



Ghrelin after chemotherapy as a prognostic predictor of progression-free survival in patients with muscle-invasive bladder cancer

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Background: Although the patients with muscle-invasive bladder cancer (MIBC) generally have poor prognosis, the utility of these biomarkers for the prediction of oncological outcomes in MIBC has not been completely explored. Ghrelin regulates processes associated with cancer, including cell proliferation, apoptosis, cell migration, cell invasion, and angiogenesis. Thus, we aimed to evaluate the impact of serum ghrelin levels on survival in MIBC.

Methods: In this study, we reviewed the clinical and pathological records of 56 patients who were diagnosed with MIBC between November 2015 and November 2019 at Gifu and Hirosaki University Hospitals. We focused on 27 patients who had received chemotherapy and collected blood samples before and after chemotherapy. Blood samples were collected before chemotherapy and after completing two cycles of chemotherapy. Serum acyl (AG) and desacyl ghrelin (DG) were measured using AG and DG enzyme-linked immunosorbent assay kits (SCETI, Tokyo, Japan), respectively.

Results: The 3-year overall and progression-free survival (PFS) rates were 82.9% and 68.3%, respectively. According to the AG level after chemotherapy, the 3-year PFS rates were 77.5% and 53.0% in patients with AG levels ≥ 1.34 and < 1.34 pg/mL, respectively ($P=0.038$). With regard to DG levels after chemotherapy, the 3-year PFS rates were 90.9% and 43.3% in patients with DG levels < 92.3 and ≥ 92.3 pg/mL, respectively ($P=0.039$). On multivariate analysis, serum AG levels were significantly associated with PFS.

Conclusions: This study suggested the usefulness of the ghrelin as a prognostic predictor of PFS in patients with MIBC.

Keywords: Muscle-invasive bladder cancer (MIBC); acyl ghrelin (AG); desacyl ghrelin (DG); predictive marker; progression-free survival (PFS)

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Introduction

Bladder cancer (BC) is the most common type of malignant tumor occurring in the urinary tract (1). In total, 25–30% of patients with BC are diagnosed with muscle-invasive BC

(MIBC) (2). Although radical cystectomy (RC) with extended pelvic lymphadenectomy is the standard treatment for MIBC (3), half of the patients with MIBC develop locoregional recurrence and/or distant metastasis, including the lymph

nodes, lung, liver, and bone, within 2 years of surgery (4–6). In addition, the 5-year overall (OS) and progression-free survival (PFS) rates in patients with MIBC are 50–70% (5,6). However, patients who are pathologically diagnosed with T3/T4 BC or lymph node involvement according to the American Joint Committee on Cancer (AJCC) Staging Manual (7) generally have poor prognosis, and their OS rate is only 10–40% (4,5).

To date, postoperative tumor recurrence and progression is a serious problem for patients with MIBC. Neoadjuvant chemotherapy, radiotherapy, and immunotherapy may reduce the possibility of MIBC recurrence and progression. However, it is difficult to develop a systemic treatment strategy for individual patients with MIBC because of the different mechanisms of tumor progression and recurrence (8). Therefore, determination of novel and effective prognostic biomarkers is needed to predict and improve the prognosis of patients with MIBC (8). Wang *et al.* reported that ribosome-binding protein 1 was associated with BC migration, invasion, and prognosis (9). Several studies have demonstrated that hematological biomarkers, including C-reactive protein (CRP), butyrylcholinesterase (BChE), neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, can help predict tumor occurrence and progression (10–12). However, the utility of these biomarkers for the prediction of oncological outcomes in MIBC has not been completely explored.

Ghrelin, a novel hormone, increases appetite and growth hormone secretion. It has two different forms—acyl (AG) and desacyl ghrelin (DG) (13). The roles of ghrelin include stimulation of food intake, promotion of carbohydrate utilization, regulation of adiposity, regulation of insulin secretion, and control of blood glucose level (14). Serum ghrelin also regulates processes associated with cancer, including cell proliferation, apoptosis, cell migration, cell invasion, and angiogenesis (15). However, the precise role of ghrelin in the regulation of a number of processes related to cancer progression, especially MIBC, is unclear (16). Thus, we aimed to evaluate the impact of serum ghrelin levels on survival in MIBC patients receiving chemotherapy.

