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Retrospective Cohort Study

Clinical Characteristics and Outcomes in CA 19-9 Negative Pancreatic Cancer

Features of CA 19-9 Negative Pancreatic Cancer

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Abstract

BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide. Tumor markers like carbohydrate antigen 19-9 (CA19-9) have proven valuable as a diagnostic tool, predictor for tumor staging and response to therapy.

AIM

Our aim was to delineate the phenotype of normal CA19-9 PDAC according to clinical features, disease staging and prognosis, as compared with high CA19-9 PDAC cases.

METHODS

We performed a retrospective single-center analysis of all PDAC cases admitted in our Gastroenterology department over a period of 30 mo, diagnosed by endoscopic ultrasound guided tissue acquisition. Patients were divided into two groups, according to CA19-9 Levels, over a threshold of 37 U/L. We performed a comparison between the two groups, with regard to demographic and clinical data, biomarkers, tumor staging and 6-month survival.

RESULTS

Altogether 111 patients were recruited, 29 having documented normal CA19-9 (< 37 U/L). In CA 19-9 negative group of patients, 20.68% had elevated levels for both CEA and CA 125, 13.79% only for CA 125, whilst 17.24% had only CEA positive. The two groups had similar demographic characteristics. Abdominal pain was more frequently reported in positive *vs* negative CA 19-9 PDAC cases (76.83% *vs* 55.17%), while smoking was slightly more prevalent in the latter group (28.04% *vs* 31.03%). Tumors over 2 cm were more frequently seen in the positive CA 19-9 group, reflecting also a higher proportion of locally advanced and metastatic neoplasia (87.7% vs. 79.3%). 6-months survival was higher for the negative CA19-9 group (58.62% *vs* 47.56%).

CONCLUSION

Elevated CA19-9 at diagnosis seems to be associated with a more pronounced symptomatology, high tumor burden and poor prognosis, compared to negative CA 19-9 PDAC cases. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Key Words: pancreatic cancer; CA 19-9; survival; Lewis; outcome

Core Tip: Given the large heterogeneity of pancreatic cancer, delineation of subgroups with different tumor biology is essential for personalized management. We outline the phenotype of CA 19-9 negative pancreatic cancer according to clinical features, disease staging and prognosis.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide, mostly due to late-stage diagnosis and resistance to chemotherapy. According to Globocan statistics 2020, pancreatic cancer has an incidence rate of 4.9/100,000 and mortality almost equal to its incidence, of 4.5/100,000 (1). In fact, while mortality rates from other types of cancer are decreasing, pancreatic cancer is the only malignancy with unfavourable trend (2).

Because of its aggressive tumor biology, early diagnosis is very important in order to maximize outcomes. Several strategies have been considered for setting an early accurate diagnosis, from case-finding tools to surveillance of high-risk patients. Alongside the imaging evaluation, there is a great interest in the development of biomarkers for optimizing the management of pancreatic adenocarcinoma (3).

The most common used biomarker for PDAC is carbohydrate antigen 19–9 (CA 19–9), which is related to Lewis blood group antigens, and has proven valuable as a diagnostic tool, but also in tumor staging, resectability and response to therapy (3). CA 19-9, also called sialylated Lewis (a) antigen, is synthesized by pancreatic and biliary ductal cells and also by other types of epithelium (stomach, colon, uterus, lung, salivary glands), which makes it a nonspecific biomarker for PDAC (4,5). Elevated CA 19-9 has been reported in both benign and malignant pathology – *Figure 1* (6,7). Expression of CA19-9 requires the presence of Lewis antigens A [Le(a+b-)] or B [Le(a-b+)], meaning that [Le(a-b-)] are theoretically non-producers of CA 19-9 (8). Lewis negative individuals ([Le(a-b-)]) lack the enzyme α1-3,4 fucosyltransferase, which is required for CA19-9 biosynthesis. This dysfunction of the Lewis gene is associated with deficient protein fucosylation, which has been involved in cancer development (9).

