Title: Serum anti-60S ribosomal protein L29 antibody as a novel prognostic marker for unresectable pancreatic cancer.

Short title: Serum anti-RPL29 in pancreatic cancer.

Authors: Shinichiro Muro, Yasuhiro Miyake, Hironari Kato, Koichiro Tsutsumi, Kazuhide Yamamoto.

Institution: Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan.

Corresponding author: Dr. Yasuhiro Miyake

Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, kita-ku, Okayama 700-8558, Japan.

E-mail: miyakeyasuhiro@hotmail.com

Fax: +81-86-225-5991

Phone: +81-86-235-7219

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Abstract

Background/Aims: Recently, we found the presence of anti-60S ribosomal protein L29 antibody (anti-RPL29) in human sera, inhibiting the proliferation of pancreatic cancer cells in vitro. We aimed to estimate the association of serum anti-RPL29 levels with clinical features in patients with unresectable pancreatic cancer.

Methods: We retrospectively reviewed 105 patients with unresectable pancreatic cancer. Serum anti-RPL29 levels were measured by the indirect enzyme-linked immunosorbent assay. The cut-off was represented by the 95th percentile in 62 healthy volunteers.

Results: Median survival time (MST) was 11.1 months in 49 patients showing serum anti-RPL29 level >cut-off and 7.4 months in 56 patients showing serum anti-RPL29 level ≤cut-off. In locally advanced disease, MST was 17.9 months in 22 patients showing serum anti-RPL29 level >cut-off and 10.0 months in 19 patients showing serum anti-RPL29 level ≤cut-off. In metastatic disease, MST was 8.7 months in 27 patients showing serum anti-RPL29 level >cut-off and 5.9 months in 37 patients showing serum anti-RPL29 level ≤cut-off. In the multivariate Cox proportional hazard model, serum anti-RPL29 level >cut-off, abdominal or back pain, performance status, and metastatic disease were identified as independent prognostic factors.

Conclusion: Serum anti-RPL29 levels may be a novel candidate for a prognostic

marker for unresectable pancreatic cancer.

Introduction

Pancreatic cancer remains one of the major unresolved health problems. First, the incidence and mortality of pancreatic cancer patients have increased. Worldwide, 302,464 patients died of pancreatic cancer in 2011 [1]. Deaths from pancreatic cancer in 2030 are projected to increase to 456,862 patients [2]. Secondly, the prognosis of pancreatic cancer has been still poor, and the 5-year survival has been reported less than 10% for last 30 years [3]. Especially, approximately 50% of the patients come to clinical attention with the metastatic disease, and their 5-year survival has been less than 3%.

For unresectable pancreatic cancer, gemcitabine had been used as a first-line agent from the second half of the 1990s to the 2000s. Median survival time (MST) in patients with the metastatic disease who were treated with gemcitabine alone or gemcitabine based combination chemotherapy has been reported to be 6-7 months [4]. Recently, a combination chemotherapy regimen consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) has shown significant effects on overall survival compared with gemcitabine monotherapy [5]. However, because of the severe toxicity of FOLFIRINOX, it cannot be applied to all patients with unresectable pancreatic cancer [6]. At present, the choice of regimen, whether FOLFIRINOX or gemcitabine based chemotherapy, depends on general conditions in each patient. As

prognostic factors for unresectable pancreatic cancer, tumor factors such as serum carbohydrate antigen (CA) 19-9 level and tumor stage, and host factors including performance status, serum C-reactive protein level, and neutrophil-lymphocyte ratio have been reported to be associated with patients' survival [7-10]. But, predicting the prognosis is not always easy, and markers reflecting the grade of spontaneous immune response to autologous cancer cells in vivo have not been elucidated yet.