We present the following article in accordance with the TREND reporting checklist (available at <http://dx.doi.org/10.21037/tau-20-1489>).

Methods

Patients

In this study, we reviewed the clinical and pathological

records of 56 patients who were diagnosed with MIBC between November 2015 and November 2019 at Gifu and Hirosaki University Hospitals. We focused on 27 patients who had received chemotherapy and collected blood samples before and after chemotherapy. Informed consent was obtained from all participants. Eligibility criteria for participation in this study were as follows: histologically confirmed MIBC without distant metastases; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; adequate bone marrow function (absolute neutrophil count $\geq 1,500/\text{m}^3$ and platelet count $\geq 100,000/\text{m}^3$), adequate renal function (creatinine < 2.0 mg/dL and/or creatinine clearance > 40 mL/min), and adequate hepatic function (total bilirubin < 1.5 mg/dL). Patients who had previously received radiation therapy to the urinary bladder or pelvis before surgery were excluded.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Gifu University (2018-212) and Hirosaki University (2017-1108) and informed consent was taken from all the patients.

Schedule of chemotherapy

All treatments were performed at our institutions. The chemotherapy regimen consisted of $1,000$ mg/ m^2 of gemcitabine (GEM) administered on days 1, 8, and 15 and 70 mg/ m^2 of cisplatin (CDDP) or carboplatin (CBDCA), with an area under the curve of 5 according to Calvert's formula, on day 2 (17). Each treatment cycle lasted for 21–28 days. The patients received at least two cycles of chemotherapy. The patients were identified as CDDP ineligible if they met at least one of the following criteria: ECOG performance status of 2, creatinine clearance < 60 mL/min, hearing loss grade ≥ 2 , neuropathy grade ≥ 2 , and/or New York Heart Association Class III heart failure (18).

Patients evaluation

The following baseline information was obtained from each patient: complete medical history, physical examination findings, ECOG performance status, abdominal and pelvic computed tomography (CT) or magnetic resonance imaging (MRI) findings, and chest radiography or CT findings.

The diagnosis of MIBC was confirmed by pathologists at our institution by reviewing the results of transurethral resection. Tumor staging was performed according to the

staging system defined in the AJCC Staging Manual (7).

All patients underwent CT or MRI every 3–6 months until disease progression according to the findings of the radiological evaluation, which was performed after administration of chemotherapy.

Measurement of blood samples

Blood samples were collected before chemotherapy and after completing two cycles of chemotherapy. Leukocyte count, neutrophil count, lymphocyte count, platelet count, lactate dehydrogenase levels, CRP levels, BChE levels, and serum ghrelin levels were measured prior to chemotherapy and after completing two cycles of chemotherapy. AG and DG levels were measured using AG and DG enzyme-linked immunosorbent assay kits (SCETI, Tokyo, Japan), respectively.

Endpoints

The primary endpoint was PFS. PFS was defined as the time from chemotherapy to the appearance of locoregional and/or distant metastasis on CT.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences software version 24.0 (IBM Corp., Armonk, NY, USA). Categorical and continuous variables were compared using the chi-square test and the Kruskal–Wallis test, respectively. Survival after chemotherapy was analyzed using the Kaplan–Meier method. Survival according to subgroup was analyzed using the log-rank test. The cutoff values for covariates were defined as the minimal value for $(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$ according to the area under the receiver operating characteristic curve (19). All P values were two sided, and a P value of <0.05 was considered significant.

