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Indole 3-acetate and response to therapy in borderline resectable or locally advanced pancreatic cancer

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Background/Aims: It was recently reported that a higher concentration of the bacterially produced metabolite indole 3-acetate (3-IAA) in blood was linked to a better response to chemotherapy in patients with metastatic pancreatic ductal adenocarcinoma (PDAC). Here, we aimed to extend these observations to patients diagnosed with non-metastatic PDAC.

Method: We measured circulating 3-IAA in samples from a prospective population-based cohort of 124 patients with borderline resectable or locally advanced PDAC, collected before initiating neoadjuvant chemotherapy. The majority (61%) of the patients were treated with FOLFIRINOX. We used univariable and multivariable Cox proportional hazards regression to estimate the association between pre-treatment 3-IAA and overall survival.

Results: The median serum 3-IAA concentration before chemotherapy was 290 (interquartile range 203–417) ng/mL. The unadjusted hazard ratio (HR) for pre-treatment $\log_2(3\text{-IAA})$ was 0.93, 95% confidence interval (CI) [0.74–1.16], $p=0.52$. When adjusting for age, ECOG, CA19-9 and tumor classification, the HR for $\log_2(3\text{-IAA})$ was 0.87, 95% CI [0.68–1.12], $p=0.28$.

Conclusion: Our findings suggest that the potentiating effect of 3-IAA observed in metastatic PDAC undergoing chemotherapy may not translate to borderline resectable or locally advanced PDAC. We recommend additional clinical validation of 3-IAA's predictive value in different categories of PDAC before implementation attempts in human studies are initiated.

KEYWORDS

pancreatic adenocarcinoma, chemotherapy - oncology, biomarkers, microbiome, indoles, 3-IAA, indole 3-acetic acid

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers, with an incidence rate of about 15 per 100 000 per year in Norway (2023) (1). About 85% of patients with PDAC are not eligible for upfront surgery with curative intent. Many of these receive neoadjuvant or palliative therapy, but unfortunately, the response rates are low (2). Biomarkers of chemotherapy response are crucial for selecting patients who are likely to benefit from treatment in a personalized way (3).

In PDAC, carbohydrate antigen 19-9 (CA19-9) is the most commonly used biomarker for therapy response, but has limited predictive power (4). Considering new methods in oncology in general, tumor characteristics like expression of specific receptors or the presence of gene rearrangements have so far been the typical candidates as biomarkers (3). The gut microbiota composition has been increasingly associated with multiple health characteristics, including the response to some cancer therapies (5, 6). Recently, Tintelnot et al. identified circulating levels of the gut microbial metabolite indole 3-acetate (3-IAA) as positively associated with response to chemotherapy in metastatic pancreatic ductal adenocarcinoma (PDAC) (7). In an elegant experimental and molecular characterization study, the mechanism of increased chemotherapeutic effect was found to depend on oxidation of 3-IAA by myeloperoxidase from neutrophils, which subsequently increased reactive oxygen species in tumor cells and hence tumor cell death. The study introduced a novel concept, whereby a metabolite produced by gut microbes is absorbed systemically and modifies chemotherapy response in the host (8). In the study, chemotherapy response in mice could even be improved by supplementing the diet with the 3-IAA precursor tryptophan, leading to increased 3-IAA levels, suggesting that a similar adjuvant approach may demonstrate clinical efficacy in humans.

Chemotherapy in metastatic PDAC is given with palliative intent, but it is the primary treatment for patients with borderline resectable or locally advanced PDAC in order to improve resection rates with curative intent (2). We therefore measured 3-IAA in a cohort of patients with borderline resectable or locally advanced PDAC before starting chemotherapy (9), aiming to investigate if elevated serum 3-IAA associates with therapeutic response in a PDAC population without distant metastases.

Patients and methods

Study design and participants

The participants included in this study were from the NORPACT-2 study (9), which was a prospective, population-based cohort of patients with borderline resectable or locally advanced PDAC enrolled at the Oslo University Hospital between 2018 and 2020. We included 124 patients who had donated blood before initiation of any chemotherapy (flowchart shown in [Supplementary Figure S1](#)). A subset of 35 (28%) patients who were available and willing to donate additional samples had a second blood draw 2–4 months after initiation of chemotherapy.

The study protocol was approved by the Regional Ethical Committee of Medical and Health Research Ethics Nord 2017/1382. To assess population concentrations of 3-IAA, $n=100$ healthy control serum samples (median age 40, female 41%) studied in the context of other studies were also included (10), approved by the Regional Ethical Committee of Medical and Health Research Ethics South-Eastern Norway 2015/2140.

