The AST/ALT (De Ritis) ratio predicts clinical outcome in patients with pancreatic cancer treated with first-line nab-paclitaxel and gemcitabine: *post hoc* analysis of an Austrian multicenter, noninterventional study

Jakob Michael Riedl, Florian Posch, Gerald Prager, Wolfgang Eisterer, Leopold Oehler, Thamer Sliwa, Klaus Wilthoner, Andreas Petzer, Petra Pichler, Eva Hubmann, Thomas Winder, Sonja Burgstaller, Markus Korger, Johannes Andel, Richard Greil, Hans-Joerg Neumann, Martin Pecherstorfer, Kathrin Philipp-Abbrederis, Angela Djanani, Birgit Gruenberger, Friedrich Laengle, Ewald Wöll and Armin Gerger

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Correspondence to:
Armin Gerger
Division of Oncology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, Graz, 8036, Austria

Center for Biomarker Research in Medicine (CBmed) Ges.m.b.H., Graz, Austria
armin.gerger@medunigraz.at

Jakob Michael Riedl
Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Florian Posch
Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Center for Biomarker Research in Medicine Ges.m.b.H., Graz, Austria

Gerald Prager
Department of Medicine I, Division of Oncology, Medical University of Vienna, Vienna, Austria

Wolfgang Eisterer
Department of Internal Medicine, Klinikum Klagenfurt am Wörthersee, Feschnigstraße Sarcoma Platform Austria, Austria

Leopold Oehler
Department of Medicine, St. Joseph Hospital, Vienna, Vienna, Austria

Thamer Sliwa
Third Medical Department, Hanusch Hospital, Vienna, Austria

Klaus Wilthoner
Landeskrankenhaus Vöcklabruck, Vöcklabruck, Austria

Andreas Petzer
Department of Internal Medicine I, Hematology with Stem Cell Transplantation, Hemostaseology and Medical Oncology, Ordensklinikum Linz-Elisabethinen, Linz, Austria

Abstract

Background: The pretreatment De Ritis ratio [aspartate transaminase (AST)/alanine transaminase (ALT)] has been shown to be an adverse prognostic marker in various cancer entities. However, its relevance to advanced pancreatic ductal adenocarcinoma (PDAC) has not yet been studied. In the present study we investigated the AST/ALT ratio as a possible predictor of treatment response and disease outcome in patients with advanced PDAC treated with first-line gemcitabine/nab-paclitaxel.

Methods: A *post hoc* analysis of a prospective, multicenter, noninterventional study was performed. A total of 202 patients with advanced PDAC treated with first-line gemcitabine/nab-paclitaxel for whom the AST/ALT ratio was measured were included in this analysis.

Results: Median and 1-year progression-free survival estimates were 4.8 months and 5.1%, respectively in patients with an AST/ALT ratio above the 75th percentile of its distribution, and 6.0 months and 18.7%, respectively in patients with an AST/ALT ratio less than or equal to this cutoff, respectively (log-rank $p = 0.004$). In univariable Cox regression, a doubling of the AST/ALT ratio was associated with a 1.4-fold higher relative risk of progression or death [hazard ratio = 1.38, 95% confidence interval (CI): 1.06–1.80, $p = 0.017$]. The prognostic association was also found in multivariable analysis adjusting for Eastern Cooperative Oncology Group performance status and lung metastases (hazard ratio per AST/ALT ratio doubling = 1.32, 95% CI: 1.00–1.75, $p = 0.047$). In treatment response analysis, a doubling of the AST/ALT ratio was associated with a 0.5-fold lower odds of objective response (odds ratio = 0.54, 95% CI: 0.31–0.94, $p = 0.020$).

Conclusions: The pretreatment serum AST/ALT ratio predicts poor disease outcome and response rate in patients with advanced PDAC treated with gemcitabine/nab-paclitaxel and might represent a novel and inexpensive marker for individual risk assessment in the treatment of pancreatic cancer.

Keywords: AST/ALT, biomarker, De Ritis ratio, gemcitabine, nab-paclitaxel, pancreatic cancer, treatment response

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Petra Pichler
Universitätsklinikum St. Pölten, Sankt Pölten, Austria

Eva Hubmann
Department of Internal Medicine 1, Hospital of the Brothers of St. John of God, Graz, Austria

Thomas Winder
Division of Oncology, Department of Internal Medicine II, Academic Teaching Hospital Feldkirch, Feldkirch, Austria

Sonja Burgstaller
Abteilung für Innere Medizin IV, Klinikum Wels-Grieskirchen, Wels, Austria

Markus Korger
Krankenhaus der barmherzigen Brüder, Eisenstadt, Austria

Johannes Andel
Landeskrankenhaus Steyr, Steyr, Austria

Richard Greil
IIIrd Medical Department, Paracelsus Medical University Salzburg, Salzburg Cancer Research Institute, Cancer Cluster Salzburg, Salzburg, Austria

Hans-Joerg Neumann
Krankenhaus der Elisabethinen, Klagenfurt, Austria

Martin Pecherstorfer
Department of Internal Medicine 2, University Hospital Krems, Karl Landsteiner Private University of Health Sciences, Krems, Austria