Recently, we reported the presence of anti-60S ribosomal protein L29 antibody (anti-RPL29) in human sera, inhibiting the proliferation of human pancreatic cancer AsPC-1 cells and Panc-1 cells via down-regulation of Wnt/β-Catenin signaling pathway [11]. RPL29 is expressed on the surface of cells and takes part in cell-cell and cell-extracellular matrix adhesion [12,13]. The expression of RPL29 is up-regulated in various types of cancers including endometrial cancer [12], hepatocellular carcinoma [14], ovarian cancer [15], and colon cancer [16]. The up-regulation of RPL29 is associated with tumor growth and local invasion [16]. Depletion of RPL29 reduces tumor angiogenesis [17], induces cell cycle arrest at G0/G1 phase [18] and cellular differentiation [19], and promotes apoptosis [12]. Thus, the presence of serum anti-RPL29 may affect the prognosis of pancreatic cancer.

In this study, we measured serum anti-RPL29 levels and estimated the association

of serum anti-RPL29 levels with clinical features in patients with unresectable pancreatic cancer.

Methods

Ethics

This study complied with the Declaration of Helsinki and was approved by the Institutional Review Board at Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Serum samples and data were collected after each subject provided written informed consent from March 2003.

Patients

We retrospectively reviewed 107 patients with unresectable adenocarcinoma of the pancreas, who admitted to Okayama University Hospital between March 2003 and December 2012. A diagnosis of pancreatic adenocarcinoma was confirmed by histological or cytological examination. All patients had no prior chemotherapy or radiotherapy. Of the 107 patients, two were transferred to other hospitals without follow-up, and were excluded from the present analysis. Thus, 105 patients were included in the present analysis. Clinical characteristics of the study population at the initiation of chemotherapy were shown in Table 1. Thirty-five (33%) patients were

female, and median age was 64 (range, 38-85) years. Forty-one (39%) patients had locally advanced disease, and the remaining sixty-four (61%) had metastatic disease.

At the initiation of chemotherapy, the patient's complete medical history and physical examination, Eastern Cooperative Oncology Group performance status [20], complete blood cell count, standard biochemical profile, electrocardiogram, chest X-rays, and computed tomography scans of the chest and upper and lower abdomen were evaluated. Tumors were staged according to the sixth edition of the tumor node metastasis (TNM) classification of the International Union against Cancer (UICC) [21]. Modified Glasgow prognostic score, neutrophil-lymphocyte ratio, and platelet-lymphocyte ratio were evaluated as previously described [9].

Serum samples at the initiation of chemotherapy were obtained from 105 patients with unresectable pancreatic cancer. Moreover, serum samples from 62 healthy volunteers (controls) were collected. Collected serum samples were stored -30°C until use.

Treatment

In patients showing obstructive jaundice due to a pancreatic head tumor, biliary drainage was achieved by endoscopic biliary drainage or percutaneous trans-hepatic cholangiodrainage before the initiation of chemotherapy.

Chemotherapy was continued until the disease progressed or the condition of patients deteriorated.

Gemcitabine monotherapy: Gemcitabine was administered at a dose of 1,000 mg/m^2 intravenously over 30 minutes on days 1, 8, 15 of a 28-day cycle.

Gemcitabine plus cisplatin: patients were treated with gemcitabine at a dose of 800 mg/m^2 plus cisplatin at a dose of 10 mg/body intravenously over 30 minutes on days 1, 8, 15 of a 28-day cycle.

Gemcitabine plus erlotinib: patients were treated with oral erlotinib 100 mg/day on days 1-28, plus gemcitabine at a dose of 1,000 mg/m^2 intravenously over 30 minutes on days 1, 8, 15 of a 28-day cycle.

S-1 monotherapy: S-1 was administered orally twice daily at a dose according to the body-surface area (BSA) (< 1.25 m^2, 80 mg/day; ≥ 1.25 to < 1.5 m^2, 100 mg/day; ≥ 1.5 m^2, 120 mg/day) on days 1-28 of a 42-day cycle.

Gemcitabine plus S-1: Patients received gemcitabine at a dose of 1,000 mg/m^2 intravenously over 30 minutes on days 1, 8 plus S-1 orally twice daily at a dose according to the BSA (< 1.25 m^2, 60 mg/day; ≥ 1.25 to < 1.5 m^2, 80 mg/day; ≥ 1.5 m^2, 100 mg/day) on days 1-14 of a 21-day cycle.

