

Analysis of risk factors for post-operative complications and prognostic predictors of disease recurrence following definitive treatment of patients with esophageal cancer from two medical centers in Northwest China

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Abstract. Evaluating the clinicopathological features of patients receiving definitive treatment for esophageal cancer may facilitate the identification of patterns and factors associated with post-operative complications, and enable the development of a surveillance strategy for surviving patients at a higher risk of disease recurrence. In the present study, clinical data from 579 patients with esophageal cancer that underwent radical resection of esophagus were collected. These patients were admitted to two medical centers in Northwest China, and information regarding the presence or absence of basic chronic diseases and post-operative results were retrospectively analyzed. The level of selected stem cell markers, including aldehyde dehydrogenase 1, CD133, integrin subunit α 6, integrin subunit β 4 and T-cell factor-4, were determined in esophageal cancer tissue samples in order

to determine whether these markers may be useful predictors of disease prognosis and recurrence. Post-operative complications in patients receiving radical resection of the esophagus included respiratory system complications, cardiovascular abnormalities and esophageal anastomotic fistulae. Diabetes, basic respiratory disease and lower pre-surgical serum albumin levels were observed to be individual risk factors associated with post-operative complications, including respiratory system complications of acute respiratory failure and pulmonary infection, cardiovascular abnormalities of atrial fibrillation and arrhythmia, as well as the development of esophageal anastomotic fistulae. Diagnosis of esophageal cancer at later stage was significantly correlated with anastomotic fistula. Molecular detection of stem cell markers for prognosis prediction was achieved by immunohistochemical and immunofluorescence staining assays. The results demonstrated that the presence of stem-like cells in cancer tissues was associated with poor disease prognosis and a high recurrence ratio. In conclusion, the results of the current study suggested that post-operative complications were more likely to occur in patients with diabetes, basic respiratory disease or lower serum albumin levels prior to surgery. Therefore, sufficient intensive peri-operative care, rigorous operative risk assessments, and the selection of the patients with early or mid-stage esophageal cancer, may decrease the risk of post-surgical complications in patients receiving radical resection of the esophagus. In addition, a high ratio of esophageal cancer stem-like cells was associated with cancer recurrence. These results suggest that an intensive surveillance strategy should be implemented in order to facilitate early detection of disease recurrence and improve the clinical management of these patients post-surgery.

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Key words: esophageal cancer, post-operative complications, cancer stem-like cells, anastomotic fistula, prognosis prediction

Introduction

As a highly lethal disease and one of the most common malignancies in China and East Asia, the therapeutic strategies and surgical procedures used to treat patients with esophageal cancer have been established and widely accepted for decades. These strategies currently consist of a multimodal treatment procedure consisting of surgery, chemoradiotherapy and neoadjuvant therapy during the peri-operative period (1,2). Surgical resection is generally recommended for the treatment of patients with early-stage esophageal carcinoma. Developments in surgical techniques and multidisciplinary treatments have improved the prognosis of patients with esophageal cancer where recurrence is often inevitable. Despite this, the mortality rate for those with esophageal cancer is ~90% (3-5). In addition, the surgical wound following transthoracic surgery is large, and this procedure typically involves a two or three-field lymphadenectomy. Patients that undergo this procedure suffer from slow wound healing, particularly when there are post-operative complications. Therefore, identifying the factors associated with disease recurrence and post-operative complications may be useful to predict treatment outcome and might improve the long-term consequences of a curative esophagectomy with radical lymph node dissection.

In Northwest China, the incidence of esophageal cancer exhibits epidemiological trends based on regional dietary habits and economic features (6). Esophageal cancer is the one of the leading causes of cancer-associated mortality in China (7-9). In the current retrospective study, the clinicopathological features of patients with esophageal cancer admitted to two medical centers affiliated to Xi'an Jiaotong University in Northwest China, which were treated with curative esophagectomy through the open rhinoplasty approach, were collected. The present study investigated which clinicopathological features were associated with disease recurrence within 2 years of surgery, as well as the characteristics associated with various post-operative complications. The aims of the current study were as follows: i) To evaluate the patterns and occurrence of post-operative complications; ii) to support the development of novel therapeutic regimens for patients with specific underlying diseases, based on the potential risk factors identified; iii) to establish the appropriate application of molecular markers of cancer stem cells identified in previous studies (10-12) as putative prognostic markers; iv) to identify which patients with esophageal cancer may require close follow-up post-surgery, and to develop optimal surveillance strategies.

Materials and methods

Patient enrollment. Between April 2010 and August 2016, 579 Chinese patients with esophageal cancer admitted to The First or Second Affiliated Hospitals of Xi'an Jiaotong University (Xi'an, China) located in Northwest China, underwent an open radical esophagectomy and reconstruction of the esophageal tract, and were evaluated and enrolled in the present study. Patients were diagnosed by pathological examination of tumor tissues obtained during surgery. Clinicopathological features, the presence of underlying chronic diseases and details of

post-operative complications were collected and summarized in Table I.