Kathrin Philipp-Abbrederis
Angela Djanani
Department of Internal Medicine I, Gastroenterology, Hepatology, Metabolism & Endocrinology, Medical University Innsbruck, Innsbruck, Austria

Birgit Gruenberger
Friedrich Laengle
Department of Surgery, Landesklinikum Wr. Neustadt, Wr. Neustadt, Austria

Ewald Wöll
Department of Internal Medicine, St. Vinzenz Hospital Zams, Sanatoriumstrasse, Zams, Austria

Introduction

With a 5-year overall survival (OS) of approximately 3% and a median OS of less than 1 year, the prognosis of pancreatic ductal adenocarcinoma (PDAC) is dismal.[1] Treatment options for advanced PDAC have been limited; for a long time, single-agent gemcitabine was the standard of care; however, significant advances of treatment response rates and disease outcome have been achieved in recent years with two novel chemotherapy regimens. In 2011 the PRODIGE study group demonstrated a 4.3-month OS benefit for the triplet chemotherapy regimen FOLFIRINOX compared with gemcitabine in patients with advanced PDAC with good performance status.[2] Then, 2 years later the MPACT trial added another treatment option, after demonstrating that the combination of nab-paclitaxel with gemcitabine was superior to gemcitabine alone in metastatic PDAC.[3] Today FOLFIRINOX and gemcitabine/nab-paclitaxel represent the standard of care in first-line therapy of patients with advanced PDAC with a good performance status. However, a considerable proportion of patients do not benefit from these highly active, but also toxic, chemotherapy regimens. Thus, it is of great importance to identify valid biomarkers that help to identify patients for treatment benefit, while sparing others from unnecessary side effects. Up to now the number of prognostic and predictive tools in advanced PDAC is limited.

The serum transaminases aspartate transaminase (AST) and alanine transaminase (ALT) are routinely measured clinical laboratory markers for liver function. In 2015, Bezan and colleagues showed that a high AST/ALT ratio (known as the De Ritis ratio) is a marker of poor prognosis in nonmetastatic renal cell carcinoma.[4] Since then, a large number of retrospective studies have examined the prognostic value of the De Ritis ratio in various cancer entities.[5–11] However, to the best of our knowledge its prognostic impact in patients with advanced PDAC has not yet been investigated.

The aim of this study was to examine the AST/ALT ratio as a potential predictive biomarker for disease outcome and treatment response in patients with advanced PDAC treated with gemcitabine/nab-paclitaxel as first-line palliative chemotherapy.

Methods

Study design and endpoints

The study was a *post hoc* analysis of a prospective multicenter noninterventional study of patients with advanced PDAC undergoing first-line systemic chemotherapy with nab-paclitaxel and gemcitabine (gemcitabine/nab-paclitaxel) at 19 academic and nonacademic centers in Austria. From March 2015 until July 2018, 242 patients were included upon initiation of gem/nab-paclitaxel, and were followed up prospectively until disease progression, death, or censoring while alive. Patients were eligible for inclusion if all of the following criteria were met: (a) advanced PDAC; (b) no previous systemic therapy for advanced disease and age $\geqslant$18 years; (c) signed informed consent; and (d) adequate liver and kidney function, defined as total bilirubin levels $\leqslant 1.5 \times$ upper limits of normal (ULN), serum AST levels $\leqslant 10 \times$ ULN and a creatinine clearance of $\geqslant$30 ml/min. Exclusion criteria included pregnant and breast-feeding women, known hypersensitivity to nab-paclitaxel, and a baseline neutrophil count $<$1.5 g/l. Patients were treated with nab-paclitaxel [125 mg/m² body surface area (BSA)] and gemcitabine (1000 mg/m² BSA) on days 1, 8 and 15 of every 4-week cycle, with the dosage being modifiable by the treating physicians in case of toxicity. This phase IV study did not interfere with routine clinical care of patients, and any treatment decisions while patients were on the study were at the discretion of the treating physicians. Patients underwent periodic imaging and treatment response assessment according to the local standard of the participating institution. Baseline and follow-up data were provided by the participating institutions and collated centrally in a study database by a contract research organization designated by the sponsor (CTM Clinical Trials Management GmbH, Vienna, Austria). For the current analysis, we used the following information from this database: (a) Baseline data on demographics including Eastern Cooperative Oncology Group (ECOG) performance status; (b) baseline laboratory data including AST, ALT, and the tumor marker CA19-9; (c) follow-up data on treatment response according to investigator-assessed Response evaluation criteria in solid tumors (RECIST) 1.1; and (d) follow-up data on disease progression, treatment discontinuation, and death.

The primary endpoint of the analysis was progression-free survival (PFS), defined as the time from the day of gemcitabine/nab-paclitaxel initiation to disease progression, treatment discontinuation for any reason, or death-from-any-cause, whichever came first. Follow-up data were truncated at 18 months after baseline. The secondary

endpoint was the investigator-assessed noncentral objective response rate during gemcitabine/nab-paclitaxel therapy, defined as a composite of complete and partial remission according to RECIST 1.1 criteria, as assessed locally by the investigators. OS was not analyzed as these data were not yet mature.

