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Abstract

Aim: Contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) parameters may be used to predict prognosis of pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumors (pNET). The aim of this study was to investigate the association between several perfusion parameters on CEH-EUS performed before treatment and survival outcome in patients with PDAC or pNET. Material and methods: Thirty patients with PDAC or pNET who underwent CEH-EUS and EUS-guided fine needle aspiration (EUS-FNA) were included. Quantitative analysis of tumor vascularity was performed using time-intensity curve (TIC) analysis-derived parameters, obtained from processing CEH-EUS recordings with a commercially available software (VueBox). Cox proportional hazards models were used to determine associations with survival outcome. Results: Median overall survival (OS) for PDAC patients was 9.61 months (95% CI: 0.1-38.7) while the median OS for pNET patients was 15.81 months (95% CI: 5.8-24.75. In a multivariate model for OS, a lower peak enhancement (HR=1.76, p=0.02) and a lower wash-in area under the curve (HR=1.06, p=0.001) were associated with worse survival outcome for patients with PDAC. Conclusions: CEH-EUS parameters may be used as a surrogate to predict PDAC aggressiveness and survival before treatment. After validation by large-scale studies, CEH-EUS perfusion parameters have the potential to be used in pretreatment risk stratification of patients with PDAC and in evidence-based clinical decision support.

Keywords: contrast enhanced harmonic endoscopic ultrasound; pancreatic ductal adenocarcinoma; pancreatic neuroendocrine tumors

Introduction

In 2030, pancreatic cancer is projected to become the second cause of cancer-related deaths after lung cancer, according to an American Association for Cancer Re- search analysis [1]. Late clinical presentation and chemoresistance are the main incriminating factors for the aggressiveness of pancreatic cancer. Currently, chemotherapy alone or in combination with radiotherapy represents the only valid option for patients with unresectable or metastatic pancreatic cancer [2]. However, some patients may experience increased toxicity rather than tumor response after therapy. Therefore, identification of prognostic markers before therapy in order to select patients with the highest chances of responding to treatment may be worthwhile.

In order to improve the outcome of this aggressive cancer, various biomarkers with prognostic or therapeutic role have been studied [3]. High resolution imaging
such as contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) with quantitative perfusion analysis may serve not only in establishing an early and accurate diagnosis, but also as a prognostic tool in order to predict survival. For instance, in colorectal cancer it has been proved that CEH-EUS can assess tumor perfusion in real-time, representing a suitable method to quantify angiogenesis and consequently to predict the response to treatment [4].

CEH-EUS uses microbubble contrast agents to quantify tissue perfusion over time. After bolus injection of microbubbles, perfusion imaging is analyzed in two phases, the early (arterial) phase and the late (venous) phase [5], with the pancreatic lesions described as hyper-, iso- or hypoenhanced. Quantitative analysis of tumor vascularity can be performed using time-intensity curve (TIC) analysis-derived parameters, obtained from processing CEH-EUS recordings with a commercially available software [6]. TIC analysis can be done for a specific region of interest (ROI) or for several regions such as inside a tumor and inside normal parenchyma. Several studies have used perfusion parameters for the differential diagnosis between benign and malignant pancreatic lesions. As an example, maximum intensity gain was the parameter that managed to differentiate with 100% sensitivity and specificity autoimmune pancreatitis from pancreatic adenocarcinoma [7]. Furthermore, other perfusion parameters such as peak enhancement, wash in rate, wash in area under the curve or uptake ratio differentiated chronic pancreatitis from pancreatic cancer [6,8].

However, the prognostic value of perfusion parameters derived from TIC analysis has not been yet evaluated. The aim of this study was to investigate the association between several perfusion parameters on CEH-EUS performed before treatment and survival outcome in patients with pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumors (PNET).

**Material and methods**

This was a retrospective study including patients with PDAC or PNET who underwent CEH-EUS and EUS-guided fine needle aspiration/biopsy (EUS-FNA/FNB) at the Research Center of Gastroenterology and Hepatology, Craiova between 2017-2019. The study was performed in accordance with standard ethical guidelines approved by the institutional review board and in accordance with the Declaration of Helsinki. The study was non-interventional and all procedures were performed according to the clinical daily management of patients with focal pancreatic masses. A convex linear-array endoscope (Pentax EG-3870UTK) and a compatible ultrasound machine (Arieta V70, Hitachi) were used in order to describe tumor characteristics (echogenicity, size, echostructure). EUS-FNA was performed using a 22-gauge needle (Expect or Acquire Boston Scientific).

Clinical and pathological characteristics were analysed by chart review. Median follow up time was 12 months. The final diagnosis was established based on the FNA/FNB cytopathology results, histological examination of the surgically resected specimens and/or clinical/radiological follow-up of at least 6 months.