In some patients, radiotherapy was targeted to the primary tumor and

administered at a total radiation dose of 50.4 Gy with a daily fraction of 1.8 Gy 5 times/week.

The response evaluation criteria in solid tumors [22] were applied to assess the response to chemotherapy.

Endpoint

The primary endpoint was overall survival, measured from the first day of chemotherapy until the date of death for any cause or December 31, 2013.

Indirect enzyme-linked immunosorbent assay

Serum anti-RPL29 levels were measured by the indirect enzyme-linked immunosorbent assay (ELISA) using Protein Detector ELISA Kit (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). All serum samples were tested in duplicate.

Briefly, 96-well U-bottom microtiter plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with 100 μl of 1 μg/ml full-length recombinant RPL29 (Abnova, Taipei, Taiwan) in phosphate-buffered saline (PBS) at room temperature for 1 hour. Unbound antigen was removed, nonspecific binding sites were blocked by incubation with 1% bovine-serum albumin (BSA) in PBS, and the wells were incubated with 100 μl of human sera diluted 1:100 in PBS with 1% of BSA for 1 hour. Following incubation, the wells were incubated with horseradish

peroxidase-labeled anti-human IgG diluted 1:1,000 in PBS with 1% of BSA, and the reaction was visualized by adding 100 μl of ABTS peroxidase substrate solution buffer (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The optical densities at 405 nm were read with a Model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). In order to avoid inter-plate variability, we used a positive serum, assigned it 2.33 OD_{405nm}, and read the optical densities of all samples against this positive serum. Intra-assay variability was found to be 5.1%.

The cut-off of serum anti-RPL29 level was represented by the 95th percentile in 62 controls.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics version 21 (IBM, Chicago, IL, USA). Continuous variables were expressed as median (range). Differences in continuous variables were evaluated by the Mann–Whitney U-test between two independent samples and the Kruskal–Wallis test among 3 independent samples. Dichotomous variables were compared by the χ^2-test. Univariate and multivariate Cox proportional hazard models were performed to identify prognostic factors, and results are presented as hazard ratios (HRs) along with their 95% confidence intervals (CIs). The variables, which showed $P < 0.2$ by univariate analysis,

were included into the multivariate analysis. Cumulative survival curves were analyzed using the Kaplan-Meier method, and the differences in the curves were tested using the log-rank test. P-values <0.05 (all two-sided) were considered significant.

Results

Characteristics of study population

Until December 31, 2013, ninety-eight (93%) patients died, and the overall MST was 8.7 months (Figure 1). MST was 15.1 months in 41 patients with locally advanced disease and 7.3 months in 64 patients with metastatic disease.

Treatment characteristics are shown in Table 2. Before the initiation of chemotherapy, biliary drainage was performed in 40 patients who had a pancreatic head tumor. Median time from the diagnosis of pancreatic cancer to the initiation of the first-line chemotherapy was 9 (range, 1-55) days and longer in 40 patients in need of biliary drainage {12 (3-55) days versus 8 (1-47) days, P = 0.034}. At the initiation of the first-line chemotherapy, 16 patients had serum CA 19-9 level less than 37 U/ml (upper limit of normal). Of 89 patients showing serum CA 19-9 level at the initiation of the first-line chemotherapy ≥37 U/ml, a decrease in serum CA 19-9 levels by at least 50% on day 56 was shown in 25 patients {2 patients with partial response (PR), 22 patients

with stable disease (SD), and one patient with progressive disease (PD)}. One patient with complete response (CR) showed serum CA 19-9 level <37 U/ml at the initiation of the first-line chemotherapy.