Laboratory methods

All laboratory analyses for AST, ALT, and CA19-9 were performed decentrally at the routine laboratories of the participating institutions. Laboratory parameters for analysis were all from the day of gemcitabine/nab-paclitaxel initiation to disease progression.

Statistical methods

All statistical analyses were performed with Stata (Windows version 15.0, Stata Corp., Houston, TX, USA) by FP. Continuous variables were reported as medians (25th–75th percentile), and count data as absolute frequencies (%). The AST/ALT ratio was dichotomized into a binary variable (necessary for PFS curve display) using two approaches: (a) Using an empirical cutoff below the 75th percentile of its distribution,[12] and (b) using a cutoff suggested by the Youden index.[13] Briefly, we first computed sensitivity and specificity for progression or death at each possible AST/ALT ratio cutoff, and then calculated the Youden index for each cutoff point as sensitivity + specificity − 1. Then, we classed patients as having an elevated AST/ALT ratio if their AST/ALT ratio was larger than the AST/ALT ratio with the highest Youden index. The objective response rate was presented as a proportion with 95% binomial exact confidence intervals (CIs), and was modeled with uni- and multivariable logistic regression. Median follow up was estimated with the reverse Kaplan–Meier method according to Schemper and Smith.[14] PFS was computed with Kaplan–Meier product limit estimators, and compared between groups using log-rank tests. Disease progression or death, whichever came first, were considered as a PFS event, and patients were censored either upon treatment discontinuation for any reason, at the last treatment date for patients still on treatment, or at the date last known on treatment in case of loss for follow up. Modeling of PFS functions was done with uni- and multivariable Cox regression models. Multivariable models included all predictor variables that were statistically significantly associated with PFS at the 5% level. The proportional hazards assumption for the AST/ALT ratio in these models was evaluated by fitting an interaction between the AST/ALT ratio and linear follow-up time. In a sensitivity analysis, we categorized the AST/ALT ratio into a four-level ordinal variable at its quartiles. The hypothesis underlying this study was formulated before the inspection or analysis of the data.

Results

Analysis at baseline

The AST/ALT ratio was available for 202 out of 242 patients, and a complete case analysis of the 202 patients was performed (Table 1). Briefly, among the 202 included patients, median age was 70 years (25th–75th percentile: 64–75, range: 43–89 years) and 46% of patients were female. Most patients had an ECOG performance status of 0 points ($n = 119$, 59%) and metastatic disease at baseline ($n = 128$, 72%).

On average, the 50 patients with an elevated AST/ALT ratio defined by an empirical cutoff at the 75th percentile (>1.23 units) had a higher prevalence of lymph node metastases than the 152 patients below this cutoff. Otherwise, the distribution of all other investigated baseline parameters was very similar between patients with and without an elevated AST/ALT ratio (Table 1).

Analysis of treatment response

During first-line treatment with gemcitabine/nab-paclitaxel, response was assessed radiographically in 143 patients (71%). The investigator-assessed radiographic objective response rate (ORR) without central review was 43% (95% binomial exact CI: 34–52) including 2 complete and 59 partial remissions (Table 2). The ORR was significantly lower in patients with an elevated AST/ALT ratio. In detail, 10 of the 35 patients with an AST/ALT ratio > 75th percentile had an ORR of 29%, compared with 51 objective responses in the 108 patients with an AST/ALT ratio lower than or equal to this cutoff (ORR = 47%), for an absolute difference in response of 19% (95% CI: 0–36, $p = 0.039$, Figure 1). In univariable logistic regression, a doubling of the AST/ALT ratio was associated with a 0.5-fold lower odds of objective response [odds ratio (OR) = 0.54, 95% CI: 0.31–0.94, $p = 0.020$]. An increased CA19-9 level emerged as the only other statistically significant univariable predictor of a higher response rate

Table 1. Baseline characteristics of the study population ($n = 202$). Distribution overall as well as by AST/ALT ratio ⩽ and >75th percentile of this variable's distribution (Q3). Data are reported as medians (25th–75th percentile) or absolute counts (%).