**CEH-EUS**

A 2-panel image with the usual conventional gray-scale B-mode EUS image on the right side and with the CEH-EUS image on the left side was used, according to established pre-settings. A low mechanical index procedure was used, with a mechanical index of 0.2 and corresponding acoustic powers. The starting point of the timer was considered the moment of intravenous contrast agent injection (SonoVue 4.8 mL).

CEH-EUS was performed during usual EUS examinations, with the whole video (T0-T60s) recorded on the embedded hard disk drive of the ultrasound system for later analysis.

**TIC analysis**

Quantitative analysis of tumor vascularity was performed using TIC analysis-derived parameters, obtained from processing CEH-EUS recordings with a commercially available software. The videos were analyzed by using the VueBox software (Bracco Suisse SA, Geneva, Switzerland) by using standard protocols already described in literature and in one of our previous studies [6].

The following quantitative parameters were calculated: peak enhancement (PE), wash-in area under the curve (Wi-AUC), rise time, mean transit time (mTT), time to peak (TTP), wash-in rate (WiR), and wash-in perfusion index (WiPI). The software also provides referenced values (expressed in percentages), aligning the set of values for the tumors regions of interest (ROI) to the parenchymal ones.

**Statistical analysis**

Cox proportional hazards models were used to determine associations with survival outcome. Hazard ratios and corresponding 95% confidence intervals were calculated.

The data were stored in excel files where the main statistical indicators were calculated and the graphs were generated. Inferential statistics were compiled using Matlab (Mathworks, USA), version 2021b. The OS curve was evaluated using the log-rank test in Kaplan-Meier analyses. Cox proportional hazards models were used to determine associations with survival outcome. Hazard ratios and corresponding 95% confidence inter-
val were calculated. Two sided p values that were <0.05 were considered to indicate statistical significance.

**Results**

Overall, 22 patients with PDAC and 8 patients with PNET were included in the study. There were 14 female and 16 male patients with a median age of 59 (38–77) years.

Seventeen (80%) patients with PDAC had metastatic disease at the moment of diagnosis while 4 (20%) patients had locally advanced disease. The median size of the pancreatic lesion was 3.3 cm. Median overall survival (OS) for patients with PDAC was 9.61 months (95%CI, 0.1-38.7). Data regarding dynamic CEH-EUS perfusion parameters values in PDAC and PNET are detailed in Table I.

Real-time qualitative analysis of the CEH-EUS data was done during each investigation by the clinician performing the procedure and the lesions were described as hypovascular, isovascular or hypervascular. The majority of the PDAC lesions were hypovascular (fig 1), whereas one tumor had an isovascular pattern. Quantitative data was further obtained from the TIC analysis (fig 2). An absolute value for each parameter in each case has been obtained, based on the value corresponding to the parenchymal ROI used as a reference (fig 3). In a multivariate model for OS, a lower peak enhancement

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**Table I. Dynamic contrast-enhanced harmonic endoscopic ultrasound perfusion parameters values in pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumors (PNET)**

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>PDAC Lesion</td>
<td>22</td>
<td>333.20</td>
<td>1776.97</td>
<td>6.55</td>
<td>42.21</td>
<td>11.16</td>
<td>99.70</td>
<td>214.08</td>
<td>3500.85</td>
<td>5333.85</td>
<td>7.58</td>
<td>47.90</td>
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<tr>
<td>SD lesion</td>
<td>22</td>
<td>13319.12</td>
<td>62037.4</td>
<td>10.18</td>
<td>187.06</td>
<td>10.76</td>
<td>12695.35</td>
<td>8069.98</td>
<td>95729.06</td>
<td>169250.49</td>
<td>26.2</td>
<td>12631.28</td>
</tr>
<tr>
<td>Normal parenchyma median</td>
<td>22</td>
<td>1617.79</td>
<td>4907.44</td>
<td>6.93</td>
<td>57.16</td>
<td>12.49</td>
<td>372.34</td>
<td>1118.74</td>
<td>6321.30</td>
<td>16562.59</td>
<td>10.14</td>
<td>162.40</td>
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<tr>
<td>SD normal parenchyma</td>
<td>22</td>
<td>24640.4</td>
<td>125081.94</td>
<td>5.31</td>
<td>159.76</td>
<td>12.52</td>
<td>4184.17</td>
<td>14852.1</td>
<td>182978.8</td>
<td>329634.64</td>
<td>10.97</td>
<td>3949.48</td>
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<tr>
<td>PNET Lesion</td>
<td>8</td>
<td>381.65</td>
<td>1506.85</td>
<td>5.16</td>
<td>38.68</td>
<td>7.35</td>
<td>112.77</td>
<td>254.39</td>
<td>2179.31</td>
<td>3576.24</td>
<td>12.43</td>
<td>58</td>
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<tr>
<td>Normal parenchyma</td>
<td>8</td>
<td>486.54</td>
<td>6687.87</td>
<td>10.7</td>
<td>166.95</td>
<td>15.59</td>
<td>120.07</td>
<td>336.64</td>
<td>9166.32</td>
<td>11265.77</td>
<td>28.75</td>
<td>25.79</td>
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Peak Enhancement (PE), Wash-in Area Under Curve (WiAUC), Rise Time (RT), mean Transit Time local (mTTI), Time To Peak (TTP), Wash-in Rate (WiR), Wash-in Perfusion Index (WiPi), Wash-out AUC (WoAUC), Wash-in and Wash-out AUC (WiWoAUC), Fall Time (FT), Wash-out Rate (WoR), Standard deviation (SD)

