

Analysis of circulating blood and tissue biopsy *PDX1* and *MSX2* gene expression in patients with pancreatic cancer

A case–control experimental study

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Abstract

Early diagnosis of pancreatic cancer (PC) is based on endoscopic ultrasound (EUS). However, EUS is invasive and requires a high level of technical skill. Recently, liquid biopsies have achieved the same sensitivity and specificity for the diagnosis of numerous pathologies, including cancer. Insulin-promoting factor 1 (*PDX1*) and Msh-homeobox 2 (*MSX2*), 2 homeotic genes, have been confirmed to be related to pancreatic oncogenesis.

The aim of this study is to establish the diagnostic utility of circulating serum levels of *MSX2* and *PDX1* expression in patients with PC.

A prospective study was conducted from January 2014 to February 2017. Patients with a suspected diagnosis of PC who underwent fine needle aspiration biopsy guided by EUS (EUS-FNA) were included in the study, in addition to non-PC control subjects. Both tissue and blood serum samples were submitted to histopathological analysis and measurement of *PDX1* and *MSX2* gene expression by means of qRT-PCR.

Patients were divided into non-PC, malignant pathology (MP), or benign pathology (BP) groups. Significant differences in both *MSX2* [2.05 (1.66–4.60) vs 0.83 (0.49–1.60), $P = .006$] and *PDX1* [2.59 (1.28–10.12) vs 1.02 (0.81–1.17), $P = .036$] gene expression were found in blood samples of PC compared with non-PC subjects. We also observed a significant increase in *MSX2* transcripts in tissue biopsy samples of patients diagnosed with MP compared with those with BP [1.98 (1.44–4.61) and 0.66 (0.45–1.54), respectively, $P = .012$]. The ROC curves indicate a sensitivity and specificity of 80% for *PDX1* and 86% for *MSX2*.

Gene expression of *MSX2* in tissue samples obtained by EUS-FNA and serum expression of *MSX2* and *PDX1* were higher in patients with PC.

Abbreviations: BP = benign pathology, cDNA = complementary deoxyribonucleic acid, Cp = crossing point, EMT = epithelial-to-mesenchymal transition, EUS = endoscopic ultrasound, EUS-FNA = fine needle aspiration endoscopic ultrasound, INR = prothrombin time, MP = malignant pathology, *MSX2* = Msh-homeobox 2, PC = pancreatic cancer, PDA = pancreatic ductal adenocarcinoma, *PDX1* = Insulin-promoting factor 1, qRT-PCR = quantitative real-time polymerase chain reaction, ROC = receiver-operating characteristic.

Keywords: biomarker, endoscopy, *MSX2*, pancreatic cancer, *PDX1*

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1. Introduction

Pancreatic cancer (PC) is the seventh most common cause of cancer mortality worldwide,^[1] with a 5-year survival rate of 5%.^[2,3] Infiltrating pancreatic ductal adenocarcinoma (PDA) is the most prevalent tumor type, making up 90% of all PC cases. According to the 2014 World Cancer Report, PC mortality is higher on the European continent (31.6%) than the rest of the world.^[4] Around 70% to 80% of patients present unresectable lesions at the time of diagnosis.^[3] The global incidence of PC based on the 2012 GLOBOCAN is 4.2 out of every 100,000 individuals.^[5] The estimated rate is lower in Mexico, with an incidence of 3.4 per 10,000 inhabitants, which is thought to be underestimated due to limited or incomplete data availability.^[6–8]

Historically, early diagnosis of PC has been difficult due to technical issues related to the physiopathology of the disease. Genetic alterations have been reported for intraductal adenocarcinoma (IDA), which is characterized by mutations in several genes, including *K-Ras*, *Cdkn2a*, *Tp53*, *Tgfb2*, *Epc1*, *Arid2*, and *Map2k4*, among others.^[9–12] Cystadenomas, intraductal papillary mucinous, and mucinous cystic neoplasms are also characterized by gene mutations in *β-catenin*, *Gnas*, *Rnf43*, and *Tp53*.^[13] In addition, inherited mutations such as those occurring in the *BRCA2*, *p16/CDKN2A*, and *PRSS1* genes might have an impact on the risk of developing PC.^[13] Previous reports indicate that *K-ras* gene mutations are most frequently observed in tissue (45–100%) and serum (77%) samples of PC patients,^[14,15] followed by mutations in the genes encoding cyclin-dependent kinase 2A (*CDKN2A*)/*p16*, tumor protein 53 (*TP53*), and SMAD family member 4 (*SMAD4*).^[9–11] Notably, *K-Ras* mutations have been detected in pre-malignant stages, which could be useful as an early detection marker for PC.^[16,17]