Enrolled patients consisted of 475 males and 104 females (mean age, 56.3±14.9 years). A total of 499 patients (86.2%) were diagnosed with squamous carcinoma, 71 patients (12.3%) with adenocarcinoma and 8 patients (1.4%) with adenosquamous carcinoma. Post-operative tumor pathologies were classified as stage I, II, III or IV, in accordance with the tumor node metastasis (TNM) classification system defined by the Union for International Cancer Control (UICC International Union Against Cancer, TNM Classification of Malignant Tumors, 7th edition, <http://meteor.aihw.gov.au/content/index.phtml/itemId/403583>). Patients with stage IV esophageal cancer were identified following surgery. Pre-operative or post-operative chemoradiotherapy, chemotherapy or radiotherapy (referred to neoadjuvant therapy) was performed in 425 patients (73.4%). Two independent pathologists performed histopathological evaluations, and patients were not diagnosed with additional malignancies. Written informed consent was obtained from all patients prior to sample collection in accordance with the Declaration of Helsinki. The study protocol and written informed consent statements were approved and supervised by the Ethics Committee of the First Affiliated Hospital and the Second Affiliated Hospital of Xi'an Jiaotong University.

Surgical procedure and therapy strategy. Patients with suspected T3 and T4 tumors received neoadjuvant therapy, whereas additional patients diagnosed with T4 tumors post-surgery were excluded from the study. Patients diagnosed with T1, T2 or T3 tumors were treated with adjuvant chemotherapy or neoadjuvant chemotherapy, which was administered following assessment by a team of independent clinicians. These patients received adjuvant chemotherapy within 2 months prior to or following surgery. Patients received docetaxel (75 mg/m²/day, used on day 1) + cisplatin (25 mg/m²/day, used on day 1 to 3) + fluorouracil (400-600 mg/m²/day, used on day 1 and 2) or cisplatin (25 mg/m²/day, used on day 1 to 3) + calcium folinate (200-400 mg/day, used on day 1 and 2) + fluorouracil (400-600 mg/m²/day, used on day 1 and 2). The radical esophagectomy procedure involved the open rhinoplasty approach, and the operative incision number was 1, 2 or 3, based on the tumor location. Radical lymphadenectomy dissection consisted of removal of the regional lymph nodes, including the middle or lower mediastinal, the peri-gastric, the superior mediastinum and the cervical nodes (three-field lymphadenectomy). During the peri-operative period patients were treated as follows (13,14): Gastrointestinal preparation and parenteral nutrition commenced at 3 days prior to surgery; patients fasted at 1 day prior to surgery; total post-operative parenteral nutrition was provided for at least 7 days, and enteral nutrition commenced with brackish water at 4 days following surgery, depending on patient recovery status. Blood samples were tested for serum albumin level at 1-3 days before surgery and 3 days after surgery. The repair of anastomosed stoma was evaluated by esophagography between 7 and 10 days following surgery. The fistula were diagnosed with clinical symptoms and physical indicators: Fever or hyperpyrexia, cough with phlegm, intense chest pain, thoracic drainage fluid changed to turbid solution, chest X-ray, upper gastrointestinal contrast examination, routine blood test for procalcitonin.

Table I. Clinicopathological features of enrolled patients with esophageal cancer that underwent radical esophagectomy.

Feature	Number
Sex (male/female)	475/104
Median age (years)	56
<65 years	239
≥65 years	340
Diabetes type II (Y/N)	108/471
Hypertension (Y/N)	202/377
Location of tumor in esophagus	
Upper	69
Middle	302
Lower	208
Histological type	
SC	499
AC	71
AS	9
Neoadjuvant or adjuvant therapy (Y/N)	425/154
Pathological tumor stage	
I	51
II	262
III	251
IV	15
Respiratory complications (Y/N)	304/275
Operation time (h)	
<4	163
≥4	416
Anastomotic fistula (Y/N)	46/533

SC, squamous carcinoma; AC, adenocarcinoma; AS, adenosquamous carcinoma; RE, radical esophagectomy.

Immunohistochemistry and immunofluorescence staining. For immunohistochemistry and immunofluorescence staining of clinical tissue samples, formalin-fixed (10% formalin for 15-30 min at room temperature), paraffin-embedded samples were prepared as 4- μ m-thick sections, washed with PBS for 15 min, and permeabilized with 0.2% Triton X-100 for 20 min. Tissue sections were subsequently blocked with BSA (cat. no. 11021037; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C for 30 min, prior to incubation with primary antibodies against CD133 (1:200, cat. no. ab19898; Abcam), aldehyde dehydrogenase 1 (ALDH1, 1:200, cat. no. sc-166362; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), T-cell factor-4 (TCF-4, 1:2,000; cat. no. 05-511; EMD Millipore, Billerica, MA, USA), ITGA6 (1:500, cat. no. HPA027582; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) or ITGB4 (1:500, cat. no. HPA036348; Sigma-Aldrich; Merck KGaA) at 4°C overnight. Tissue sections were then incubated with Alexa Fluor 594-conjugated goat anti-rabbit IgG (H+L) secondary antibody (1:1,000, cat. no. A-11012; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. The nuclei were counterstained with DAPI (1:10,000, cat. no. 4084; Cell Signaling

Technology, Inc., Danvers, MA, USA) at 37°C for 10 min. The fluorescence images were obtained using a fluorescence microscope. Immunohistochemistry and immunofluorescence staining was scored using a semi-quantitative scoring system, which was based on the staining intensity and the proportion of positive cells by analyzing 10 fields of view for each sample. The labels of +, ++, +++ and ++++ were used to denote 1-10%, 10-20%, 20-50% and >50% positive cells, respectively.