Serum anti-RPL29 level

Serum anti-RPL29 levels at the initiation of the first-line chemotherapy are shown in Figure 2. Serum anti-RPL29 levels were higher in patients with unresectable pancreatic cancer than controls {0.51 (range, 0.04-1.90) OD_{405nm} versus 0.30 (range, 0.09-0.56) OD_{405nm}; P <0.0001}; however, there was no difference in serum anti-RPL29 levels between 41 patients with locally advanced disease and 64 patients with metastatic disease {0.54 (range, 0.04-1.16) OD_{405nm} versus 0.50 (range, 0.13-1.90) OD_{405nm}; P = 0.70}. The cut-off of serum anti-RPL29 level was defined 0.53 OD_{405nm}, based on the 95th percentile in 62 controls. Twenty-two (54%) of 41 patients with locally advanced disease and 27 (42%) of 64 patients with metastatic disease showed serum anti-RPL29 level >cut-off.

Serum anti-RPL29 and clinical characteristics

Associations of serum anti-RPL29 level with clinical characteristics are shown in Table 3. At the initiation of the first-line chemotherapy, patients with serum anti-RPL29 level ≤cut-off showed peripheral platelet count $\geq 300\times10^3/mm^3$, platelet-lymphocyte

ratio ≥150, and serum CA 19-9 level ≥500 U/ml more frequently. Age, performance status, tumor stage, neutrophil-lymphocyte ratio, serum C-reactive protein level, and modified Glasgow prognostic score were not associated with serum anti-RPL29 level. Response to gemcitabine plus cisplatin was better in patients showing serum anti-RPL29 level >cut-off. MST was 11.1 months in 49 patients showing serum anti-RPL29 level >cut-off and 7.4 months in 56 patients showing serum anti-RPL29 level ≤cut-off (Figure 3A, log-rank test; P <0.0001). In locally advanced disease, MST was 17.9 months in 22 patients showing serum anti-RPL29 level >cut-off and 10.0 months in 19 patients showing serum anti-RPL29 level ≤cut-off (Figure 3B, log-rank test; P = 0.0063). On the other hand, in metastatic disease, MST was 8.7 months in 27 patients showing serum anti-RPL29 level >cut-off and 5.9 months in 37 patients showing serum anti-RPL29 level ≤cut-off (Figure 3C, log-rank test; P = 0.012).

In this study, there were differences in the first-line chemotherapy regimens between 49 patients showing serum anti-RPL29 level >cut-off and 56 patients showing serum anti-RPL29 level ≤cut-off. So, we estimated associations of serum anti-RPL29 level with survival in 75 patients treated with gemcitabine alone as the first-line chemotherapy. MST was 11.1 months in 29 patients showing serum anti-RPL29 level >cut-off and 6.2 months in 46 patients showing serum anti-RPL29 level ≤cut-off (Figure

4A, log-rank test; P = 0.0009). The response to gemcitabine was similar between these two groups (CR/PR/SD/PD: 0/1/21/7 versus 1/1/26/18; P = 0.48). In locally advanced disease, MST was 20.7 months in 13 patients showing serum anti-RPL29 level >cut-off and 10.0 months in 17 patients showing serum anti-RPL29 level ≤cut-off (Figure 4B, log-rank test; P = 0.0056). On the other hand, in metastatic disease, MST was 9.6 months in 16 patients showing serum anti-RPL29 level >cut-off and 5.2 months in 29 patients showing serum anti-RPL29 level ≤cut-off (Figure 4C, log-rank test; P = 0.075).

Prognostic factor

In 105 patients with unresectable pancreatic cancer, univariate analysis identified serum anti-RPL29 level (>0.53 OD_{405nm} versus ≤0.53 OD_{405nm}; P <0.0001), abdominal or back pain (yes versus no; P = 0.0015), performance status (0 versus 1+2; P = 0.010), tumor location (head versus body, body/tail, tail; P = 0.0075), tumor spread (metastatic versus locally advanced; P = 0.0008), neutrophil-lymphocyte ratio (≥5.0 versus <5.0; P = 0.016) as prognostic factors (Table 4). Age, gender, platelet-lymphocyte ratio, serum C-reactive protein level, and modified Glasgow prognostic score were not significantly associated with patients' prognosis. In the multivariate analysis, we identified serum anti-RPL29 level {>0.53 OD_{405nm}; HR 0.46 (0.29-0.73), P = 0.0009}, abdominal or back pain {yes; HR 1.74 (1.07-2.84), P = 0.027}, performance status {0; HR 0.48

(0.30-0.75), P = 0.0014}, and tumor spread {metastatic; HR 2.34 (1.48-3.70), P = 0.0003} as independent prognostic factors (Table 4).

