INTRODUCTION

Pancreatic cancer (PC) is the fourth leading cause of cancer death in the world (1). The mortality rate for PC is projected to increase dramatically and, eventually, become the second leading cause of cancer-related deaths before 2030 (2). There were no major changes in 5-year relative survival to PC among European regions and countries over time. Overall, 5-year relative survival increased from 5% (1997 – 2001) to 6% (2005 – 2007) (3).

Chronic pancreatitis (CP) is a progressive inflammatory condition of the pancreas, which eventually leads to pancreatic fibrosis with irreversible changes in the parenchyma and pancreatic ducts leading to a gradual impairment of exocrine and endocrine insufficiency (4, 5). The main etiological factor of CP is chronic alcohol abuse (4-6). CP is a known risk factor for pancreatic cancer (5-7). According to the latest research, there is a three-fold to five-fold increase in the risk of PC in patients with previous CP (diagnosed > 2 years before) (7, 8).

A tumor marker has been defined, as a naturally occurring molecule that is measured in serum or plasma, or other body fluids or tissue extracts, or paraffin-embedded tissue to identify the presence of cancer and to assess a patient’s prognosis, or to
monitor a patient’s response to therapy with the overall goal of improving the clinical management of the patient (9). The tumor markers could be endogenous products of active metabolic malignant cells, products of newly switched genes, which remained unexpressed in early life, or newly acquired antigens at cellular and sub-cellular levels. The appearance of tumor markers and their concentration is related to the genesis and growth of malignant tumors in patients (10, 11).

CA19-9 is the only validated tumor marker with accepted clinical use in pancreatic cancer (9, 12). CA19-9 is related to the Lewis blood group antigens and only patients belonging to the Le (α– β–) or Le (α– β+) blood groups will express the CA19-9 antigen. Le (α– β–) phenotypes occur in 5 – 10% of the population lacking the enzyme 1,4-fucosyl transferase required for antigen epitope production and as such limits the use of CA19-9 as a universally applicable biomarker (12, 13). Elevated levels of this marker are observed in many benign liver and bile disorders such as cholelithiasis, cholecystitis, hepatitis, liver cirrhosis, as well as pancreatitis, and jaundice, which is the most important source of false-positive results of the CA19-9. Its level is also elevated in other malignancies such as cholangiocarcinoma, gallbladder cancer, hepatocellular carcinoma, colorectal cancer, esophagus, lung, and ovarian cancer (12-16). CA19-9 serum levels have a sensitivity and specificity of 79 – 81% and 82 – 90% respectively for the diagnosis of pancreatic cancer in symptomatic patients, but are not useful as a screening marker because of low positive predictive value (0.5 – 9.0%) (14).

Metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes responsible for the degradation of the extracellular matrix (ECM) and the basal membrane (BM) of the vessels (17, 18). MMPs occur naturally and have physiologic functions, including organogenesis, angiogenesis, cell migration, fertilization, wound healing, and inflammatory response. Changes in MMPs activity have also been observed in many pathologies: autoimmune diseases, gastric and duodenal ulcers, hepatic cirrhosis, Crohn’s disease, myocardial infarction, atherosclerosis, aneurysms, neurodegenerative diseases, and cancers (17-26).

Two of the most important enzymes from this group are matrix metalloproteinase-2 (gelatinase A) MMP-2 and matrix metalloproteinase-9 (gelatinase-B) MMP-9, whose main substrates are collagen type I, IV, V, VII, X, XI, XIV, gelatine, fibronectin, laminin, aggrecan, osteonectin, vitronectin, decorin, cascin TNF precursor, pro-TGF-β and MBP (myelin basic protein) (27). One of the most important functions of gelatinases is the degradation of collagen type IV, the main component of BM, including vascular BM (28). BM and components of ECM degradation by gelatinases make for one of the most important steps in the process of angiogenesis. Angiogenesis, in turn, plays an important role in cancer growth, progression, and tumor cell dissemination (22, 29, 30).

These two metalloproteinases may play a significant role in the pathogenesis of PC and can be used as a marker for differentiating PC and tumors in the course of the CP. Also, metalloproteinases may play a potential role as a marker of neoplastic advancement and the presence of cancer dissemination, however, there is little sufficient data to confirm this statement. This study aimed to compare the concentration of metalloproteinases: MMP-2, MMP-9, CA19-9, and CEA in patients with pancreatic cancer, chronic pancreatitis, and the control group in serum and peritoneal cavity.

**MATERIAL AND METHODS**

**Patients**

The study has been performed in a group of 90 patients including 51 (56.66%) females and 39 (43.33%) males with diagnosed pancreatic tumor and chronic pancreatitis complications selected for surgical treatment at the Department of Gastrointestinal Surgery Silesian Medical University in Katowice (Poland) and a control group, which was recruited among patients operated for non-inflammatory cholelithiasis, between August 2014 and August 2015.

The study protocol was approved by the ethics committee, (KNW/0022/KBi/64/14), and informed consent was obtained from each patient. The exclusion criteria included patients who had undergone oncological therapy before the surgical treatment and patients with other than adenocarcinoma or inflammatory tumor lesions in the pancreas. There was no case of pancreatic cancer in the CP group. All specimens obtained during surgery were confirmed histopathologically.

Group 1 consisted of 30 patients with PC (F:M = 19:11), group 2 consisted of 30 patients with CP (F:M = 10:20), and group 3 - CG consisted of 30 patients (F:M = 22:8). The average age of patients in the PC group was 67.0 ± 10.0 (27 – 77); in the group with CP it was 56.5 ± 9.5 (25 – 72) and 48.0 ± 24.5 (26 – 73) in the CG group (Table 1).

**Experimental procedures**

Comparison of MMP-2, MMP-9, CA19-9 and CEA concentrations in PC, CP, and CG serum samples were performed. Comparison of MMP-2, MMP-9, CA19-9 and CEA concentrations in PC, CP and CG intraperitoneal fluid samples or intraperitoneal lavage fluid were also performed.

Additional divisions of PC group were performed to evaluate relationships between MMP-2, MMP-9 and CA19-9 serum and peritoneal fluid concentrations and chosen histopathological features such as tumor stage in the TNM/UICC classification and presence of distant metastases. Histopathological characteristics of the PC group was shown in Table 2.

All histopathological examinations were performed utilizing routine methods. Additionally, in PC conventional cytological examination of intraperitoneal fluid samples or intraperitoneal lavage fluid were also performed (Table 3). Since hyperbilirubinemia will spuriously elevate CA19-9, in our study, we performed the analysis of the correlation between the concentrations of MMP-2, MMP-9 and CA19-9, and the level of bilirubin. Also, we analyzed the PC group in terms of clinical data.

