

# Alcohol Dehydrogenase (ADH) and Aldehyde Dehydrogenase (ALDH) as Candidates for Tumor Markers in Patients with Pancreatic Cancer

Wojciech Jelski<sup>1</sup>, Emilia Kutylowska<sup>2</sup>, Magdalena Laniewska-Dunaj<sup>1</sup>, Maciej Szmitkowski<sup>1</sup>

1) Department of Biochemical Diagnostics, Medical University of Białystok; 2) Analytical Laboratory, General Hospital in Wysokie Mazowieckie, Poland

## Abstract

**Background:** Various alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) exist in the pancreas. Moreover, ADH and ALDH are present in pancreatic cancer cells. The activity of ADH class III isoenzymes is significantly higher in cancerous than in healthy tissues. The expression of these enzymes in cancer cells is reflected by increased enzyme activity in the sera and thus could be helpful for diagnosing pancreatic cancer. The aim of this study was to investigate the potential role of ADH and ALDH as tumor markers for pancreatic carcinoma. **Methods:** Serum samples were taken from 165 patients with pancreatic cancer and 166 healthy controls. Total ADH activity and class III and IV isoenzymes were measured by photometric and ALDH activity, ADH I and II by the fluorometric method. **Results:** There was a significant increase in the activity of ADH III isoenzyme (14.03 mU/l vs 11.45 mU/l;  $p < 0.001$ ) and total ADH activity in the sera of pancreatic cancer patients compared to the control. The diagnostic sensitivity for ADH III was 70%, specificity 76%, positive and negative predictive values were 79% and 71% respectively. Area under ROC curve for ADH III was 0.64. **Conclusion:** The results suggest a potential role for ADH III as a marker of pancreatic cancer.

## Key words

ADH isoenzymes – ALDH – pancreatic cancer – tumor markers.

## Introduction

Pancreatic carcinoma is a malignant tumor and one of

the most lethal human cancers. The etiology and molecular pathology of this disease is still not really understood. It is characterized by a relatively late stage diagnosis, rapid clinical progression, and very poor patient's survival [1]. Therefore, it is very important to find markers that detect a malignant cell transformation at an early stage. Numerous studies have shown that alcohol dehydrogenase (ADH) is present in pancreatic tissue and performs many important physiological functions [2, 3]. ADH exists in multiple molecular forms which have been grouped into several classes [4]. The characterization of ADH isoenzyme activities in the pancreas has shown that four classes of isoenzymes can be differentiated. Among the ADH isoenzymes the highest activity in the pancreas is represented by isoenzymes of class III. In addition to that, the pancreas possesses aldehyde dehydrogenase (ALDH) activity, which catalyzes the oxidation of acetaldehyde to acetic acid [2]. In our previous study, we demonstrated that ADH and its isoenzymes and ALDH are also present in the pancreatic cancer cells [5]. Moreover, the activity of class III isoenzymes was significantly higher in the cancer tissue than in healthy pancreatic parenchyma. The differing enzyme activities in cancer tissue are reflected by enhanced enzyme activities in the corresponding serum [6]. The total ADH activity has been elevated in the sera of patients with pancreatic carcinoma. The increase in total ADH activity, correlated with class III ADH, seems to be caused by the release of this isoenzyme from the cancer cells.

In the current study, which is a continuation of our previous investigations, we defined the diagnostic performance such as diagnostic sensitivity, specificity, predictive value for positive (PVPR) and negative results (PVNR), and receiver-operating characteristics (ROC) curve of tested enzymes. These data may be used in the evaluation of ADH and ALDH as candidates for tumor markers in pancreatic cancer patients

## Methods

### Patients

The study protocol was approved by the Human Care

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Address for correspondence:

Wojciech Jelski M.D.  
Department of Biochemical  
Diagnostics Medical University  
Białystok, Poland  
Email: wjelski@umwb.edu.pl

Committee of the Medical University in Bialystok, Poland. All patients gave informed consent for the examination.

