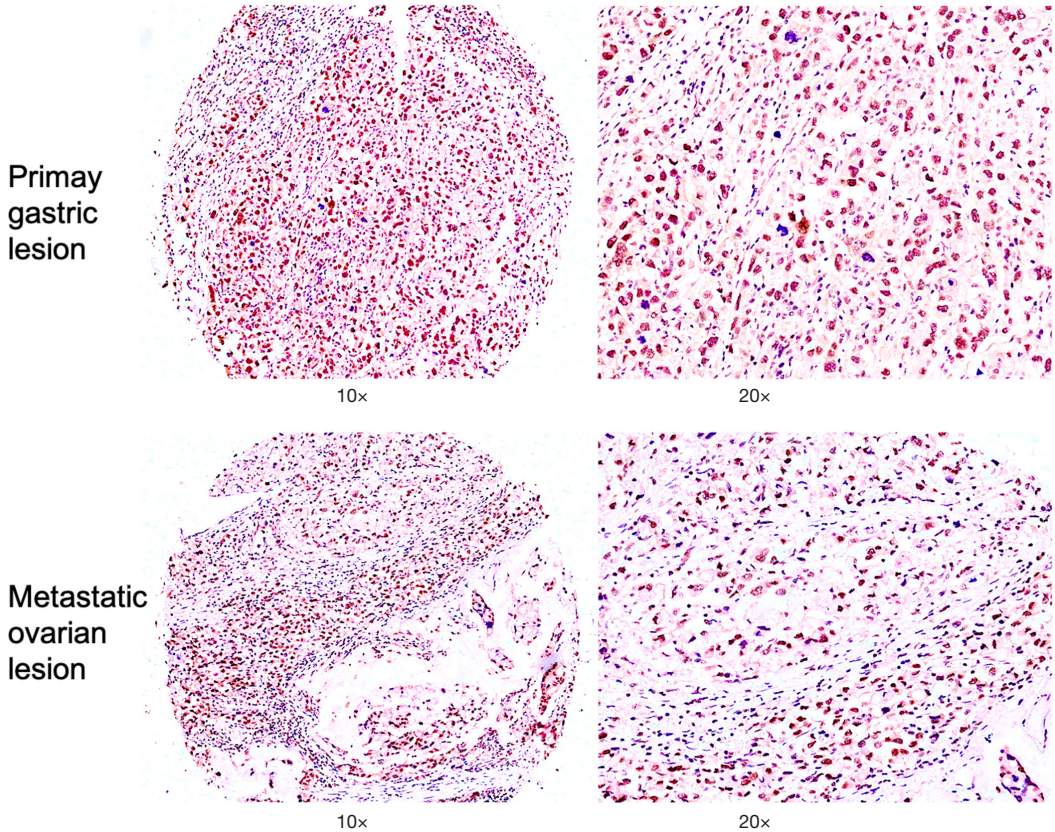

Figure S1 Representative immunohistochemical staining of ERβ on paired tumors (primary gastric tumor *vs.* metastatic ovarian tumor) of operated GC patients with MOM. Erβ, estrogen receptor β; GC, gastric cancer; MOM, metachronous ovarian metastasis.

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