Peer-reviewed version available at *Cancers* **2018**, *10*, 454; doi:10.3390/cancers10110454

Article

Severe neutropenia is associated with better clinical outcomes in patients with advanced pancreatic cancer who received modified FOLFIRINOX therapy

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Received: date; Accepted: date; Published: date

Abstract: Modified FOLFIRINOX is effective for advanced pancreatic cancer but frequently causes severe neutropenia. The present study was designed to investigate the influence of severe neutropenia on clinical outcomes in advanced pancreatic cancer patients receiving modified FOLFIRINOX. Fifty-one advanced pancreatic cancer patients who received modified FOLFIRINOX during January 2014 and May 2018 were subjects of the present study. Adverse events, including neutropenia, were graded according to the Common Terminology Criteria for Adverse Events, version 4.0. Median overall survival (OS) was determined as the primary endpoint, while median time to treatment failure (TTF), overall response rate (ORR), and the incidence of other adverse events were measured as secondary endpoints. Severe neutropenia (grade≥3) occurred in 39 patients (76.4%), in which high level of total bilirubin (>0.6mg/dL) was a significant risk as assessed by a multivariate logistic regression analysis. Median duration of OS was significantly longer in patients with severe neutropenia than in those without it (15.2 months versus 7.2 months, p=0.032). Moreover, there was a significant correlation between OS and the grade of neutropenia (r=0.306, p=0.029). ORR tended to be higher, though not significantly, in patients with severe neutropenia. In contrast, the incidence rates of other adverse events were not different between the two groups. Severe neutropenia is an independent predictor of prognosis in advanced pancreatic cancer patients received modified FOLFIRNOX therapy.

Keywords: modified FOLFIRINOX; severe neutropenia; overall survival; overall response rate; time to treatment failure; advanced pancreatic cancer

1. Introduction

Pancreatic cancer reveals extremely poor prognosis and represents the fourth leading cause of cancer-related mortality in the world. Ten-year (4.6% for mail and 4.8% for female) and five-year (7.0% for mail and 5.9% for female) survival rates of pancreatic cancer are worst among various malignancies in Japan [1,2]. There have been few effective therapies for pancreatic cancer and, therefore, development of more effective chemotherapy is urgently required to improve poor outcomes of this malignancy.

FOLFIRINOX, a combination chemotherapy consisting of oxaliplatin, irinotecan, 5-Fluorouracil (5-FU), and leucovorin, has been shown to significantly prolong the survival and becomes a standard chemotherapy for advanced pancreatic cancer. Conroy et al. showed a clinical superiority of FOLFIRINOX therapy over gemcitabine monotherapy in respect of overall survival (OS), progression-free survival (PFS), and tumor response rate (RR) in patients with metastatic pancreatic cancer [3]. However, grade 3-4 neutropenia occurs more frequently in FOLFIRINOX than

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in gemcitabine (45.7% versus 21.0%, p <0.001). A high incidence of grade 3-4 neutropenia (77.8%) was also observed in Japanese patients receiving FOLFIRINOX for metastatic pancreatic cancer [4].

To reduce the incidence of toxicites associated with FOLFIRINOX, the chemotherapy regimen has been modified by omission of bolus injection of 5-FU and/or reduction of the dose of irinotecan without reducing the clinical response [5–9]. The incidence of grade 3-4 neutropenia is reduced to 47.8% by modification of FOLFIRINOX regimen (oxaliplatin 85 mg/m2, irinotecan 150 mg/m2, 5-FU infusion 2400 mg/m2 over 46-h, no bolus 5-FU) [7]. More importantly, a meta-analysis showed that the modified FOLFIRINOX is as effective as the original FOLFIRINOX regimen with similar tumor response rate (32% versus 33%, P=0.879), rate of 11-month survival (47% versus 50%, p=0.38), rate of 6-month PFS rate (47% versus 53%, p=0.38) and less frequency in grade 3-4 adverse events [10].

