Dynamic changes in tumor blood flow during chemotherapy: Implications in customized treatment for pancreatic cancer

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is the most lethal malignancy, and the optimal selection of neoadjuvant chemotherapy (NAC) remains a critical clinical challenge. Tumor blood flow (TBF) is associated with chemotherapeutic response. However, the role of TBF in regimen-specific outcomes remains unclear. Thus, this association was investigated in the present study. A total of 58 patients with PDAC who underwent pancreatectomy after NAC between 2011 and 2023 were retrospectively analyzed. TBF was quantified using contrast-enhanced computed tomography. Patients were stratified into high and low pre-treatment TBF groups, and the associations between TBF, chemotherapy regimen [gemcitabine plus S-1 (GS), gemcitabine plus nab-paclitaxel (GnP) and modified FOLFIRINOX (mFOLFIRINOX)] and therapeutic pathological response were evaluated. Changes in TBF after treatment and intra-tumoral vascular characteristics were also assessed. A total of 35 (60.3%) and 23 (39.7%) patients were categorized into the low and high TBF groups, respectively. The prevalence of a therapeutic pathological response of at least grade II was greater in the high TBF group (P=0.027). A higher pre-treatment TBF was associated with an improved therapeutic pathological response in patients receiving mFOLFIRINOX (P=0.010) but not in those receiving GnP or GS. In patients with low pre-treatment TBF, GnP therapy increased TBF post-treatment (P=0.004), whereas mFOLFIRINOX and GS did not. Furthermore, post-treatment TBF was correlated with the number of intra-tumoral patent vessels (r=0.486; P<0.001). In conclusion, TBF may serve as a predictive biomarker for selecting neoadjuvant chemotherapy regimens for PDAC. High pre-treatment TBF was associated with improved efficacy of mFOLFIRINOX, whereas GnP may be beneficial for tumors with initially low TBF by improving tumor perfusion during therapy.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal malignancies, characterized by an extremely poor prognosis (1,2). In recent years, neoadjuvant chemotherapy has become the standard treatment for borderline resectable PDAC (3-5). Moreover, even in patients initially diagnosed with unresectable PDAC, surgical resection following a favorable response to chemotherapy has been shown to improve survival outcomes (6). Additionally, several studies suggest that neoadjuvant chemotherapy may also be beneficial for patients with resectable PDAC (7,8). These findings demonstrate the important role of chemotherapy in the multidisciplinary management of PDAC.

Several chemotherapeutic regimens are currently available for the treatment of PDAC. However, their efficacies vary among patients. In some cases, tumor progression has been observed despite treatment, resulting in loss of resectability (9). Therefore, the development of personalized treatment strategies based on tumor characteristics is becoming an important consideration for optimizing the selection of chemotherapy for PDAC.

In a previous study, we found that a higher tumor blood flow (TBF) prior to treatment was associated with a better response to neoadjuvant chemotherapy (10). However, that study was limited by its small sample size and lack of stratification according to the chemotherapy regimen. Furthermore, PDAC generally possesses an intense stromal pathology surrounding cancer cells, which is called desmoplastic stroma (2,11). Desmoplastic stroma leads to high intra-tumoral tissue pressure, blood vessel collapse, and decreased tumor perfusion (12). Chemotherapy may affect desmoplastic stroma by reducing the number of cancer cells. Furthermore, the previous report described the experience of tumor molecular and cellular alterations in PDAC (13). Therefore, it is important to evaluate not only pre-treatment TBF but also

post-treatment TBF. Although one study examined changes in TBF after chemotherapy (14), a comparison between pre- and post-treatment TBF according to chemotherapy regimen has not been explored.

The aim of this study was to evaluate the association between pre-treatment TBF and histopathological therapeutic response stratified by chemotherapy regimen and to assess changes in TBF before and after chemotherapy. In addition, intra-tumoral vessels, which may affect the TBF, were evaluated using immunostaining.

Materials and methods

Patient selection. We conducted a retrospective review of the electronic medical records at Shiga University of Medical Science Hospital between January 2011 and December 2023 to identify patients who underwent pancreatectomy following preoperative chemotherapy or chemoradiotherapy for PDAC. The medical records of the patients were accessed for the purpose of this study in April 2024. A total of 58 consecutive patients who underwent contrast-enhanced computed tomography (CECT) before the initiation of preoperative therapy were included. Clinical and pathological data were collected for the analysis.

This study was approved by the Ethics Committee of Shiga University of Medical Science (approval number R2017-171) and was conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all the patients or their legal representatives on an opt-out basis.

CECT examinations protocol and image analysis. Three-phase CECT examinations were performed using a 64-detector row computed tomography (CT) scanner (Aquilion CX Edition; Canon Medical Systems Corporation, Tochigi, Japan). An intravenous bolus of 100 ml of nonionic iodinated contrast material (Iopamiron®, Bayer Schering Pharma, Berlin, Germany) was administered at a dose of ≥600 mgI/kg body weight and an injection rate of 3 ml/s through the median cubital vein. Scans were acquired with a slice thickness of 5 mm according to institutional protocols. Imaging was performed at 35 s (arterial phase), 70 s (portal venous phase), and 130 s (late phase) after contrast injection.