Serum samples were taken for routine biochemical investigations from 165 patients (97 males and 68 females, mean age 64 years; range 45 – 77 years; mean age of men 66, range 45 – 77; mean age of women 61, range 51 – 73 years) with histologically proven pancreatic cancer. Tumors were classified in accordance with the staging of the 5th International Union Against Cancer (UICC). The patients were divided into four groups: 36 patients with stage I, 44 – with stage II, 51 – with stage III and 32 with stage IV cancer. Of the patients with stage IV cancer, 13 had liver metastasis, 10 had bone metastasis, and 9 lung metastasis.

Pre-treatment staging procedures included physical and biological examinations, chest X-ray and computerized tomography (CT). None of the patients had received chemo- or radiotherapy prior to serum collection. All of the patients drank alcohol only occasionally and self-reported intake was < 25 g of ethanol per week. Data were collected by using a standardized questionnaire during a face-to-face interview. The interview was conducted by trained interviewers. Using a standardized questionnaire, trained interviewers collected demographic characteristics, family history and cigarette, alcohol and area consumption from study subjects. Information on habitual substance use included whether the subject had been a habitual area chewer, cigarette smoker or alcoholic beverage drinker in his or her lifetime, what year the subject started and quitted, the duration of consumption and the daily amount and type of alcoholic beverage consumed.

Enzyme activities were also assayed in the sera of 166 healthy persons (100 men, 66 women, aged 46 – 75 years) as a control group. The healthy controls were recruited from the same geographical location. The control group was selected from healthy community residents who had attended the hospital for routine physical check-ups at the Department of Preventive Medicine. Control subjects were volunteers and were defined as those having normal results of all physical and blood examinations including normal imaging by CT. Controls and cases were recruited over a period of 4 years. None of them consumed any drugs and alcohol.

#### **Assessment of total ADH activity**

Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate [6, 7]. The reduction in NDMA was monitored at 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

#### **Assessment of total ALDH activity**

Aldehyde dehydrogenase activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphthaldehyde to the fluorescent 6-methoxy-2-naphthoate [6, 8]. The fluorescence was read at an excitation wavelength of 310 and an emission wavelength of 360 nm on a Shimadzu RF-5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

#### **Assessment of class I and II ADH isoenzymes**

Class I and II ADH isoenzyme activities were measured using fluorogenic substrates (4-methoxy-1-naphthaldehyde for class I and 6-methoxy-2-naphthaldehyde for class II) in reduction reaction according to Wierzbowski et al [6, 9]. The measurements were performed on a Shimadzu RF-5301 spectrofluorophotometer at excitation wavelength of 316 nm for both substrates and emission of 370 nm for class I and 360 nm for class II isoenzymes.

#### **Assessment of class III ADH isoenzyme**

The activity of class III ADH isoenzyme was estimated by the photometric method with formaldehyde as a substrate [6, 10]. The reduction in NAD was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

#### **Assessment of class IV ADH isoenzyme**

Class IV ADH isoenzyme activity was measured using a photometric method with m-nitrobenzaldehyde as a substrate [6, 11]. The oxidation of NADH was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

#### **Diagnostic performance calculation**

The diagnostic criteria, such as the diagnostic sensitivity, specificity, predictive and negative value and the ROC curve, were determined using GraphRoc Program for Windows (University of Turku, Turku, Finland) [12].

#### **Statistical analysis**

A preliminary statistical analysis (chi-square test) revealed that ADH and ALDH activities did not follow a normal distribution. Consequently, the Wilcoxon's test was used for statistical analysis. Data were presented as median, range and mean values. Statistically significant differences were defined as comparisons resulting in  $p < 0.05$ .

## **Results**

The activities of ADH, ALDH and isoenzymes of ADH in the sera are presented in Table I. The comparison of ADH isoenzymes activities showed that the high difference was evidenced in class III ADH. The median activity of this class isoenzyme in the total cancer group increased about 22 % (14.03 mU/l) in comparison with the control level (11.45 mU/l). This increase was statistically significant ( $p < 0.001$ ). The other tested classes of ADH isoenzymes had higher activities in the sera of patients with cancer, but the differences were not statistically significant. The total ADH activity was significantly higher (32 %) in patients with pancreatic cancer than in the healthy subjects ( $p < 0.001$ ). The median total activity of ADH was 753 mU/l in patients group and 514 mU/l in control group. The analysis of ALDH activity did not show a significant difference between the total tested group and healthy persons.