Results

Patient characteristics

The baseline characteristics of the patients are listed in *Table 1*. Fifteen patients did not provide written informed consent for this study and data for 4 patients were missing. All patients were diagnosed with muscle-invasive urothelial carcinoma based on the results of histological examination

of the specimens obtained via transurethral resection. A total of 23 (85.2%) patients underwent RC after chemotherapy, 3 (11.1%) received adjuvant chemotherapy after RC. Of the patients who received adjuvant chemotherapy, two received GEM and CDDP and one was administered GEM plus CBDCA. The median number of adjuvant chemotherapy cycles was two. One 86-year-old patient (3.7%) received 4 cycles of GEM and CBCDA as palliative therapy.

The demographic data of patients who were classified into two groups according to the status of locoregional and/or distant metastasis are listed in *Table 2*.

Oncological outcomes

The 3-year OS and PFS rates were 82.9% and 68.3%, respectively. After the follow-up period, 5 (18.5%) patients died of BC, 1 (0.4%) patient died of malignant lymphoma, and 1 (0.4%) patient died of acute pan-peritonitis. Conversely, all patients who did not undergo RC were still alive at the end of the follow-up period. The median follow-up period in patients who did not undergo RC was 10.5 months. Eight (29.6%) patients developed clinical recurrences. The most common site of clinical recurrence was the lymph nodes [4 patients (50%)]. The other recurrence sites were locoregional [2 patients (7.4%)] and the lungs [2 patients (7.4%)].

According to the AG level after chemotherapy, the 3-year PFS rates were 77.5% and 53.0% in patients with AG levels ≥ 1.34 and < 1.34 pg/mL, respectively ($P=0.038$; *Figure 1*). With regard to DG levels after chemotherapy, the 3-year PFS rates were 90.9% and 43.3% in patients with DG levels < 92.3 and ≥ 92.3 pg/mL, respectively ($P=0.039$; *Figure 2*). According to CRP level, the 3-year PFS rates were 79.8% and 35.7% in patients with CRP levels of < 0.475 and ≥ 0.475 mg/dL, respectively ($P<0.001$; *Figure 3*). In patients treated with GEM and CDDP and GEM and CBDCA, the 3-year PFS rates were 99.3% and 41.3%, respectively ($P=0.020$; *Figure 4*). On multivariate analysis, serum AG levels were significantly associated with PFS (*Table 3*).

Discussion

To our knowledge, the current study is the first to evaluate the relationship between serum ghrelin levels and oncological outcomes in patients with MIBC who received chemotherapy. In our previous study, we identified BChE as a potential biomarker for the prediction of MIBC

Table 1 Patient characteristics (n=27)

Covariates	
Age (years, median, interquartile range)	71 (64–74)
Gender (number, %)	
Male	25 (92.6)
Female	2 (7.4)
Histological findings (number, %)	
Urothelial carcinoma	26 (96.3)
Small cell carcinoma	1 (3.7)
Clinical Tumor stage (number, %)	
T2	12 (44.4)
T3	13 (48.2)
T4a	2 (7.4)
Clinical nodal status (number, %)	
Negative	25 (92.6)
Positive	2 (7.4)
Body mass index (kg/m ² , median, interquartile range)	
Pre-chemotherapy	24.1 (21.7–27.5)
Post-chemotherapy	24.2 (20.9–26.9)
Chemotherapy regimen (number, %)	
Gemcitabine and cisplatin	15 (55.6)
Gemcitabine and carboplatin	12 (44.4)
Leucocyte count (/μL, median, interquartile range)	
Pre-chemotherapy	6,665 (5,835–8,520)
Post-chemotherapy	5,435 (4,700–7,575)
Neutrophil count (/μL, median, interquartile range)	
Pre-chemotherapy	4,455 (3,330–5,963)
Post-chemotherapy	3,355 (2,390–5,028)
Lymphocyte count (×10 ³ /μL, median, interquartile range)	
Pre-chemotherapy	1,771 (1,401–2,175)
Post-chemotherapy	1,600 (995–2,173)
Platelet count (/μL, median, interquartile range)	
Pre-chemotherapy	2,345 (199.5–3,967.5)
Post-chemotherapy	283.5 (190.5–3,967.5)
lactate dehydrogenase (U/L, median, interquartile range)	
Pre-chemotherapy	178 (168–210)
Post-chemotherapy	205 (185–231)