Evaluation of patients post-surgery. Enrolled patients were followed up at 6-month intervals for 24 months, commencing at 3 months following surgery. Post-operative complications were primarily classified as the following: Cardiac, including atrial fibrillation and arrhythmia as a result of non-atrial fibrillation disorders; respiratory, including atelectasis or acute respiratory failure, pneumonia or pulmonary empyema; or fistulae (15). Post-operative complications were identified between 1 and 30 days following surgery. Complications including myocardial infarction, diaphragmatic hernia, cerebrovascular accidents, deep vein thrombosis and additional rare events were excluded. Disease recurrence was confirmed by clinical examinations, including radiography, computed tomography (CT) and positron emission tomography (PET)/CT scans, as well as endoscopy procedures, such as an ultrasound endoscopy. The type of disease recurrence was not classified in the present study, with loco-regional, hematogenous and mixed-type esophageal cancer types all considered as disease recurrence. CT scans and tumor biomarkers (carcinoembryonic antigen, cancer antigen (CA) 19-9 and CA-125) were assessed at least twice a year following surgery. PET/CT scans were employed for patients with a high risk of disease recurrence, or those with strong evidence of recurrence from clinical examinations. Endoscopies were performed annually, or when patients were considered to have developed anastomotic stenosis as a result of edema or anastomotic recurrence. The development of a second primary cancer post-surgery was not considered as recurrence.

Statistical analysis. The association between post-operative complications, disease recurrence, the expression of stem-like cell markers and clinicopathological features were assessed using the two-tailed χ^2 test or Fisher's exact test. The independent factors associated with clinicopathological features and the expression of stem-like cell markers were evaluated using logistic regression analysis and Cox regression analysis. Statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA, USA) or the Microsoft Office 2010 Excel software (Microsoft Corporation, Redmond, WA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Geographical distribution of enrolled patients. Patients admitted to The First Affiliated Hospital of Xi'an Jiaotong University (Xi'an Hospital) and The Second Affiliated Hospital of Xi'an Jiaotong University (Northwest Hospital) located in Northwest China, were enrolled in the present study. These hospitals are associated with the National Health and Family Planning Commission of China (Ministry of Public Health,

Table II. Geographical distribution of enrolled patients.

Geographical location	Hospital 1	Hospital 2	Total (%)
Northern Shaanxi	95	41	136 (23.5)
Guanzhong region	17	11	28 (4.8)
Southern Shaanxi	113	38	151 (26.1)
Shanxi province	36	3	39 (6.7)
Henan province	20	5	25 (4.3)
The Ningxia Hui autonomous region	55	3	58 (10.0)
Gansu province	48	7	55 (9.5)
Sichuan province	19	0	19 (3.3)
Qinghai province	46	5	51 (8.8)
Additional provinces	15	2	17 (2.9)
Total	464	115	579 (100.0)

Hospital 1, The First Affiliated Hospital of Xi'an Jiaotong University; Hospital 2, The Second Affiliated Hospital of Xi'an Jiaotong University.