The tendency of ADH III activity to increase in accordance with the advance of disease was observed after the analysis of the activity of particular ADH isoenzymes

**Table I.** ADH and ALDH activity in the sera of patients with pancreatic cancer depending on the carcinoma stage

Tested group	ADH total	ALDH total	ADH I	ADH II	ADH III	ADH IV
	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean	Median Range Mean
Total group n=165	753* 317 – 1923 731 ± 164 p < 0.001	3.34 1.49 – 7.16 3.12 ± 1.23 p = 0.254	3.11 1.13 – 6.43 2.95 ± 1.38 p = 0.378	14.91 6.13 – 23.72 14.05 ± 6.51 p = 0.286	14.03* 7.24 – 21.81 13.65 ± 5.92 p < 0.001	5.78 2.97 – 12.03 5.31 ± 2.05 p = 0.402
Stage I n=36	602* 317 – 1482 588 ± 135 p < 0.001	2.66 1.49 – 5.06 2.31 ± 1.05 p = 0.345	2.87 1.13 – 5.36 2.67 ± 1.59 p = 0.409	14.66 6.13 – 22.15 13.94 ± 6.02 p = 0.301	13.31* 7.24 – 20.55 13.08 ± 5.79 p < 0.001	5.40 2.97 – 11.35 5.02 ± 2.18 p = 0.377
Stage II n=45	725* 387 – 1543 707 ± 143 p < 0.001	3.81 1.88 – 6.15 3.66 ± 1.85 p = 0.462	3.02 1.55 – 5.69 2.79 ± 1.33 p = 0.344	14.84 6.45 – 22.86 14.31 ± 7.03 p = 0.344	13.77* 7.68 – 20.94 13.60 ± 7.22 p < 0.001	5.71 3.16 – 11.69 5.28 ± 2.41 p = 0.211
Stage III n=52	806* 504 – 1827 795 ± 178 p < 0.001	2.94 1.65 – 6.71 3.01 ± 1.59 p = 0.283	3.45 1.81 – 6.07 3.22 ± 1.55 p = 0.308	15.04 6.76 – 23.05 14.73 ± 7.62 p = 0.205	14.15* 7.93 – 21.07 13.87 ± 6.74 p < 0.001	5.88 3.34 – 11.92 5.65 ± 2.72 p = 0.205
Stage IV n=32	898* 571 – 1923 855 ± 203 p < 0.001	3.58 2.02 – 7.16 3.32 ± 1.11 p = 0.231	3.62 1.95 – 6.43 3.45 ± 1.09 p = 0.487	15.18 6.90 – 23.72 14.90 ± 7.06 p = 0.273	14.44* 8.16 – 21.81 14.11 ± 6.07 p < 0.001	5.94 3.48 – 12.03 5.81 ± 2.33 p = 0.306
Control n=166	514 276 – 1626 498 ± 133	3.17 1.35 – 7.01 3.08 ± 1.45	3.01 1.08 – 6.23 2.80 ± 1.31	14.62 5.94 – 22.17 14.08 ± 6.25	11.45 6.63 – 20.18 11.12 ± 5.32	5.41 2.72 – 11.07 5.18 ± 2.60

Data are expressed as median (range) in mU/l; \* Statistical significance ( $p < 0.05$ ) compared to the control group

depending on the progression stage of the carcinoma. Significantly higher ADH class III activity was found in the cancer patients regardless of their stages in comparison with the control group. The other isoenzymes did not exhibit marked changes in activity among patients at various advancing stages of the tumor. The serum level of total ADH activity was significantly higher in the cancer patients group (each stage) compared with the control group. A tendency toward an increase in the total ADH activity together with the progression of the carcinoma was also observed. In contrast, the activity of ALDH did not show the significant one-way changes.