The occurrence of severe adverse events, including neutropenia [11,12], mucositis [13], neurotoxicity [14]and diarrhea[15], during cancer chemotherapy results in the impairment of patient quality of life, and leads to the therapy interruption or dose reduction, which may interfere with the therapeutic effect due to the decrease in relative dose intensity. For instance, the incidence of grade 4 neutropenia is significantly higher, while median PFS and OS are significantly shorter, in non-small cell lung cancer patients receiving irinotecan with UGT1A1*6 homozygous mutation than in those with heterozygous mutation or wild-type allele[16]. The incidence rates of diarrhea and neutropenia are significantly higher, while median OS tends to be shorter, in metastatic colorectal cancer patients who received irinotecan with UGT1A1*28 heterozygous or homozygous mutations [17].

On the contrary, several investigators have shown that myelosuppression such as neutropenia becomes a surrogate for better survival in patients receiving cancer chemotherapy. In patients with metastatic colorectal cancer who receive FOLFOX therapy, the incidences of mild (grade1-2) and severe (grade3-4) neutropenia are associated with improved survival [18]. In patients with advanced pancreatic cancer receiving gemcitabine alone or in combination with other anticancer drugs, the median OS is significantly longer in patients with early onset of neutropenia than in those without neutropenia [19]. When comparing the survival effect of gemcitabine monotherapy among metastatic pancreatic patients with grade 3 neutropenia, those with grade 1-2 neutropenia and those without neutropenia, median survival time is also prolonged as the grade of neutropenia is elevated [20]; however, it is still uncertain whether the occurrence of adverse events causes favorable or unfavorable influence on the clinical outcomes, especially in pancreatic cancer patients receiving chemotherapy.

In present study, the relationship between the incidence of severe neutropenia and OS was investigated in patients with advanced pancreatic cancer who received modified FOLFIRINOX as the first-line chemotherapy.

2. Results

2.1. *Comparison of demographics between patients with and without severe neutropenia*

The overall incidence rate of severe neutropenia (grade≥3) was 76.5% (39/51). To determine the risk for severe neutropenia, demographics of patients were compared between patients who had severe neutropenia and those who did not. Patients with severe neutropenia showed significantly higher serum levels of alanine aminotransferase (P=0.017) and total bilirubin (p=0.020) as compared with those without severe neutropenia (Table1). Moreover, patients with severe neutropenia tended to be older than those without severe neutropenia (p=0.095). The relative dose intensities (RDI) of irinotecan (p=0.034), oxaliplatin (p<0.001) and 5-FU (p<0.001) were significant lower in patients with severe neutropenia than in those without it. At the start of chemotherapy, no significant difference was found in body surface area, body mass index, hemoglobin, the numbers of white blood cells, neutrophil, and platelet, between the two groups.

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Table 1. Comparison of demographics between patients with and without grade≥3 neutropenia.

	Without neutropenia (grade≥3) *n*=12		With neutropenia (grade≥3) *n*=39		*p*-value
Sex (female / male)	41.7	(5 / 7)	41.0	(16 / 23)	1.000 a)
Age (mini-max)	61.2	(54 - 71)	64.1	(49 - 74)	0.095 b)
Heterozygous for UGT1A1*6 or *28 (with/without)	43.3	(4 / 8)	38.5	(15 / 24)	1.000 a)
Height (cm)	162.1	± 5.3	161.7	± 8.1	0.152 c)
Body weight (kg)	53.6	± 8.0	58.0	± 9.4	0.892 c)
Body surface area (m^2)	1.55	± 0.11	1.60	± 0.15	0.297 c)
Body mass index	20.3	± 2.5	22.1	± 2.9	0.730 c)
Aspartate aminotransferase (IU/L)	22.8	± 10.8	28.6	± 18.2	0.304 c)
Alanine aminotransferase (IU/L)	20.1	± 11.3	37.1	± 37.9	0.017 c)
Serum creatinine (mg/dL)	0.63	± 0.19	0.70	± 0.19	0.331 c)
Total bilirubin (mg/dL)	0.55	± 0.18	0.80	± 0.35	0.020 c)
Neutrophil (/ μL)	5,667	± 3978	3,593	± 1,110	0.101 c)
White blood cells (/ μL)	7,770	± 4289	5,482	± 1,211	0.094 c)
Hemoglobin (g/dL)	11.8	± 1.0	12.3	± 1.0	0.148 c)
Platelet (10^4 / μL)	22.3	± 6.8	27.6	± 38.3	0.635 c)
HbA1c (%)	6.51	± 0.48	6.45	± 1.89	0.844 c)
Relative dose intensity					
Irinotecan	0.84	± 0.13	0.73	± 0.14	0.034 c)
Oxaliplatin	0.74	± 0.15	0.49	± 0.14	<0.001 c)
5-Fluorouracil	0.83	± 0.13	0.65	± 0.12	<0.001 c)