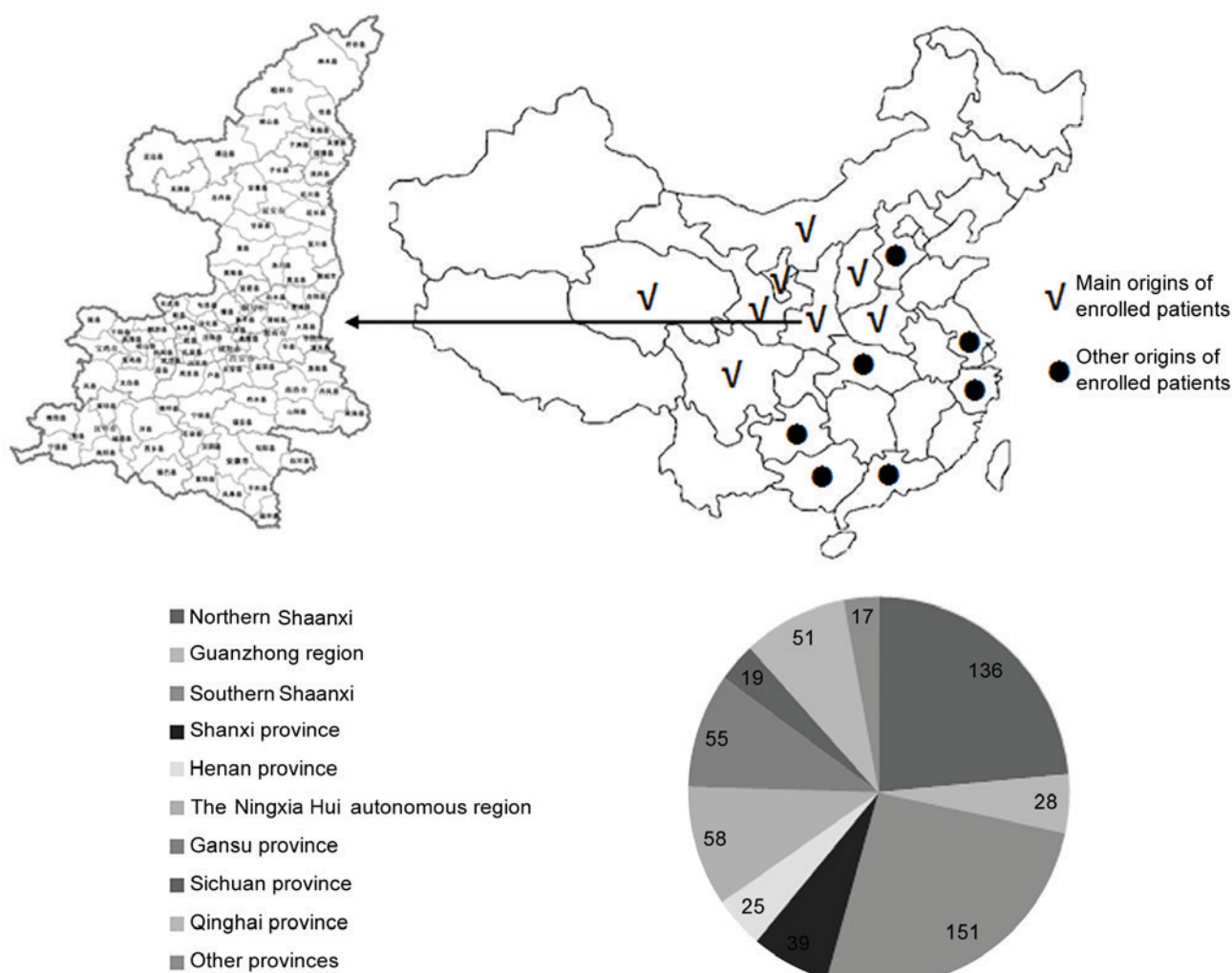


Figure 1. The geographical distribution patterns of enrolled patients. The origins of enrolled patients were indicated with a black tick or a filled black circle. The relative geographical distribution of enrolled patients is indicated in a pie chart, and the majority of enrolled patients that received an esophagectomy were from Northern and Southern Shaanxi province, where individuals tend to consume a diet rich in pickled and solid food with low fiber content.

Beijing, China), which supports surrounding provinces and cities. Enrolled patients were referred to either of these two

hospitals following the strict three-level patient transfer system or two-way referral system, and the geographical distribution

Table III. Respiratory system complications in patients with that underwent a radical esophagectomy (n=579).

A, Atelectasis/ARF			
Feature	Patients with	Patients without	P-value
Sex (male/female)	2/3	473/101	0.014
Age (<65/≥65 years)	1/4	238/336	0.332
Diabetes type II (Y/N)	5/0	103/471	<0.001
Hypertension (Y/N)	1/4	201/373	0.483
URD (Y/N)	4/1	300/274	0.216
DO (<4/≥4 h)	1/4	162/412	0.684
BF (Y/N)	1/4	14/560	0.014
EAS (Y/N)	4/1	42/532	<0.001

B, Pneumonia/empyema

Feature	Patients with	Patients without	P-value
Sex (male/female)	25/6	450/98	0.835
Age (<65/≥65 years)	11/20	228/320	0.501
Diabetes type II (Y/N)	21/10	87/461	<0.001
Hypertension (Y/N)	13/18	189/359	0.397
URD (Y/N)	28/3	276/272	<0.001
DO (<4/≥4 h)	6/25	157/391	0.263
BF (Y/N)	7/24	8/540	<0.001
EAS (Y/N)	24/7	22/526	<0.001

ARF, acute respiratory failure; URD, underlying respiratory diseases; DO, duration of operation; BF, bronchopleural fistulae; EAS, esophageal anastomotic fistulae.

of patients is indicated in Table II and Fig. 1. Analysis of the regional features indicated that the majority of patients were from North and South Shaanxi (Fig. 1 and Table II) (16), and the high incidence rates of esophageal cancer in North and South Shaanxi are consistent with the dietary habits of individuals residing there, compared to the ratio of middle Shaanxi province.

Cardiac and respiratory system post-operative complications. Two enrolled patients without underlying respiratory or cardiovascular diseases suffered a myocardial infarction, one of which did not survive. These patients were subsequently excluded from the study. The association between specific respiratory complications and patient characteristics, including gender, average age, underlying diseases and the presence of anastomotic fistulae was then determined (Table III). In addition, the association between cardiac complications and the same patient characteristics were determined (Table IV). The results demonstrated that type II diabetes mellitus and underlying respiratory and cardiac diseases were associated with the incidence of respiratory system complications post-surgery (Tables III and IV). In addition, respiratory system complications contributed to an increased risk of the development of anastomotic fistulae. Hypertension was significantly correlated with post-operative cardiac disorders, which may have

contributed to the development of esophageal anastomotic fistula (Table IV).

Risk factors for the development of anastomotic fistulae following esophagectomy. The development of anastomotic fistulae is one of the most serious complications following an esophagectomy procedure, and is associated with a reduction in patient quality of life, surgery failure, increased monetary expenditure on medical care and a high risk of unexpected mortality. Novel treatments and reliable clinical management strategies that aim to prevent fistula development or facilitate patient recovery are important considerations of research (17-19). In the present study, fistulae were diagnosed according to the clinical symptoms, physical indicators and an esophagography, and atypical cases were confirmed by endoscopy analysis.