**Table 1. Patients and groups (PC, pancreatic carcinoma; CP, chronic pancreatitis; CG, control group).**

<table>
<thead>
<tr>
<th></th>
<th>PC</th>
<th>CP</th>
<th>CG</th>
<th>Together</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>19/11</td>
<td>10/20</td>
<td>22/8</td>
<td>51/39</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.0 ± 10.0</td>
<td>56.5 ± 9.5</td>
<td>48.0 ± 24.5</td>
<td>0.05**</td>
<td></td>
</tr>
</tbody>
</table>

Female/Male: Chi square exact test: PC versus CP (p < 0.01) PC versus CG (NS) CP versus CG (p < 0.01).

Age (years): Kruskall-Wallis test: PC versus CP (p < 0.01) PC versus CG (p < 0.01) CP versus CG (NS).

*Chi square exact test; ** Kruskall-Wallis test.
Serum and peritoneal fluid analysis

All blood samples were obtained at fasting early in the morning, and the sera were immediately separated by centrifugation and stored at –80ºC until use. Peritoneal fluid samples were taken immediately after laparotomy or laparoscopy in CG. When ascites was present, 20 ml was taken and stored at –80ºC until use. In cases without ascites, the peritoneal cavity was lavaged with a 100 ml saline solution and the samples were collected 10 minutes later.

Plasma and peritoneal fluid MMP-2 were assayed by Enzyme Linked Immuno Sorbent Assay (ELISA), using the MMP-2 Human ELISA Kit (Wuhan Fine Biological Technology Co.). Plasma and peritoneal fluid MMP-9 were assayed by Enzyme Linked Immuno Sorbent Assay (ELISA), using the MMP-9 Human ELISA Kit (Wuhan Fine Biological Technology Co.).

Plasma and peritoneal fluid CA19-9 and CEA concentration were assayed by immunoradiometric assay (IRMA) according to the manufacturer’s instructions.

**Table 2.** Histopathology - characteristic of the pancreatic cancer tissue samples: tumor stage in the TNM/UICC classification.

<table>
<thead>
<tr>
<th>Histopathology of the pancreatic cancer</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Tis, N0, M0</td>
<td>2</td>
</tr>
<tr>
<td>IA T1, N0, M0</td>
<td>1</td>
</tr>
<tr>
<td>IB T2, N0, M0</td>
<td>1</td>
</tr>
<tr>
<td>IIA T3, N0, M0</td>
<td>1</td>
</tr>
<tr>
<td>IIB T1-3, N1, M0</td>
<td>3</td>
</tr>
<tr>
<td>III T1-3, N2, M0</td>
<td>7</td>
</tr>
<tr>
<td>IV M1</td>
<td>15</td>
</tr>
</tbody>
</table>

**Statistical analysis**

The distribution type of continuous variables was determined with the use of the Shapiro-Wilk test. The Student's t-test or analysis of variance were used for a detailed comparative analysis of the continuous variables of the obtained results in the case of normal distribution. The Mann-Whitney U test, the Kruskal-Wallis test, and the median test were used if the variable distribution was different from normal. Post hoc tests (Tukey’s RIR test) were used to determine significant relationships after the analysis of variance. In the case of discrete variables, the obtained results were compared using the chi-square test of independence and Fisher’s exact test if the sample size was small. The correlation between the variables was tested using Spearman’s rank correlation test. The level of statistical significance, for which the differences were considered statistically significant, was adopted for the value of p < 0.05.

**RESULTS**

There were no statistically significant differences in MMP-2 concentrations in serum between groups. The concentration of MMP-2 was significantly higher in PC intraperitoneal fluid samples compared to the CG (p < 0.01) and in PC intraperitoneal fluid samples compared to the CG (p < 0.01). The concentration of MMP-2 intraperitoneal fluid samples was similar in the PC group.

**Table 3.** Cytology of intraperitoneal lavage in pancreatic cancer group.

<table>
<thead>
<tr>
<th>Cytology</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of cancer cells</td>
<td>4</td>
<td>13.3%</td>
</tr>
<tr>
<td>Absence of cancer cells</td>
<td>26</td>
<td>86.7%</td>
</tr>
</tbody>
</table>

**Table 4.** Comparison of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) serum and intraperitoneal lavage fluid concentrations in pancreatic cancer (PC), chronic pancreatitis (CP) and control group (CG) serum samples. (S, serum; PL, peritoneal lavage; NS, not significant).

<table>
<thead>
<tr>
<th></th>
<th>PC median ± interquartile range</th>
<th>CP median ± interquartile range</th>
<th>CG median ± interquartile range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 S (ng/ml)</td>
<td>32.1 ± 50.0 (10.2 – 188.0)</td>
<td>34.6 ± 48.2 (10.2 – 125.4)</td>
<td>29.7 ± 27.64 (4.6 – 142.0)</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-2 PL (ng/ml)</td>
<td>10.8 ± 9.2 (4.6 – 38.0)</td>
<td>9.5 ± 10.8 (2.4 – 69.0)</td>
<td>0.49 ± 0.83 (0.22 – 1.45)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MMP-9 S (ng/ml)</td>
<td>173 ± 138.0 (39.0 – 267.0)</td>
<td>120 ± 158.5 (23.1 – 453.0)</td>
<td>92.6 ± 33.0 (32.45 – 238.0)</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 PL (ng/ml)</td>
<td>17.3 ± 14.7 (7.5 – 75.0)</td>
<td>15.9 ± 25.5 (5.7 – 74.4)</td>
<td>1.56 ± 1.91 (0.85 – 2.52)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CA 19-9 S (IU/ml)</td>
<td>460 ± 2122 (4.27 – 21000.0)</td>
<td>13.0 ± 14.1 (0.1 – 227.0)</td>
<td>6.19 ± 11.0 (0.1 – 28.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CA 19-9 PL (IU/ml)</td>
<td>79.2 ± 679.5 (1.27 – 4720.8)</td>
<td>178.4 ± 618.1 (1.73 – 1100.15)</td>
<td>9.68 ± 35.16 (1.38 – 36.54)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CEA S (ng/ml)</td>
<td>3.36 ± 5.77 (0.18 – 66.2)</td>
<td>2.27 ± 1.93 (0.49 – 14.0)</td>
<td>1.48 ± 1.7 (0.13 – 4.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CEA PL (ng/ml)</td>
<td>1.66 ± 4.2 (0.1 – 27.2)</td>
<td>1.36 ± 1.04 (0.15 – 6.56)</td>
<td>0.32 ± 0.28 (0.1 – 0.9)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Kruskall Wallis test. Post hoc tests: MMP-2 PL: PC versus CP (NS); PC versus CG (p < 0.01); CP versus CG (p < 0.01). MMP-9 PL: PC versus CP (NS); PC versus CG (p < 0.01); CP versus CG (p < 0.01). CA 19-9 S: PC versus CP (< 0.01); PC versus CG (p < 0.01); CP versus CG (NS). CA 19-9 PL: PC versus CP (NS); PC versus CG (p < 0.01); CP versus CG (p < 0.01). CEA S: PC versus CP (NS); PC versus CG (p < 0.01); CP versus CG (NS). CEA PL: PC versus CP (NS); PC versus CG (p < 0.05); CP versus CG (p < 0.05).
and CP groups. There were no statistically significant differences in MMP-9 concentrations in serum between groups. The concentration of MMP-9 was significantly higher in PC intraperitoneal fluid samples compared to the CG (p < 0.01) and in CP intraperitoneal fluid samples compared to the CG (p < 0.01). The concentration of MMP-9 intraperitoneal fluid samples was similar in the PC and CP groups. The concentration of CA19-9 was significantly higher in PC serum samples compared to the CP (p < 0.01) and PC serum samples compared to the CG (p < 0.01). The concentration of CA19-9 serum samples was similar in the PC and CP groups and in the PC and CG groups. The concentration of CA19-9 was significantly higher in PC intraperitoneal fluid samples compared to the CG (p < 0.01). The concentration of CA19-9 intraperitoneal fluid samples was similar in the PC and CP groups (Table 4).