a) Fisher's exact probability test, b) Mann-Whitney *U*-test, c) *t*-test

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2.2. Risk factors for severe neutropenia

ROC analysis indicated that the cut-off values for age, alanine aminotransferase and total bilirubin were 64.5-year-old, 39 mg/dL and 0.6 mg/dL, respectively, as assessed by Youden index method. The area under curves (AUC) for age, alanine aminotransferase and total bilirubin were 0.660 [95% confidence interval (CI):0.487–0.833], 0.600 (95% CI: 0.416–0.784) and 0.749 (95% CI: 0.594–0.904), respectively. Among these factors, a multivariate logistic regression analysis showed that total bilirubin level>0.6 mg/dL [odds ratio (OR) = 6.62, 95% CI = 1.17–37.21; P=0.032] was found to be a significant risk for severe neutropenia (Table 2).

Table 2. Logistic regression analyses for the risk of grade≥3 neutropenia in pancreatic cancer patients receiving FOLFIRINOX therapy.

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age > 64 year-old	2.87 (0.73-11.19)	0.128	2.19 (0.50-9.54)	0.294
Alanine aminotransferase > 39 mg/dL	3.79 (0.43-33.20)	0.228	1.08 (0.08-13.68)	0.953
Total bilirubin > 0.6 mg/dL	7.63 (1.73-33.58)	0.070	6.62 (1.17-37.21)	0.032

Odds ratio (OR) and 95% confidence intervals (CI) were indicated.

2.3. Comparison of the incidence of other adverse events than neutropenia between patients with and without severe neutropenia

No significant differences in the incidence rates of such adverse events as nausea (grade≥2), vomiting (grade≥1), oral mucositis (grade≥2), dysgeusia (grade≥2), peripheral neuropathy (grade≥2), diarrhea (grade≥2), and thrombocytopenia (grade≥2) were observed between patients with and without severe neutropenia (Table3).

Table 3. Comparison of the incidence of other adverse events (grade≥2) between patients with and without grade≥3 neutropenia.

	Without neutropenia (*n*=12)		With neutropenia (*n*=39)		*p*-value
	%	(presence/absence)	%	(presence/absence)	
Nausea	58.3	(7 / 5)	53.8	(21 / 18)	1.000
Vomiting	8.3	(1 / 11)	10.3	(4 / 35)	1.000
Oral mucositis	16.7	(2 / 10)	20.5	(8 / 31)	1.000
Dysgeusia	8.3	(1 / 11)	25.6	(10 / 29)	0.422
Peripheral neuropathy	41.7	(5 / 7)	28.2	(11 / 28)	0.481
Diarrhea	33.3	(4 / 8)	25.6	(10 / 29)	0.715
Leukopenia	33.3	(4 / 8)	100	(39 / 0)	<0.001

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Thrombocytopenia	16.7	(2 / 10)	25.6	(10 / 29)	0.706

Data were statistically analyzed by Fisher's exact probability test.

2.4. Comparison of the efficacy of modified FOLFIRINOX between patients with and without severe neutropenia

Median OS was significantly longer in patients with severe neutropenia than in those without it [15.2 months (95% CI: 9.8–20.5) versus 7.2 months (95% CI: 5.0–9.5); Hazard ratio (HR): 0.44 (0.03–0.92); P=0.032] (Figure 1).