Table 1 (continued)**Table 1** (continued)

Covariates	
C-reactive protein (mg/dL, median, interquartile range)	
Pre-chemotherapy	0.140 (0.049–0.475)
Post-chemotherapy	0.357 (0.075–1.133)
Butyrylcholinesterase (U/L, median, interquartile range)	
Pre-chemotherapy	278 (214–301)
Post-chemotherapy	248 (207–321)
Serum acyl-ghrelin levels (pg/mL, median, interquartile range)	
Pre-chemotherapy	1.48 (1.13–2.51)
Post-chemotherapy	1.38 (0.82–1.97)
Serum des-acyl ghrelin levels (pg/mL, median, interquartile range)	
Pre-chemotherapy	54.02 (8.85–124.97)
Post-chemotherapy	100.89 (44.95–147.99)
Follow-up period (months, interquartile range)	42.9 (10.8–46.6)

patient prognosis despite normal liver function with or without hepatic involvement (10). BChE may also be physiologically relevant for the hydrolysis of ghrelin (14). Additionally, BChE may be a key regulator of ghrelin in the peripheral circulation with widespread impacts (20). On the contrary, ghrelin affects physiological functions and pathological states, including insulin release, adiposity, energy homeostasis, and the development and progression of several types of cancers (20). Therefore, we investigated the association between serum ghrelin levels and PFS in patients with MIBC.

Ghrelin is a 28-amino-acid peptide that stimulates the release of pituitary growth hormone. It was discovered as an intrinsic ligand for the growth hormone-secreting receptor in 1999 (13,20). Ghrelin is mainly produced in the stomach. It has two forms—AG, which is catalyzed by ghrelin O-acyltransferase, whereas DG has no such modification (21). In general, the blood concentration of DG is approximately 4–5 times higher than that of AG (22). AG is recognized as an orexigenic peptide and has several characteristic functions, including secretion of growth hormone, promotion of food intake, gastrointestinal motility, secretion of gastric acid, weight gain, and strong anti-inflammatory action (13,23–25). Conversely, DG is an anorexigenic peptide, and its mechanism of action

Table 2 Clinical characteristics before and after chemotherapy (n=27)

	The patients with CP after chemotherapy (N=8)	The patients without CP after chemotherapy (N=19)	P value
Age (years, median, interquartile range)	73 (68–79)	69 (59–73)	0.091
Gender (number, %)			
Male	7 (87.5)	18 (94.7)	0.531
Female	1 (12.5)	1 (4.3)	
Histological findings (number, %)			
Urothelial carcinoma	8 (100)	18 (94.7)	0.527
Small cell carcinoma	0	1 (4.3)	
Clinical Tumor stage (number, %)			
T2	4 (50.0)	8 (42.1)	0.981
T3	3 (37.5)	10 (52.6)	
T4a	1 (12.5)	1 (4.3)	
Clinical nodal status (number, %)			
Negative	7 (87.5)	18 (94.7)	0.531
Positive	1 (12.5)	1 (4.3)	
Chemotherapy regimen (number, %)			
GC	2 (25.0)	13 (68.2)	0.039
GCarbo	5 (75.0)	6 (31.6)	
Body mass index (kg/m ² , median, interquartile range)			
Pre-chemotherapy	25.6 (18.1–27.3)	23.9 (21.8–28.0)	0.714
Post-chemotherapy	24.9 (18.9–27.3)	23.6 (20.9–27.2)	0.504
Leucocyte count (/μL, median, interquartile range)			
Pre-chemotherapy	6,075 (5,188–8,320)	6,865 (4,725–7,005)	0.385
Post-chemotherapy	4,865 (4,608–8,535)	5,895 (4,725–7,005)	0.969
Neutrophil count (/μL, median, interquartile range)			
Pre-chemotherapy	4,450 (3,175–5,895)	4,455 (3,340–6,018)	0.926
Post-chemotherapy	3,335 (2,428–5,688)	3,355 (2,262–4,743)	0.806
Lymphocyte count (/μL, median, interquartile range)			
Pre-chemotherapy	1,431 (1,093–1,758)	1,965 (1,598–2,354)	0.023
Post-chemotherapy	974 (798–2,155)	1,675 (1,400–2,189)	0.116
Platelet count (×10 ³ /μL, median, interquartile range)			
Pre-chemotherapy	241.5 (167.3–271)	234 (2,067.5–3,437.5)	0.203
Post-chemotherapy	156 (99.2–310.5)	325 (2,237.5–4,642.5)	0.021