Variable	*n* (% missing)	Overall (*n* = 202)	AST/ALT ratio ⩽ Q3 (*n* = 152)	AST/ALT ratio > Q3 (*n* = 50)	*p*-value*
Demographic characteristics					
Age (years)	202 (0%)	70 (64–75)	70 (64–75)	72 (65–75)	0.361
Female	202 (0%)	92 (46%)	74 (49%)	18 (36%)	0.118
BMI (kg/m²)	202 (0%)	24.2 (21.2–27.2)	24.0 (20.9–27.2)	24.4 (22.5–27.2)	0.822
ECOG performance status	202 (0%)	/	/	/	0.363
—0	/	119 (59%)	92 (61%)	27 (54%)	/
—1	/	72 (36%)	52 (34%)	20 (40%)	/
—2	/	10 (5%)	8 (5%)	2 (4%)	/
—3	/	1 (<1%)	0 (0%)	1 (2%)	/
Smoking status	153 (24%)	/	/	/	0.441
Ex-smoker	/	48 (31%)	34 (29%)	14 (38%)	/
Never-smoker	/	67 (44%)	54 (47%)	13 (35%)	/
Current smoker	/	38 (25%)	28 (24%)	10 (27%)	/
Current alcohol abuse status	133 (34%)	/	/	/	0.714
—Occasionally	/	65 (49%)	50 (51%)	15 (44%)	/
—Multiple times per week	/	7 (5%)	5 (5%)	2 (6%)	/
—Never	/	61 (46%)	44 (44%)	17 (50%)	/
Tumor characteristics					
TNM (M)	178 (12%)	/	/	/	0.302
—M0	/	34 (19%)	28 (21%)	6 (14%)	/
—M1	/	128 (72%)	98 (72%)	30 (71%)	/
—MX	/	16 (9%)	10 (7%)	6 (14%)	/
Pancreatic/biliary stent at baseline	190 (6%)	44 (23%)	35 (24%)	9 (20%)	0.689
Prior resection of primary tumor	193 (4%)	39 (20%)	33 (22%)	6 (13%)	0.190
Prior adjuvant chemotherapy	197 (2%)	30 (15%)	23 (15%)	7 (15%)	0.942
Metastases at baseline**	/	/	/	/	/
—Liver metastases	182 (10%)	126 (69%)	92 (68%)	34 (72%)	0.592
—Lung metastases	173 (14%)	50 (29%)	36 (28%)	14 (31%)	0.704
—Lymph node metastases	165 (18%)	62 (38%)	39 (32%)	23 (52%)	**0.019**
—Bone metastases	156 (23%)	12 (8%)	7 (6%)	5 (13%)	0.186

(Continued)

Table 1. (Continued)

Variable	*n* (% missing)	Overall (*n* = 202)	AST/ALT ratio ⩽ Q3 (*n* = 152)	AST/ALT ratio > Q3 (*n* = 50)	*p*-value*
—Peritoneal metastases	163 (19%)	33 (20%)	22 (18%)	11 (26%)	0.310
—Other metastases	157 (22%)	32 (20%)	24 (21%)	8 (20%)	0.872
Laboratory parameters					
AST (U/l)	202 (0%)	30 (22–50)	29 (21–47)	35 (25–68)	**0.023**
ALT (U/l)	202 (0%)	31 (21–55)	34 (24–63)	25 (13–40)	**0.0006**
AST/ALT ratio	202 (0%)	0.99 (0.78–1.24)	0.90 (0.69–1.06)	1.64 (1.35–2.1)	**<0.0001**
CA19-9 (kU/l)	180 (11%)	2186 (131–11,987)	2136 (71–11,987)	2207 (205–11,000)	0.793

*p-values were from rank-sum tests [association between AST/ALT ratio groups and continuous variables (such as age)], Chi-square tests [association between AST/ALT ratio groups and categorical variables with an expected cell count of ⩾5 (such as smoking status)], and Fisher's exact tests [association between AST/ALT ratio groups and categorical variables with an expected cell count <5 (such as ECOG performance status)].
**Metastases variables are nonexclusive, that is patients could have one or more of these metastases locations.
ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; CA 19-9, tumor marker cancer antigen 19-9; ECOG, Eastern Cooperative Oncology Group performance status; Q3, 75th percentile of the AST/ALT ratio distribution; TNM, tumor node metastasis classification.

Table 2. Enumeration of investigator-assessed radiographic response groups (*n* = 143). Radiographic response was assessed by the participating centers, that is no centralized RECIST 1.1 assessment was performed. Radiographic response data were only available for 143 of the 202 patients. The ORR was defined as a composite of complete or partial remission. The DCR was defined as a composite of ORR and SD.

Radiographic response group	*n* (%)
CR	2 (1%)
PR	59 (29%)
SD	57 (28%)
PD	25 (12%)
NE	59 (29%)
ORR (CR + PR)	61 (43%)
DCR (CR + PR + SD)	118 (83%)

CR, complete remission; DCR, disease control rate; NE, response not assessed; ORR, objective response rate; PD, progressive disease; PR, partial remission; SD, stable disease.

(OR = 1.15, 95% CI: 1.05–1.25, $p = 0.002$), while a better ECOG performance status was numerically but not statistically significantly associated with poor response (Table 3). After multivariable adjustment for CA19-9 levels, the association between an elevated AST/ALT ratio and poor ORR prevailed in terms of magnitude of association, although the strength of association was not statistically significant (adjusted OR for a doubling in the AST/ALT ratio = 0.59, 95% CI: 0.34–1.03, $p = 0.064$, Table 3).

Analysis of the primary endpoint: PFS

Patients were followed up for a median interval of 7.9 months (range: 2 days–18 months, 95% CI: 6.4–8.8 months), cumulating in 1204 patient years at risk of progression or death. Overall, 75% and 25% of the cohort were followed for at least 5.3 and 15.5 months, respectively. During follow up, we observed 87 disease progressions and 39 deaths without previously confirmed disease progression. A total of 11 patients discontinued treatment due to toxicity (Supplementary Table 1). The median PFS was 5.5 months (95% CI: 5.1–6.3, Supplementary Figure 1).