CT images were independently reviewed by two experienced surgeons, each with >10 years of expertise in pancreatic imaging, who were blinded to all clinical and pathological data. Tumor and aortic attenuation values were measured during each phase (non-enhanced, arterial, portal venous, and late) using a picture archiving and communication system (ShadeQuest/ViewR-DG; Yokogawa Medical Solutions Corporation, Tokyo, Japan). In cases of discrepancies, a consensus was reached through a joint review.

Tumor attenuation values (TAV) and aortic attenuation values (AAV) were determined following a previously described methodology (10,15). Regions of interest were drawn to encompass as much of the tumor and aorta as possible, while avoiding their margins. For tumor measurements, cystic or vascular regions were excluded. The average of three measurements per phase was recorded. In all cases, the peak aortic enhancement occurred at 35 s.

TBF was calculated using the maximum slope method [10, 14]:

$$TBF = \frac{[maximum\ TAV\ (HU)] - [minimum\ TAV\ (HU)]}{35\ (s) \times [maximum\ AAV\ (HU)]} \times 100\ (s^{-1})$$

The previous study demonstrated that TBF ≥0.36 s^{-1} provides prediction of the destruction of over 50% of tumor cells in PDAC (10). Hence, the patients were classified into two groups according to the pre-treatment TBF: the high TBF group (TBF ≥0.36 s^{-1}) and the low TBF group (TBF <0.36 s^{-1}).

Assessment of therapeutic pathological response. The therapeutic pathological response was graded according to the Japanese classification of pancreatic carcinoma by the Japan Pancreas Society: Eight edition (16). The evaluations were performed by two specialized pancreatic pathologists who were blinded to the clinical outcomes. Hematoxylin and eosin-stained sections from the largest cross-section of the tumor were assessed.

Assessment of intra-tumoral vessels by CD31 immunostaining. The tissue specimens were cut into 4 μm slices from 10% formalin-fixed and paraffin-embedded blocks. The specimens were then deparaffinized and rehydrated, followed by antigen retrieval by heating the slides in distilled water at 98˚C for 45 min with an antigen retrieval solution (Immunosaver®, Nisshin EM, Tokyo, Japan). The slides were then treated in 3% H_2O_2 in methanol for 10 min at 25˚C to block endogenous peroxidase activity. To prevent nonspecific protein binding, the slides were treated with Blocking One Hist (Nacalai Tesque) and incubated overnight with a monoclonal antibody against CD31 (1:50, MA5-16337, Invitrogen, CA, USA). Subsequently, they were incubated with horseradish peroxidase-labeled polymer-conjugated secondary antibody [Simple Stain MAX PO (MULTI); Nichirei Bioscience] at 25˚C for 30 min. The antigen was visualized by diaminobenzidine staining (#415172; Nichirei Bioscience) chromogen for 2-5 min. Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were prepared by excluding the primary antibodies.

The tumor sections were examined under a microscope. Image analysis was performed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The CD31-positive area ratio was determined by evaluating five randomly selected fields, excluding the adenocarcinoma ductal structures. For vessel quantification, five high-power fields (200x magnification) were selected and patent (open-lumen) vessels were visually counted.

Clinical data collection and statistical analysis. The patient characteristics were compared between the low and high TBF groups. Categorical variables are expressed as numbers and percentages (%), whereas continuous variables are expressed as medians with interquartile ranges. Fisher's exact test (for categorical variables) and the Mann-Whitney U test (for continuous variables) were used to evaluate significant differences between the two groups. The Jonckheere-Terpstra trend test was used to examine the trends. The Wilcoxon signed rank test was used to evaluate significant difference between pre-treatment and post-treatment TBF in each regimen. Spearman's rank correlation analysis was performed to

Table I. Clinical features in the high and low pre-treatment TBF groups.