Table 2 (continued)

Table 2 (continued)

	The patients with CP after chemotherapy (N=8)	The patients without CP after chemotherapy (N=19)	P value
Neutrophil-to-lymphocyte ratio (% , interquartile range)			
Pre-chemotherapy	2.9 (2.10–5.58)	2.10 (1.67–3.06)	0.236
Post-chemotherapy	2.9 (1.54–5.88)	1.97 (1.37–2.90)	0.806
Platelet-to-lymphocyte ratio (% , interquartile range)			
Pre-chemotherapy	150.2 (122.8–202.3)	140.5 (86.9–186.0)	0.617
Post-chemotherapy	134.4 (103.2–289.3)	151.8 (122–311.7)	0.924
lactate dehydrogenase (U/L, median, interquartile range)			
Pre-chemotherapy	174 (133–228)	178 (170–193)	0.493
Post-chemotherapy	191 (140–217)	214 (197–239)	0.665
C-reactive protein (mg/dL, median, interquartile range)			
Pre-chemotherapy	0.5 (0.07–0.59)	0.13 (0.04–0.40)	0.105
Post-chemotherapy	0.4 (0.05–2.65)	0.36 (0.09–0.95)	0.522
Butyrylcholinesterase (U/L, median, interquartile range)			
Pre-chemotherapy	251 (160–297)	283 (225–303)	0.203
Post-chemotherapy	236 (196–248)	289 (225–327)	0.053
Acyl ghrelin (pg/mL, median, interquartile range)			
Pre-chemotherapy	1.26 (0.26–2.38)	1.59 (1.20–2.51)	0.298
Post-chemotherapy	1.14 (0.07–1.98)	1.53 (0.90–2.01)	0.496
Desacyl ghrelin (pg/mL, median, interquartile range)			
Pre-chemotherapy	40.06 (15.62–110.44)	109.06 (0.01–153.53)	0.854
Post-chemotherapy	107.26 (51.82–169.41)	84.03 (19.90–142.39)	0.339

CP, cancer progression; GC, gemcitabine and cisplatin; GCarbo, gemcitabine and carboplatin.

is different from that of AG, including promotion or suppression of food intake (an anti-arteriosclerotic effect), suppression of inflammatory cell infiltration, protection against muscle atrophy, and neuroprotection (23,24).

Ghrelin expression was recently identified in a wide range of normal tissues, including those in the stomach, duodenum, colon, hypothalamus, pituitary, pancreas, placenta, lung, cardiomyocytes, ovaries, and testes (15). Similarly, ghrelin is expressed in various malignant tumor tissues, including those in digestive tract, lung, thyroid, breast, prostate, testicular, pancreatic, and renal carcinomas (15). Grönberg *et al.* investigated the expression of ghrelin and obestatin protein in 137 patients with breast cancer (26). Immunoreactivity of ghrelin and obestatin was

positively correlated with the patients' estrogen receptor status, but it was negatively associated with tumor size, tumor grade, and Ki-67 index (26). Therefore, ghrelin could be a useful prognostic marker for breast cancer due to the correlation between expression levels of ghrelin and survival rates (26). Colorectal cancer cells produce significantly more ghrelin than normal colonocytes (27). Therefore, the tissue expression levels of ghrelin may be highly correlated with the development of more advanced tumors (27). In genitourinary cancer, ghrelin expression has also been observed in the sites of renal cell carcinoma metastasis, including the bones, lungs, adrenal glands, and intestines (28). In prostate cancer cell lines, ghrelin and DG inhibited the proliferation of DU-145 cells, displayed