PFS was significantly shorter in patients with an elevated AST/ALT ratio. In detail, median and 1-year PFS estimates were 4.8 months (95% CI: 2.9–5.7) and 5% (95% CI: 0.4–20.2) in patients with an AST/ALT ratio >75th percentile of its distribution, and 6.0 (95% CI: 2.9–5.7) months and 18.7% (95% CI: 10.2–29.3) with an AST/ALT ratio less than or equal to this cutoff, respectively (log-rank $p = 0.004$, Figure 2). Similar results were observed when using an AST/ALT

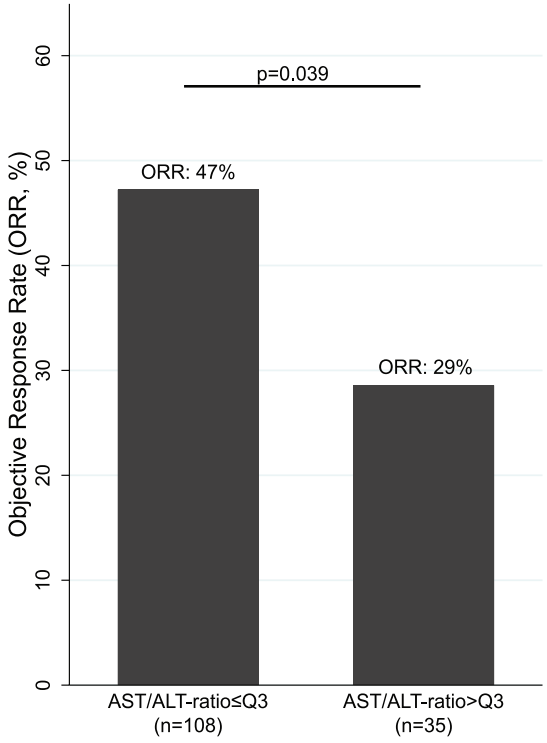

Figure 1. Investigator-assessed radiographic ORR according to the AST/ALT ratio ($n = 143$). Radiographic response data were only available for 143 of the 202 patients. The ORR was defined as a composite of complete or partial remission. A new cutoff for elevated AST/ALT ratio at the 75th percentile of its distribution (Q3) was defined for this subset of patients. The *p*-value is from a Chi-square test.
ALT, alanine transaminase; AST, aspartate transaminase; ORR, objective response.

ratio cutoff at the 48th percentile as suggested by the Youden index (Supplementary Figure 2).

In univariable Cox regression, a doubling of the AST/ALT ratio was associated with a 1.4-fold relative increase in the risk of progression or death [hazard ratio (HR) = 1.38, 95% CI: 1.06–1.80, $p = 0.017$]. We did not observe evidence for non-proportionality of hazards in this model (interaction *p*-value of AST/ALT ratio with linear follow-up time = 0.176), although the interaction hazard ratio of the AST/ALT ratio with linear follow-up time suggested that the adverse prognostic impact of an elevated AST/ALT ratio on poor PFS becomes slightly weaker over time (HR = 0.94 per month of follow-up time). Other univariable predictors of PFS were an ECOG performance status >2 (associated with poor PFS), and lung metastases (associated with favorable PFS, Table 4). In multivariable analysis adjusting for ECOG performance status and lung metastases, the prognostic association between an elevated AST/ALT ratio and poor PFS prevailed (adjusted HR per doubling = 1.32, 95% CI: 1.00–1.75, $p = 0.047$, Table 4).

Sensitivity analyses

Treating the AST/ALT ratio as a four-level ordinal variable separated by AST/ALT ratio quartiles, only those patients with an AST/ALT ratio >the 75th percentile experienced significantly shorter PFS, whereas the PFS of patients with an AST/ALT ratio in the first, second, and third quartile of its distribution was comparable (Table 4, Figure 3). In a second sensitivity analysis, we observed that patients without a documented radiographic response assessment ($n = 59$) had a significantly higher AST/ALT ratio than the 143 patients with a documented response assessment (median: 1.15 units *versus* 0.93 units, rank-sum $p = 0.0001$). Likewise, PFS was significantly poorer in patients with an undocumented radiographic response assessment (HR = 2.91, 95% CI: 1.96–4.33, $p < 0.0001$, Supplementary Figure 3).

Discussion

The present *post hoc* study used prospectively collected data from a noninterventional phase IV trial in order to investigate the predictive impact of the serum AST/ALT ratio in patients with advanced PDAC treated with gemcitabine/nab-paclitaxel. We found that a high AST/ALT ratio is significantly associated with poor disease outcome expressed as PFS. In addition, the AST/ALT ratio also emerged to be a strong predictor of treatment response to combination chemotherapy with gemcitabine/nab-paclitaxel. These findings indicate that the AST/ALT ratio might represent a novel valuable tool for risk assessment in the treatment of patients with advanced PDAC.