Aside from the above-mentioned genes, some reports have indicated that homeotic genes such as insulin promoter factor 1 (*PDX1*) and the transcription factor Msh-homeobox 2 (*MSX2*) are related to PC. In the first case, it has been shown that *PDX1* directly regulates acinar cell identity, resisting the formation of PanIN-derived PDA. Thus, upon neoplastic transformation, *PDX1* becomes an oncogenic factor.^[18] In the second case, *MSX2* was shown to be a transcription factor related to the epithelial-to-mesenchymal transition (EMT), with elevated expression noted in several tumors of epithelial origin.^[19] The expression of *MSX2* has also been related to PDA aggressiveness through induction of EMT via the BMP4-related signaling pathway.^[20] Together, these findings highlight the potential use of *MSX2* and *PDX1* as potential biomarkers for use in PC diagnostics.

The aim of this study was to evaluate the diagnostic utility of *MSX2* and *PDX1* expression in the circulating blood of patients with PC.

2. Materials and methods

2.1. Study population

A prospective case–control study was conducted between January 2014 and February 2017. Patients (>18 years old) with a suspected diagnosis of PC with solid or cystic lesions, without a contraindication to the procedure, platelet count >50,000, and a pro-thrombin time (INR) <1.5 were included in the study. All patients authorized their participation in the study by providing informed signed consent. The protocol was reviewed and

authorized by the Ethics and Research Committees. Clinical follow-up of patients with neoplasms was carried out for 6 months after the diagnosis.

Patients with a lesion in the pancreas diagnosed by at least 1 imaging study and histologically confirmed by a biopsy sample obtained by endoscopic ultrasound (EUS) were considered cases. Tissue biopsies were processed to measure the mRNA expression of *PDX1* and *MSX2* genes. Control (non-PC) pancreas tissue was obtained from cadaveric donors for liver transplantation.

2.2. Aspiration biopsy guided by endoscopic ultrasound

Endosonographic studies were performed under sedation with a FUJI EG-530UT convex linear instrument attached to a SU-8000 console (Fujifilm Corporation, Minato-Ku, Tokyo, Japan) or with a GF-UCT140 (Olympus Corp., Tokyo, Japan) device attached to an Aloka SSD-console 5500 (Aloka Ltd., Tokyo, Japan). Biopsy collection was conducted with 19G, 22G and/or 25G needles (Wilson-Cook Echotip Ultra, Winston-Salem, NC or Boston Scientific, MA). Following ultrasonography screening, once the lesion was located, the transducer was placed in a stable position in front of the lesion and a needle was inserted through the gastrointestinal wall with the stylet in position only during the first pass, then the stylet was removed and suction was applied. An average of 2 to 3 passes were performed per patient with a “fan” technique. The tissue obtained was used for histopathological evaluation and for the extraction of total RNA.

After the procedure, the patients remained under surveillance for at least 2 hours to assess for complications. The EUS procedures were conducted by 2 endosonographers with experience in the field (>2500 procedures), assisted by an anesthesiologist and performed under continuous monitoring.

2.3. Peripheral blood sample collection

Blood samples were collected using K2-EDTA tubes (BD-Vacutainer; Franklin Lakes, NJ). Control blood samples were collected from healthy patients who attended the endoscopy service for routine procedures. The plasma was separated from the globular package by centrifugation at 3000 rpm for 10 minutes at room temperature.

2.4. Total RNA extraction and RT-qPCR

Total RNA was extracted from whole blood or biopsy samples with the Tripure Isolation Reagent (Roche Diagnostics GmbH, Mannheim, Germany). Total RNA enrichment and integrity were assessed by 260-nm wavelength absorbance and denaturing agarose gel electrophoresis, respectively. Ratios of 260/280 nm and 260/230 nm above 1.8 and 1.5, respectively, were used as indicators of RNA purity. Once evaluated, RNA was kept at -70°C until use. As the pancreas samples were too small to obtain enough RNA, cDNA was synthesized using 1 µg of total RNA, a mix of random hexamers (60 µM), and anchored-oligo-dT 18 (50 µM) primers, according to the Transcriptor First-Strand cDNA Synthesis Kit guidelines (Roche Diagnostics GmbH). The resulting single-stranded cDNA was amplified by real-time polymerase chain reaction (qPCR) in duplicate, using TaqMan hydrolysis probes (Roche Diagnostics GmbH) and the Light-Cycler TaqMan Master kit (Roche Diagnostics GmbH), according to the manufacturer’s instructions. The primers used in this study are as follows: pancreatic and duodenal homeobox 1