As CA 19-9 secretion is dependent on the Lewis antigen expression, undetectable false negative results can occur in Lewis antigen-negative individuals, meaning [Le(a−b−)] non-expressors (10). This could represent a cause of delayed diagnosis in these patients and also a pitfall in screening strategies based on CA 19-9. While red cell phenotyping for Lewis antigen status would provide insight in such situations, this is not routinely done in clinical practice. However, despite the relationship between CA 19-9 secretion and Lewis antigen status, not all Lewis (-) individuals with PDAC are non-secretors of CA 19-9, which makes CA 19-9 retain its diagnostic utility at least partially even in this patient category (11–13) – *Figure 2.*

Given the large heterogeneity of PDAC, delineation of subgroups with different tumor biology is considered of paramount importance for personalized management. Currently available literature is inconsistent regarding the clinical features and outcomes of patients with CA 19-9 or Lewis negative PDAC. Some authors have shown a better prognosis, while others have revealed worse outcomes compared to high CA 19-9 PDAC (14,15). Our aim was to delineate the phenotype of CA 19-9 negative PDAC according to clinical features, disease staging and prognosis, as compared with high CA 19-9 PDAC cases.

MATERIALS AND METHODS

2.1. Study design and patient population

We performed a retrospective analysis of patients admitted to our Gastroenterology Department during a period of 30 mo, from Jan 2019 to Jul 2021, who were diagnosed with PDAC by endoscopic ultrasound guided tissue acquisition. Demographic, clinical, laboratory work-up and imaging data were collected from patients' medical records. Staging was carried out based on pancreatic-protocol CT scan, according to International Association of Pancreatology (IAP) criteria for resectability – resectable, borderline resectable, locally advanced or metastatic disease (16). Regarding tumor location, we grouped cases into lesions extended to head, uncinate and neck of the pancreas, comprising one set, and tumors of the body and tail, representing another set. A 6 mo follow-up aiming at assessing survival was carried out either by reaching out to the general practitioner/oncologist, either by contacting the patient/patient's family by phone. Patients with missing data according to items assessed in this research were excluded from analysis. Also, patients lost from follow-up were also excluded, as survival could not be determined.

For the purpose of this study, we divided patients into two groups, according to CA 19-9 Levels. A threshold was set at 37 U/L and patients were classified as CA 19-9 negative or normal (for values < 37 U/L) – group A and CA 19-9 positive (≥ 37 U/L) – group B. We then compared the two groups according to demographic and clinical data, biomarkers, tumor staging and 6-month survival.

2.2. Statistics

Data analysis was carried out using SPSS Statistics 25 software Armonk, NY, USA. Continuous variables are reported as mean and categorical variables are reported as count and percentage. Comparison among the two groups was done using chi square tests for categorical variable and a two-sample t-test for continuous variables, at a significance of $\alpha = 0.05$.

RESULTS

Altogether 111 patients were analysed for the purpose of this study, 29 of them having documented normal CA 19-9 (< 37) and 82 being CA 19-9 positive (≥ 37). Demographic data, tumor characteristics and outcomes among the two groups are summarized in *Table 1.*

With regard to gender distribution, a male predominance was seen in the study cohort (75/111, 67.5%), mostly owing to a higher male:female ratio in group B (2.4:1). Median age similar between the two groups, 64 and 67 years respectively.

Considering at risk behaviour among patient population, a higher proportion of smokers was seen in group A (31.03% *vs* 28.04%), while heavy alcohol consumption was seen slightly more frequent in group B (23.17% *vs* 20.68%). Concerning the symptoms, abdominal pain was more prevalent in patients from group B (76.83% *vs* 55.17%), while weight loss and jaundice were noted in similar proportions in both patient groups. Also, diabetes mellitus was seen in about one third of patients in both groups (34.48% *vs* 34.14%).

Average value of CA 19-9 was 16904.85 for group B, compared with 8.48 for group A. In this latter group of patients, 20.68% had elevated levels for both CEA and CA 125, 13.79% only for CA 125, whilst 17.24% had only CEA positive. For both groups analysed, most tumors (62.06% - group A, 57.31% - group B) were located in the head or uncinate process, with the remaining 37.93% and 42.68% respectively developed in the body or tail region. Regarding tumor size, there were no significant differences among the two groups in tumors over 4 cm, but a higher proportion of lesions under 2 cm was reported in group A (10.34% *vs* 2.43%) while tumors sized 2-4 cm were more frequently seen in group B (64.63% *vs* 58.62%).