First described in 1957 by the Italian pathologist Fernando De Ritis as an enzymatic test for viral hepatitis,[15] the ratio of the serum transaminases AST and ALT has received attention as a potential prognostic biomarker in a variety of malignancies in recent years.[4–11] Bezan and colleagues were the first to demonstrate that a high quotient of AST/ALT is associated with poor survival in non-metastatic renal cell carcinoma patients. They proposed the elevated aerobic glycolysis and pyruvate production observed in cancer cells (the Warburg effect) as a potential biological

Table 3. Uni- and multivariable logistic regression models of investigator-assessed radiographic ORR ($n = 143$). Radiographic response data were only available for 143 of the 202 patients. The ORR was defined as a composite of complete or partial remission. All variables that were statistically significant predictors of response in univariable analysis were included in multivariable analysis.

Variable	Univariable analysis		Multivariable analysis	
	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
AST/ALT ratio (per doubling)	0.54 (0.31–0.94)	0.029	0.59 (0.34–1.03)	0.064
Age (per 5 years increase)	0.96 (0.79–1.18)	0.719	/	/
Female sex	1.44 (0.74–2.80)	0.288	/	/
BMI (per 5 kg/m² increase)	1.06 (0.73–1.53)	0.760	/	/
ECOG: 0 points	Ref.	Ref.	/	/
—ECOG: 1 point	0.84 (0.41–1.72)	0.641		
—ECOG: 2 points	0.41 (0.04–4.14)	0.453		
TNM M1 or TNM MX	2.55 (0.99–6.54)	0.052	/	/
Stent at baseline	0.53 (0.21–1.33)	0.177	/	/
Resection of primary tumor	0.66 (0.28–1.54)	0.331	/	/
Liver metastases	2.05 (0.94–4.46)	0.072	/	/
Lung metastases	0.69 (0.31–1.50)	0.347	/	/
Lymph node metastases	0.90 (0.42–1.95)	0.788	/	/
Bone metastases	0.38 (0.08–1.93)	0.243	/	/
Peritoneal metastases	0.88 (0.33–2.34)	0.800	/	/
Other metastases	0.41 (0.15–1.15)	0.091	/	/
CA19-9 (per doubling)	1.15 (1.05–1.25)	0.002	1.14 (0.07–0.48)	0.003

ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; CA 19-9, tumor marker cancer antigen 19-9; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group performance status; ORR, objective response rate; TNM, tumor node metastasis classification.

explanation for these findings.[16] Considering that the ATP production of mitochondrial oxidative phosphorylation is 18 times more efficient than aerobic glycolysis it may seem elusive why cancer cells rely on aerobic glycolysis as their main energy source. However recent studies suggest that cancer cells adapt their energy metabolism in order to increase the generation of glycolytic metabolites needed as biomass for cell growth and proliferation while compensating for the less efficient energy production by increasing glucose import.[17] Besides glucose the amino acid glutamine supplies most of the substrates and free energy necessary to fuel anabolic processes. In a recently published article, Son and colleagues described a new pathway of glutamine utilization and demonstrated that human pancreatic cancer cells are strongly dependent on glutamine. The aspartate transaminase GOT1 plays a key role in the regulation of this metabolic pathway and is essential for pancreatic cancer cell growth.[18] Correspondingly high levels of serum AST have been shown to be a marker of poor prognosis in a large cohort of patients with advanced PDAC.[19] In contrast, high levels of serum ALT, an enzyme primarily expressed in liver cells, seem to be associated with favorable pancreatic cancer outcome.[20] We therefore hypothesized that the ratio of AST/ALT might represent an even more accurate predictive biomarker in pancreatic cancer.

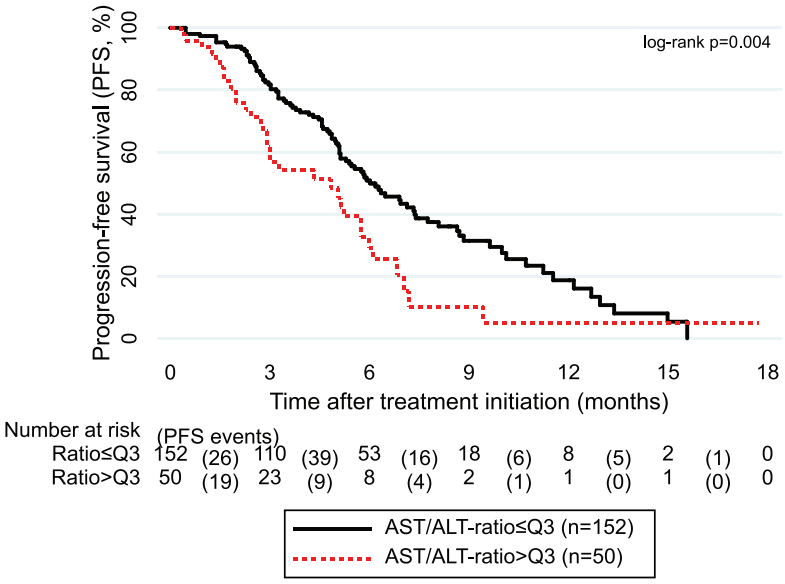

Figure 2. PFS according to AST/ALT ratio ($n = 202$). PFS estimates were computed with Kaplan–Meier estimators, and the numbers below the *x*-axis represent a risk table with the number of PFS events occurring between the respective intervals in round brackets. Patients with an elevated AST/ALT ratio were defined as having an AST/ALT ratio >75th percentile of this variable's distribution (Q3).
ALT, alanine transaminase; AST, aspartate transaminase; PFS, progression-free survival.