Fistulae were observed in 46 patients (7.9%), two of which did not survive due to the development of a respiratory infection and severe hemorrhage. The mean time of fistula development following surgery was 6.5 ± 3.3 days. The association between anastomotic fistula development and different clinicopathological characteristics were statistically analyzed and the results are presented in Table V. Diabetes, preoperative level of serum albumin and pathological tumor stage were identified to be significantly associated with the

Table IV. Post-operative cardiovascular disorders in study participants.

A, Atrial fibrillation			
Feature	Patients with	Patients without	P-value
Sex (male/female)	32/19	443/85	<0.001
Age (<65/≥65 years)	12/39	227/301	0.007
Hypertension (Y/N)	36/15	166/362	<0.001
URD (Y/N)	30/21	274/254	0.344
DO (<4/≥4 h)	15/36	148/380	0.834
BF (Y/N)	11/40	4/524	<0.001
EAS (Y/N)	17/34	29/499	<0.001
B, Arrhythmia			
Feature	Patients with	Patients without	P-value
Sex (male/female)	23/7	452/97	0.431
Age (<65/≥65 years)	10/20	229/320	0.364
Hypertension (Y/N)	21/9	181/368	<0.001
URD (Y/N)	15/15	289/260	0.778
DO (<4/≥4 h)	8/22	155/394	0.853
BF (Y/N)	2/28	13/536	0.149
EAS (Y/N)	8/22	38/511	<0.001

AF, atrial fibrillation; URD, underlying respiratory diseases; DO, duration of operation; BF, bronchopleural fistulae; EAS, esophageal anastomotic fistulae.

development of anastomotic fistula following radical esophagectomy ($P<0.001$).

Association between disease recurrence and the expression of stem cell markers. Disease recurrence was defined as esophageal tumor development within 2 years following the radical esophagectomy procedure (1,20-22). The levels of stem-like cell markers ALDH, CD133, integrin subunit α 6 (ITGA6), integrin subunit β 4 (ITGB4) and TCF-4, were determined to evaluate their potential value as predictive markers of disease prognosis (11,12). Disease recurrence was observed in 212 patients (36.6%), and the recurrence ratio and the time-course of disease recurrence are presented in Fig. 2. Patient characteristics potentially associated with disease recurrence are presented in Table VI and Figure 3A-C, with a particular focus on the correlation between recurrence and the expression of ALDH, CD133, ITGA6 and ITGB4 stem cell markers.

ALDH1, CD133, ITGA6, ITGB4 and TCF-4 expression was detected by immunohistochemical or immunofluorescence analysis of patient tissue samples in order to obtain the most accurate results. However, the results were presented with different testing number, as not all the tissues of tumor were available. The results demonstrated significant differences among groups with different histological types, adjuvant therapy strategies and pathological stages (Table VI and Fig. 4). An increased number of ALDH1 (Fig. 4A), CD133 (Fig. 4B) and TCF-4 (Fig. 4C) positive stem-like cells were

significantly associated with disease recurrence, as the results of IHC and immunofluorescence presented (Fig. 4D-F). This suggests that these stem cell markers may be useful indicators of disease recurrence following radical esophagectomy.

Discussion

Esophageal cancer is one of the most common cancers in China and is the fourth highest cause of cancer-associated mortality (23-25). The five-year overall survival rate varies from 15 to 25%, and no effective treatments are currently available (24). The prevalence of esophageal cancer in East Asia is primarily due to poor dietary habits, alcohol consumption, tobacco chewing and smoking and physical inactivity (11). Worldwide, esophageal cancer is a lethal disease, with morbidity and mortality rates increasing due to the development of metastases and disease recurrence (26,27). Treatment strategies for patients with esophageal cancer have improved considerably over recent decades. Esophagectomy and esophago-gastrostomy procedures performed via the esophageal bed are conventional surgical procedures; however, the subsequent sizeable wound and physical structure alternations inevitably lead to a lower quality of life. The factors that influence efficient patient recovery and affect the risk of developing post-surgical complications, as well as the identification of groups of patients that would benefit most from surgery are important considerations. Therefore, an improved understanding of the risk factors associated with post-operative

Table V. Factors associated with the development of anastomotic fistula following radical esophagectomy.

Feature	Acceptable recovery	Fistula	Total	Fistula/total (%)	P-value
Sex					0.487
Male	439	36	475	7.6	
Female	94	10	104	9.6	
Age (years)					0.119
<65	225	14	239	5.9	
≥65	308	32	340	9.4	
Diabetes type II (Y/N)	91/442	17/29	108/471	15.7/6.2	<0.001
Hypertension (Y/N)	183/350	19/27	202/377	9.4/7.2	0.341
PreLSA (g/l)					<0.001
<35	227	33	260	12.7	
≥35	306	13	319	4.1	
PosLSA (g/l)					0.093
<35	97	19	116	16.3	
≥35	236	27	463	5.8	
URD (Y/N)	281/252	23/23	304/275	7.6/8.4	0.723
PSI					0.802
1	125	12	137	8.8	
2	305	24	329	7.3	
3	103	10	113	8.8	
DO (h)					0.166
<4	146	17	163	10.4	
≥4	387	29	416	7.0	
HT					0.105
SC	463	36	499	7.2	
AC+AS	70	10	80	12.5	
PTS					<0.001
I	46	5	51	9.8	
II	236	26	262	9.9	
III+IV	251	15	266	5.6	

PreLSA, preoperative level of serum albumin; PosLSA, postoperative level of serum albumin; URD, underlying respiratory diseases; PSI, patterns of surgery incision; DO, duration of operation; HT, histological type; SC, squamous carcinoma; AC, adenocarcinoma; AS, adenosquamous carcinoma; PTS, pathological tumor stage.