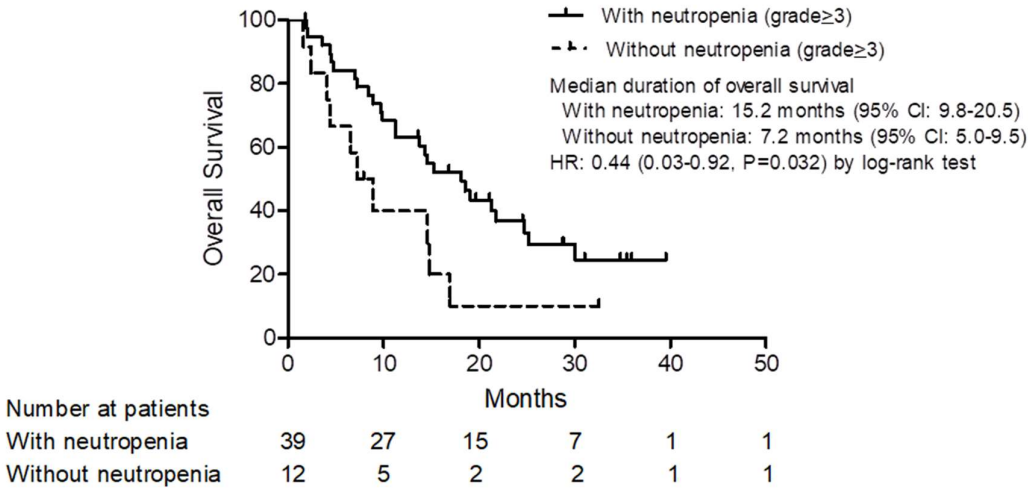

Figure 1. Kaplan-Meier estimates for comparison of overall survival in pancreatic cancer patients receiving modified FOLFIRINOX therapy who experienced with or without grade≥3 neutropenia.

The median TTF also tended to be longer in patients with severe neutropenia than in those without it [7.0 months (95% CI: 1.9–24.5) versus 3.7 months (95% CI: 2.0–12.1); HR: 0.49 (0.22–1.09), p=0.079] (Table 4). Interestingly, there was a significant correlation between the grade of neutropenia and the OS (r=0.306, p=0.029, Figure 2A), while TTF tended to correlate with the grade of neutropenia (r=0.259, p=0.066, Figure 2B).

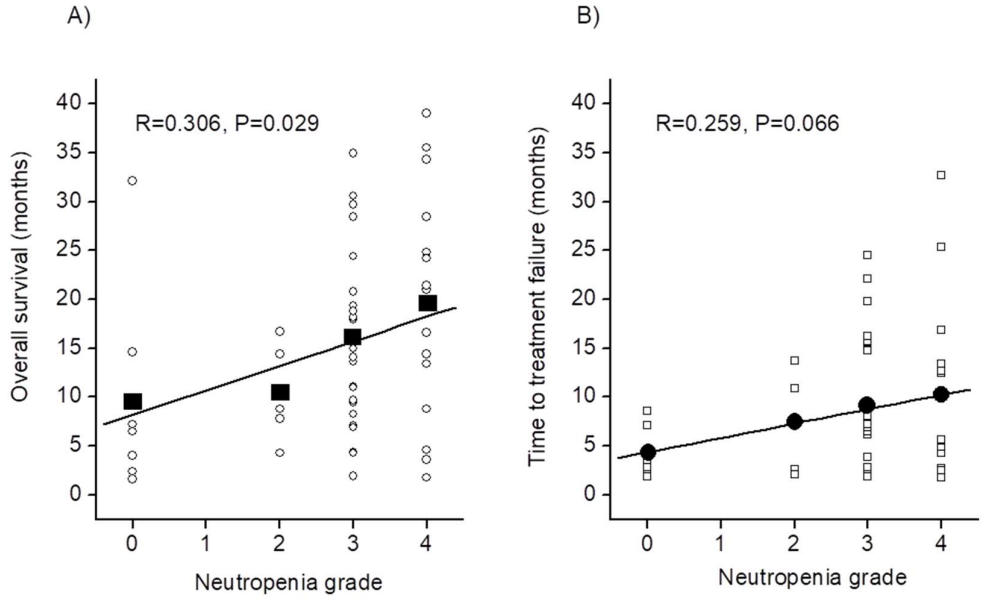

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Figure 2. Relationship between the grade of neutropenia and overall survival (A) or time to treatment failure (B) in patients with advanced pancreatic cancer who received modified FOLFIRINOX therapy.

