JOP. J Pancreas (Online) 2008; 9(1):37-45.

CASE REPORT

A Case of Pancreatic Cancer with Formation of a Mass Mimicking Alcoholic or Autoimmune Pancreatitis in a Young Man. Possibility of Diagnosis by Hypermethylation of Pure Pancreatic Juice

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ABSTRACT

Context Mass-forming pancreatitis can be divided into two distinct types: alcoholic and autoimmune. There have been some cases of an ambiguous diagnosis although care was taken to differentiate between alcoholic mass-forming pancreatitis, focal type autoimmune pancreatitis and pancreatic cancer.

Case report We report a case of pancreatic cancer mimicking alcoholic or autoimmune pancreatitis with the formation of a mass in a 32-year-old man with a history of heavy drinking. Although both serum immunoglobulin G and immunoglobulin G4 levels were normal, many serum auto-antibodies, including the antinuclear antibody, were detected. After he stopped drinking, abdominal computed tomography showed a pancreatic head mass 28 mm in diameter with little and weak enhancement in the early and delayed phases, respectively. Endoscopic retrograde cholangiopancreatography showed an obstruction of the main pancreatic duct in the pancreatic head and marked stenosis of the lower common bile duct. Although a percutaneous ultrasound-guided pancreatic biopsy demonstrated no evidence of autoimmune pancreatitis, he was treated with prednisolone to test the efficacy of steroid therapy. However, the pancreatic mass became enlarged after steroid therapy, and he underwent surgery during which the mass was found to be pancreatic cancer. Although the patient was treated with gemcitabine, he died 5 months after surgery. We retrospectively assessed DNA hypermethylation in the patient's pure pancreatic juice obtained on admission. We observed hypermethylation of the cancer-specific gene *tissue factor pathway inhibitor 2* (*TFPI2*).

Conclusion This finding suggests that if the DNA hypermethylation of pure pancreatic juice had been assayed before steroid therapy, it would have supported the diagnosis of pancreatic cancer, and steroid therapy could have been avoided.

INTRODUCTION

Mass-forming pancreatitis can be divided into two distinct types: alcoholic and autoimmune [1]. Autoimmune pancreatitis is a new entity characterized by diffuse enlargement of the pancreas, diffusely irregular narrowing of the main pancreatic duct, increased levels of serum gammaglobulin, immunoglobulin G

(IgG) or immunoglobulin G4 (IgG4), the presence of antibodies, and severe lympho-plasmacytic infiltration and fibrosis of the pancreas [2, 3, 4, 5, 6, 7, 8]. Autoimmune pancreatitis accompanied by the formation of a mass has often been misdiagnosed as pancreatic cancer [9, 10, 11, 12]. There have been some cases of an ambiguous diagnosis although care was taken to differentiate between alcoholic mass-forming pancreatitis, focal type autoimmune pancreatitis and pancreatic cancer [13, 14]. We herein report the case of a young man with pancreatic cancer mimicking alcoholic or autoimmune pancreatitis with the formation of a mass and many auto-antibodies. In addition, we described the usefulness of hypermethylation analyses of pure pancreatic juice in the diagnosis of pancreatic cancer.

CASE REPORT

In January 2002, a 32-year-old man consulted a local hospital because of back and epigastric pain. Although he was a heavy drinker (100 g/day ethanol for 12 years), he had no history of pancreatitis. His serum level of pancreatic enzymes was high, and abdominal computed tomography (CT) showed pancreatic swelling, suggesting acute alcoholic pancreatitis. Although his symptoms disappeared when he stopped drinking and was treated with conservative therapy, his pancreatic head remained swollen. He was referred to our hospital for closer examination and treatment.
Biochemical tests showed elevation of his pancreatic enzymes, including amylase (170 IU/L; reference range: 40-113 IU/L), lipase (109 IU/L; reference range: 11-53 IU/L) and elastase I (760 ng/dL; reference range: 100-400 ng/dL). Autoimmune antibodies were present, including antinuclear antibody (ANA, x320; reference range: 0-20), anti-DNA antibody (7 IU/mL; reference range: 0-6 IU/mL), anti-thyroglobulin antibody (TgAb, 5.5 IU/mL; reference range: 0-0.7 IU/mL), anti-thyroid peroxidase antibody (TPOAb, 3.6 IU/mL; reference range: 0-0.2 IU/mL) and anti-Ro/SS-A antibody (141.3 index; reference range: 0-10 index) as well as rheumatoid factor (26 IU/mL; reference range: 0-20 IU/mL), although the patient did not express the symptoms related to these markers for the screening of autoimmune pancreatitis. Both IgG and IgG4 were normal as were the serum tumor markers carcino-