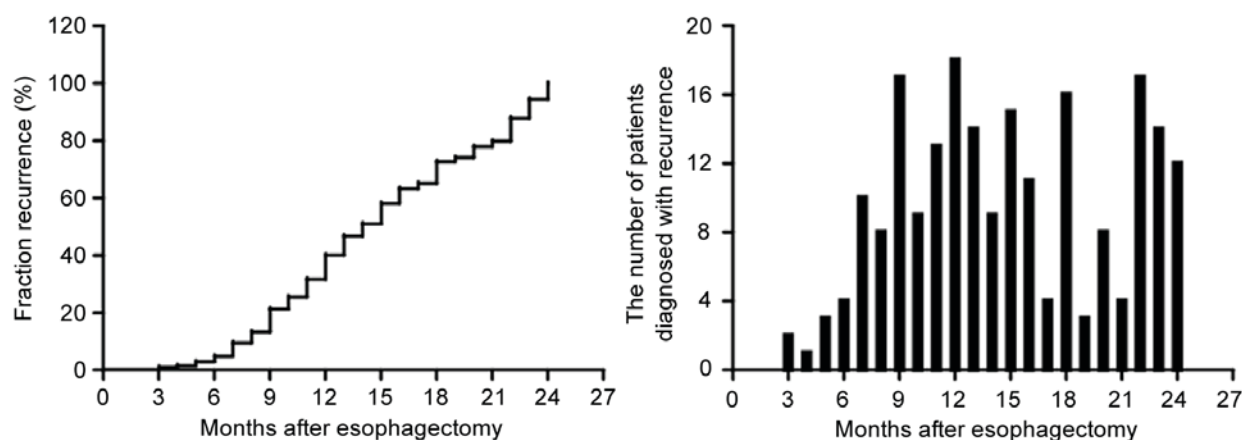


Figure 2. Disease recurrence following curative esophagectomy with radical lymph node dissection. Time-course of disease recurrence following the radical esophagectomy (left plot), and the number of patients that developed disease recurrence each month (right plot). Recurrence was defined as esophageal tumor development within 2 years following the surgical procedure.

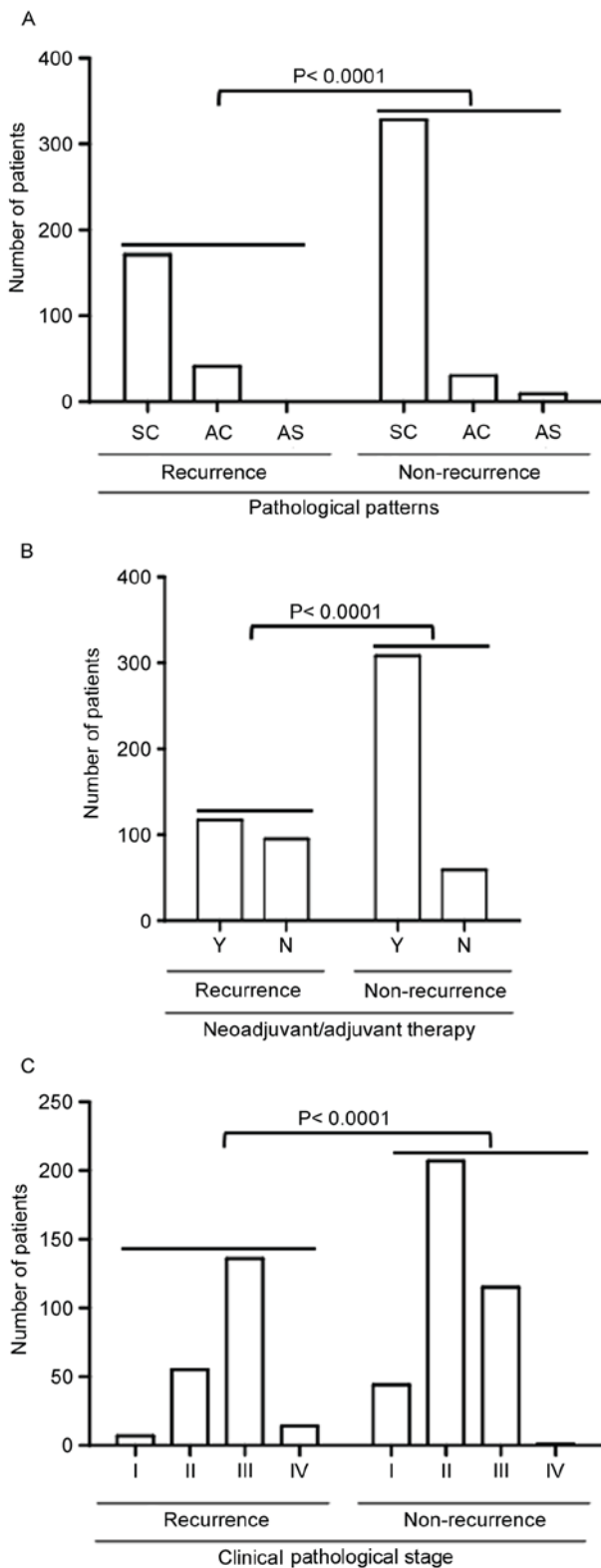


Figure 3. Clinicopathological features potentially associated with disease recurrence following curative esophagectomy with radical lymph node dissection. (A) Histological type, (B) neoadjuvant or adjuvant therapy and (C) pathological stage were assessed for their potential association with disease recurrence. Patients grouped by disease recurrence demonstrated significant differences among these three clinicopathological features. SC, squamous carcinoma; AC, adenocarcinoma; AS, adenosquamous carcinoma.