(*PDX1*, NM_000209.3), forward 5'-AAGCTCACGCGTG-GAAAG-3', reverse 5'-GCCGTGAGATGTACTTGTGAA-3'; Msh-homeobox 2 (*MSX2*, NM_002449.4), forward 5'-CATGATGGATGCTTGTTC-3', reverse 5'-TGGCTGGTACT-GCCTTCG-3'; and beta-actin, (*ACTB*, ENST00000331789.2) as the constitutively expressed gene, forward 5'-CAACCGCGA-GAAGATGAC-3', reverse 5'-GTCCATCACGATGCCAGT-3'. The reaction was performed using a LightCycler-480 II apparatus (Roche Diagnostics LTD, Rotkreuz, Switzerland) under the following conditions: 1 cycle of 95°C for 10 minutes; followed by 45 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 1 second.

2.5. qPCR data analysis

Raw qPCR data were analyzed according to the standard curve method.^[21] Briefly, serial 0.2-fold dilutions starting with 100 ng of input RNA were prepared to obtain a dynamic range of PCR amplification product. The crossing point (C_p) and the log₁₀-based amount of RNA (nanograms) for each dilution were plotted, and the linear regression and coefficient of determination (R^2) were obtained. These data were correlated with the log₁₀-based input amount of RNA (nanograms) to interpolate and calculate the real concentration of curve input points, which were found to have a high correlation coefficient (0.9980). Once the curves were ready, sample data were interpolated on the curve, and the amount of RNA was calculated and used to normalize the control and PC-positive samples. The data were then compared, using control patients as the basal expression (1-fold).

2.6. Statistical analysis

Data distribution for each variable were analyzed with the Kolmogorov–Smirnov test. Qualitative data are expressed as frequencies and percentages, while quantitative data are expressed as mean \pm standard deviation (SD) for parametric data and median (25th–75th percentiles) for nonparametric data. The Mann–Whitney test was used to assess the differences between groups. Analysis of the correlation between variables was conducted by applying the Pearson correlation test. A receiver operating characteristic (ROC) analysis was carried out for *MSX2* and *PDX1* serum gene expression in comparison with the control group. The area under curve (AUC), sensitivity, specificity, positive predictive values (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR) were also calculated. All of the statistical analyses were conducted using SPSS software 25.0 (SPSS, Chicago, IL), and graphs were generated with GraphPad Prism software V7.0 (GraphPad Software, San Diego, CA). All tests were 2-tailed and considered significant at $P < .05$.

3. Results

3.1. Clinical characteristics of the participants

The inclusion criteria allowed us to initially recruit 83 patients with diagnostic suspicion of PC, from whom 49 were excluded due to insufficient tissue sample. Thus, 34 patients with pancreatic lesions were included in the final analysis. We also included 13 non-PC participants (non-PC). Of the patients with pancreatic lesions, 23 (67%) were women and 11 (33%) were men, and the average age was 60 ± 16 years. The baseline characteristics are summarized in Table 1.

Table 1

Clinical feature and tumor characteristics of participants.

Anthropometric	N (%)
Men	11 (33)
Women	23 (67.6)
Age	60 (27–93)*
Background	
Smoking	7 (20.6)
Alcoholism	4 (11.8)
Mellitus diabetes	4 (11.8)
Clinical features	
Anemia	19 (55.9)
Weight-loss	21 (61.8) [†]
Jaundice	18 (52.9)
Laboratories	
Hemoglobin, g/dL	12.4 \pm 1.8
Hematocrit (%)	37 \pm 5.1
Leukocytes, 10 ⁶ /mL	8.4 \pm 3.5
Platelets	264.6 \pm 99.4
Total bilirubin, mg/dL	6.8 \pm 15.1
Alkaline phosphatase, U/L	316 \pm 287.1
Tumor	n (%)
Solid	30 (69.8)
Cystic	4 (9.3)
Size, mm	36.8 \pm 12.5
Pathology	n (%)
Adenocarcinoma	23 (92.3)
Acinar tissue without alterations	2 (5.8)
Fibrin and polymorphonuclear	1 (2.9)
Ampuloma	1 (2.9)
Ductal carcinoma	2 (5.9)
Solid pseudopapillary tumor	1 (2.9)
Chronic pancreatitis	2 (5.8)
Neuroendocrine tumor	1 (2.9)
Intestinal epithelium	1 (2.9)
Type	
Malign	16 (60.5)
No malign (benign)	8 (18.5)
Status	
Deceased, n (%)	23 (53.5)

* Mean age is given in years and the min-max interval is shown.

[†] More than 5% of body weight loss. Data presented as mean \pm standard deviation.