In 75 patients treated with gemcitabine alone as the first-line chemotherapy, univariate analysis identified serum anti-RPL29 level, abdominal or back pain, performance status, tumor location, tumor spread, and neutrophil-lymphocyte ratio as prognostic factors. In the multivariate analysis that included serum anti-RPL29 level, abdominal or back pain, performance status, tumor location, tumor spread, neutrophil-lymphocyte ratio, serum bilirubin level, and serum CA 19-9 level, we identified serum anti-RPL29 level {>0.53 OD_{405nm}; HR 0.53 (0.29-0.98), P = 0.043} and tumor spread {metastatic; HR 2.31 (1.34-3.95), P = 0.0023} as independent prognostic factors.

Discussion

Recently, glycosylation of IgG, which influences IgG effector function, has been reported to be associated with the prognosis of malignant tumors including pancreatic cancer [23,24]. This indicates that serum IgG of cancer patients may contain some autoantibodies associated with the disease progression, and autologous tumor can become a target of anti-tumor immune response in vivo. Until now, many

autoantibodies against autologous tumor-associated antigens (TAAs) have been reported. In pancreatic cancer, the presence of serum anti-MUC1 antibodies has been shown to be associated with the better prognosis [25], but associations of serum antibodies to autologous TAAs with the prognosis have not been fully elucidated yet.

Ribosomes are essential components of the protein translation machinery, and are composed of more than 80 unique large and small ribosomal proteins. Some ribosomal proteins control cell proliferation, and autoantibodies to some ribosomal proteins may exist in human sera and influence cell proliferation. Previously, autoantibodies to ribosomal protein LP0 (RPLP0), which exists in the cytoplasmic and on the surface of cells and takes part in the regulation of protein synthesis, have been revealed to exist in human sera of patients with head and neck cancer [26] and patients with breast cancer [27]. Anti-RPLP0 antibodies induce cell cycle arrest at sub-G1 phase and promote apoptosis [28]; however the associations of serum anti-RPLP0 antibodies with the prognosis of cancer patients have not revealed yet.

In this study, the prognosis of patients with unresectable pancreatic cancer, who were mainly treated with gemcitabine alone or gemicitabine based combination chemotherapy, did not differ from those reported previously [4]. And, performance status, tumor stage, and abdominal or back pain were identified as prognostic factors for

unresectable pancreatic cancer. These results are consistent with those of the previous reports [7-9]. Thus, clinical features of patients with unresectable pancreatic cancer included into this study seem to be common. On the other hand, serum anti-RPL29 level, which was elevated in some patients with unresectable pancreatic cancer, was associated with their survival as an independent prognostic factor. Patients showing serum anti-RPL29 levels >cut-off seem to have longer survivals compared with the general patients with unresectable pancreatic cancer treated with gemcitabine based chemotherapy [4]. To our knowledge, this study is the first to focus on serum anti-RPL29 as a prognostic marker for unresectable pancreatic cancer. Serum anti-RPL29 level may be useful for predicting the prognosis of unresecatble pancreatic cancer.

Immune response to autologous tumor has been shown to improve the prognosis in vivo [29]. In peripheral blood from pancreatic cancer patients, circulating tumor cells are frequently detected [30]. So, spontaneous immune response to autologous pancreatic cancer cells may be induced. The expression of RPL29 is up-regulated in various types of cancers including pancreatic cancer [12,14-16,18]. In this study, serum anti-RPL29 levels, which were elevated in 47% of the patients with unresectable pancreatic cancer compared with healthy volunteers, were associated with their survival. So, RPL29 may

be an autologous TAA, and serum anti-RPL29 levels may reflect the grade of spontaneous immune response to autologous cancer cells. On the other hand, neutrophil-lymphocyte ratio, which reflects systemic inflammatory response [9], was identified as a prognostic factor by the univariate analysis, but the multivariate analysis including serum anti-RPL29 level did not show the association of neutrophil-lymphocyte ratio with patients' survival. This may indicate that markers reflecting the grade of spontaneous immune response to autologous cancer cells rather than systemic inflammatory response are suitable for predicting the prognosis of unresectable pancreatic cancer.