different treatments, enable the development of personalized therapeutic strategies and generate effective therapeutics in the treatment of patients with esophageal cancer. In addition, this may satisfy the growing demand for health care despite the limited resources (28).

The aim of the present study was to identify potential risk factors associated with the development of complications following radical esophagectomy, and to investigate factors associated with disease recurrence. To achieve this, 579 patients from two regional medical centers were enrolled and underwent a radical esophagectomy procedure. From the results, the status of current treatments on esophageal cancer, and the life-threatening and permanently incapacitating complications were identified. The identified factors may provide a useful tool to assess patient prognosis, improve the management of clinical therapy, and facilitate the development of individualized treatment plans and novel examination of biomarkers of stem cells. Although factors associated with post-radical esophagectomy surgery complications and the predictors of disease recurrence have been reported previously (29-31), an extensive investigation of these factors, and whether stem cell markers may be used as predictors of disease recurrence-free survival has not yet been determined.

In the present study, patients with esophageal cancer from multiple regions of China were enrolled, the majority of which resided in Northwest China where individuals consume meat and dried food. Therefore, the present study was limited by a lack of an extensive epidemiological investigation of each region. Analysis of post-operative complications revealed that post-surgical respiratory system complications were associated with underlying diabetes and respiratory diseases. Respiratory complications involved the development of bronchopleural fistulae and esophageal anastomotic fistulae, which were likely due to hypoxemia and infection in the thorax. In addition, cardiac disorders were more likely to occur in patients diagnosed with hypertension, which was demonstrated to be associated with an increased likelihood of underlying cardiovascular disease. Furthermore, a significant association between atrial fibrillation and the development of esophageal anastomotic fistulae was observed, which may have been due to disruption of blood stream dynamics and the development of minute thrombi. Risk factors associated with the development of anastomotic fistulae following esophagectomy and esophagogastrotomy through the esophageal bed included low pre-operative levels of serum albumin, pre-existing diabetes and a high pathological stage. These indicators may enable clinicians to make an informed decision regarding the treatment of patients with esophageal cancer, and prevent these complications following radical esophagectomy procedures. However, one notable limitation of the present study was that the effects of different strategies for enteral nutrition and adjuvant chemoradiotherapy were not considered or analyzed, which may have influenced patient recovery and post-operative responses.

Previous research has investigated the role of stem-like cells in multiple malignancies (32-34). Cancer stem cells are a subpopulation of 'seed cells' that are involved in cancer development, and contribute to disease recurrence and distant metastases (33). Pluripotency-associated signaling pathways and stemness-associated non-coding genes have been studied for their novel and crucial roles in cancer initiation,

complications and the identification of markers of disease prognosis may facilitate the selection of suitable candidates for

Table VI. Association between clinicopathological features, stem cell marker expression and disease recurrence following radical esophagectomy.

Feature	Recurrence		P-value	χ^2
	Yes	No		
Histological type				
SC	171	328	<0.0001	20.000
AC	41	30		
AS	0	9		
Neoadjuvant/adjuvant therapy (Y/N)	117/95	308/59	<0.0001	N/A
Pathological tumor stage				
I	7	44	<0.0001	93.241
II	55	207		
III	136	115		
IV	14	1		
Relative ALDH intensity				
+/++	51	9	0.0276	7.179
+++	21	10		
++++	9	7		
Relative CD133 intensity				
+/++	38	6	0.0244	7.427
+++	29	14		
++++	23	15		
Relative ITGA6 intensity				
+/++	31	4	0.0856	4.916
+++	26	10		
++++	8	5		
Relative ITGB4 intensity				
+/++	29	5	0.0514	5.938
+++	16	11		
++++	19	11		
Relative TCF-4 intensity				
+	21	2	0.0313	8.851
++	32	6		
+++	42	23		
++++	10	3		

SC, squamous carcinoma; AC, adenocarcinoma; AS, adenosquamous carcinoma; ALDH, aldehyde dehydrogenase 1; ITGA6, integrin subunit α 6; ITGB4, integrin subunit β 4; TCF-4, T-cell factor-4.

development, progression and distant recurrence. However, little is known about non-coding genes and stem-like cells in esophageal cancer (35-38). The present study investigated whether the expression of specific stem-like cell markers may be used to predict patient prognosis following esophagectomy. The expression of four potential stem-like cell markers and one pluripotent signaling pathway factor were analyzed. Together with different histological subtype, the adjuvant therapy strategy and pathological stage, ALDH1, CD133 and TCF-4 expression were demonstrated to be prognostic indicators. Therefore, intensive surveillance should be provided to patients by detecting these stem cell-associated markers, even weighed against costs and potential side effects.