3.2. Findings of endoscopic ultrasonography

EUS allowed the diagnosis of PC in 30 out of 34 (88.2%) patients, of whom 26 (90%) corresponded to malignant pathology. The histopathological results confirmed that 24 of the 26 (92.3%) were malignant adenocarcinomas, with ampuloma and malignant neuroendocrine tumor representing the remaining 7.7% of cases (Table 1). Of the remaining 4 patients who were not diagnosed with PC by EUS-FNA, 2 were found to have normal acinar tissue, and the other 2 had chronic pancreatitis. The overall average lesion size was 36.8 ± 12.5 mm. In terms of tumor type, we observed that 30 of 34 (88.2%) were solid lesions, while the remaining 4 (11.8%) were cystic lesions. All of patients with malignant neoplasms presented vascular invasion, mainly to the portal vein, which was the affected vessel in 17 cases. The most frequently used needle for the biopsy was 22G. The average number of passes was 2 (range 1–3) per procedure, and there were no reported complications.

3.3. Tissue biopsy *MSX2* and *PDX1* gene expression

In order to make a reliable comparison, we first determined *MSX2* and *PDX1* gene expression in the non-PC group (data not shown). We then use those values as the basal expression (1-fold). The group of patients with a pancreatic lesion was divided into 2 groups depending on the histopathological findings. The first group included patients with malignant pathology (MP, n=26) and the second group were patients with a benign pathology (BP, n=8) (Table 1).

We observed that *MSX2* expression was increased in patients with a pancreatic lesion compared with the non-PC group [1.72 (0.87–3.70) and 1.07 (0.29–1.63), respectively], although this result did not reach statistical significance ($P=.17$; Fig. 1B). Analysis of *PDX1* transcript levels also showed that there was no difference between the PC and non-PC groups [0.52 (0.16–0.96) and 0.79 (0.71–1.49), respectively, $P=.23$; Fig. 1A].

When this comparison was performed with the BP and MP groups, we observed a significant difference in *MSX2* transcript expression [0.66 (0.45–1.54) and 1.98 (1.44–4.61), respectively, $P=.012$; Fig. 1D]. The same comparison for *PDX1* revealed that this transcript was also increased in the MP group compared with

the BP group [0.62 (0.20–1.20) and 0.36 (0.15–0.65), respectively, $P=.23$; Fig. 1C]. In order to determine whether any of these mRNA expression differences remained evident when data from the BP group were removed, we compared data from the non-PC and MP groups. In this analysis, we observed that the *MSX2* transcript was upregulated in the MP group, while *PDX1* gene expression did not differ between the 2 groups of patients (Supplementary Figure 1A and B, <http://links.lww.com/MD/D43>).

3.4. Circulating blood expression of *MSX2* and *PDX1*

The analysis showed that both transcripts were significantly elevated in the PC group compared with the non-PC group [*MSX2*, 2.05 (1.66–4.60) vs 0.83 (0.49–1.60), $P=.006$; *PDX1*, 2.59 (1.28–10.12) vs 1.02 (0.81–1.17), $P=.036$; Fig. 2A, B]. The comparison between MP and BP groups revealed that *PDX1* expression was increased in the MP group [2.74 (1.35–11.08) vs 1.53 (-2.06 to 5.64)], but not significantly ($P=.23$; Fig. 2C). *MSX2* expression was similar in both groups [2.02 (1.58–3.57) vs 2.25 (0.44–5.62), $P=.92$; Fig. 2D]. In contrast to the tissue biopsy findings, both *MSX2* [2.02 (1.58–3.57) vs 0.83 (0.49–1.60),