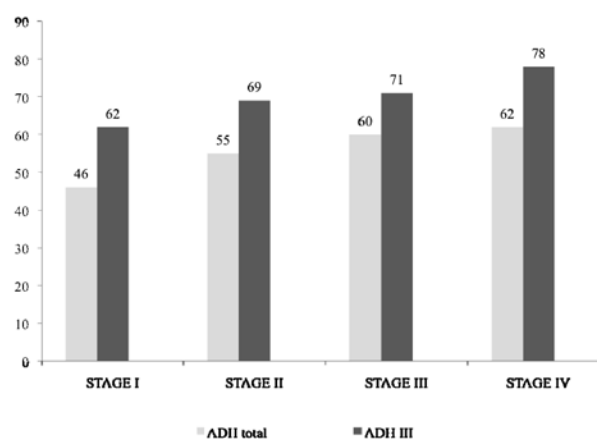
Table II shows the performance parameters of diagnostic criteria for ADH total and ADH III. The sensitivity (70%) and specificity (76%) of ADH III were higher than for the ADH total. Both the PVPR and PVNR were also the highest for the class III of ADH isoenzymes. PVPR and PVNR for ADH III were 79% and 71% respectively. The sensitivity of ADH III was not statistically different, although there was a tendency toward increased sensitivity in the more advanced stages of the tumors (Fig. 1).

The relationship between diagnostic sensitivity and specificity was illustrated by a ROC curve (Fig. 2). It shows that the area under the ROC curve for ADH III (0.64) was higher than the ROC area of the ADH total (0.57).

## Discussion

Pancreatic cancer is the fourth leading cause of cancer

mortality in the world, with only a minimum of patients surviving 5 years [13]. The prognosis for patients with pancreatic cancer is strongly correlated with the pathologic stage at the time of diagnosis. Consequently, it is of utmost importance to find markers which could detect a malignant transformation as early as possible. These substances should be synthesized and excreted by tumor cells [14]. In our previous study, we have shown that the activity of human ADH is significantly higher in pancreatic carcinoma tissue compared to normal pancreatic tissue whereas the activity of ALDH does not differ in both tissues [5]. Moreover, results

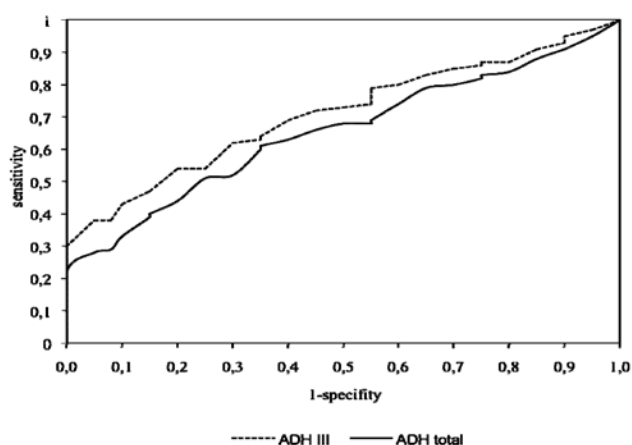


**Fig 1.** Sensitivities of ADH total and ADH III in pancreatic cancer according to tumor stage.

**Table II.** Diagnostic characteristics for ADH total and ADH III in pancreatic cancer.

Tested enzymes	Cut-off mU/l	Sensitivity (%)	Specificity (%)	Predictive positive value (%)	Predictive negative value (%)
ADH total	1315	57	65	70	66
ADH III	18.27	70	76	79	71

The cut-off points were obtained from a study of a health population (95th percentile)



**Fig 2.** ROC curves for ADH III and ADH total. ADH III, Area = 0.6457, SE= 0.0527; ADH total, Area = 0.5784, SE= 0.0611

of the same study showed that only the activity of class III ADH (the most important isoenzymes of ADH in the pancreas) is significantly higher in pancreatic malignancy compared to normal pancreatic cells. The other tested classes of isoenzymes had higher activities in cancer tissue than in normal pancreatic cells, but differences were not statistically significant [5]. The increased enzyme activity in pancreatic cancer is associated with an increase of their corresponding activity in the serum. We have shown that the serum total ADH activity varied in relation to the course of pancreatic cancer.