Factors	Low TBF group (n=35)	High TBF group (n=23)	P-value
Sex, male	25 (71.4)	11 (47.8)	0.098
Age, years	65.0 (62.5, 71.5)	69.0 (61.0, 72.0)	0.556
Resectability			0.286
Resectable	14 (40.0)	8 (34.8)	
Borderline-resectable	10 (28.6)	11 (47.8)	
Unresectable	11 (31.4)	4 (17.4)	
Pre-treatment CEA, ng/dl	4.0 (2.5, 5.5)	4.0 (3.2, 7.2)	0.643
Pre-treatment CA19-9, U/l	114 (33, 215)	114 (33, 215)	0.279
Pre-treatment DUPAN-2, U/l	270 (74, 1,400)	145 (37, 1,020)	0.698
Pre-treatment tumor size, mm	32.4 (28.0, 44.0)	26.2 (23.5, 37.3)	0.057
Pre-treatment regimen			>0.999
GS	14 (40.0)	9 (39.1)	
GnP	11 (31.4)	7 (30.4)	
mFOLFIRINOX	10 (28.6)	7 (30.4)	
Pre-treatment period, months	3.0 (2.0, 5.0)	3.0 (2.0, 4.0)	0.453
Chemoradiotherapy, yes	3 (8.6)	2 (8.7)	>0.999
Post-treatment CEA, ng/dl	4.7 (2.4, 5.7)	3.6 (2.9, 6.4)	0.905
Post-treatment CA19-9, U/l	41 (23, 77)	33 (18, 59)	0.279
Post-treatment DUPAN-2, U/l	98 (25, 338)	25 (25, 92)	0.044[a]
Post-treatment tumor size, mm	26.2 (19.0, 33.5)	22.1 (17.5, 27.5)	0.046[a]
RECIST classification			0.427
Progressive disease	1 (2.9)	1 (4.3)	
Stable disease	23 (65.7)	11 (47.8)	
Partial response	11 (31.4)	11 (47.8)	
Surgical procedure, PD	18 (51.4)	21 (91.3)	0.004[a]
Pathological response, at least grade II	8 (22.9)	12 (52.2)	0.027[a]

[a]Statistically significant. Data are presented as the median and interquartile range for continuous data and as the number and percentage for categorical data. TBF, tumor blood flow; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; DUPAN-2, duke pancreatic monoclonal antigen type 2; GS; gemcitabine + S-1; GnP, gemcitabine + nab-paclitaxel; mFOLFIRINOX, modified FOLFIRINOX; PD, pancreaticoduodenectomy; RECIST, Response Evaluation Criteria in Solid Tumors.

evaluate the relationship between the intra-tumoral vessels and the post-treatment TBF. In two-tailed tests, P<0.05 was considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R software (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria) (17) and Bell Curve for Excel software version 3.23 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

Comparison of clinical features between high and low TBF groups. A total of 58 patients were enrolled in the study. Of these, 35 (60.3%) were categorized into the low TBF group and 23 (39.7%) into the high TBF group. The treatment regimens included gemcitabine plus S-1 (GS, n=23), gemcitabine plus nab-paclitaxel (GnP, n=18), and modified FOLFIRINOX (mFOLFIRINOX, n=17). The clinical characteristics of each group are summarized in Table I. There were no significant differences between the groups in pre-treatment tumor size or tumor marker levels, including carcinoembryonic antigen, carbohydrate antigen 19-9 (CA19-9), and Duke pancreatic monoclonal antigen type 2 (DUPAN-2). Additionally, no significant differences were observed in pre-treatment duration, treatment regimen, or resectability classification. Furthermore, the pre-treatment duration showed no significant differences between low and high TBF groups according to the treatment regimen (P=0.708 in GS, P=0.138 in GnP, and P=0.768 in mFOLFIRINOX, respectively). The patients who received chemoradiotherapy did not show significant differences between low and high TBF groups (8.6% vs. 8.7%, P=1.000). However, post-treatment tumor size (P=0.046) and DUPAN-2 (P=0.044) were significantly lower in the high TBF group. Moreover, pancreaticoduodenectomy was performed significantly more frequently in the high TBF group (P=0.004). Although no significant difference was found in the RECIST classification between groups, the prevalence of a therapeutic pathological response of Grade II or higher was significantly greater in the high TBF group (P=0.027). The overall survival