Analysis of tumor staging revealed there were more resectable (13.79% *vs* 7.31%) or borderline resectable tumors (6.89% *vs* 4.87%) in group A, while locally advanced and metastatic tumors were predominant in group B (24.39 *vs* 20.68%, 63.41 %vs 48.62%). 6-months survival was higher in group A – 58.62%, compared to group B - 47.56%.

We further performed a subgroup analysis according to gender, taking into account the male predominance of our study cohort. While there were more men with elevated CA 19-9 than women (77.33% *vs* 66.67%), proportion of locally advanced or metastatic tumors was higher in subgroup B females than males (95.83% vs. 84.48%). Regarding symptomatology, abdominal pain was more frequent in group B for both genders, but the difference seen with group A was higher for males (72.41% *vs* 47.06%) than females (87.5% *vs* 66.67%), without being statistically significant. We also conducted an analysis according to an age threshold set at 65 years: while advanced tumors were seen more in subgroup A less than 65 years of age compared to over 65 (86.7% *vs* 71.4%), in group B 90.5% of elderly patients had locally advanced or metastatic neoplasia compared to 83.9% in those under 65 years. 6 mo survival was similar in subgroups A and B according to the 65 years threshold (57.1% and 49% for patients under 65 years, 60% and 45.2% respectively for those 65 or more).

DISCUSSION

CA 19-9 is the most widely used biomarker for PDAC, but its major drawbacks are represented by false positive results in benign inflammatory conditions and extra-pancreatic neoplasms, and by false negative results in Lewis negative individuals, which comprise about 10% of the Caucasian population (5). However, in several aspects CA 19-9 remains a valuable biomarker for PDAC management, from screening and diagnosis to treatment response, prognosis and recurrence – *Figure 3* (9,17–21).

In our study, we enrolled PDAC patients and divided them into two groups: CA 19-9 positive (*n* = 82) and CA 19-9 negative (*n* = 29), according to a threshold of 37 U/mL. 6 mo survival was better in the CA 19-9 negative patients (58.62% *vs* 47.56%), reflecting a lower proportion of locally advanced and metastatic disease in this group. This could be explained by triggering of imaging studies in patients with elevated CA 19-9 Leading to an early stage diagnosis and thus a better prognosis, while in patients with negative CA 19-9 further investigations are often deferred due to lack of concern, leading to delayed diagnosis in advanced stages and poorer prognosis.

Some authors have proposed genotyping Lewis antigen along with CA 19-9 dosing in order to improve its diagnostic accuracy (22,23), but recent studies have shown that CA19-9 retains its utility even in Lewis(-) individuals - in the study by Luo *et al* (11), CA 19-9 values over 37 U/L were seen in 27.4% of Lewis (-) patients and areas under the receiver operating characteristic curve (AUROCs) for the diagnostic accuracy of CA19-9 were similar in Lewis (-) PDAC patients compared to all PDAC patients (0.842 *vs* 0.898). This was also shown by Kwon *et al* (14), who also found that not all Lewis(-) PDAC patients are non-secretors of CA 19-9 – in this study, 172/375 (45.87%) of patients in the Le(a−b−) group had a serum CA 19-9 over 37 U/mL. The paradoxical elevation of CA 19-9 in Lewis negative individuals might be explained by partial secretion of the protein, which can be detected by enzymatic immunoassays or by cross-reactivity of the antibodies used for CA 19-9 dosing; treating the collected specimen with blocking agents has been proposed as a method to eliminate interference with heterophilic antibodies (5,13,24). Therefore, PDAC prognosis is different if patients are stratified according to either CA 19-9, or to Lewis antigen.