One-year survival was also slightly and not significantly higher in patients with neutropenia than in those without it [71.8% versus 41.7%; OR: 3.56 (95% CI: 0.93–13.65), p=0.085]. There were no significant differences in the tumor response rates between the two groups (35.9% versus 16.7%, p=0.296 for ORR; 76.9% versus 66.7%, p=0.474 for DCR) (Table 4).

Table 4. Comparison of median time to treatment failure and tumor response between patients with grade≥3 neutropenia and those without neutropenia after treatment with FOLFIRINOX for pancreatic cancer.

	Without neutropenia (*n*=12)		With neutropenia (*n*=39)		*p*-value
Median time to treatment failure (months, 95% CI)	3.7	(2.0-12.1)	7.0	(1.9-24.5)	0.079
Tumor response rate (%)					
Response rate (CR+PR)	16.7	(2 / 12)	35.9	(14 / 39)	0.296
Disease control rate (CR+PR+SD)	66.7	(8 / 12)	76.9	(30 / 39)	0.474
One-year survival (%)	41.7	(5 / 12)	71.8	(28 / 39)	0.085

Data were statistically analyzed by Fisher's exact probability test.

2.5. Factors involving survival prolongation as assessed by Cox proportional hazard analyses

Univariate analysis indicated that neutropenia (grade≥3) (HR: 0.492; 95% CI: 0.252–0.959, p=0.037) and oral mucositis (grade≥2) (HR: 0.423; 95% CI: 0.195–0.919, p=0.030) were significant factors that prolong median OS among various adverse events that occurred frequently during modified FOLFIRINOX therapy (Table5), while multivariate analysis showed that only neutropenia (grade≥3) was found to be a significant factor that extends median OS (HR: 0.266; 95% CI: 0.122–0.580, p=0.001).This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Table 5. Cox proportional hazard analysis for the risk of the overall survival among various adverse events in pancreatic cancer patients receiving FOLFIRINOX therapy.

Adverse events	Univariate analysis			Multivariate analysis		
	HR	(95% CI)	*p*-value	HR	(95% CI)	*p*-value
Neutropenia	0.492	(0.252 - 0.959)	0.037	0.266	(0.122 - 0.580)	0.001
Peripheral neuropathy	0.595	(0.324 - 1.090)	0.093	0.508	(0.246 - 1.050)	0.068

Nausea	1.203	(0.687 - 2.110)	0.518	1.160	(0.606 - 2.220)	0.653
Oral mucositis	0.423	(0.195 - 0.919)	0.030	0.482	(0.185 - 1.253)	0.134
Dysgeusia	0.773	(0.393 - 1.520)	0.455	1.063	(0.461 - 2.448)	0.887
Diarrhea	0.667	(0.353 - 1.262)	0.214	0.482	(0.214 - 1.085)	0.078

Hazard ratio (HR) and 95% confidence intervals (CI) were indicated.

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3. Discussion

FOLFIRINOX or modified FOLFIRINOX is the first-line chemotherapy for advanced pancreatic cancer; however, these regimens cause a number of severe adverse events such as neutropenia. Actually, a large portion of patients (76.5%, 39/51 patients) experienced grade 3-4 neutropenia during the course of modified FOLFIRINOX therapy in the present study. The occurrence of severe adverse events may cause the therapy interruption or reduction in the dose of chemotherapy drugs, thereby resulting in an impairment of clinical outcomes. In the present study, the incidence of severe neutropenia led to the dose reduction, and thus, RDIs for irinotecan, oxaliplatin and 5-FU were all significantly lower in patients with severe neutropenia than in those with grades 0-2 neutropenia.