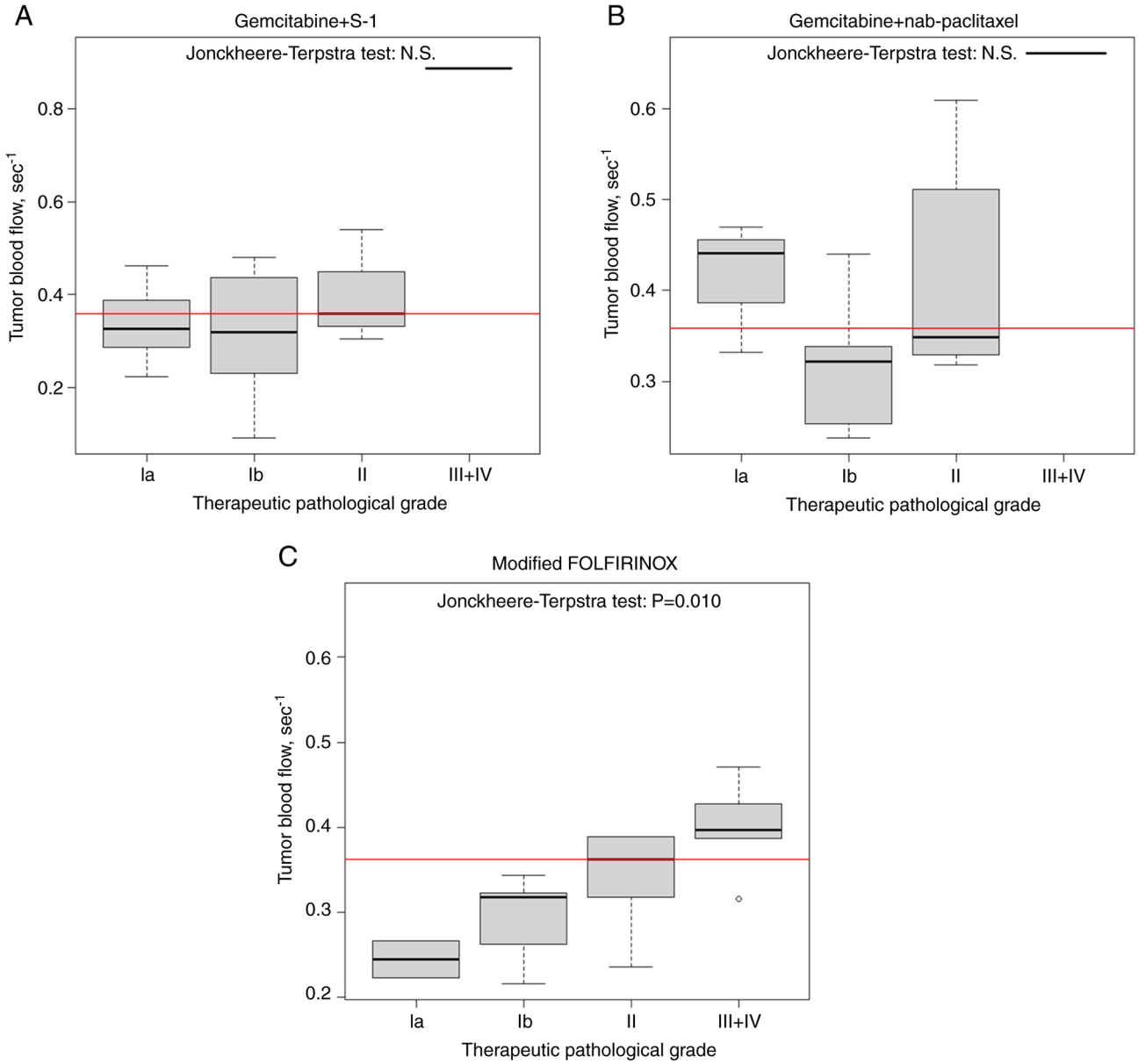

Figure 1. Association between TBF and therapeutic pathological response in each regimen. The red line indicates the cutoff borderline of high and low TBF groups. (A) Gemcitabine + S-1. (B) Gemcitabine + nab-paclitaxel. (C) Modified FOLFIRINOX. N.S., not significant; TBF, tumor blood flow.

was significantly better in the high TBF group than that in the low TBF group (not reached vs. 27 months, P=0.043). Furthermore, the progression-free survival was better in the high TBF group than that in low TBF group (55 months vs. 10 months, P=0.002).

Association between TBF and therapeutic pathological response in each regimen. Fig. 1 illustrates the association between TBF and therapeutic pathological responses. In the mFOLFIRINOX group, a higher TBF was significantly associated with a better therapeutic pathological response, as assessed using the Jonckheere-Terpstra test (P=0.010). In contrast, TBF was not associated with therapeutic response in patients receiving GnP (P=0.490) or GS (P=0.387). Fig. 2 shows the therapeutic pathological response according to regimen in the low and high TBF groups. In the low TBF group, approximately 90% of patients treated with GnP achieved a therapeutic pathological response of Grade Ib or higher, compared to 80% of patients treated with mFOLFIRINOX and approximately 65% of those treated with GS. These findings suggest that GnP is more effective than mFOLFIRINOX or GS in patients with a low TBF. Conversely, in the high TBF group, mFOLFIRINOX demonstrated greater efficacy than GnP or GS.

Between January 2024 and August 2025, we treated few new cases; Case 1: Patient with resectable PDAC who received mFOLFIRINOX for 2 months, followed by radical resection. The TBF was 0.43, and the therapeutic pathological response was Grade II. Case 2: Patient with resectable PDAC who received GnP for 2 months, followed by radical resection. The TBF was 0.24, and the therapeutic pathological response was Grade II. Case 3: Patient with resectable PDAC who received GS for 2 months, followed by radical resection. The TBF was 0.43, and the therapeutic pathological response was