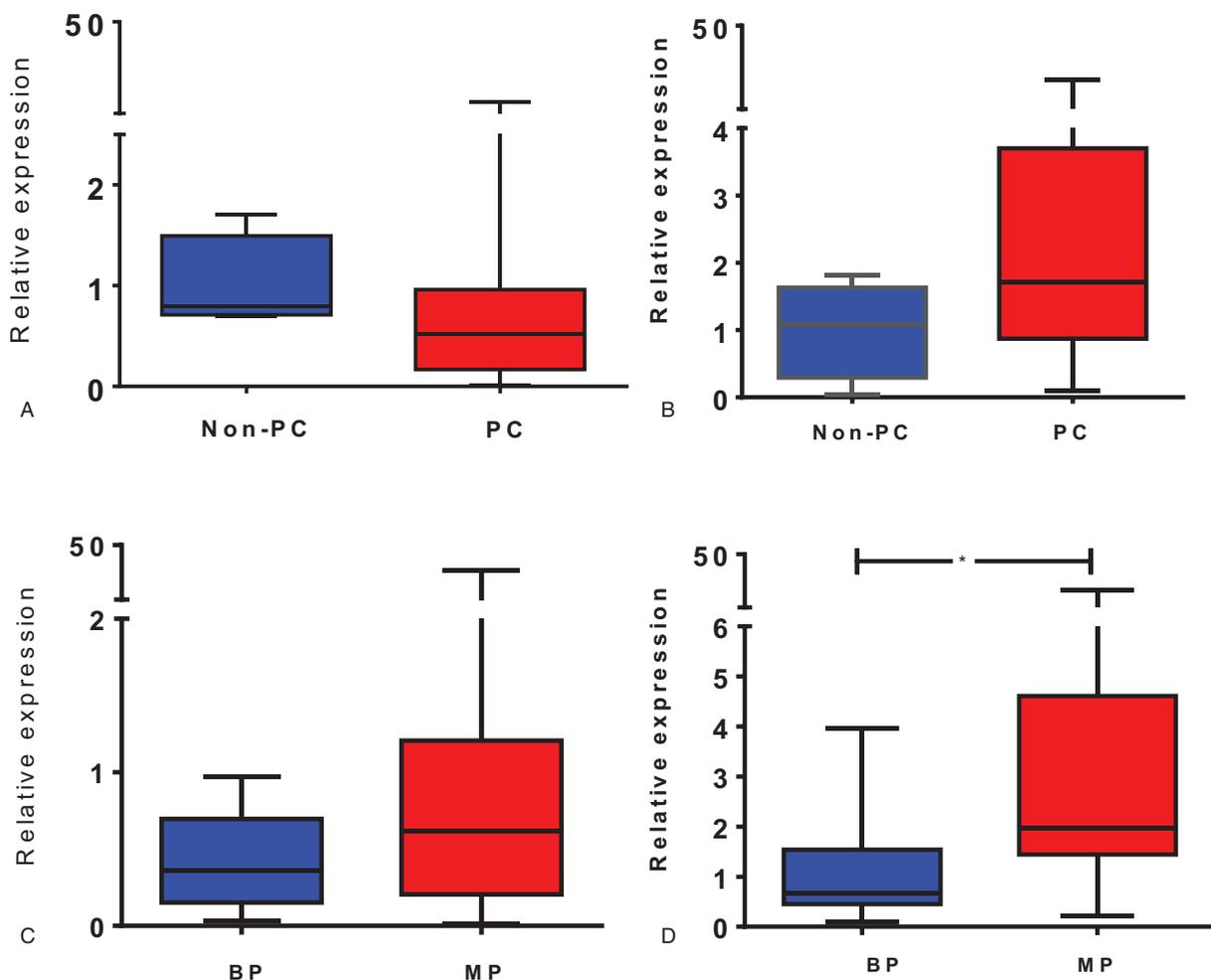


Figure 1. Transcript expression of *PDX1* and *MSX2* in tissue biopsies were evaluated in pancreatic cancer (PC) and non-PC subjects (A, B), and in PC patients diagnosed with benign pathology (BP) and malignant pathology (MP) (C, D). The plots represent the median and percentile (25–75%) for comparisons between the indicated groups for *PDX1* (A, C) and *MSX2* (B, D). Data were evaluated using the nonparametric *t* test and the Mann–Whitney post-hoc test. * $P < .05$ versus BP.