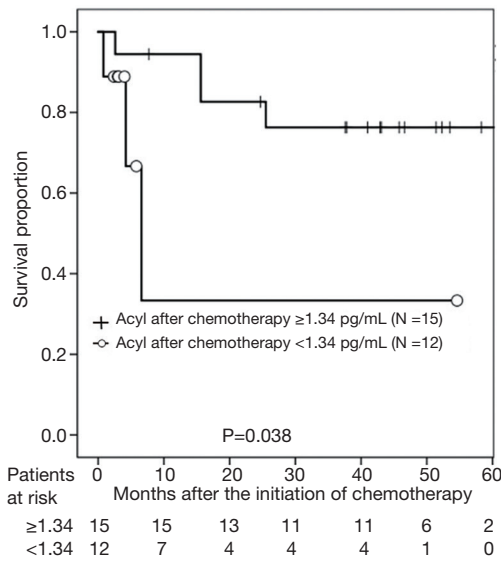


Figure 1 Kaplan–Meier estimates of progression-free survival (PFS) in patients with muscle-invasive bladder cancer after chemotherapy according to acyl ghrelin (AG) levels. The PFS rate was significantly higher in the patients with AG levels ≥ 1.34 pg/mL than in those with AG levels < 1.34 pg/mL ($P=0.038$).

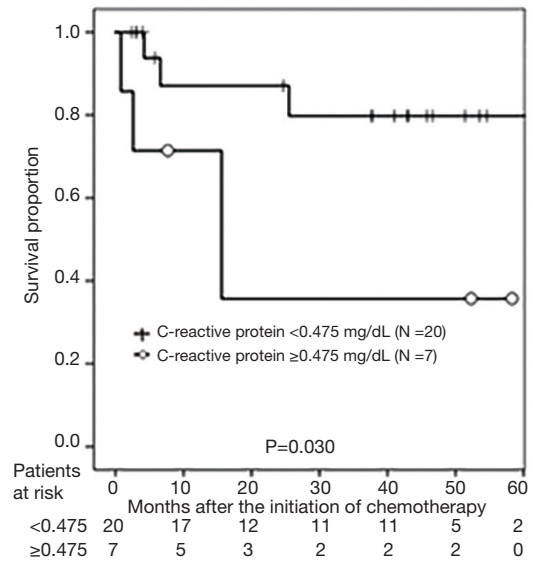


Figure 3 Kaplan–Meier estimates of progression-free survival (PFS) in patients with muscle-invasive bladder cancer before chemotherapy according to C-reactive protein (CRP) levels. The PFS rate was significantly higher in the patients with CRP levels < 0.475 mg/dL than in those with CRP levels ≥ 0.475 mg/dL ($P=0.030$).

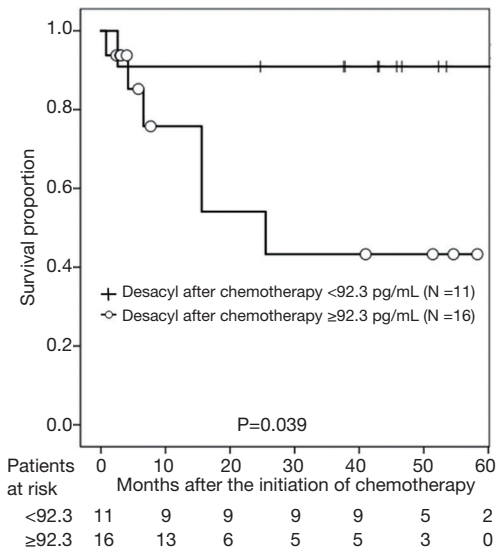


Figure 2 Kaplan–Meier estimates of progression-free survival (PFS) in patients with muscle-invasive bladder cancer after chemotherapy according to desacyl ghrelin (DG) levels. The PFS rate was significantly higher in the patients with DG levels < 92.3 pg/mL than in those with DG levels ≥ 92.3 pg/mL ($P=0.039$).