Moreover, we have found in our present study that the serum levels of ALDH were not significantly elevated in patients with pancreatic cancer in comparison to healthy controls. In addition, the activity of ALDH in the serum of cancer patients seems to be disproportionally low as compared to ADH activity. This would suggest that there is a much higher ethanol-oxidizing activity and considerably less acetaldehyde-oxidizing activity.

The present study is a continuation of our previous investigations. Higher levels of ADH in patients with cancer might result from enzyme release by cancer cells and could be helpful for the diagnosis of pancreatic cancer. The diagnostic criteria for disease markers are sensitivity, specificity and area under the curve (AUC). The ideal marker should possess a very high specificity, i.e. not detectable in healthy subjects, and a very high sensitivity, i.e. be very early on or when only a few cancer cells are present. The criteria for 100% sensitivity and 100% specificity have not yet been fulfilled by any of the known tumour markers. The established tumor markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9)

showed high specificity (100%) and CA 19-9 (a commonly used tumor marker for pancreatic carcinoma) exhibited a high diagnostic sensitivity – 74 % [15]. The CA19-9 and CEA are still the most commonly used biomarkers in pancreatic cancer. CA 19-9 has recently shown encouraging prognostic usefulness in patients with post-resectional CA 19-9 values  $\leq 180$  and 90 U/ml, respectively [16]. Kim et al showed that the preoperative CA19-9 level might be a useful predictor of early distant metastasis of pancreatic cancer [17]. However, the role of CA19-9 kinetics as a prognostic tool in patients treated with palliative first-line chemotherapy remains contradictory. While several studies reported a significant improvement in overall survival for patients that show decreasing CA19-9 values between 20% and 89%, a recently published large study conducted by Hess et al could not confirm any robust correlation between a CA 19-9 biomarker decline and overall survival [18-20]. Several previous analyses have shown elevated concentrations of circulating cytokines such as macrophage-colony stimulating factor (M-CSF), stem cell factor (SCF), granulocyte-colony stimulating factor (G-CSF), and interleukin 3 (IL-3) in patients with pancreatic cancer [21, 22]. The diagnostic sensitivity of SCF in pancreatic carcinoma patients was the highest (98%) among all tested hematopoietic cytokines. In our study the sensitivity of ADH III (70%) is higher than the sensitivity of CEA (37%) and G-CSF (19%), M-CSF (67%), IL 3 (62%), but lower than CA19-9 (77%). The sensitivity of the ADH total (57%) is higher than the sensitivity of CEA and G-CSF. The diagnostic specificity of the ADH III and ADH total is lower than specificity of the CA19-9, CEA and hematopoietic cytokines [21]. The PVPR indicates the probability with which a tumor is present in the case of positive test results. The PVNR predicts the probability of an existing tumor in the case of negative test results. In this investigation, ADH III has rather high predictive values for positive and negative results (79% and 71%, respectively).

The most important criterion for tumour markers is the sensitivity/specificity diagram, namely the ROC curve. The area under the ROC curve indicates the clinical usefulness of a tested tumor marker. A larger area under the ROC curve corresponds to a better tumour marker. In this study the ADH III (0.64) area under the ROC curve was lower than for the tumour markers CA19-9 (0.914), CEA, (0.909), and SCF (0.902) but higher than for G-CSF (0.513) [22].

The total ADH activity is higher in cancer cells derived from different organs compared to healthy tissues. Previously we found that serum activities of ADH I and ADH total were higher in colorectal cancer patients than in healthy subjects [23]. According to our results, we also demonstrated that the

activity of class IV ADH (main class in the stomach) in the serum changes in relation to the course of gastric cancer [24]. Consequently, ADH I and ADH IV could be helpful for the diagnosis of colorectal and gastric cancer, respectively.

To the best of our knowledge, this is the first study showing the whole spectrum of diagnostic characteristics of ADH and ALDH in pancreatic cancer patients. These results demonstrate the potential clinical role for ADH (ADH III, respectively) as a marker for pancreatic cancer but further confirmation by prospective studies is warranted.

### Conflicts of interests

None to declare.

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