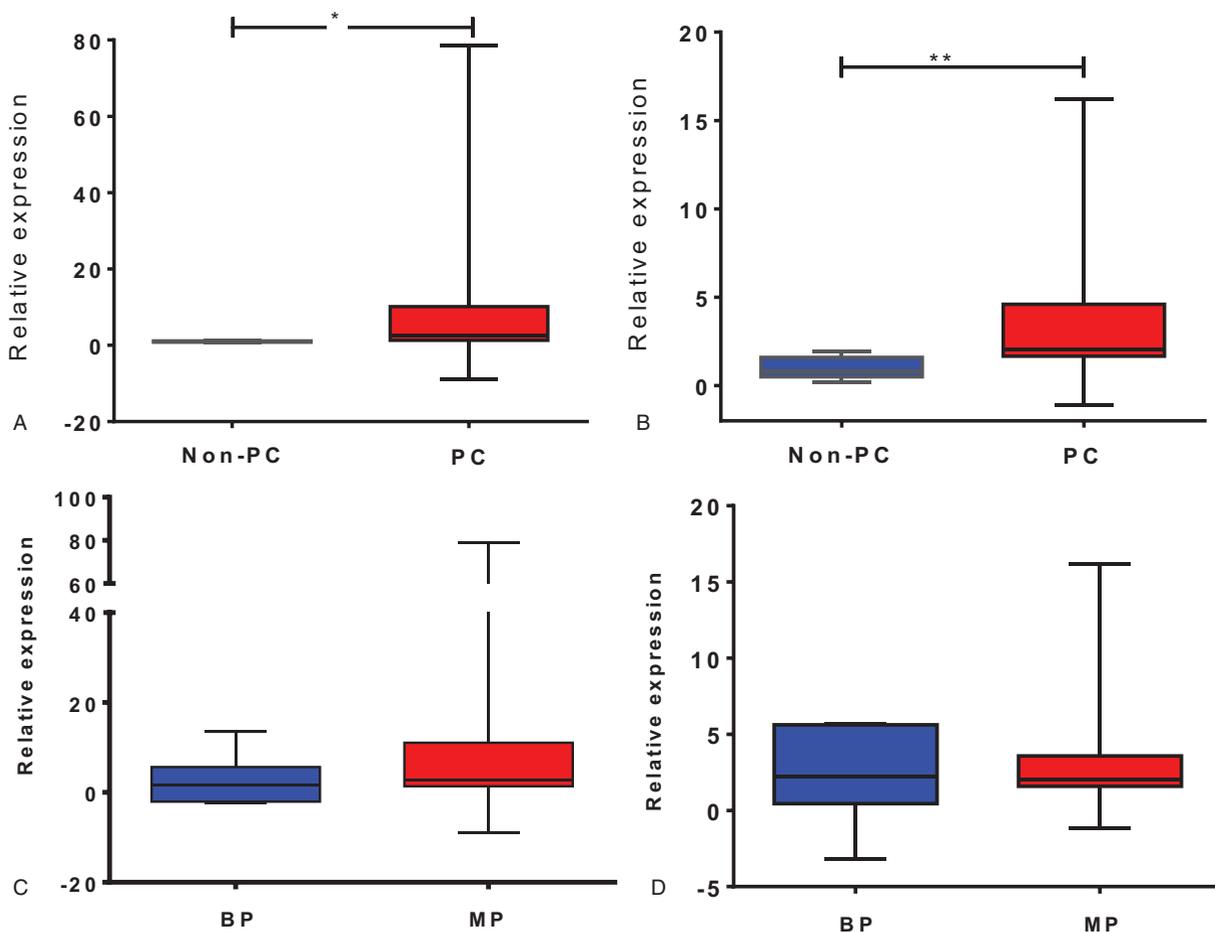


Figure 2. Transcript expression of *PDX1* and *MSX2* in circulating blood samples were evaluated in pancreatic cancer (PC) and non-PC subjects (A, B), and in PC patients diagnosed with benign pathology (BP) and malignant pathology (MP) (C, D). The plots represent the median and percentile (25–75%) for comparisons between the indicated groups for *PDX1* (A, C) and *MSX2* (B, D). Data were evaluated using the nonparametric *t* test and the Mann–Whitney post-hoc test; * $P < .05$, † $P < .01$.

$P = .012$] and *PDX1* [2.74 (1.35–11.08) vs 1.02 (0.81–1.17), $P = .021$] transcripts were significantly higher in the blood samples of the MP than in the non-PC group (Supplementary Figure 1C and D, <http://links.lww.com/MD/D43>).

3.5. Correlation analysis between laboratory parameters and gene expression data

We performed a correlation analysis between laboratory findings and *PDX1* or *MSX2* mRNA expression in both tissue and blood samples. Data are presented in Table 2, respectively. We observed no significant associations between the tested parameters.

3.6. Diagnostic utility of *PDX1* or *MSX2* gene expression for pancreatic cancer

Receiver operating characteristic curves were generated to determine whether the gene expression data could be used to diagnose PC in our group of patients. The AUC for gene expression in blood samples was 0.800 [95% confidence interval (95% CI) 0.660–0.940], $P = .034$] for *PDX1* (Fig. 3A) and 0.827 (95% CI 0.686–0.967, $P = .021$] for *MSX2* (Figure 3B).

The sensitivity and specificity values for using the blood *PDX1* transcript level to discriminate between the non-PC and PC groups were both 80%, with a small cut-off value of a 1.05-fold change. The PPV and NPV for *PDX1* gene expression in blood were 96% and 60%, respectively, with an accuracy of 80%, while the sensitivity and specificity of blood *MSX2* gene expression were 86% and 80%, respectively, with a cut-off value of a 1.36-fold change. The PPV and NPV for *MSX2* gene expression were 96% and 50%, respectively, with an accuracy of 86%. The odds ratio (OR) for PC in patients whose *MSX2* gene expression levels were >1.36 -fold change was 26 (95% CI 2.28–2.95, $P = .006$). Similarly, the OR for PC in patients with *PDX1* gene expression >1.05 -fold change was 16 (95% CI 1.5–1.70, $P = .017$).