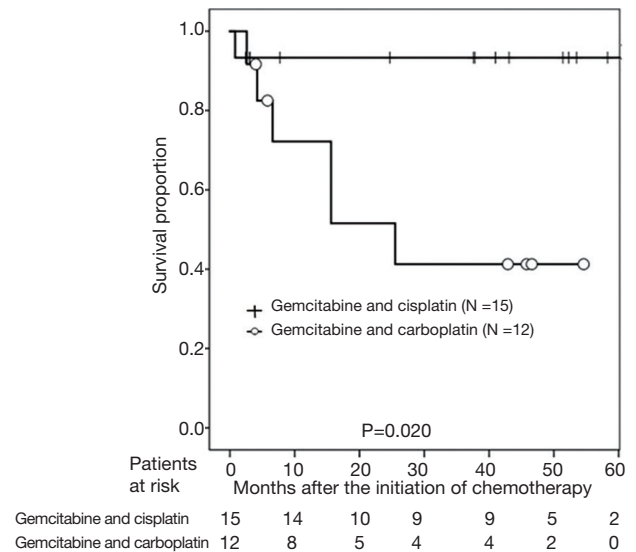


Figure 4 In the patients treated with gemcitabine and cisplatin and gemcitabine and carboplatin, the 3-year progression-free survival rates were 99.3% and 41.3%, respectively ($P=0.020$).

Table 3 Multivariate analysis according to progression-free survival (n=27)

Covariates	Odds ratio	95% CI	P
AG after chemotherapy	0.053	0.003–0.820	0.036
DG after chemotherapy	1.007	0.984–1.031	0.536
Chemotherapy regimen	0.116	0.009–1.419	0.092
CRP before chemotherapy	2.709	0.755–9.722	0.126

CI, confidence interval; AG, acyl ghrelin; DG, desacyl ghrelin; CRP, C-reactive protein.

a biphasic effect in PC-3 cells, and were effective against LNCaP cells (29).

In this study, the CRP levels before chemotherapy, serum ghrelin (AG and DG) levels after chemotherapy, and the GEM plus CDDP regimen were significantly associated with PFS. Most patients with cancer cachexia have higher total plasma ghrelin levels than those without cachexia (29). Although the mechanisms underlying the increase in ghrelin levels in patients with cachexia are unclear, patients with cachexia do not show an increased appetite despite the elevation in ghrelin levels (30). Therefore, the provision of systemic chemotherapy, especially GEM and CDDP, in patients with MIBC may not only improve the oncological outcomes but also the general condition of the patients. In addition, patients with MIBC receiving chemotherapy may have improved anti-inflammatory function, resulting in the activation of AG based on multivariate analysis findings. However, a small number of patients were enrolled in this study, therefore, GEM plus CDDP chemotherapy may have affected the improvement with oncological outcomes compared to that in GEM and CDBCA chemotherapy or changes in ghrelin levels.

The present study has few limitations. First, as this study was a retrospective study, there is an inherent potential for bias. Second, this study has a key weakness in that a small number of patients were enrolled and the follow-up period was relatively short.

Conclusions

This study suggested the usefulness of the ghrelin as a prognostic predictor of PFS in patients with MIBC. Further studies are warranted to determine the usefulness of serum ghrelin as a potential biomarker to predict oncological outcomes, especially OS, in patients with MIBC. Further research is also necessary to determine the role of AG and

DG in MIBC.

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Footnote

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Data Sharing Statement: available at <http://dx.doi.org/10.21037/tau-20-1489>

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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 Institutional Review Board of Gifu University (2018-212) and Hirosaki University (2017-1108) and informed consent was taken from all the patients.

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