Traditional Biomarkers in Patients with Pancreatic Cancer Staged by Computed Tomography and Endoscopic Ultrasound: Is There Still a Role in the Molecular Era?

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Abstract: Serum carbohydrate antigen 19-9 (Ca19-9) is the only approved biomarker approved for the screening and diagnosis of pancreatic cancer (PC), but its value remains controversial. The aim of our study is to evaluate the role of CA 19-9 in the management of PC patients in jaundiced patients staged by both Computed Tomography (CT) and Endoscopic Ultrasound (EUS). Additionally, we evaluated traditional cholestasis marker behavior. Medical records of 73 patients have been retrospectively reviewed. We considered tumor size, tumor stage, CA 19-9, cytolysis, and cholestasis biomarkers. All patients underwent CT scan for staging. EUS +/- fine-needle biopsy (FNB) was performed in doubtful cases. Median alkaline phosphatase (ALP) and y-glutamyltransferase (GGT) levels were significantly lower compared to baseline after the biliary drainage (204 vs. 465 U/L, p < 0.0001, 204 U/L vs. 608.5, p < 0.0001, respectively), whilst no differences were observed for CA 19-9 levels. CA 19-9 showed significant association with the tumor stage in the pre-drainage setting. CT and EUS showed a low agreement in estimating tumor size (mean difference 4.8 mm 95% LoA −10.82–20.38). We did not find any significant correlation between CA 19-9 and bilirubin levels (r = −0.05, p = 0.7). In our cohort, survival rate was lower in patients with higher CA 19-9 levels (log rank p = 0.007). CA 19-9 has some limitations as a biomarker in the PC setting, thus it cannot address the treatment strategy alone. Nonetheless, it provides valuable information, and is not replaceable for the time being.

Keywords: pancreatic cancer; CA 19-9; biomarkers; EUS; tumor staging

1. Introduction

Pancreatic cancer (PC) is the third cause of cancer-related deaths in Northern America and the fourth in Europe [1]. Despite the global improvements in imaging diagnosis and oncological treatments, PC is still characterized by high mortality and recurrence rate. Surgery is the only curative treatment [2], although, in about the 80% of cases, PC is unresectable at the time of diagnosis. Even surgical resections with positive margins have the same prognosis of patients with unresectable disease treated with chemotherapy only [3].

The leading causes of this poor prognosis may be found firstly in the genomic instability of this kind of tumor and its anatomical position deep in the abdomen, which leads to a lack of clinical signs in the early phases, and secondly in the absence of specific and reliable biomarkers. The majority of PC arise from the head or the uncinate process of the organ, whilst about 15–20% are locate in the body or tail [1,4]. PC clinical presentation includes jaundice, abdominal pain, steatorrhea, weight loss, and new onset diabetes. According to the tumor localization, the clinical presentation may vary. Head PC usually present with biliary obstruction symptoms and are likely to be found at earlier stages compared to body/tail tumors. Moreover, transabdominal ultrasound is generally the first imaging used to investigate patients presenting with abdominal pain, weight loss and/or diarrhea, but it shows a very low sensitivity to detect PC [3] and this can contribute to diagnostic delays.
Serum carbohydrate antigen (CA) 19-9, a Lewis blood group antigen expressed by epithelial cells, is the only biomarker for PC approved by the Food and Drug Administration (FDA). CA 19-9 can be used to assess response to chemotherapy, to identify patients with poor prognosis, and to guide treatment decision [1]. However, it is not recommended for screening, and its value as an early diagnostic tool remains controversial [5–7]. Indeed, CA 19-9 is undetectable or normal in the 5–10% of the population with the Lewis antigen-negative phenotype [8,9], and it can also elevate in conditions such as chronic pancreatitis, biliary obstructions, and other gastrointestinal (GI) tumors [10]. It is well known that CA 19-9 is influenced by serum bilirubin [11,12]. For this reason, in clinical practice, this tumor marker is often considered unreliable in patients with jaundice, even if in the literature there are no strong evidences to support this common belief.

The low specificity of CA 19-9 and the poor prognosis of patients with advanced disease explain the urge to find new biomarkers and diagnostic tools that can help physicians to detect PC in the early stages [13]. Some studies showed that combining different serum biomarkers can increase both sensitivity and specificity for PC diagnosis [14–16]. Recently, research efforts shifted to liquid biopsy and new markers such as cell-free DNA, microRNA, exosomes, and circulating tumor cells which are currently being investigated for detection of disease and monitoring response to treatment [8,15,17]. Despite all the mentioned promising results, such innovations are still far from being included in clinical practice.