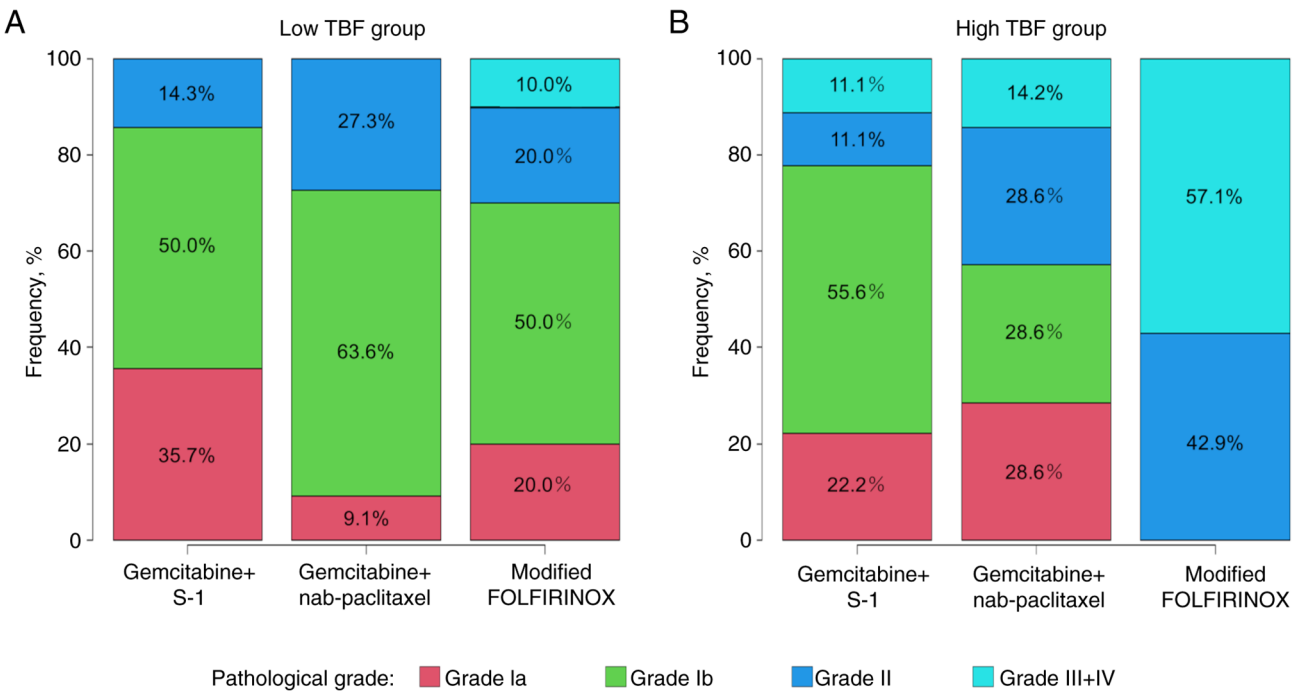

Figure 2. Frequency of each therapeutic pathological response grade by regimen in the low and high TBF groups. (A) Low TBF group. (B) High TBF group. TBF, tumor blood flow.

Grade Ia. The medical records of these patients were accessed in August 2025.

Change in TBF following treatment in each regimen. The changes in TBF before and after treatment for each regimen are shown in Fig. 3. No significant differences were observed between the pre- and post-treatment TBF values for mFOLFIRINOX and GS. However, the post-treatment TBF significantly increased after GnP treatment (P=0.018). Furthermore, among patients in the low TBF group, post-treatment TBF significantly increased after GnP treatment (P=0.004), whereas TBF remained unchanged in patients treated with mFOLFIRINOX (P=0.445) or GS (P=0.397).

Association between the post-treatment TBF and the intra-tumoral vessels. We further evaluated intra-tumoral vessels using CD31 immunostaining (Fig. 4). The number of intra-tumoral patent vessels was significantly higher in the high post-treatment TBF group (P=0.006). However, the areas of the intra-tumoral vessels were not significantly different between the two groups (P=0.428). Regarding the correlation between post-treatment TBF and intra-tumoral vessels, post-treatment TBF did not correlate with the area of intra-tumoral vessels. However, a higher post-treatment TBF was significantly associated with a greater number of intra-tumoral patent vessels (Spearman rank correlation coefficient, r=0.486; P<0.001).

Discussion

In this study, we identified three clinically significant findings. First, higher TBF before treatment was associated with better therapeutic efficacy of mFOLFIRINOX, whereas pre-treatment TBF did not influence outcomes in patients receiving GnP or GS. Second, in cases with low pre-treatment TBF, TBF improved after treatment with GnP, yet remained unchanged in patients treated with mFOLFIRINOX or GS. Finally, the TBF was positively correlated with the number of intra-tumoral patent vessels. Recently, neoadjuvant chemotherapy has become the standard therapeutic option for PDAC in recent years (3-7). However, the optimal pre-operative therapeutic regimen remains unclear. Considering the heterogeneous nature of the pancreatic tumor microenvironment (2), personalizing chemotherapy based on individual tumor characteristics may improve clinical outcomes. Our findings suggest that TBF may serve as a useful indicator for selecting an appropriate chemotherapy regimen for PDAC.