This study indicates that, in patients treated with gemcitabine alone as the first-line chemotherapy, serum anti-RPL29 level is not associated with the best overall response, but those showing elevated serum anti-RPL29 levels have better prognosis. Thus, induction of immune response to RPL29 may be effective to suppress the disease progression. On the other hand, in this study, T cell response to RPL29 was not investigated. Hereafter, in order to develop RPL29-based immunotherapy, it will be necessary to investigate the association between T cell response to RPL29 and the prognosis in patients with unresectable pancreatic cancer.

In pancreatic cancer, Wnt/beta-catenin signaling, apoptosis signaling, and

regulation of G1/S phase transition are core signaling pathways responsible for pancreatic tumorigenesis, and genetic alterations in these core signaling pathway are nearly universal [31]. RPL29 has been found one of candidate targets of Wnt/beta-catenin signaling [14]. Additionally, silencing of RPL29 up-regulates the expression of p21, inhibitor of cell cycle progression through G1/S phase, and p53, inducer of apoptosis [19]. Thus, RPL29 may be a candidate target molecule for pancreatic cancer therapy although further studies are required.

This study had several limitations. First, this study was a retrospective, single-center study. Second, many patients were treated with gemcitabine alone as the first-line chemotherapy. Some were treated with gemcitabine plus cisplatin or gemcitabine plus erlotinib. But, none was treated with FOLFIRINOX. Hereafter, FOLFIRINOX are speculated to become the standard regimen for unresectable pancreatic cancer. In order to confirm our findings, a multicenter prospective validation study, registered with the UMIN Clinical Trials Registry (number: UMIN000011478), is under execution.

In conclusion, this study indicates that some patients with unresectable pancreatic cancer show elevated serum anti-RPL29 levels, and that serum anti-RPL29 levels are associated with the prognosis of unresectable pancreatic cancer, independent of

performance status, tumor stage, and serum CA19-9 level. Thus, serum anti-RPL29 level may be a novel candidate for a prognostic marker for unresectable pancreatic cancer, although further studies are required.

Disclosure Statement

The authors have no disclosures relevant to this publication.

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Figure legends

Figure 1. Kaplan-Meier curve stratified by tumor spread.

Figure 2. Serum anti-RPL29 levels in study populations. Closed circles show serum anti-RPL29 level >cut-off. Open circles show serum anti-RPL29 level ≤cut-off. The cut-off was represented by the 95th percentile in 62 healthy volunteers. (= 0.53 OD_{405nm}).

Figure 3. Kaplan-Meier curve stratified by serum anti-RPL29 level in overall 105 patients with unresectable pancreatic cancer (A), 41 patients with locally advanced disease (B), and 64 patients with metastatic disease (C).

Figure 4. Kaplan-Meier curve stratified by serum anti-RPL29 level in 75 patients treated with gemicitabine alone as the first chemotherapy (A), 30 patients with locally advanced disease (B), and 45 patients with metastatic disease (C).

Table 1. Patient characteristics.