Outcome, definitions and diagnostic criteria

Borderline resectable or locally advanced PDAC was diagnosed according to the National Comprehensive Cancer Network (NCCN) criteria, version 2, 2017 (2). Distant metastases were ruled out using computed tomography (CT) scans of the abdomen and chest. Fine-needle aspiration cytology or biopsy by endoscopic ultrasound was required to confirm PDAC. The chemotherapy regimen was decided by the treating medical oncologist at the local hospital.

The primary outcome was defined as overall survival, and time to the outcome was defined as the time from blood draw, which was done in conjunction with initiation of primary chemotherapy. A secondary outcome was included where participants were censored at their time of surgical resection (after start of primary chemotherapy) where applicable.

Targeted liquid chromatography-tandem mass spectrometry

Baseline and follow-up serum was sampled and stored according to a standardized procedure at Oslo University Hospital. Serum was left to coagulate for at least 30 minutes, followed by centrifugation at $2450 \times g$ for 15 minutes and frozen and stored at -80°C . Targeted metabolite analysis of 3-IAA was performed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform, which is also used for measuring B-vitamins and human and bacterial tryptophan metabolites at BEVITAL (www.bevital.no) as described in Midttun et al. (11).

Briefly, 3-IAA was added to the established assay using indole-2,4,5,6,7- d_5 -3-acetic acid (3-IAA d_5), obtained from C/D/N Isotopes Inc (Quebec, Canada), as internal standard. The retention times were 4.93 min (3-IAA) and 4.91 min (3-IAA d_5). The analytes were detected in positive-ion multiple reaction monitoring (MRM) mode, using the ion-pairs of 176.2/130.2 m/z and 181.1/134.1 m/z for 3-IAA and 3-IAA d_5 , respectively.

Statistics

Surviving proportions were visualized by plotting Kaplan-Meier survival curves of quartiles of 3-IAA. Cox proportional hazards models were used to estimate 3-IAA's (\log_2 -transformed) association with overall survival. We included ECOG performance status (modeled as a continuous variable), CA19-9

(log₂-transformed), tumor classification (locally advanced or borderline resectable) and age in a multivariable Cox model.

To test for differences in distributions of continuous variables we used the Wilcoxon rank-sum test for independent observations and the Wilcoxon signed-rank test for paired observations. Distributions are given as median (interquartile range, IQR).

For any variable used in the analyses, data were missing in less than 5% of the participants and missing data were therefore not imputed. All data processing, visualization and statistical analyses were performed in R.

Results

In total, 124 patients (median 68 years, 47% male) with borderline resectable (n=68) or locally advanced PDAC (n=56) were included (Table 1). Seventy-six (61%) patients were treated with fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX), 26 (21%) with gemcitabine plus nab-paclitaxel, and 22 (18%) with gemcitabine monotherapy or other chemotherapy regimens.

The median 3-IAA concentration before chemotherapy was 290 (IQR 203–417) ng/mL. This was within the range of concentrations observed in n=100 healthy individuals analyzed in the context of another study (median 377 (IQR 303–464) ng/mL). Serum 3-IAA was positively correlated with age (Spearman's $\rho=0.33$, $p<0.001$; Supplementary Figure S2A) but not with tumor diameter or serum CA19-9 (Supplementary Figures S2B, C). Pre-treatment 3-IAA was similar in the different chemotherapy groups (Supplementary Figure S2D).

We found no statistically significant differences in pre-treatment 3-IAA concentrations according to response as defined by CT scan or CA19-9 decline (Figures 1A, B). The 3-IAA concentration was numerically higher in patients who later had surgical resection and in those alive after one year, but the difference was not statistically significant (Figures 1C, D). In participants with a second sample taken, 3-IAA concentrations were on average increased after chemotherapy (median 265 nmol/L before and 328 nmol/L after), but the increase was not statistically significant ($p=0.24$, Supplementary Figure S2E).