4. Discussion

According to our data, *MSX2* and *PDX1* transcript levels in circulating blood samples were higher in patients with malignant pancreatic lesions than in controls (non-PC patients).

The use of biomarkers for the diagnosis of biliopancreatic diseases, including PC, has been previously described.^[22–24] Bartsch et al^[25] showed that the circulating levels of *LCN2* and

Table 2
Correlation between *PDX1* and *MSX2* gene expression in pancreatic tissue and serum, and routine laboratory parameters in patients with pancreatic cancer.

Parameter	Pancreatic tissue		Peripheral blood	
	<i>PDX1</i> gene expression		<i>r</i>	<i>P</i>
	<i>r</i>	<i>P</i>		
Age	-0.048	.786	-0.004	.984
Tumor size	-0.224	.203	-0.082	.667
Hemoglobin	0.126	.478	0.165	.383
Hematocrit	0.115	.522	0.127	.511
Leukocytes	-0.037	.836	-0.066	.734
Platelets	0.363	.035*	0.271	.147
Total bilirubin	-0.099	.585	-0.027	.888
Alkaline phosphatase	-0.077	.669	-0.102	.592

Parameter	<i>MSX2</i> gene expression		<i>r</i>	<i>P</i>
	<i>r</i>	<i>P</i>		
	Age	-0.064	.727	0.104
Tumor size	0.306	.088	-0.051	.789
Hemoglobin	-0.022	.906	0.174	.356
Hematocrit	0.058	.757	0.26	.172
Leukocytes	-0.057	.763	0.028	.885
Platelets	-0.044	.81	0.11	.561
Total bilirubin	-0.092	.623	0.295	.121
Alkaline phosphatase	0.048	.798	-0.077	.686

Association analysis among variables was conducted using Pearson correlation test.
 * *P* < .05 versus non-PC.

TIMP1 transcripts could be useful for the early diagnosis of PC. Here, we report that the abundance of *MSX2* and *PDX1* transcripts are good serum biomarkers for discriminating between non-PC and PC patients. Our results indicate that both genes are significantly upregulated in the peripheral blood samples of patients with PC. We also found that the transcript abundance of *PDX1* and *MSX2* was positively correlated between peripheral blood and tissue gene expression. To our knowledge, no previous reports have suggested the use of *PDX1* as a biomarker in nondiagnosed PC patients. Moreover, as *PDX1*

transcript expression has not been estimated in healthy patients, our data extended the analysis by using blood samples from healthy participants in order to gain an insight into the correlation between tissue expression and that observed in peripheral blood.

Marzioni et al^[26] reported a significant increase in *PDX1* transcript expression in 54 PC biopsies. However, our findings are in contrast to those of Marzioni et al^[26] in terms of tissue *PDX1* expression, as we observed diminished expression of this transcript in our biopsies. Differences between the studies could arise due to the method used to normalize and analyze the raw PCR data. While Marzioni et al^[26] used the expression of the MIA.PaCa-2 pancreatic cell line as the basal expression, we used tissue and circulating blood samples from non-PC subjects as calibrators. Data from a study by Igarashi et al^[27] using cholangiocarcinoma samples reported similar *PDX1* expression to that of PC samples, including the values reported in our study.

Although we did not observe elevated *PDX1* transcript levels in PC biopsies when compared with non-PC samples, these data are consistent with the dual role of *PDX1*, likely undergoing a transition from a tumor-suppressive to oncogenic gene, promoting the carcinogenesis-associated EMT process.^[18] Moreover, our findings are in line with the notion that *PDX1* expression is downregulated in order for epithelial cells to undergo EMT and acquire metastatic properties.^[18] In contrast, Koizumi et al^[28] reported that survival of PC patient was longer when *PDX1* expression was directly downregulated in pancreatic tissue, while Park et al^[29] found no differences in the survival rate between low and high *PDX1* protein expression in situ in a series of paraffin-embedded samples derived from PC patients.

On the contrary, we observed increased levels of *PDX1* transcript in malignant compared with the nonmalignant tissue and blood samples, which strongly suggests that *PDX1* expression might effectively be a more specific indicator for differentiating between these 2 conditions (Figs. 1 and 2 and Supplemental Figure 1, <http://links.lww.com/MD/D43>).