Nevertheless, it was noteworthy that the patients with grade 3-4 neutropenia showed significantly longer survival than those without severe neutropenia in the present study. Severe neutropenia was also a significant factor for better survival and the OS correlated well with the grade of neutropenia. In addition, there was a tendency of correlation between the grade of neutropenia and TTF. Other clinical responses, including OS rate at one-year, ORR, and DCR, also tended to be better for patients with severe neutropenia than for those without it. Therefore, severe neutropenia may become a surrogate marker for the survival effect of modified FOLFIRINOX therapy in patients with advanced pancreatic cancer. Our data were generally consistent with a previous report indicating that patients with severe neutropenia after treatment with gemcitabine-containing chemotherapy for advanced pancreatic cancer show significantly longer overall survival than those without it [19]. The survival effect of gemcitabine monotherapy for metastatic pancreatic cancer is better for patients with grade 3 neutropenia than those with lower grades of neutropenia [20].

A question raised here is why the incidence of severe neutropenia yielded better survival effect in pancreatic cancer patients who received modified FOLFIRINOX therapy. One of the mechanisms of this favorable result might be suppression of neutrophils themselves because these cells play a critical role in growth of tumors by promoting the acceleration of angiogenesis and suppressing the antitumor immune response [21,22]. Increase in neutrophils, which reflects systemic inflammation, might promote tumor progression by providing an advantageous environment for invasion and promotion of pancreatic cancer cells. Several studies have revealed that low neutrophil-to-lymphocyte ratio is a good predictor of prognosis in patients with pancreatic cancer [23,24]. Whether modified FOLFIRINOX therapy can also decrease an infiltration of neutrophils in pancreatic tumor tissue in patients with neutropenia should be examined in future studies.

We also presume that myelosuppression occurring in severe neutropenic patients contributes to improve the prognosis of the patients. Myeloid-derived suppressor cells (MDSCs), which reveal immunosuppressive action in tumor microenvironment via inhibition of CD4+ T cell proliferation [25], are accumulated in tumor cells and peripheral blood as the disease is progressed or the stage is advanced in pancreatic cancer [26–30]. Reduction of the number of MDSCs is one of the key antitumor mechanisms of certain chemotherapy agents, including 5-FU [31], because this agent prevents the accumulation of MDSCs in patients with pancreatic cancer [32]. Taken together, it is suggested that the suppressive effect of 5-FU included in FOLFIRINOX regimen on MDSCs in tumor microenvironment is more potent in patients with severe neutropenia than in those without it, which may recover the cytotoxic action of T lymphocytes and cause survival prolongation.

In the present study, total bilirubin level>0.6 mg/dL was a significant risk for severe neutropenia. Therefore, we should think about the possibility that biological activity of cytotoxic drugs, including irinotecan, was increased in patients with severe neutropenia because elevated bilirubin has critical effects on metabolism of such drugs [33]. Moreover, the patients showing severe neutropenia might have similar genetic predisposition for drug metabolism, which is

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associated with similar features of clinical course [34]. Although RDIs for irinotecan, oxaliplatin and 5-FU were lower in patients with severe neutropenia, there is a possibility that these doses could exert antitumor effects in the present study. Biological activity and/or blood concentration of cytotoxic agents might be relatively higher in severe neutropenic patients; however, future studies are needed to evaluate these hypotheses.

4. Materials and Methods

4.1. Patients

A total of 69 patients with advanced pancreatic cancer received first-line modified FOLFIRINOX in our outpatient chemotherapy clinic during a period between January 2014 and May 2018. Among them, 18 patients were excluded from the present study, since they were treated with reduced initial doses of irinotecan because of the homozygous mutation in UGT1A1 genes such as UGT1A1*28/*28, UGT1A1*6/*6 and UGT1A1*28/*6 (n=2), poor performance status (≥2 by the Eastern Cooperative Oncology Group) (n=9), or limited duration of therapy less than three cycles (n=7), thus, the remaining 51 patients were the subjects of the present study. Data were obtained from electronic medical record in our hospital and analyzed retrospectively.