Comparative analysis of the concentration of MMP-2, MMP-9, and CA19-9 in serum and intraperitoneal fluid in all
study groups was performed. The concentration of MMP-2 was significantly higher in serum compared to the intraperitoneal fluid in the PC group (p < 0.01). The concentration of MMP-9 was significantly higher in serum compared to the intraperitoneal fluid in the PC group (p < 0.01). In the CP group, the concentration of MMP-2 was significantly higher in serum compared to the intraperitoneal fluid (p < 0.01). The concentration of MMP-9 was significantly higher in serum compared to the intraperitoneal fluid in the CP group (p < 0.01). In the CG group, the concentration of MMP-2 was significantly higher in serum compared to the intraperitoneal fluid (p < 0.01). The concentration of MMP-9 was significantly higher in serum compared to the intraperitoneal fluid in the CG group (p < 0.01). There was no significant difference between the concentration of CA19-9 in the intraperitoneal fluid compared to the serum in the CG group.

The significant correlation between increased MMP-2 and CA19-9 serum concentration and concentration of these markers in intraperitoneal fluid in the PC group was observed (Figs. 1 and 2). There was no significant correlation between increased serum MMP-9 concentration and its intraperitoneal fluid concentration.

During the analysis, we evaluated serum and intraperitoneal fluid concentration of MMP-2, MMP-9, and CA19-9 in the PC group depending on the tumor stage in the TNM/UICC classification and distant metastases. The patients were divided into three subgroups due to the small number of patients with the lowest cancer advancement: 0 – II (8 patients, 26.7%), III (7 patients, 23.3%), and IV (15 patients, 50%) tumor stages. A statistically significant increase in the serum concentration of MMP-2 between groups 0 – II and IV was observed (p < 0.05) (Table 5, Fig. 3). There were no statistically significant differences between groups 0 – II and III and III and IV in the serum concentration of MMP-2. There were no statistically significant differences between serum concentration of MMP-9 and CA19-9 and tumor stages, despite the difference in CA19-9 concentrations between the groups: the lowest concentrations were observed in stages 0 – II and the highest in stages IV.

There were no statistically significant differences in the intraperitoneal fluid of MMP-2 and MMP-9 concentrations depending on the tumor stage in the TNM/UICC classification. A statistically significant increase in the intraperitoneal fluid concentration of CA19-9 between group 0 – II and IV was observed (p < 0.05) (Table 6, Fig. 4). There were no statistically significant differences between serum concentration of MMP-9 and CA19-9 and tumor stages, despite the difference in CA19-9 concentrations between the groups: the lowest concentrations were observed in stages 0 – II and the highest in stages IV.

There were statistically significant differences in serum concentration of MMP-2 (p < 0.05) and CA19-9 (p < 0.05)

### Table 5. Matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and carbohydrate antigen 19-9 (CA19-9) serum concentration in pancreatic cancer (PC) group depending on tumor stage. (NS, not significant).

<table>
<thead>
<tr>
<th>TNM classification</th>
<th>MMP-2 Median, Interquartile range</th>
<th>MMP-9 Median, Interquartile range</th>
<th>CA 19-9 Median, Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – II</td>
<td>23.6 ± 20.8</td>
<td>138 ± 150.0</td>
<td>33.75 ± 558.1</td>
</tr>
<tr>
<td>III</td>
<td>28.2 ± 51.01</td>
<td>66 ± 84.0</td>
<td>250.9 ± 1022.8</td>
</tr>
<tr>
<td>IV</td>
<td>50.2 ± 83.0</td>
<td>129 ± 144.0</td>
<td>2080.0 ± 10855.0</td>
</tr>
<tr>
<td>Kruskal-Wallis test</td>
<td>p = 0.44</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

The only difference in MMP-2 serum level is between stage 0 – II and stage IV (post hoc test), (p < 0.05). NS, not significant.
Table 6. Metalloproteinases MMP-2, MMP-9 and carbohydrate antigen 19-9 (CA 19-9) intraperitoneal fluid concentration in pancreatic cancer group depending on tumor stage.

<table>
<thead>
<tr>
<th>TNM classification</th>
<th>MMP-2 Median, Interquartile range</th>
<th>MMP-9 Median, Interquartile range</th>
<th>CA 19-9 Median, Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-II</td>
<td>7.2 ± 12.6</td>
<td>12.6 ± 22.2</td>
<td>10.45 ± 59.16</td>
</tr>
<tr>
<td>III</td>
<td>11.6 ± 33.4</td>
<td>16.05 ± 27.9</td>
<td>55.13 ± 4715.14</td>
</tr>
<tr>
<td>IV</td>
<td>12.8 ± 32.4</td>
<td>18.0 ± 67.5</td>
<td>564.0 ± 3952.83</td>
</tr>
</tbody>
</table>

Kruskal Wallis test NS $p = 0.34$

The only difference in CA 19-9 intraperitoneal fluid level is between stage 0 – II and stage IV (post hoc test) ($p < 0.05$). NS, not significant.
between M0 (no distant metastases) and M1 (distant metastases) groups. There were no significant differences in MMP-9 and CEA serum concentrations between M0 and M1 groups. There were no statistically significant differences in intraperitoneal fluid concentration of MMP-2, MMP-9, and CA19-9 between M0 and M1 groups. A statistically significant increase in the intraperitoneal fluid concentration of CEA was observed in the case of distant metastases (p < 0.01). The serum concentrations of MMP-2 and CA19-9 and concentration of the CEA in the intraperitoneal fluid were statistically significantly higher in the M1 group compared to the concentrations in the M0 group. (Table 7, Figs. 5-7).

In the PC group, the MMP-2, MMP-9, and CA19-9 serum and intraperitoneal fluid concentration was analyzed depending on the positive cytology examination in the intraperitoneal fluid. There was no statistically significant correlation between the concentration of the tested marker both in the serum and in the intraperitoneal fluid and the presence of neoplastic cells found in the cytological examination of the intraperitoneal fluid in patients with PC.