Our findings also showed that *MSX2* was upregulated in MP biopsies when compared with their respective controls (Fig. 1D). The opposite was true when this transcript was evaluated in the

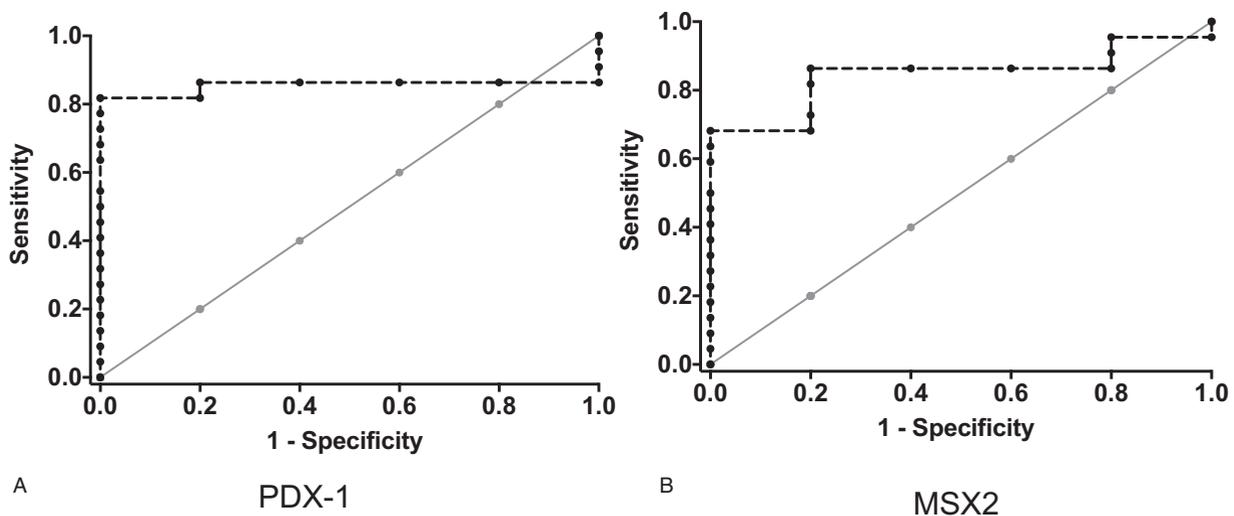


Figure 3. Receiver operator characteristic (ROC) curves for discriminating between nonpancreatic cancer (PC) and PC groups based on *PDX1* (A) or *MSX2* (B) transcript expression in circulating blood (dotted interrupted lines).

peripheral blood, where PC was significantly different when compared with non-PC samples, while BP and MP were not different. Our results are similar to those reported by Satoh et al,^[30] who reported a diagnostic accuracy of 79.3% for the expression of *MSX2* in patients with suspected PDAC. Satoh et al^[31] suggested that *MSX2* expression is related to the differentiation process for carcinoma cells rather than carcinogenesis, because experimentally induced *MSX2* overexpression results in an aggressive PC phenotype. The increased expression of *MSX2* in our biopsies could also be related to the metastatic phenotype and EMT process observed in previous studies.^[32,33]

Despite the differences among studies, they all conclude that *MSX2* expression is increased in PC at the tissue level, and *MSX2* could be a useful potential biomarker in PC diagnosis when associated with histological or clinical data. An important difference to consider, however, is that while brushing pancreatic stenosis or a surgical procedure were the chosen methods for specimen recovery in these studies, all samples used in our study were obtained by EUS-FNA. Indeed, EUS-FNA is the most frequently used tissue sampling method for histological diagnosis in PC. The total mass of tissue obtained by EUS-FNA is larger than brushing, which is an important factor to consider in terms of gene expression quantitation.

Together, these data suggest that *PDX1* and *MSX2* are differentially and consistently expressed in peripheral PC blood samples, meaning that these gene products could potentially be used as biomarkers for PC. In support of this notion, our ROC analysis of *PDX1* and *MSX2* expression demonstrated an ability to distinguish between the healthy and PC groups. Our data indicate that the sensitivity and specificity for *PDX1* were both 80%, and the ROC curve analysis indicates that the sensitivity and specificity for *MSX2* were 86% and 80%, respectively. The PPV and NPV were estimated for both transcripts. We found equal values for PPV (96%), but the NPVs for *PDX1* and *MSX2* were 60% and 50%, respectively. These results indicate that *PDX1* expression seems to be more reliable for discriminating between PC and healthy patients. Furthermore, *MSX2* has also been found to be elevated in PDA.^[30,31] To our knowledge, the present study is the first to report both *MSX2* and *PDX1* expression levels in tissue and peripheral blood, providing an additional tool for a more complete approach for the diagnosis of patients with suspected PC.

In conclusion, the pancreatic gene expression of *MSX2* in tissue samples obtained by EUS-FNA, as well as blood *MSX2* and *PDX1* expression levels, are higher in patients with PC.

Author contributions

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