According to the guidelines, Computed Tomography (CT) is the first-line imaging modality for diagnosis and staging of PC; while abdominal Magnetic Resonance Imaging (MRI) and Endoscopic Ultrasound (EUS) are recommended when CT is not conclusive or contraindicated. Furthermore, EUS fine-needle biopsy (FNB) is the chosen procedure for malignancy confirmation [1]. Actually, Canto et al. and Yoshida et al. [18,19] showed that EUS performs better than CT for detecting small pancreatic lesions and lymphnode involvement [20]. On the other hand, a unique imaging technique cannot always guarantee an accurate tumor staging, therefore, an approach consisting in an initial CT followed by EUS for inconclusive cases represents the most reliable and cost-effective strategy for resectability assessment [20].

The aim of our study was to delineate the role of CA 19-9 in jaundiced patients with PC in the era of molecular and technological developments with a focus on its relationship with new staging techniques, such as EUS. We opted for selecting only PC located in the head or uncinate process in order to investigate the relationship between CA 19-9 and jaundice, without bias related to the tumor localization. Secondary outcomes were the evaluation of the usefulness of the traditional cytolyis and cholestasis indexes and the staging agreement of EUS and CT for pancreatic cancer.

2. Patients and Methods

2.1. Patients

In this retrospective study, we included jaundiced patients with tumors located in the head or uncinate process of the pancreas, and those who were referred to our Center for Endoscopic Retrograde Cholangiopancreatography (ERCP)-guided biliary drainage between June 2021 and June 2022. We only considered patients with jaundice and in cases where the PC diagnosis was confirmed by histological evaluation.

The exclusion criteria were loss to follow-up before one year from diagnosis; missing data in the laboratory tests before the drainage procedure; concomitant GI tumor; failure of endoscopic biliary drainage; no imaging/lesion measurement available at the time of the procedure, and absence of written consent.

2.2. Methods

Medical records of the patients enrolled were retrospectively reviewed. We collected data about medical history, tumor size, tumor stage, treatment strategy, CA 19-9, and the other serum biomarkers measured before any biliary drainage, like direct bilirubin, aminotransferases, γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP). Serum
biomarkers have been measured within 7 days from the ERCP. We also collected biomarker levels after the procedure, when available, within 10 days after the procedure. PC was classified according to the TNM criteria. All patients underwent Total Body CT scan. EUS +/- FNB was used for staging in doubtful cases, and also to achieve histological confirmation of diagnosis in patients judged unfit for curative or palliative surgery. We evaluated outcomes at 1 year from diagnosis.

The study was performed according to the Declaration of Helsinki. All patients admitted in our Unit were asked to provide informed written consent to allow the use of anonymized data for research purposes.

2.3. Statistical Analysis

Continuous data are presented as median and interquartile range (IQR), whereas categorical data are presented as absolute values and percentage. The Wilcoxon’s test for paired data was used to compare CA 19-9, ALP, and GGT measurements before and after the biliary drainage. To analyze the relationship of CA 19-9 and the other biomarkers’ serum concentrations with tumor stage we used the Kruskall–Wallis test. Spearman’s rank correlation coefficient was used to evaluate the association between tumor size and both CA 19.9 and direct bilirubin levels. We investigated the agreement between CT and Endoscopic Ultrasound EUS using the Bland Altman plot analysis. Survival time has been estimated by using the Kaplan–Meier with log-rank test to calculate differences among the groups. \( p < 0.05 \) in a two-tailed test was considered statistically significant.

Analyses were performed with GraphPad Prism version 10.2.2 software.

3. Results

3.1. Baseline Patient Characteristics

In this study we included 91 patients; among them, 6 patients were excluded for inconclusive results or other types of cancer than adenocarcinoma at the histological evaluation; 5 for lack of data before the endoscopic drainage; 1 for unsuccessful ERCP; 2 were excluded for colorectal cancer history; 4 patients were lost to follow up. Thus, we included in the analysis data from 73 patients.

Patients were mostly male (\( n = 38, 52.1\% \)), median age was 77 years (range 32–95), of which 21 (28.8%) were diagnosed with stage IV; 57 (78%) patients underwent EUS for staging completeness, and it was necessary to perform EUS-guided sampling in 49 patients for malignancy confirmation.

The overall characteristics of the study population are illustrated in Table 1.

Table 1. Main characteristics of the study population.