First, we conducted a treatment response analysis. Here we observed a strong association between the AST/ALT ratio and response to chemotherapy. Patients with an elevated AST/ALT ratio had a significantly worse treatment response. To the best of our knowledge, we are the first to investigate the association of the AST/ALT ratio with chemotherapy response in patients with cancer. Further research is needed to clarify the predictive role of the AST/ALT ratio in other cancer entities.

Interestingly, ORRs to gemcitabine/nab-paclitaxel in our study cohort were markedly higher than those reported in the phase III MPACT trial. However, this result has to be interpreted with caution, as response rates were not assessed in 29% of our patients. In order to clarify the origin of this unexpected finding we conducted a PFS analysis of patients with missing radiologic response assessment. Here we observed that patients without a documented response assessment had poor PFS, indicating that they likely came off study before the radiologic response assessment due to clinical progression or death. Also, no centralized review of responses was performed, which creates potential for overestimating the ORR. For example, in the phase III MPACT trial, response rates by investigator assessment and centralized review were 23% and 29%, respectively. Due to the real-world setting of this trial, a selection bias might also have contributed to the altered response rates. In a second analysis we used the subgroup of patients without response assessment as an exploratory cohort for our biomarker analysis. We found that patients without a response assessment had a significantly higher AST/ALT ratio, than those included in the primary analysis. Considering the poor prognosis in this patient subgroup, these findings support the prognostic value of the AST/ALT ratio.

In our primary endpoint analysis, the AST/ALT ratio emerged as an independent marker for disease outcome in patients with PDAC treated with gemcitabine/nab-paclitaxel. Previous studies have mostly used receiver operating characteristic (ROC) analysis in order to locate an optimal cutoff for patient dichotomization. In the present study we applied three different approaches to analyze the association of the AST/ALT ratio with disease outcome indicated as PFS. First, we used an empirical cutoff at the 75th percentile of the AST/ALT level. Here we observed that patients with an elevated AST/ALT ratio above 1.23 units had a 1.2 month shorter PFS and a 14% lower 6-month PFS rate than those with an AST/ALT in the lower three quartiles. In the present study, patients with an AST/ALT ratio in the upper quartile had poor disease outcome, whereas no statistically

Table 4. Uni- and multivariable Cox proportional hazards regression models of progression-free survival ($n = 202$). All variables that were statistically significant predictors of response in univariable analysis were included in multivariable analysis.

Variable	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
AST/ALT ratio (per doubling)	1.38 (1.06–1.80)	0.017	1.32 (1.00–1.75)	0.047
AST/ALT ratio: Q1	Ref.	Ref.	/	/
AST/ALT ratio: Q2	1.08 (0.65–1.79)	0.762	/	/
AST/ALT ratio: Q3	1.27 (0.78–2.07)	0.344	/	/
AST/ALT ratio: Q4	1.97 (1.21–3.20)	0.006	/	/
Age (per 5 years increase)	1.04 (0.93–1.16)	0.528	/	/
Female	0.76 (0.54–1.09)	0.138	/	/
BMI (per 5 kg/m² increase)	1.09 (0.90–1.32)	0.367	/	/
ECOG: 0 points	Ref.	Ref.	Ref.	Ref.
—ECOG: 1 point	1.72 (1.18–2.49)	0.004	1.49 (0.99–2.25)	0.056
—ECOG: ⩾2 points	5.05 (2.46–10.37)	<0.0001	7.94 (3.59–17.56)	<0.0001
TNM M1 or TNM MX	1.22 (0.71–2.11)	0.476	/	/
Stent at baseline	1.27 (0.84–1.92)	0.376	/	/
Resection of primary tumor	1.30 (0.77–2.18)	0.321	/	/
Liver metastases*	1.08 (0.72–1.61)	0.713	/	/
Lung metastases*	0.45 (0.28–0.73)	0.001	0.51 (0.32–0.82)	0.006
Lymph node metastases*	0.87 (0.58–1.31)	0.508	/	/
Bone metastases*	0.89 (0.43–1.84)	0.759	/	/
Peritoneal metastases*	1.16 (0.72–1.87)	0.539	/	/
Other metastases*	1.25 (0.78–2.02)	0.355	/	/
CA19-9 (per doubling)	1.03 (0.98–1.07)	0.252	/	/

*Metastases variables are nonexclusive, that is patients could have one or more of these metastatic locations.
ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; CA 19-9, tumor marker cancer antigen 19-9; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group performance status; Q1, 1st quartile of the AST/ALT ratio distribution; Q2, 2nd quartile of the AST/ALT ratio distribution; Q3, 75th percentile of the AST/ALT ratio distribution; Q4, 4th quartile of the AST/ALT ratio distribution; TNM, tumor node metastasis classification.