Parameter	Number of patients (%)
Age at diagnosis (years)	
<65	53 (50)
≥65	52 (50)
Gender	
Female	35 (33)
Male	70 (67)
Symptoms	
Abdominal pain	62 (59)
Back pain	26 (25)
Performance status	
0	40 (38)
1	59 (56)
2	6 (6)
Tumor location	
Head	55 (52)
Body	27 (26)
Body/tail	2 (2)
Tail	21 (20)
Tumor spread	
Locally advanced	41 (39)
Metastatic	64 (61)
Sites of metastases	
Lung	6 (6)
Liver	45 (42)
Intra-abdominal	12 (11)
Bone	1 (1)
UICC stage	
IIa	3 (3)
IIb	3 (3)
III	35 (33)
IV	64 (61)
Body mass index	
<25.0	93 (89)
≥25.0	12 (11)

White blood cell count (/mm^3)	
<9000	93 (89)
≥9000	12 (11)
Neutrophil count (/mm^3)	
<5000	78 (74)
≥5000	27 (26)
Lymphocyte count (/mm^3)	
<2000	89 (85)
≥2000	16 (15)
Hemoglobin level (g/dl)	
<13.0 (male), <11.5 (female)	49 (47)
≥13.0 (male), ≥11.5 (female)	56 (53)
Platelet count (×10^3/mm^3)	
<300	83 (79)
≥300	22 (21)
Neutrophil-lymphocyte ratio	
<5.0	89 (85)
≥5.0	16 (15)
Platelet-lymphocyte ratio	
<150	43 (41)
≥150	62 (59)
Bilirubin (mg/dl)	
<2.0	97 (92)
≥2.0	8 (8)
Albumin (g/dl)	
<3.5	27 (26)
≥3.5	78 (74)
C-reactive protein (mg/dl)	
<1.0	75 (71)
≥1.0	30 (29)
Creatinine (mg/dl)	
<0.9	91 (87)
≥0.9	14 (13)
Modified Glasgow prognostic score	
0	78 (74)
1	25 (24)

2	2 (2)
CA 19-9 (U/ml)	
<37 (ULN)	16 (15)
≥37 and <500	36 (35)
≥500	53 (50)

CA19-9, carbohydrate antigen 19-9; UICC, the International Union against Cancer; ULN, upper limit of normal.

Table 2. Treatment.

Parameter	Number of patients (%)
Biliary drainage before chemotherapy	
Yes	40 (38)
No	65 (62)
Time from diagnosis to chemotherapy	
<10days	54 (51)
≥10 days	51 (49)
First-line chemotherapy	
Gemcitabine	75 (71)
Gemcitabine + Cisplatin	24 (23)
Gemcitabine + erlotinib	5 (5)
S-1	1 (1)
Second-line chemotherapy	
Gemcitabine + S-1	9 (9)
S-1	37 (35)
Radiation therapy	2 (2)
Best overall response	
CR	1 (1)
PR	3 (3)
SD	69 (66)
PD	32 (30)
CA 19-9 decrease on day 56	
≥50%	25 (24)
<50%	61 (58)
Baseline level <37 U/ml (ULN)	16 (15)
Missing	3 (3)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ULN, upper limit of normal.

Table 3. Serum anti-RPL29 level and clinical characteristics.