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>N = 73</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male/Female)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38 (52.1)</td>
</tr>
<tr>
<td>Female</td>
<td>35 (47.9)</td>
</tr>
<tr>
<td>Age</td>
<td>78 (71–84)</td>
</tr>
<tr>
<td>Previous biliary surgery</td>
<td></td>
</tr>
<tr>
<td>Cholecistectomy</td>
<td>7 (9.6)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>16 (21.9)</td>
</tr>
<tr>
<td>II</td>
<td>24 (32.9)</td>
</tr>
<tr>
<td>III</td>
<td>12 (16.4)</td>
</tr>
<tr>
<td>IV</td>
<td>21 (28.8)</td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>N = 73</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endoscopic procedures (EUS/ERCP)</strong></td>
<td></td>
</tr>
<tr>
<td>EUS</td>
<td>57 (78.1)</td>
</tr>
<tr>
<td>EUS-FNB</td>
<td>49 (85.9)</td>
</tr>
<tr>
<td>ERCP</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Plastic stent</td>
<td>8 (11.4)</td>
</tr>
<tr>
<td>Partially covered metallic stent</td>
<td>9 (13)</td>
</tr>
<tr>
<td>Fully covered metallic stent</td>
<td>44 (62.9)</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>13 (18)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>28 (38)</td>
</tr>
<tr>
<td>Best supportive care</td>
<td>32 (44)</td>
</tr>
<tr>
<td><strong>Follow-up at 1 year</strong></td>
<td></td>
</tr>
<tr>
<td>Deaths</td>
<td>41 (56.2)</td>
</tr>
<tr>
<td>Alive</td>
<td>32 (43.8)</td>
</tr>
</tbody>
</table>

Data are shown as median (25th and 75th percentiles) and absolute value (%).

3.2. Serum Biomarkers According to Biliary Drainage and Cancer Stage

Median values after the biliary drainage were significantly lower compared to baseline measurements for both GGT (204.5 U/L vs. 608.5, \( p < 0.0001 \)) and ALP (204 vs. 465 U/L, \( p < 0.0001 \)), whereas we did not observe any statistically significant difference between CA 19-9 values pre- and post-endoscopic drainage (83.2 vs. 178, \( p = 0.24 \)) (Figure 1).

In the pre-drainage setting, concentrations of direct bilirubin and ALT did not differ by tumor stage (\( p = 0.25 \) and \( p = 0.38 \), respectively) whilst ALP, GGT, and CA 19-9 levels showed significant association (\( p = 0.01, p = 0.02, p = 0.002 \), respectively) (Figure 2). On the other hand, when we evaluated these serum biomarkers after the endoscopic drainage, neither ALP (\( p = 0.23 \)) nor GGT (\( p = 0.068 \)) were significantly associated to the tumor stage. We did not perform CA 19-9 levels analysis because of the shortage of data available post drainage.

Figure 1. Wilcoxon test. Serum marker levels at diagnosis and after the biliary drainage. A. Alkaline phosphatase (ALP) (A) and \( \gamma \)-glutamyltransferase (GGT). (B) Levels decreased significantly after the biliary drainage (\( p < 0.0001 \)). No difference was observed for the serum carbohydrate antigen CA 19-9 (C) levels (\( p = 0.24 \)).
Lesion measurements by EUS were available for 50 patients. In those cases, CT and EUS showed a low agreement in estimating tumor size (mean difference 4.8 mm; 95% limits of agreement $-10.82$-$20.38$) (Figure 3). No systematic over- or under-estimation was observed. Although low, we noticed a statistically significant correlation between CA 19-9 levels with both CT ($r = 0.4, p = 0.0017$) and EUS ($r = 0.3, p = 0.039$) measures. We did not find any statistically significant correlation between direct bilirubin concentrations with CT ($r = 0.1, p = 0.3$), neither with EUS ($r = 0.06, p = 0.7$) tumor size measurements. CA 19-9 and bilirubin levels appeared to be not correlated ($r = -0.05, p = 0.7$).

More than half of our study population had potentially resectable or borderline resectable disease at the time of diagnosis. Only 13 patients (18%) underwent surgery, mainly because older patients were deemed unfit for surgery. Twelve patients (16%) with stage I-IIA received ERCP palliation with positioning of a fully covered metal stent just like patients with advanced disease due to fragility status. At the end of follow-up, 41 patients (56.2%) had died. Median survival was 3 months. When we grouped patients according to...
CA 19-9 serum levels, we noticed that survival decreased significantly with the increase in CA 19-9 levels (log rank $p = 0.007$, Figure 4).