To determine the specificity and sensitivity of MMP-2, MMP-9, CA19-9, and CEA serum and intraperitoneal fluid concentrations in the prediction of PC, ROC analysis was performed. It has been demonstrated that the intraperitoneal fluid concentration of MMP-2 and MMP-9 and serum CA19-9 and CEA concentration showed significant sensitivity and specificity in PC prediction. The diagnostic specificity of MMP-2 (57%) and MMP-9 (68%) in the intraperitoneal fluid was lower than classical tumor marker CA19-9 (90%) and CEA (86%) in the serum. Similar results were revealed for predictive values, but only for CA19-9 PPV (positive predictive values) was higher than for other markers. (Table 8, Fig. 8).

Additionally, ROC analysis was performed to determine the specificity and sensitivity of MMP-2, MMP-9, CA19-9, and CEA serum and intraperitoneal fluid concentrations in the prediction of cancer dissemination. It has been demonstrated that the serum concentration of MMP-2 and MMP-9 and serum and intraperitoneal fluid of CA19-9 and CEA concentration showed significant sensitivity and specificity in PC prediction. The highest diagnostic specificity was revealed for serum CA19-9 (93%) concentration. A similar result was revealed for PPV – CA19-9 (89%). More results show in Table 9 and Fig. 9.

At the time of surgery 8 (26.7%) patients in the PC group and 2 (6.7%) patients in the CP group had jaundice. The concentration of bilirubin was significantly higher in PC serum samples compared to the CP group (p < 0.01) and to the CG group (p < 0.01). The concentration of bilirubin serum samples was similar in the CP and CG groups (Table 10). There was no statistically significant correlation between the serum and intraperitoneal fluid concentration of MMP-2 and MMP-9 and intraperitoneal fluid concentration of CA19-9 and bilirubin level. There was a statistically significant correlation between serum concentration of the CA19-9 and bilirubin level (p < 0.05).

Newly diagnosed diabetes was diagnosed in 8 (26.7%) patients in the PC group. Endocrine insufficiency was present in 10 (33.3%) patients in the CP group at the time of surgery and manifested in diabetes. The concentration of glucose was

Table 7. Matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) serum and intraperitoneal fluid concentration in pancreatic cancer group depending on distant metastases.

<table>
<thead>
<tr>
<th>Test</th>
<th>M0 (median)</th>
<th>M1 (median)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 S</td>
<td>25.9</td>
<td>68.613</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>MMP-2 PL</td>
<td>12.286</td>
<td>15.42</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 S</td>
<td>107.571</td>
<td>129.0</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 PL</td>
<td>17.1</td>
<td>18.0</td>
<td>NS</td>
</tr>
<tr>
<td>CA 19-9 S</td>
<td>1062.506</td>
<td>2080.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CA 19-9 PL</td>
<td>459.567</td>
<td>564.0</td>
<td>NS</td>
</tr>
<tr>
<td>CEA S</td>
<td>3.594</td>
<td>4.39</td>
<td>NS</td>
</tr>
<tr>
<td>CEA PL</td>
<td>1.014</td>
<td>3.75</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

U Mann Whitney test. S, serum; PL, peritoneal lavage; NS, not significant.

![Fig. 6. Carbohydrate antigen 19-9 (CA19-9) serum concentration depending on distant metastases.](image-url)
Table 8. Matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) as statistically significant PC predictors. (S – serum, PL – peritoneal lavage).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>AUC</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 PL</td>
<td>0.809</td>
<td>4.6</td>
<td>1</td>
<td>0.568</td>
<td>0.698</td>
<td>1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MMP-9 PL</td>
<td>0.77</td>
<td>11.4</td>
<td>0.831</td>
<td>0.684</td>
<td>0.676</td>
<td>0.839</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CA 19-9 S</td>
<td>0.916</td>
<td>26.1</td>
<td>0.793</td>
<td>0.902</td>
<td>0.852</td>
<td>0.860</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CEA S</td>
<td>0.702</td>
<td>3.66</td>
<td>0.467</td>
<td>0.867</td>
<td>0.636</td>
<td>0.765</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

S, serum; PL, peritoneal lavage.

Fig. 7. Carcinoembryonic antigen (CEA) intraperitoneal fluid concentration depending on distant metastases.

Fig. 8. Matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) concentrations levels in intraperitoneal fluid and carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA) in sera as a pancreatic cancer (PC) predictor.
significantly higher in PC serum samples compared to the CG (p < 0.01) and in CP serum samples compared to the CG (p < 0.01). The concentration of glucose serum samples was similar in the PC and CP groups. (Table 10). There were no statistically significant differences between the concentration of MMP2, MMP9, and CA19-9 and glucose level.

The concentration of glucose serum samples was similar in the PC and CP groups. (Table 10). There were no statistically significant differences between the concentration of MMP2, MMP9, and CA19-9 and glucose level.

The pain was the most common symptom in both PC (56.7%) and CP (60%) groups. Exocrine pancreatic insufficiency occurred in 6 (20%) patients in the CP group and had manifested mainly in diarrhea and pale or clay-colored stools. Eighteen (60%) of patients in the CP group were chronic tobacco smokers.

The observed intraperitoneal fluid concentrations of the MMP-2, MMP-9, and CA19-9 were significantly higher in the PC and CP groups compared to CG. There were no statistically significant differences between intraperitoneal fluid concentrations of the MMP-2, MMP-9, and CA19-9 and glucose level.

Table 9. Matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) as statistically significant predictors of PC dissemination.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>AUC</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 S</td>
<td>0.743</td>
<td>29.4</td>
<td>0.8</td>
<td>0.571</td>
<td>0.667</td>
<td>0.727</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MMP-9 S</td>
<td>0.710</td>
<td>75</td>
<td>0.867</td>
<td>0.571</td>
<td>0.684</td>
<td>0.80</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Ca19-9 S</td>
<td>0.723</td>
<td>2080</td>
<td>0.533</td>
<td>0.926</td>
<td>0.889</td>
<td>0.632</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Ca19-9 PL</td>
<td>0.718</td>
<td>399.3</td>
<td>0.667</td>
<td>0.846</td>
<td>0.833</td>
<td>0.688</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CEA S</td>
<td>0.705</td>
<td>3.15</td>
<td>0.800</td>
<td>0.643</td>
<td>0.706</td>
<td>0.750</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CEA PL</td>
<td>0.840</td>
<td>0.56</td>
<td>0.867</td>
<td>0.786</td>
<td>0.813</td>
<td>0.846</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

S, serum; PL, peritoneal lavage.