First, we found that patients with higher pretreatment TBF showed better pathological responses to mFOLFIRINOX. Previous study has reported that therapeutic responders showed significantly higher TBF when assessed using perfusion computed tomography, which allows accurate evaluation of TBF (14). The findings of our study are consistent with these results, supporting the notion that higher TBF is associated with a better response to therapy in PDAC. Although our study evaluated TBF using conventional contrast-enhanced CT, which is considered less accurate than perfusion CT for assessing TBF, we obtained similar findings. Importantly, conventional contrast-enhanced CT is more widely available and routinely performed in clinical practice compared with perfusion CT, which highlights the potential clinical applicability of our results. Furthermore, prior study did not perform analyses according to treatment regimen, and their cohorts included patients treated with both gemcitabine- and 5-fluorouracil (5-Fu)-based therapies. In contrast, our study demonstrated the relationship between TBF and preoperative therapy according to each regimen, which may provide new

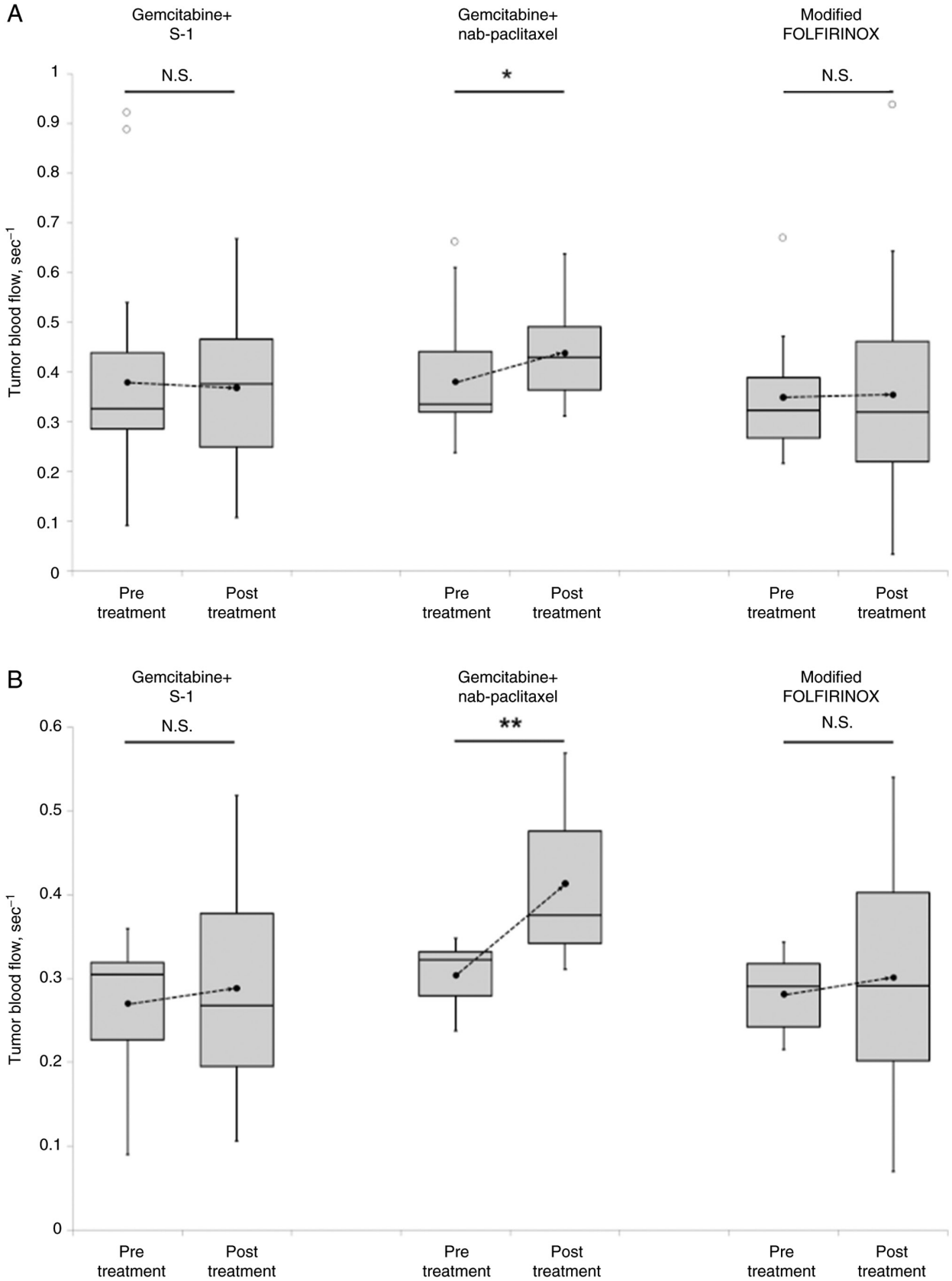

Figure 3. Changes in TBF before and after treatment for each regimen. (A) All patients. (B) Patients with low TBF. The horizontal lines in the boxes indicate median values. The circle dots indicate mean values. *P<0.05, **P<0.01 (Wilcoxon signed rank test). N.S., not significant; TBF, tumor blood flow.

information for guiding the selection of personalized optimal therapy for patients with PDAC. Nevertheless, both previous report and our study are limited by relatively small sample sizes, and further large-scale investigations are needed to validate these findings and to clarify the role of TBF in treatment stratification. One of the hallmark pathological features