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*Fig 1. Grey scale ultrasound (a) and contrast-enhanced harmonic endoscopic ultrasound for pancreatic ductal adenocarcinoma in arterial (b) and venous phase (c)*
(HR=1.76, p=0.02) and a lower wash-in area under the curve (HR=1.06, p=0.001) were associated with worse survival outcome for patients with PDAC. No statistically significant association was found between the other perfusion parameters and the overall survival of patients with PDAC.

Regarding patients with pNET, 3 patients (37%) had metastatic disease and received systemic therapy while 5 patients (63%) had local disease and underwent surgical resection. Median OS for patients with PNET was 17.28 months (95% CI: 10.9-20.7). All PNET lesions were described as hypervascular on CEH-EUS (fig 4). None of the perfusion parameters was correlated with survival for this subgroup of patients.

**Discussion**

The availability of ultrasound contrast agents has increased the utility of EUS in characterizing pancreatic lesions by allowing for the description of tumor vascularity, which is useful not only for differential diagnosis, but also for assessing the relationship between the tumor and the surrounding vessels. CEH-EUS has been shown in several studies to be useful in distinguishing focal pancreatic tumors [6]. Additionally, CEH-EUS offers a higher diagnostic accuracy than MRI or CT for small pancreatic lesions [9].

Perfusion parameters can be determined once CH-EUS recordings have been post-processed using spe-

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**Fig 2.** Time-intensity curve (TIC) for a) pancreatic neuroendocrine tumors and b) pancreatic ductal adenocarcinoma with patterns that are markedly different for instance peak intensity, time to peak

**Fig 3.** VueBox main screen with selection of ROI in a case of a) pancreatic neuroendocrine tumors and b) pancreatic ductal adenocarcinoma

**Fig 4.** Grey scale ultrasound (a) and contrast-enhanced harmonic endoscopic ultrasound for pancreatic neuroendocrine tumors, arterial phase (b) venous phase (c)
tients were separated into two groups based on intratumoral blood flow changes following treatment. They noticed that patients with abundant intratumoral blood flow responded significantly better to treatment, and that intratumoral blood flow changes following treatment were associated with prognosis (p=0.006).

CEH-EUS has been recommended as a method for predicting response to therapy due to its ability to describe changes in tumor vascularity. Studies have shown that angiogenesis is an important factor that influences the prognostic of solid tumors [12,13]. Changes in tumor vascularity under CE ultrasonography (CE-US) were employed for evaluating the effectiveness of chemotherapy. Sofuni et al [14] employed CEUS in patients treated with chemotherapy for unresectable pancreatic cancer. They determined correlation between quantitative tumor perfusion using TIC analysis and PNET grading [17].

All prior research, however, employed qualitative interpretation of CEH-EUS data to predict survival and tumor response in PDAC patients. Our study is the first to investigate the relationship between various perfusion parameters measured on CEH-EUS before therapy and survival outcomes in patients with PDAC or PNET. Only two parameters, the peak enhancement and wash-in area under the curve, were shown to be associated with survival in PDAC patients. Lower levels of these factors were linked to a lower chance of survival. However, the small number of patients included in this study, as well as the retrospective methodology, are significant limitations. Another limitation to be mentioned is that ROI placement on pancreatic tumor is highly operator-dependent and represents a major cause of divergent test results. ROI size and placement vary to include the entire tumor or various parts of it and this may affect the results. Despite various published studies, there is no clear consensus on how to perform this procedure.

More data regarding the prognostic value of the CEH-EUS perfusion parameters in patients with PDAC will be obtained from our ongoing prospective, multicentric trial. The main aim of the PEACE study is to assess the changes in tumor vascularity using CEH-EUS before and 2 months after treatment initiation in patients with unresectable, locally advanced/metastatic pancreatic cancer and to examine the correlation between vascular changes and treatment response, progression-free survival, and overall survival.

**Conclusion**

CEH-EUS parameters may be used as a surrogate to predict PDAC aggressiveness and survival before treatment. After validation by large-scale studies, CEH-EUS perfusion parameters have the potential to be used in pre-treatment risk stratification of patients with PDAC and in evidence-based clinical decision support.

**Conflict of interest:** none

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