Parameter	Number of patients (%)		P
	Anti-RPL29 >cut-off n = 49	Anti-RPL29 ≤cut-off n = 56	
Age at diagnosis, ≥65 years	22 (45)	30 (54)	0.38
Gender, female	17 (35)	18 (32)	0.78
Abdominal or back pain	31 (63)	33 (59)	0.65
Performance status, 0	19 (39)	21 (38)	0.89
Tumor location, head	28 (57)	27 (48)	0.36
Tumor spread, metastatic	27 (55)	37 (66)	0.25
Body mass index, ≥25.0	7 (14)	5 (9)	0.39
White blood cell count, ≥9000/mm^3	8 (16)	4 (7)	0.24
Neutrophil count, ≥5000/mm^3	12 (24)	15 (25)	0.79
Lymphocyte count, ≥2000/mm^3	10 (20)	6 (11)	0.17
Anemia	27 (55)	22 (39)	0.11
Platelet count, ≥300×10^3/mm^3	6 (12)	16 (29)	0.040
Neutrophil-lymphocyte ratio, ≥5.0	7 (14)	9 (16)	0.80
Platelet-lymphocyte ratio, ≥ 150	24 (49)	38 (68)	0.049
Bilirubin, ≥2.0 mg/dl	4 (8)	4 (7)	0.84
Albumin, <3.5 g/dl	15 (31)	12 (21)	0.28
C-reactive protein, ≥1.0 mg/dl	16 (33)	14 (25)	0.39
Creatinine, ≥0.9 mg/dl	5 (10)	9 (16)	0.38
Modified Glasgow prognostic score, 1+2	15 (31)	12 (21)	0.28
CA 19-9, ≥500 U/ml	19 (39)	34 (61)	0.025
Biliary drainage before chemotherapy, yes	21 (43)	19 (34)	0.35
Time from diagnosis to chemotherapy, ≥10 days	27 (55)	24 (43)	0.21
First-line chemotherapy			
Gemcitabine alone	29 (59)	46 (82)	0.0094
Gemcitabine + Cisplatin	19 (39)	5 (9)	0.0003
Gemcitabine alone or Gemcitabine + Cisplatin	48 (98)	51 (91)	0.13
Best overall response, PD			
Any chemotherapy	7 (14)	25 (45)	0.0007
Gemcitabine alone	7/29 (24)	18/46 (39)	0.18
Gemcitabine + Cisplatin	0/19 (0)	2/5 (40)	0.0040
Gemcitabine alone or Gemcitabine + Cisplatin	7/48 (15)	20/51 (39)	0.0060
CA 19-9 decrease on day 56, ≥50%	15/39 (38)	10/47 (21)	0.081

The cut-off value of serum anti-RPL29 level is 0.53 OD_{405nm}. Anti-RPL29, anti-60S ribosomal protein L29 antibody; CA19-9, carbohydrate antigen 19-9; PD, progressive disease.

Table 4. Cox proportional hazard model.

Parameter	Univariate		Multivariate	
	HR (CI)	P	HR (CI)	P
Anti-RPL29, >0.53 OD_{405nm}	0.41 (0.26-0.64)	<0.0001	0.46 (0.29-0.73)	0.0009
Age at diagnosis (years), ≥65	1.20 (0.79-1.82)	0.39	—	—
Gender, female	1.27 (0.82-1.97)	0.29	—	—
Abdominal or back pain	2.07 (1.32-3.24)	0.0015	1.74 (1.07-2.84)	0.027
Performance status, 0	0.57 (0.37-0.88)	0.010	0.48 (0.30-0.75)	0.0014
Tumor location, head	0.58 (0.38-0.86)	0.0075	0.87 (0.57-1.34)	0.53
Tumor spread, metastatic	2.05 (1.35-3.13)	0.0008	2.34 (1.48-3.70)	0.0003
Body mass index, ≥25.0	0.91 (0.48-1.71)	0.77	—	—
Anemia	0.99 (0.67-1.49)	0.98	—	—
Neutrophil-lymphocyte ratio, ≥5.0	1.99 (1.14-3.47)	0.016	1.60 (0.91-2.82)	0.10
Platelet-lymphocyte ratio, ≥150	1.03 (0.69-1.55)	0.89	—	—
Bilirubin, ≥2.0 mg/dl	0.57 (0.25-1.30)	0.18	0.52 (0.21-1.25)	0.14
C-reactive protein, ≥1.0 mg/dl	1.09 (0.70-1.71)	0.69	—	—
Creatinine, ≥0.9 mg/dl	0.94 (0.53-1.70)	0.85	—	—
mGPS, 1+2	1.03 (0.65-1.63)	0.91	—	—
CA19-9, ≥500 U/ml	1.35 (0.90-2.01)	0.15	0.94 (0.62-1.44)	0.79
Biliary drainage	0.93 (0.62-1.39)	0.71	—	—
Time from diagnosis to chemotherapy, ≥10 days				
	0.83 (0.55-1.24)	0.36	—	—
Gemcitabine alone (first-line)	1.12 (0.72-1.72)	0.62	—	—

Anti-RPL29, anti-60S ribosomal protein L29 antibody; CA19-9, carbohydrate antigen 19-9; CI, 95% confidence interval; HR, hazard ratio; mGPS, modified Glasgow prognostic score.