A literature search of studies assessing PDAC outcomes according to CA 19-9 and Lewis antigen status has shown inconsistent results – *Table 2* (11,14,25–40). While low CA 19-9 PDAC has been associated with better prognosis, some have shown that Lewis negative PDAC harbours a more aggressive tumor biology and has a poorer outcome (15). Discordant results might be due to different patient populations, different timeframes while studies have been conducted and not least to overlap of Lewis negative with detectable CA 19-9 PDAC patients. Some authors have concluded that the usefulness of the 37 U/mL threshold for CA19-9 is more appropriate for PDAC diagnosis than predicting prognosis. However, others have proven strong correlation of CA 19-9 with tumor burden, survival and recurrence (41,42).

In order to better predict outcomes, some have proposed measuring other markers such as CA 242, CA 50, CEA, CA 125 or periostin, complementary to CA 19-9 for PDAC (43–48). Such additional markers proved its usefulness especially in PDAC with normal

CA 19-9 Levels, such as CEMIP, apolipoprotein A-I and transferrin (49,50). Lee at al have shown that CEMIP (also called KIAA1199) had a diagnostic yield of 86.1% in CA 19-9 negative PDAC and the combination of CEMIP + CA 19-9 had a significant improved AUROC over CA 19-9 alone (0.94 *vs* 0.89, p < 0.0001) (49). In our study, 34.47% of CA 19-9 negative PDAC cases had elevated levels of CA 125, 37.92% for CEA and 20.68% for both. Concerning the patients with negative CA 19-9 and positive CA 125 and CEA, 83.33% had metastatic disease at the time of the diagnosis and only 50% survived at 6 mo.

Similar results were seen in the paper by Luo *et al* – in Lewis negative patients, high values of CEA were seen in 63.8% of patients and of CA125 in 51.1% (39), which concluded that CEA and CA 125 should be routinely measured for PDAC. Considering the metastatic burden and survival among 853 pancreatic cancer patients, Liu *et al* have shown that Lewis negative PDAC constitutes an aggressive tumor subtype, with low secretion of CA19-9 and high secretion of CA125 (15). In line with Luo *et al*, others have highlighted the fact that CEA and CA 125, similar to CA 19-9, can be also used to monitor therapeutic response (39,51).

Interestingly, several papers have shown that CA 19-9 and the other biomarkers are upregulated early in the course of PDAC development – up to 2 years before clinical diagnosis, and can be used to detect preclinical pancreatic cancer (52,53). This could be useful for screening strategies of high-risk groups, keeping in mind that Lewis (-) individuals might be missed by this approach. Moreover, clinicians should take note that CA 19-9 is also of limited value in the follow-up of Lewis negative patients, in order to avoid erroneous decisions in PDAC management.

The current study has several limitations. Patients recruited in this study are from a hospital-based setting, which had either an acute presentation (jaundice, pancreatitis) or were referred for diagnostic procedures. Also, we acknowledge the lack of Lewis antigen genotyping in our study population, which might have provided further insight into PDAC outcomes according to both CA 19-9 and Lewis antigen status. Another

important limitation is the sample size, which makes very difficult to obtain a statistically significant analysis.

CONCLUSION

In our study, patients with negative CA 19-9 had a better prognosis than those with values over 37 U/L. Elevated CA19-9 at diagnosis seems to be associated with a more pronounced symptomatology and higher tumor burden. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

ARTICLE HIGHLIGHTS

Research background

CA 19-9 is the most widely used biomarker for pancreatic ductal adenocarcinoma (PDAC), but it's use is hindered by both false-positive and false-negative results.

Research motivation

There are inconsistent results regarding the outcome of CA 19-9 negative PDAC cases.

Research objectives

We aimed to delineate the phenotype of negative CA 19-9 PDAC according to clinical features, disease staging and outcome.

Research methods

Retrospective single-center analysis of PDAC cases over a period of 30 mo.

Research results

Among 111 recruited patients, 29 had normal CA19-9. Patients with elevated CA 19-9 had higher tumor burden and more advanced staging. 6-months survival was higher for the negative CA19-9 group (58.62% *vs* 47.56%).

Research conclusions

Negative CA 19-9 PDAC has a better prognosis than PDAC with high CA 19-9 values. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Research perspectives

Negative CA 19-9 PDAC cases warrant indepth analysis of tumor biology, to assess if there is indeed a different phenotype of neoplasia.

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