Table 10. Glucose and bilirubin concentration in pancreatic cancer (PC), chronic pancreatitis (CP) and control croup (CG) serum samples.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>PC median ± interquartile range (min. - max.)</th>
<th>CP median ± interquartile range (min. - max.)</th>
<th>CG Median ± interquartile range (min. - max.)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>123 ± 42.5 (80 – 301)</td>
<td>119 ± 75.5 (93 – 361)</td>
<td>76 ± 24 (76.00 – 146.00)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.86 ± 1.34 (0.32 – 20.91)</td>
<td>0.69 ± 0.53 (0.45 – 21.83)</td>
<td>0.95 ± 1.18 (0.1 – 1.81)</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

*Kruskall Wallis test. Post hoc tests:
Bilirubin: PC versus CP (p < 0.01), PC versus CG (p < 0.01), CP versus CG (NS)
Glucose: PC versus CP (NS), PC versus CG (p < 0.01), CP versus CG (p < 0.01)
concentrations of the MMP-2, MMP-9, and CA19-9 in PC and CP groups. The revealed serum concentration of the MMP-2 and MMP-9 in the PC, CP, and CG were significantly higher compared to the intraperitoneal fluid. There was no significant correlation between serum and intraperitoneal fluid concentration of the MMP-2, MMP-9, and CA19-9 and the presence of cancer cells in the intraperitoneal fluid conventional cytological examination. The elevated preoperative intraperitoneal fluid concentration of MMP-2 and MMP-9 and serum concentration of CA19-9 and CEA showed significant sensitivity and specificity in PC prediction. The preoperative serum concentrations of MMP-2 and MMP-9, serum, and peritoneal fluid concentrations of CA19-9 and CEA showed a statistically significant effect on predicting cancer dissemination. The serum concentration of MMP-2 increases depending on changing tumor stages in the TNM/UICC classification and the presence of distant metastases. The intraperitoneal fluid concentration of CA19-9 increases depending on changing tumor stages in the TNM/UICC classification and the serum CA19-9 concentration increases depending on the presence of distant metastases.

DISCUSSION

Pancreatic cancer has the highest malignancy and the worst prognosis among cancers of the gastrointestinal tract (3, 31). Independent risk factors of PC include: obesity, smoking tobacco, chronic pancreatitis, hereditary pancreatitis, high-fat diet, and diabetes (7, 31, 32, 33). At the time of diagnosis, only 15 – 20% of patients with PC are eligible for surgical resection (34). The reason for this is the aggressive biology of pancreatic cancer and late diagnosis due to a lack of clinical symptoms in the early stages of the disease (34). The average survival rate for PC in European countries is 26% at one year and 7% at 5 years since diagnosis and is the lowest among all malignant gastrointestinal tumors (3).

CP has a poor prognosis, with the mortality rate being approximately two-fold higher than that in the general population (35). One of the causes of CP may be recurrent acute pancreatitis. Markers that influence the inflammatory process are investigated, including pancreatici-associated protein-1 (PAP-1), which inhibits induced cell death and apoptosis of pancreatic acinar cells (36). The indication for surgery in CP patients are severe pains with no response to pharmacological treatment, common bile duct stenosis, duodenal obstruction, pseudocysts of the pancreas, narrowing or blockage of the pancreatic duct, and the high risk of PC or the inability to distinguish cancer from an inflammation tumor based on standard diagnostic tests (37, 38).

An ideal tumor marker should be highly sensitive, specific, reliable with high prognostic value, organ specificity and it should correlate with tumor stages (11). Unfortunately, none of the tumor markers reported to date have above ideal characteristics. It is not specific to a single malignancy. Every tumor marker is specific to a group of malignancies or a single organ. The malignant process is known to elaborate a group of cancer markers (11, 39). The source of cancer markers, that may be investigated, including pancreatitis-associated protein-1: adiponectin and leptin (40). Unintentional weight loss associated with cancer cachexia may be evidence of stage IV of PC, as demonstrated on the activating A plasma levels, which can be useful biomarker for identifying patients with metastatic disease (41).

CA19-9 is the most extensively studied and currently the gold-standard biomarker for pancreatic cancer diagnosis in symptomatic patients. Limitations such as non-specific expression in several benign and malignant diseases, false-negative results in sialyl Lewis negative individuals, false-positive elevation in the presence of obstructive jaundice, and poor sensitivity limit the universal applicability of serum CA19-9 (12, 14).

In recent years, researchers expressed great interest in the group of zinc-dependent proteolytic enzymes called metalloproteinases. The published data suggests that metalloproteinases contribute to the genesis of many types of cancer. They play an important role in the progression of cancer by stimulating the growth of cancer cells, infiltration of surrounding tissues and destruction of ECM, migration of cancer cells, the formation of metastases, and new blood vessels (22, 30, 42). Similar results are achieved following intraperitoneal inflammatory reaction induced by repeated infusions of the dialysis fluid accelerates the senescence of the mesothelial cells, which may result in fibrosis and neoangiogenesis within the peritoneum. This new findings with the effect of glutathione precursor on the aging of human peritoneal mesothelial cells in vitro, also reflecting some aspects of peritoneal lavage concentration of metalloproteinases presented in our study (43).

The role of gelatinases as markers of PC is not established yet. Ellenrieder et al. in his clinical trial showed overexpression of MMP-2 in pancreatic cancer tissue. Expression and activation of MMP-2 were also strongly associated with the extent of the desmoplastic reaction in pancreatic cancer tissues. MMP-2 expression was also found in a small number of chronic pancreatitis tissue samples (44). Zhai et al. in his research showed that the expression of MMP-2 was significantly increased in pancreatic carcinoma tissues relative to paracarcinoma tissues. Additionally, the expression of MMP-2 was significantly higher in pancreatic carcinomas with higher preoperative serum CA19-9 levels, poor histological grade, perineural invasion, lymph node metastasis, distant metastasis, and advanced histological stage (45). Harvey et al. showed that patients with overexpression of MMP-9 in pancreatic cancer tissue had a trend toward poorer survival than those who did not express it (46). In his research, Lekstan et al. studied activity and concentrations of matrix metalloproteinases 2 and 9 and the concentrations of their tissue inhibitors (TIMP 1 and 2) in the PC and CP tissue homogenates. The authors showed a higher concentration of both gelatinases and their inhibitors in PC tissue compared to CP. However, a statistically significantly higher concentration of MMP-9 and TIMP-2 in both groups was obtained compared to MMP-2 and TIMP-1 (47). Using gelatin zymography Yokoyama et al. revealed the presence of active MMP-2 and pro MMP-2 in pancreatic juice. Active MMP-2 was significantly higher in pancreatic juice in the PC group than in CP and CG in this study (48). Nagakawa et al. showed a correlation between MMP-2 and MMP-9 overexpression in pancreatic cancer tissue and the occurrence of liver metastases and cancer infiltration on large-sized (> 201 um) veins (49).