There are a number of limitations of the present study. The acquisition of retrospective data introduced bias, and the statistical power of the analyses may have been weak, as the number of patients in each subgroup was relatively small. In addition, the study included data collected from patients admitted to two hospitals, which may limit the generalizability of the results. Furthermore, the identification of factors associated with predicting patient prognosis among different groups did not account for differences in adjuvant treatment, which should be investigated in a future prospective study.

In conclusion, the current study investigated the significance of specific markers and clinicopathological features in predicting patient recovery following radical esophagectomy.

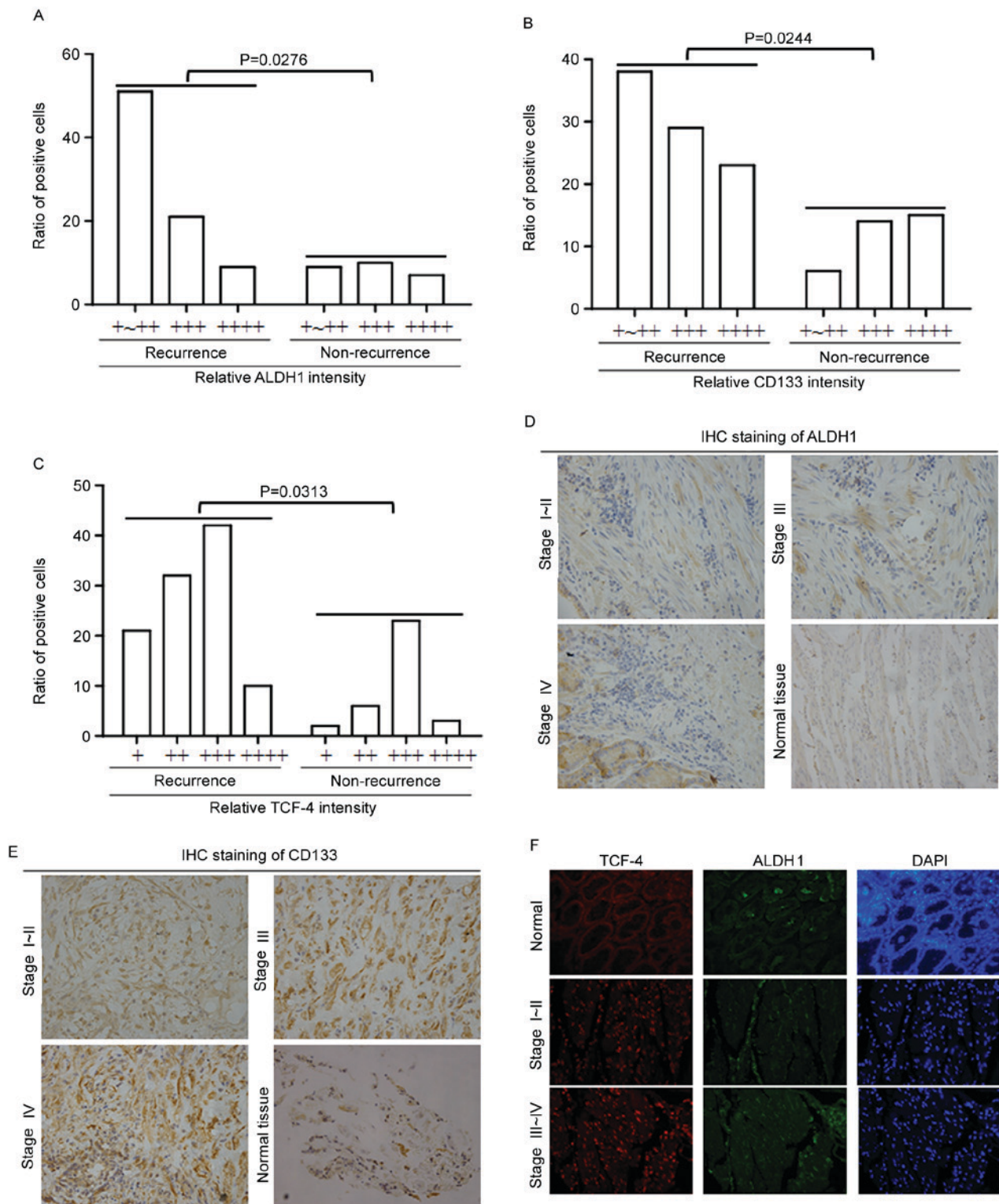


Figure 4. Evaluating the role of stemness markers in predicting disease recurrence. A cohort of stem cell markers was assessed for their potential role in predicting disease recurrence within 2 years following curative esophagectomy with radical lymph node dissection. The relative staining intensities of (A) ALDH1, (B) CD133 (C) and TCF-4 were determined. Representative images of immunohistochemical staining for (D) ALDH1, (E) CD133, and (F) representative images of immunofluorescence staining for ALDH1 and TCF-4 are presented. ALDH1, aldehyde dehydrogenase 1; TCF-4, T-cell factor-4.

The comprehensive analysis of detecting underlying diseases and applying novel stemness markers will be instructional in analyzing the cost-effectiveness and prognosis prediction of esophageal cancer treatment. In addition, future research

should evaluate the stemness phenotype and imbalanced cell division, also known as asymmetric division, which may be useful markers for clinical diagnosis and the prediction of disease recurrence (35,39-42).

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