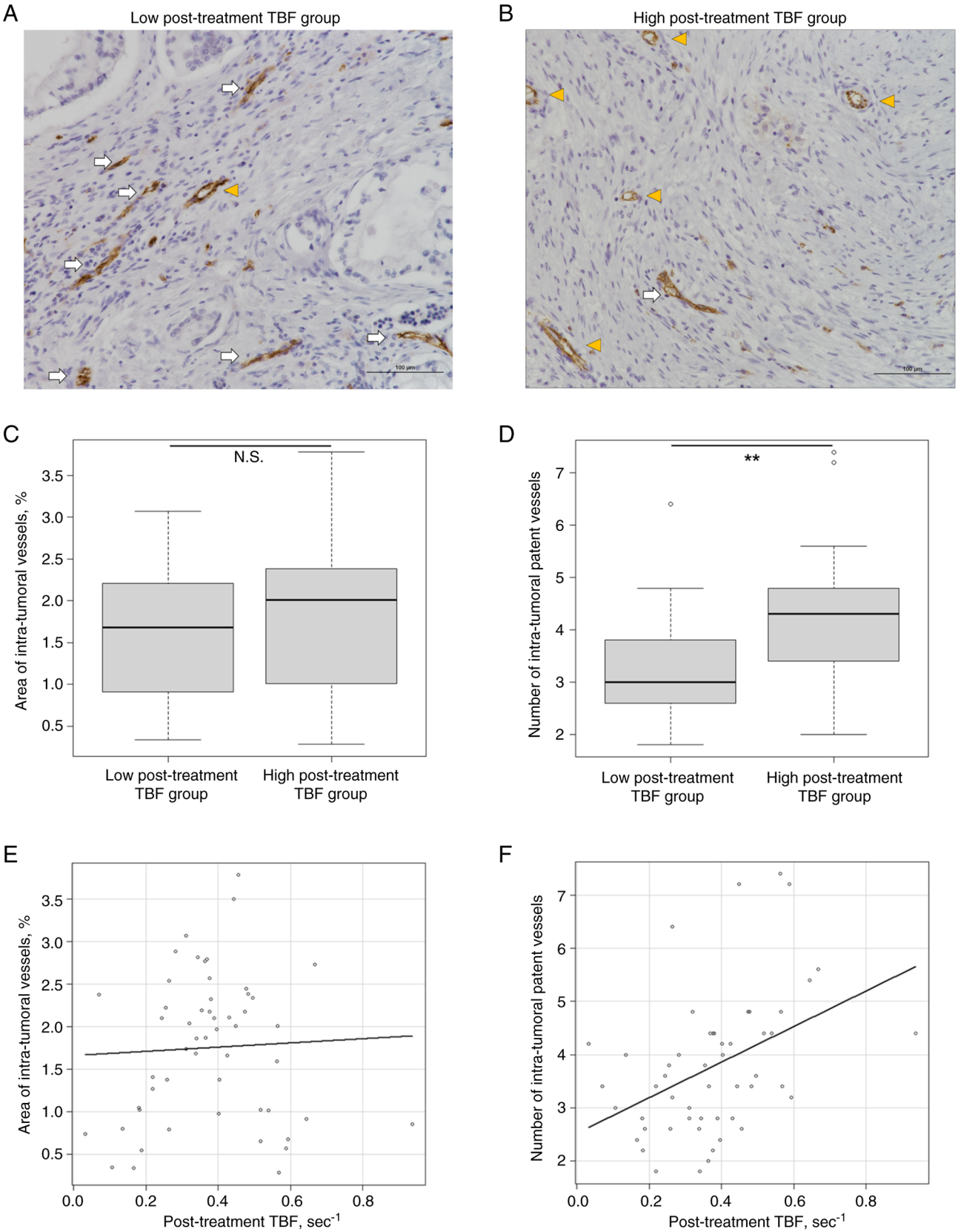

Figure 4. Assessment of intra-tumoral vessels. (A) Representative images of immunohistochemistry for CD31 in human pancreatic ductal adenocarcinoma in the low post-treatment TBF group. Orange arrowheads denote vessels that have a clearly visible lumen (patent vessels). White arrows denote closed vessels. Scale bar, 100 μm. (B) Representative images of immunohistochemistry for CD31 in human pancreatic ductal adenocarcinoma in the high post-treatment TBF group. Orange arrowheads denote vessels that have a clearly visible lumen (patent vessels). White arrows denote closed vessels. Scale bar, 100 μm. (C) Comparison of the area of intra-tumoral vessels in the high and low post-treatment TBF groups. (D) Comparison of the number of intra-tumoral patent vessels in the high and low post-treatment TBF groups. (E) Scatter diagram showing the relationship between the post-treatment TBF and the area of intra-tumoral vessels. These two factors were not correlated, with a Spearman's rank correlation coefficient of 0.044 (P=0.754). (F) Scatter diagram showing the relationship between the post-treatment TBF and the number of intra-tumoral patent vessels. These two factors are positively correlated, with a Spearman's rank correlation coefficient of 0.486 (P<0.001). **P<0.01 (Mann-Whitney U test). N.S., not significant; TBF, tumor blood flow.

of PDAC is the dense desmoplastic stroma surrounding tumor cells (2,11), which increases intra-tumoral pressure, compresses blood vessels, and reduces perfusion. This, in turn, impairs drug delivery to tumor (12). Previous studies have also reported a relationship between stromal density and TBF (18), supporting the notion that tumors with high TBF levels may permit more effective drug delivery, leading to improved therapeutic outcomes.