Only a few studies on serum gelatinases concentration in patients with PC and CP are available but their results are conflicted. In the case of research on MMP-2 Łukaszewicz-Zajęc et al. showed that the concentration of MMP-2 was higher in the PC group compared to CG, however lower than in the CP group, but cited authors did not achieve statistical significance (52). Similar results were also obtained by Smigielski et al. Author showed a higher preoperative concentration of MMP-2 in the PC group, both in the group of patients with resectable tumors (stage I and II) and in the group of patients in stage III and IV compared to CG but did not obtain statistical significance (24). In contrast, Singh et al. in his research found that plasma
MMP-2 concentration in patients with PC was significantly higher than those with CP and CG (54). Iki et al. investigated the circulating level of MMP-2 and MMP-9 in sera from Syrian golden hamsters into which hamster pancreatic duct adenocarcinoma tissues had been transplanted subcutaneously. Researchers showed significantly higher levels of serum MMP-2 and MMP-9 in hamsters with PC than in CG and demonstrated a significant correlation between tumor growth and serum MMP-2 and MMP-9 levels (55).

In our material, no statistically significant difference in MMP-2 concentrations in serum between groups was observed. Statistical analysis for each of the tested markers, both in serum and intraperitoneal fluid, was performed for each group separately. The results are comparable with the literature data (24, 52). The highest concentration of MMP-2 in the intraperitoneal fluid was observed in the PC group, while the lowest in CG. Statistical significance, compared to the level of MMP-2, in the PC with the CG group and the CP with CG was obtained. No statistically significant difference between the PC and CP was observed. The level of intraperitoneal fluid MMP-2 was comparable. However, this result can't be compared to the literature, because we didn’t find the description of gelatinases in the peritoneal fluid in patients with PC, CP and healthy controls in the available sources. According to the literature data, this was the first study evaluating MMP-2 and MMP-9 concentrations in peritoneal fluid in patients with PC and CP. So far, only a few studies have been performed on the concentration of tumor markers in the intraperitoneal fluid in pancreatic and gastric cancer but concerned only the CA19-9 and CEA concentration. It is noteworthy that the level of MMP-2 concentrations both in the serum and in the intraperitoneal fluid is comparable in the PC and CP groups and it is impossible to differentiate malignant from benign lesions based on its concentration, but it is statistically significantly lower in healthy subjects. Only a few data on the concentration of gelatinases in tissue homogenates are available. Yamaguchi et al. in 2005 obtained the long-term increase in MMP-2 activity in their experiment conducted on rats with induced chronic pancreatitis characterized by disturbances in the continuity of type IV collagen along with BM and atrophy of pancreatic lobules (56). On the other hand, Ng et al. found a decreased activity of MMP-2 and MMP-9 in pancreatic homogenates of animals with pancreatitis induced by ligation of the pancreatic ducts and intraperitoneal administration of cerulein. The authors concluded that the decreased activity of gelatinases in induced pancreatitis is one of the factors responsible for pancreatic fibrosis (57). Lekstan et al. showed a higher concentration of both gelatinases and their inhibitors in PC tissue compared to CP. However, the authors obtained statistically significantly higher levels of MMP-9 and TIMP-2 in both groups compared to MMP-2 and TIMP-1 (47).

As in the case of MMP-2, only a few studies on the concentration of MMP-9 in the serum and plasma of patients with PC and CP are available in the medical literature, and their results are inconsistent. Joergensen et al. in his prospective case-control study demonstrated that circulating MMP-9 and TIMP-1 were inferior to CA19-9 as markers for detecting pancreatic ductal adenocarcinoma and did not improve the diagnostic accuracy when combined with CA19-9. He also showed that the median level of MMP-9 in PC was not statistically different from that found in CG (50). In contrast, Mroczko et al. found that patients with PC had statistically higher serum levels of MMP-9, TIMP-1, and tumor markers (CA19-9, CEA) as compared with patients with CP and CG and elevated preoperative concentration of MMP-9 was a significant independent prognostic factor for the patients’ survival (51). Comparable results to Mroczko obtained by Tian et al. Author showed that serum MMP-9 levels were significantly higher in patients with PC than those with CP and CG (53). Smigielski et al. also showed a significantly higher preoperative level of MMP-9 in the PC group in comparison to CG. At the same time, the authors showed that the level of MMP-9 was significantly lower in the CP group than in the PC group (24).

In our material, no statistically significant difference in MMP-9 concentrations in serum between groups was observed. The results are comparable with the literature data; however, it should be emphasized that in the research of Jorgenstein et al. the CG was not uniform. The CG included patients with CP, healthy people, patients with gallstone disease, and other benign diseases, as well as patients with diagnosed neoplasms of other organs, including gastric cancer and lung cancer (50). The highest concentration of MMP-9 in the intraperitoneal fluid was observed in the PC group, while the lowest in CG. Statistical significance, compared to the level of MMP-9, in the PC with the CG group and the CP with CG was obtained. No statistically significant difference between the PC and CP was observed. The level of intraperitoneal fluid MMP-9 was comparable. This result can't be applied to the literature as in the case of MMP-2, due to the current lack of description of the above relationship.

Preoperative CA19-9 concentration has a high prognostic value and correlates with the tumor stage. The CA19-9 concentration above 1000 U/ml is almost 100% specific for the PC (39). In our material, mean serum CA19-9 median was significantly higher in PC serum samples compared to the CP and to the CG. The obtained results correspond to the literature data, which indicates a significant increase in the CA19-9 marker in advanced pancreatic neoplasms (12, 14, 15).

There are only a few reports in the literature on the evaluation of CA19-9 in the intraperitoneal fluid in patients with PC. Hoskovec et al. compared the concentrations of CA19-9 in the serum and the intraperitoneal cavity in 20 patients with PC. The authors showed that the concentration of the CA19-9, both in the serum and in the intraperitoneal fluid, increases with increasing cancer stages and that the higher level of CA19-9 and CEA correlates with a higher stage of PC, worse prognosis and more frequent recurrence of the neoplastic process (10). Midwinter et al. performed immunocytochemical examination of mesothelial cells obtained during the examination of intraperitoneal fluid in patients with PC (22 patients), CP (3 patients), and gallbladder stones (7 patients) using antibodies against CEA and CA19-9. Only one patient with CP had cells that showed weak positivity for CEA, the remainder showed no positivity for CA19-9 and CEA. Thirteen patients with PC had a positive reaction for CA19-9 or CEA. In the cited research positive immunocytochemical reaction in the PC group was observed only in patients with locally advanced or metastatic disease (58). It is difficult to relate the results obtained by the cited authors to the results obtained in the analyzed material due to the discussed only patients with PC in the first study and an unrepresentative group of patients with CP in the second study. In the presented material, an increase CA19-9 concentration in the intraperitoneal fluid was obtained both in the PC and CP groups. A statistically significant difference was obtained in both groups by comparing them with the CG, in which the concentration of CA19-9 was within the normal range. CEA has a relatively low sensitivity and specificity in the diagnosis of PC (59). The limited usefulness of the CEA in the diagnosis of PC results from an increase in its concentration only in III and IV stages of PC, when surgical resection is impossible or when distant metastases are present (10, 60, 61). In the presented material the differences between the PC and CG group
were statistically significant. The difference may result from the high percentage of patients in the tumor stage III and IV in the PC group. There are only a few reports in the literature on the evaluation of CEA in the intraperitoneal fluid in patients with PC (10, 58). The study by Hoskovec et al. showed an increase in CEA concentration in 20% of patients with PC in serum, and in 43% in the intraperitoneal fluid (10). Takahashi et al. found a positive polymerase chain reaction with the reverse transcription of CEA mRNA in 24% of patients with PC with a negative peritoneal lavage fluid cytology (62). As for CA19-9, it is difficult to relate the results obtained in the analyzed material to received by the cited authors due to the discussed only patients with PC and used the PCR method while in the presented material radioimmunological methods were used. Statistically significant increase of CEA concentration in intraperitoneal fluid was obtained both in the PC and CP groups compared to CG in analyzed material.