significant difference in PFS was observed in patients with an AST/ALT ratio in the first, second or third quartile. This leads us to the hypothesize that the AST/ALT ratio might be a particularly valuable tool in identifying high risk patients with pancreatic cancer. Using the Youden index for patient dichotomization, we found consistent results with a significant PFS advantage for the low AST/ALT ratio subgroup. In a last step, we conducted a Cox regression analysis using the AST/ALT ratio as a continuous variable. We demonstrated that a doubling of the AST/ALT ratio was associated with a 1.4-higher relative risk of disease progression or death. Interestingly besides good

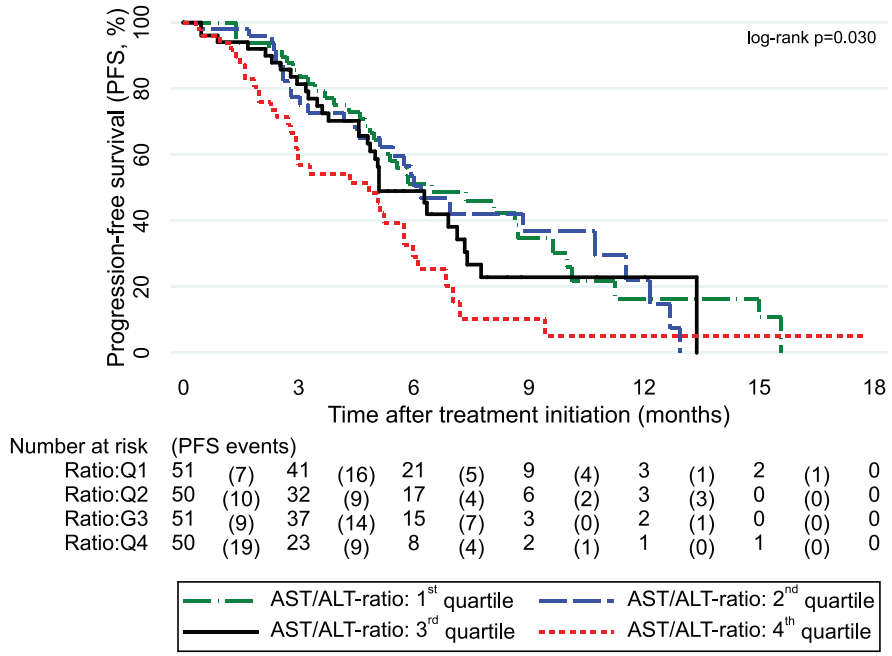

Figure 3. PFS according to AST/ALT ratio quartiles ($n = 202$). PFS estimates were computed with Kaplan–Meier estimators, and the numbers below the *x*-axis represent a risk table with the number of PFS events occurring between the respective intervals in round brackets.
ALT, alanine transaminase; AST, aspartate transaminase; PFS, progression-free survival.

performance status also the presence of lung metastases appeared to be a statistically significant marker of favorable prognosis in our cohort. These findings are in contrast with a biomarker analysis of the MPACT trial in which the presence of lung metastases did not have an impact on PFS but was associated with shorter OS.[21]

Some limitations should be discussed. One crucial point is the lack of OS data in our study, as dates of death were immature at the time of analysis. However, a recent trial has demonstrated that PFS correlates strongly with OS in advanced PDAC, rendering it a valid surrogate marker for our analysis.[22] Therefore, we can reasonably expect that the AST/ALT ratio will also be a valid prognostic marker for OS in patients with PDAC treated with gemcitabine/nab-paclitaxel. Second, no other biomarker data, including the well-established inflammatory blood-based marker neutrophil–lymphocyte ratio, were available for the current analysis and we thus cannot analyze the relationship between alterations in AST/ALT and other biomarkers indicative of, for example, inflammation. Further limitations are the absence of an independent validation cohort and the *post hoc* study design of our biomarker analysis. We therefore encourage other study groups to externally validate our findings in comparable patient cohorts with advanced PDAC in prospectively conducted biomarker studies.

Conclusion

In this *post hoc* analysis of a multicenter noninterventional study of patients with advanced PDAC undergoing first-line systemic chemotherapy with nab-paclitaxel and gemcitabine the pretreatment serum AST/ALT ratio emerged as a valid predictive marker for disease outcome and treatment response. These findings indicate that the AST/ALT ratio might represent a novel valuable tool for risk assessment and treatment stratification in patients with advanced PDAC treated with gemcitabine and nab-paclitaxel.

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Conflict of interest statement

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare that they have no competing interests.

Ethics approval and consent to participate

All patients provided written informed consent before being enrolled into the study, and the study protocol was approved by the pertinent ethics committees of the participating institutions before patient enrollment (National Lead Institutional Review Board at Ethics Committee of Medical University Innsbruck, EK-Nr.: AN2015-0028 346).

Patient involvement

Patients were neither involved in the design or conduct of this study, nor in the writing of this manuscript.

Transparency declaration

The lead authors (JMR and AG) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files). Statistical analysis code is available on request from FP. The dataset analyzed during the current study cannot be shared under the current protocol and ethics committee approval.

Supplemental material

Supplemental material for this article is available online.

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