The median survival time was 499 days (95% confidence interval (CI) 437–597). Twenty-six (21%) patients were event-free at their last follow-up date. We stratified participants into quartiles by pre-treatment 3-IAA concentrations but found no significant differences in survival between the groups (Figure 1E), a finding supported by a Cox model where 3-IAA (log₂) had a HR=0.93, 95% CI [0.74–1.16], $p=0.52$. The model estimates were similar in the subgroups of borderline resectable or locally advanced cancer. Since 41 (33%) of the participants eventually had surgical resection of their primary tumor, which could influence survival, we evaluated the prognostic value of 3-IAA when censoring these cases at their time of surgery. Also here, we found no evidence of an association between 3-IAA and overall survival (Supplementary Figure S3). Finally, using the full cohort, we adjusted 3-IAA for age, ECOG, CA19-9 and tumor classification, where 3-IAA (log₂) had an adjusted HR=0.87, 95% CI [0.68–1.12], $p=0.28$.

Discussion

In the present study, serum 3-IAA concentration was not associated with response to chemotherapy or overall survival in a population-based cohort of borderline resectable or locally advanced PDAC starting chemotherapy. The results suggest that high 3-IAA levels do not predict chemotherapy response in borderline resectable or locally advanced PDAC.

TABLE 1 Baseline characteristics of the study population.

Characteristics	N = 124
Age	68 (60, 73)
Sex, male	58 (47%)
Body mass index, median (IQR)	23.9 (21.0, 27.0)
Performance status (ECOG)	
0	80 (65%)
1	40 (32%)
>1	4 (3.2%)
Charlson comorbidity index (- for tumor/age)	
0	62 (50%)
1	43 (35%)
>1	19 (15%)
Anatomic tumor classification	
Borderline resectable	68 (55%)
Locally advanced	56 (45%)
CA19-9, kU/L	
0-500 kU/L	70 (59%)
500-1000 kU/L	19 (16%)
≥1000 kU/L	20 (17%)
Non-producer	
Hyperbilirubinemia	2 (1.7%)
Missing	6 (4.8%)
Biliary stent	
	67 (54%)
Tumor location	
Head	101 (81%)
Body/Tail	23 (19%)
Tumor size (CT), mm	
	35 (29, 43)
Primary chemotherapy	
Fluorouracil, leucovorin, irinotecan, and oxaliplatin	76 (61%)
Gemcitabine plus nab-paclitaxel	26 (21%)
Gemcitabine monotherapy	18 (15%)
Other	4 (3%)

Continuous variables are shown as median (IQR). CA19-9, Carbohydrate antigen 19-9; ECOG, Eastern Cooperative Oncology Group.

Data availability statement

Data are not deposited in a public repository due to data privacy regulations in Norway and lack of participant consent. However, data are available on request, if the aim of the analysis is covered by the consent signed by the participants, following an amendment to the ethics approval and a data transfer agreement. Requests to access the datasets should be directed to JH, j.e.r.hov@medisin.uio.no.

Ethics statement

The studies involving humans were approved by Regional Ethical Committee of Medical and Health Research Ethics Nord (ref 2017/1382) and Regional Ethical Committee of Medical and Health Research Ethics South-East (ref 2015/2140). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PB: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. IF: Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. EK: Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. SY: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. AM: Investigation, Methodology, Writing – original draft, Writing – review & editing. PU: Investigation, Methodology, Writing – original draft, Writing – review & editing. KL: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. JH: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

Authors AM and PU were employed by the company BEVITAL AS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2024.1488749/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Flow chart showing standard-of-care for this patient group and the timing of blood samples in the present study.

SUPPLEMENTARY FIGURE 2

(A–C) Plots of baseline 3-IAA concentrations versus age, largest tumor diameter (by CT-scan) and CA19-9, all measured at baseline (before primary chemotherapy). The bivariate associations were tested using Pearson's (*r*) and Spearman's (*ρ*) correlation. The red lines show the linear regression fits with shaded 95% confidence intervals. (D) Distribution of pre-treatment 3-IAA by chemotherapy regimen (FOLFIRINOX versus all other). Statistical significance was tested using a Mann-Whitney U test. (E). Distributions of 3-IAA before and after primary chemotherapy, with lines indicating changes from before to after. Statistical significance was tested using a paired Wilcoxon signed-rank test. 3-IAA, 3-indoleacetic acid; FFX, FOLFIRINOX.

SUPPLEMENTARY FIGURE 3

Kaplan-Meier survival curves for overall survival, censoring participants who underwent surgical resection at their times of surgery. Patients were categorized by their baseline 3-IAA concentration (quartiles). The number of patients at risk at the indicated time points is shown below the plot. The results from a univariable Cox model for $\log_2(3\text{-IAA})$ is shown as an inset. 3-IAA, indole 3-acetate; CI, confidence interval; HR, hazard ratio.

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