The comparative analysis of MMP-2 and MMP-9 concentration between blood serum and intraperitoneal fluid showed a statistically significant higher serum concentration in all study groups. The concentration of CA 19.9 was significantly higher in serum compared to the intraperitoneal fluid in the PC group. The concentration of CA 19.9 was significantly higher in the intraperitoneal fluid compared to the serum in the CG group. There was no significant difference between the concentration of CA 19.9 in the intraperitoneal fluid compared to the serum in the CG group. These results can’t be applied to the literature due to the current lack of description of the above relationships. CA19-9 concentration in the intraperitoneal fluid was assessed by Hoskovec et al. The authors showed an increase in serum CA19-9 concentration in 66% of patients in stage I and II of PC, while in the peritoneal fluid an increase in concentration was achieved in 33% of patients. At stage III and IV, an increase in serum CA19-9 concentration was observed in 85% of patients, and in the intraperitoneal fluid in 66% of patients. The serum concentration of CA19-9 at PC was higher compared to the intraperitoneal fluid, but the authors didn’t determine whether statistical significance was achieved (10). It is worth emphasizing that in the case of both gelatinases in all discussed groups, their concentration in blood serum was higher than in the fluid obtained from the peritoneal cavity.

At the same time, the analysis of correlation of the increase in the activity of MMP-2, MMP-9, and CA19-9 in the intraperitoneal fluid of patients with PC, CP, and people with CG was performed concerning the increase in their concentration in blood serum. In the PC group, a positive correlation was found between the concentration of MMP-2 and CA19-9. The positive correlation may result from the participation of MMP-2 in neangiogenesis, BM degradation, migration of neoplastic cells, and the formation of metastases, and thus an increase in its activity in the intraperitoneal fluid (17, 22). A parallel increase in the concentration of CA19-9 in the serum and the intraperitoneal fluid was shown in the study by Hoskovec et al. (10). The result of the cited authors is comparable with the result obtained in the presented material.

The concentration of MMP-2, MMP-9, and CA19-9 depending on increasing tumor stage in the TNM/UICC classification and distant metastases were examined separately in the PC group. A statistically significant increase in the serum concentration of MMP-2 between the lowest-level group 0 – II and group IV was observed. A higher concentration of MMP-2 in the serum were observed in highly advanced neoplasms. No statistically significant difference between MMP-9 and CA19-9 concentration depending on increasing tumor stage was observed. Lukaszewicz-Zajac et al. received different results regarding the concentration of MMP-2. The authors didn’t show a statistically significant difference between the concentration of MMP-2 in the serum and the tumor stages, however, in the cited study, the authors didn’t show a statistically significant difference in the concentration of CA19-9 and tumor stages, which is consistent with the results obtained in the discussed material (52). Singh et al. also didn’t show a statistically significant correlation between the concentration of MMP-2 and the increasing tumor stage (54). The results in the presented material correspond with the work of Joergensen, which also showed no correlation between the concentration of MMP-9 and CA19-9 and tumor stage.

There were no statistically significant differences in intraperitoneal fluid MMP-2 and MMP-9 concentrations depending on the tumor stage in the TNM/UICC classification. A statistically significant increase in the intraperitoneal fluid concentration of CA19-9 between group 0 – II and IV was observed. A higher concentration of CA19-9 in the intraperitoneal fluid was observed in highly advanced neoplasms. The obtained results correspond with the work of Hoskovec. The authors of the cited study obtained an increase in CA19-9 concentration in the intraperitoneal fluid in 33% of patients with stage I and II, and in 60% of patients with stage III and IV (10).

There were statistically significant differences in serum concentration of MMP-2 and CA19-9 and intraperitoneal concentration of CEA between groups 0 and M1. There were no statistically significant differences in intraperitoneal fluid concentration of MMP-2, MMP-9, and CA19-9 between M0 and M1 groups. There were no statistically significant differences in intraperitoneal fluid concentration of MMP-2, MMP-9, and CA19-9 between M0 and M1 groups. A statistically significant increase in the serum concentration of CEA was observed in the case of distant metastases. In the study by Lukaszewicz-Zajac et al., the concentration of serum MMP-2 and CA19-9 were elevated in the M1 group compared to M0, but no statistically significant differences were obtained (52). Mroczko showed the increased serum concentration of CEA and CA19-9 in the M1 group compared to M0, but the cited authors also didn’t obtain statistically significant differences (51). The results obtained in the presented material are comparable to the results obtained by the cited authors, however, it should be emphasized that the results obtained in the presented material are statistically significant. On the other hand, the cited authors showed that the serum CEA concentration was statistically significantly higher in the M1 group compared to M0. In the presented material, no statistically significant differences were obtained in CEA serum concentration between M0 and M1 groups, while a significantly higher CEA concentration was found in the M1 intraperitoneal fluid group compared to M0. Similar results were obtained by Havlík et al. The authors found a positive correlation between CEA transcript levels in peritoneal lavage in PC patients and the clinical stage (significantly higher level of CEA transcript in stage IV) and the radicality of resection when comparing patients after radical resection R0 and after non-radical R1/R2 resections (63). The study by Hoskovec also showed an increase in CEA concentration in the intraperitoneal fluid in 43% of PC patients with stage III and IV, compared to the serum with an increase in the same stages only in 20% of patients. There was no evidence of an increase in CEA concentration in stage I and II patients, both in serum and intraperitoneal fluid (10). Whereas Takahashi comparing CEA mRNA(+) patients with CEA mRNA(-) patients in the peritoneal fluid found no statistically significant difference depending on blood and lymph vessels invasion, presence of lymph node metastases (N1), presence of distant metastases (M1), the radicality of surgical resection, nerve fibers invasion and the tumor stages (62).
of the intraperitoneal fluid in patients with PC with the serum and intraperitoneal fluid concentration of the MMP-2, MMP-9 and CA19-9 was performed. There were no statistically significant differences in all performed correlations. According to the AJCC (American Joint Committee on Cancer) positive PLC (peritoneal lavage cytology) is recognized as stage IV of PC, but there is still much controversy about the sensitivity of this study. In PC, malignant cancer cells have been identified in 7 – 30% of peritoneal washings (64). Clark et al. in their study confirmed the presence of cancer cells in 20% of patients in material obtained during diagnostic laparoscopy and diagnostic PLC in patients with PC confirmed in BAC. Over 25% of patients, who have initially been diagnosed with locally advanced PC (stage III) based on CT after finding cancer cells in PLC were re-qualified to stage IV (65). Hirabayashi et al. obtained positive PLC in 11% potentially resectable PC cases, while Iwagami et al. obtained positive PLC in 12.8% of patients who underwent surgery for left-sided PC (66, 67). On the other hand Midwinter et al. found no neoplastic cells in the cytological examination of the peritoneal fluid in 22 patients with PC while using the immunocytochemical examination they found the presence of CEA and CA19-9 antigens in the mesothelial cells of the of intraperitoneal fluid in patients with advanced, unresectable PC (58). Also, in recent reports, Takahashi et al. showed no neoplastic cells in the cytological examination of the peritoneal fluid in 237 patients with PC after preoperative radiochemotherapy, but CEA mRNA was found in the peritoneal fluid in 24% of PC patients with the negative cytological test (62). In contrast to the Midwinter and Takahashi in the presented material in 4 (13.33%) patients cancer cells were found in a conventional cytological examination, which is comparable with the previously cited literature data (64-67).