The present study was carried out in accordance with the guideline for human studies adopted by the ethics committee of the Gifu University Graduate School of Medicine and notified by the Japanese government (Institutional review board approval No.26-156). In view of the retrospective nature of the study, the need for informed consent from subjects was not mandated.

4.2. Chemotherapy

Patients were treated with modified FOLFIRINOX every 2 weeks, consisting of 2-h bolus injection of oxaliplatin at 85 mg/m2, 2-h bolus injection of l-leucovorin at 200 mg/m2, 90-min bolus injection of irinotecan at 150 mg/m2, followed by continuous infusion of 5-FU for 46 h at 2400 mg/m2 without bolus 5-FU.

4.3. Assessment of adverse events

The adverse events included hematological toxicities such as neutropenia, leukopenia, thrombocytopenia, and non-hematological toxicities, including nausea, vomiting, oral mucositis, dysgeusia, peripheral neuropathy and diarrhea. The symptom of adverse events was graded according to the Common Terminology Criteria for Adverse Events version 4.0. [35]. The incidence rates of other adverse events than neutropenia associated with modified FOLFIRINOX were compared between patients with and without severe neutropenia.

4.4. Risk analysis for grade>3 neutropenia

Demographics of patients who received modified FOLFIRINOX were compared between patients who experienced severe neutropenia and those who did not. Subsequently, risk factors for severe neutropenia were examined by univariate and multivariate logistic regression analyses. The cut-off values for age, alanine aminotransferase and total bilirubin were assessed by the Youden index method in the receiver operating characteristic curve (ROC) analysis, in which the index was calculated as the maximum value of (sensitivity+specificity-1), according to methods described elsewhere [36].

4.5. Efficacy of chemotherapy

The OS was assessed as the primary indicator of the efficacy of modified FOLFIRINOX. The tumor response rates and the time to treatment failure (TTF) were assessed as the secondary indicators for the efficacy of modified FOLFIRINOX. OS was defined as the duration from the start of therapy to the death. The tumor response was evaluated on the computed tomography scan as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), according to the Response Evaluation Criteria in Solid Tumors guideline version 1.1.[37]. The overall

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response rate (ORR) was defined as CR plus PR, while the disease control rate (DCR) as CR plus PR plus SD. TTF was defined as the duration from the start of therapy to the end of the therapy.

4.6. Statistical analyses

Data were analyzed by using IBM SPSS version 22 (IBM Japan Ltd., Tokyo, Japan) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). p-values less than 0.05 were considered significant. For comparison of the demographics of patients between two groups, t-test was used for parametric analysis, while chi-square test or Mann-Whitney U-test was used for non-parametric analyses. For comparison of the incidence of adverse events and tumor response between two groups, chi-square test or Fisher's exact probability test was carried out. Kaplan-Meier estimate was used to analyze OS, and statistically compared by Mantel-Cox log-rank test. Cox proportional hazard analysis was performed to assess prognostic factors of OS. The square of coefficient of correlation (r) was calculated as a measure showing linearity of the relationship between the two variables.

5. Conclusions

The results of the present study showed the first evidence that severe neutropenia was a significant factor for better survival in patients with advanced pancreatic cancer who received modified FOLFIRINOX regimen. Incidence of severe neutropenia might become a useful index for the survival effect in pancreatic cancer patients receiving modified FOLFIRINOX therapy.

Author Contributions: Conceptualization, Hironori Fujii; Data curation, Yunami Yamada, Daichi Watanabe, Hiroko Kato-Hayashi and Koichi Ohata; Formal analysis, Yunami Yamada and Hironori Fujii; Investigation, Yunami Yamada and Daichi Watanabe; Methodology, Hironori Fujii; Validation, Ryo Kobayashi; Writing – original draft, Yunami Yamada and Hironori Fujii; Writing – review & editing, Yunami Yamada, Hironori Fujii, Daichi Watanabe, Hiroko Kato-Hayashi, Koichi Ohata, Ryo Kobayashi, Shinya Uemura, Takuji Iwashita, Masahito Shimizu and Akio Suzuki.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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