Conversely, no correlation was observed between pre-treatment TBF and therapeutic response in patients receiving GnP or GS. This led us to hypothesize that changes in TBF during treatment may contribute to therapeutic response. Indeed, when we compared TBF before and after treatment, we observed that TBF improved in GnP-treated patients with an initially low TBF, but remained unchanged in those treated with mFOLFIRINOX or GS. These results suggest that GnP may improve tumor perfusion during treatment, potentially enhancing drug delivery and efficacy, even in poorly perfused tumors. The previous study showed GnP reduce fibrillar collagen matrix (19). Thus, GnP may reduce intra-tumoral pressure and compresses blood vessels, subsequently improve tumor perfusion. On the other hand, in the nude mice gastric cancer model, conventional dose of 5-Fu and prodrug of 5-Fu did not reduce cancer-associated fibroblast (20). Thus, mFOLFIRINOX and GS may not improve tumor perfusion. Therefore, mFOLFIRINOX may be more suitable for tumors with a high pre-treatment TBF, whereas GnP may be preferable for tumors with a low pre-treatment TBF. As for GS, there were differences in clinical background compared to mFOLFIRINOX or GnP group, such as shorter treatment duration and a greater number of patients with low pre-treatment CA19-9 levels. However, it was difficult to describe the reason behind the lack of correlation between TBF and treatment efficacy in GS group only from these factors. In the previous report, molecular and cellular alterations in PDAC were shown to occur during treatment, suggesting that a single time-point analysis of tumor characteristics may be insufficient (13). To achieve personalized treatment, it may be important to repeatedly assess molecular and cellular information and intervene promptly in accordance with tumor evolution. Therefore, future investigations are warranted.

Additionally, we investigated the association between TBF and intra-tumoral vessels using CD31 immunostaining. Although TBF did not correlate with the area of the intra-tumoral vessels, a higher TBF was associated with a greater number of intra-tumoral patent vessels. Generally, PDAC are characterized by dense desmoplastic stroma (2,11). Therefore, both patent and occlusive vessels were observed in these tumors. However, a previous report evaluated only the area of intra-tumoral vessels (18). Our study was the first, to the best of our knowledge, to evaluate patent intra-tumoral vessels. In GnP-treated patients, TBF improved post-treatment, suggesting dynamic changes in intra-tumoral vascular patency. The predictive value of TBF for chemotherapeutic response is likely due to its effect on the efficiency of intra-tumoral drug delivery.

This study had several limitations. First, this was a retrospective analysis with a limited small sample size. Second, variations in neoadjuvant treatment duration among patients could not be controlled. Third, owing to the lack of pre-treatment specimens, we were unable to directly evaluate the correlation between pre-treatment TBF and pathological features. Because all tissue analyses were performed on post-treatment specimens, treatment-induced changes could not be ruled out. Nonetheless, we believe that the correlations observed between post-treatment TBF and pathological features are biologically plausible. Future *in vivo* studies are warranted to investigate these associations under controlled conditions.

In conclusion, we demonstrated an association between TBF and the therapeutic response to each chemotherapy regimen for PDAC. Furthermore, we observed that the changes in TBF during treatment varied depending on the chemotherapy regimen. These findings suggest that TBF may serve as a useful biomarker for guiding the selection of neoadjuvant chemotherapy for PDAC. However, larger prospective studies are needed to validate these results.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HMa, TMa, YT, HMo, NN, TS, RO, ST, KT, MK, SK, TMi and MT contributed to the study conception and design. HMa performed data analysis and interpretation. HMa and TMa assessed the immunostaining. HMa and YT evaluated tumor blood flow using computed tomography. HMo, NN, TMa and TS sectioned the pathological tissue blocks, prepared the slides and performed the immunostaining. HMo, NN, TMa, TS, RO, ST, KT, MK, SK, TMi and MT performed surgery and postoperative management. The first draft of the manuscript was written by HMa, and TMa, YT, HMo, NN, TMa, TS, RO, ST, KT, MK, SK, TMi and MT commented on previous versions of the manuscript. HMa, TMa and MT confirmed the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shiga University of Medical Science (approval no. R2017-171; Otsu, Japan) and was conducted in accordance with the principles outlined in The Declaration of Helsinki. Informed consent was obtained from all the patients or their legal representatives on an opt-out basis.

Patient consent for publication

Consent for publication of the study was obtained via the opt-out approach.

Competing interests

The authors declare that they have no competing interests.

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