To determine the specificity and sensitivity of MMP-2, MMP-9, CA19-9, and CEA serum and intraperitoneal fluid concentrations in the prediction of PC, ROC analysis was performed. It has been demonstrated that the intraperitoneal fluid concentration of MMP-2 and MMP-9 and serum CA19-9 and CEA concentration showed significant sensitivity and specificity in PC prediction. These results correspond with the work of Lukaszewicz-Zajac et al. in which the diagnostic sensitivity of serum concentration of MMP-2 in detecting PC was lower than for CA19-9 and CEA and the result was not statistically significant. On the other hand, when determining the sensitivity of the predictive value it was shown that both CA19-9 and CEA in the serum had a statistically significant influence in predicting PC, with the marker CA19-9 having the highest PPV, which was also shown in the presented material (52). These results also correspond with the work of Joergensen et al. in which the sensitivities of MMP-9 in the serum in detecting PC was 58.82%, respectively, with specificities of 34.6% and was definitely inferior to CA19-9 as a marker for detecting PC. The authors also showed that even the combination of the three tested markers MMP-9, TIMP-1, and CA19-9 does not statistically increase the possibility of detecting PC in comparison with the CA19-9 alone (50).

Also, Poruk et al. showed that the sensitivity of serum concentration of the CA19-9 in predicting PC reaches 81.7%, while the specificity is 82% at the cut-off values of 37 U/ml (68). However, a different result was obtained by Mroczko et al., who showed that the diagnostic sensitivity for TIMP-1 serum concentration for predicting PC is higher than that of the classic markers CA19-9 and CEA, and also clearly increases in association with the concentration of MMP-9 (51). The statistically significant results obtained in the presented material, which show that the concentration of MMP-2 and MMP-9 in the intraperitoneal fluid influences the prediction of the PC presence, just like several others described above, can’t be compared to literature because we did not find the description of the following correlation in the available sources.

The ROC analysis was performed to determine the specificity and sensitivity of MMP-2, MMP-9, CA19-9, and CEA serum and intraperitoneal fluid concentrations in the prediction of cancer dissemination too. It has been demonstrated that the serum concentration of MMP-2 and MMP-9 and serum and intraperitoneal fluid of CA19-9 and CEA concentration have a statistically significant effect on the prediction of cancer advancement and the presence of distant metastases. The results in the presented material, concerning the serum concentration of CA19-9 and CEA as predictors of cancer advancement and dissemination are comparable with the literature data. Van Manen et al. reported the predictive value of CEA and CA19-9 levels for predicting advanced PC in 352 patients. Both tumor markers were independent predictors for advanced PC resulting in PPV of 83.3%, 73.6%, and 91.4% for CEA, CA19-9, and combined, respectively (60). The statistically significant results obtained in the presented material, which show that the serum concentration of MMP-2 and MMP-9 and concentration of CA19-9 and CEA in the intraperitoneal fluid has an influence on the prediction of the cancer advancement and dissemination, just like several others described above, can’t be compared to literature, because we didn’t find the description of the following correlation in the available sources. In the early stages of cancer differentiation (stage I and II), it makes it possible to achieve 5-year survival in over 30% of patients (60). Preoperative, accurate determination of the cancer stage, especially cancer dissemination, would allow avoiding many unnecessary laparotomies, and in cases of borderline resectability, first, use induction chemotherapy to regress the tumor infiltration into blood vessels and surrounding tissues. Despite the progress made in preoperative diagnostics modalities, especially in dedicated pancreatic protocol CT (computed tomography) with dual-phase contrast enhancement and thin axial section, recommended by the NCCN (National Comprehensive Cancer Network) guidelines and EUS (endoscopic ultrasound) and EUS-guided tissue sampling, these methods are still not perfect (69, 70). At the same time, due to its low sensitivity cytological examination of the intraperitoneal fluid in patients with PC obtained by diagnostic laparoscopy, is not a good marker of dissemination, especially in the absence of morphological features of dissemination (58, 62, 64, 66, 67). For this reason, it is necessary to search for new tumor markers whose sensitivity and specificity would allow for recognition of PC in the first stages of the disease, when radical surgery is still possible to be performed, markers that would allow distinguishing malignant tumors from benign pancreatic diseases, which can be treated conservatively, and finally markers that would allow to accurately determine the stage of PC advancement. MMP-2 and MMP-9 were also examined in this matter (24, 50-52, 54, 55). Despite its imperfections, tumor marker CA19-9 is still the only marker recognized and approved for general use in PC.

In conclusion, it should be emphasized that differentiation between pancreatic cancer and chronic pancreatitis based on the available diagnostic methods is not currently possible and represents a major challenge for modern science. The role of presented metalloproteinases as tumor markers of pancreatic cancer requires further study and exclusion of other causes that may lead to alterations in their concentrations in cancers.

Abbreviations: AJCC, American Joint Committee on Cancer; BM, basal membrane; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CG, control group; CP, chronic pancreatitis; CT, computed tomography; ECM, extracellular matrix; EUS, endoscopic ultrasound; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; NCCN, National Comprehensive Cancer Network; NPV,
negative predictive value; PPV, positive predictive value; PC, pancreatic cancer; PLC, peritoneal lavage cytology.

D. Dranka-Bojarowska and A. Lewinski contributed equally to this work.

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