![Figure 4. Kaplan–Meier curve. (A) Overall survival at 12 months. (B) Survival according to CA 19-9 subgroup levels at 12 months.](image)

4. Discussion

PC is a leading cause of cancer death, and its incidence has dramatically risen in the last few decades, particularly in the so-called developed countries. Moreover, those numbers are expected to double in the next years due to the ageing of the population [21]. Nowadays, the overall 5-year survival is about 10% [22]. The silent nature of PC and the absence of curative treatment for advanced stages are responsible for such a poor prognosis. Early diagnosis appears to be the only means to improve PC outcomes at present. If liquid biopsies and MiRNAs represent a promising future, CA 19-9 is still the only marker approved for use in clinical practice. Traditionally, CA 19-9 levels are considered unreliable in the case of jaundice. Several studies faced the problem of CA 19-9 levels’ interpretation in a cholestasis setting with conflicting outcomes [10,23].

In our retrospective cohort of jaundiced PC patients, we showed no correlation between CA 19-9 and hyperbilirubinemia. These results are consistent with those reported by other studies [24–26]. After jaundice relief, median values of ALP and GGT significantly decreased, whereas CA 19-9 appeared to be not significantly influenced by biliary drainage. Indeed, the ALP and GGT levels’ association with tumor stage of jaundiced patients was not confirmed in the post-biliary drainage setting, making these cholestasis indexes unreliable biomarkers. We could not analyze the relationship of CA 19-9 with tumor stage after jaundice relief, due to the paucity of data in the post-drainage setting; however, if CA 19-9 levels are not influenced by jaundice (see above), we can speculate that the significant association of CA 19-9 levels and tumor stage is valid before and after the obstruction relief.

In the present study, we demonstrated a correlation between CA 19-9 and tumor size, measured by both EUS and CT. As known, CA 19-9 is proportional to the amount of neoplastic tissue (including primary tumor or metastasis). This explains the connection of this marker with both tumor stage and tumor size. On the contrary, bilirubin levels were not influenced by the size or stage of the tumor, probably because the localization in the head/uncinated process was responsible for biliary tree compression regardless of the size. These results proved to be independent of the staging technique (i.e., CT vs. EUS) and this is a very interesting finding in the era of technological development and individualized therapy where a multimodal approach is often necessary in order to decide the best treatment strategy for each patient.

In our analysis, CT and EUS demonstrated a relative agreement in estimating tumor size with a mean bias of 4.8 mm and levels of agreement ranging from $-10.82$ to $20.38$ mm. However, we did not observe systematic over- or under-estimation and only a few times were there discrepancies in the measurement. More importantly, differences in tumor size
did not cause any treatment strategy changes. Some studies showed a low agreement between EUS and other imaging modalities [27,28] and a high operator dependency of EUS [29]. On the other hand, Leeds et al. [30] reported that EUS and CT are interchangeable techniques for assessing pancreatic cysts size, and Rahman et al. [31] showed that EUS and CT are both valuable techniques to assess pancreatic cancer resectability. It is acknowledged that EUS is an effective tool for identification of small pancreatic masses and for evaluation of vascular invasion and lymph node involvement. With reference to these supposed discrepancies, indeed, we can argue that, compared to CT, the role of EUS in PC staging is additional and not replaceable. Furthermore, a perfect agreement between imaging modalities in estimating size is crucial for follow-up but it is not so essential for cancer staging.

Finally, CA 19-9 levels correlated with patient survival. In our study, higher CA 19-9 levels were associated to lower survival probability at 12 months. According to previous studies, these results support the prognostic role of CA 19-9 even in advanced disease [32,33].

There are some limitations to our study. First, it is a single-center study with a retrospective design. Therefore, we had a relatively small-size population and the availability of laboratory data was conditioned by clinical application. In addition, not all patients underwent EUS. However, we adopted accurately selected inclusion criteria, and our population was well balanced: all disease stages were adequately represented, offering an actual panorama of PC management in clinical practice. All EUS procedures were performed by a unique highly trained endoscopist with the merit to overcome the problem of the high dependency on EUS operators. CA 19-9 carries another limit itself because Lewis antigen-negative patients cannot express it. We intentionally chose to also include in our analysis patients with negative CA 19-9 values to assess its prognostic value and avoid bias in the survival curve.

In conclusion, CA 19-9 is not a perfect tumor marker and PC treatment cannot be decided based on CA 19-9 levels alone, but it provides some valuable information even in a jaundice setting. It should be routinely assessed at the time of diagnosis regardless of the bilirubin levels and clinicians must be aware of its limitations. As new biomarkers are far from being included in clinical practice, an approach combining advanced imaging and endoscopic modalities with CA 19-9 measurement may help physicians choose the treatment strategy with a higher likelihood of success. As expected, cholestasis indexes are unreliable biomarkers in a PC setting.

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**Data Availability Statement:** Data available on request from the authors.

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

**References**


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