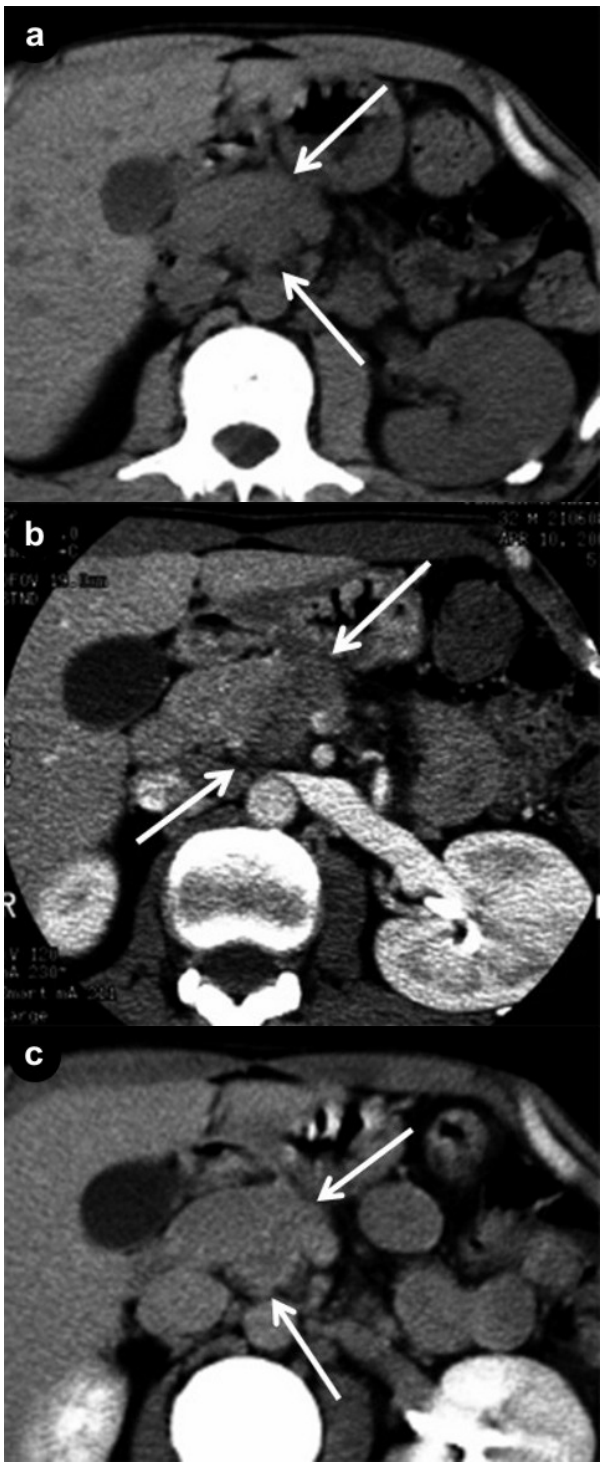

Figure 1. Abdominal computed tomography showing a low density mass 28 mm in diameter in the pancreatic head (**a.**), with little and weak enhancement in the early **(b.**) and delayed phases (**c.**), respectively (arrows).

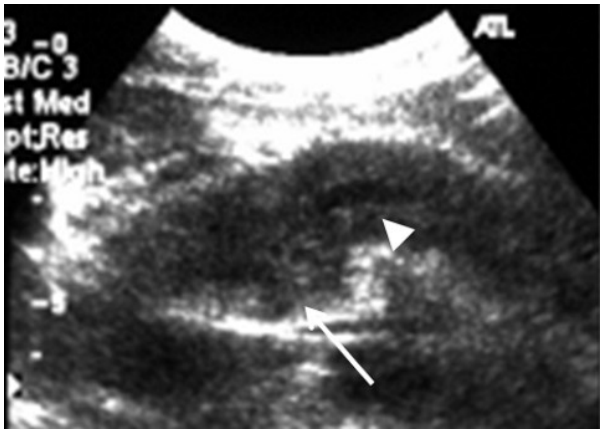

Figure 2. Abdominal ultrasound showing a low echoic mass (arrow) with dilatation of the mild main pancreatic duct (arrow head) in the pancreatic head.

embryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9) and DU-PAN 2.
Abdominal CT showed a low density mass 28 mm in diameter in the pancreatic head with little and weak enhancement in the early and delayed phases, respectively (Figure 1). Abdominal ultrasound (US) revealed a low echoic mass with mild main pancreatic duct dilatation (Figure 2), and endoscopic ultrasonography (EUS) showed a low echoic mass with penetrating a duct sign 30 mm in diameter (Figure 3). Endoscopic retrograde cholangiopancreatography (ERCP) revealed obstruction of the main pancreatic duct in the pancreatic head and marked stenosis of the lower common bile duct (Figure 4), and magnetic resonance cholangiopancreatography (MRCP) showed a mildly dilated main pancreatic duct upstream from the stricture (Figure 5a). Malignant cells were not detected, and K-*ras* and *p53* mutations were not found in pure pancreatic juice collected by endoscopic cannulation [15, 16, 17, 18]. A percutaneous US-guided pancreatic biopsy was performed to confirm the histological

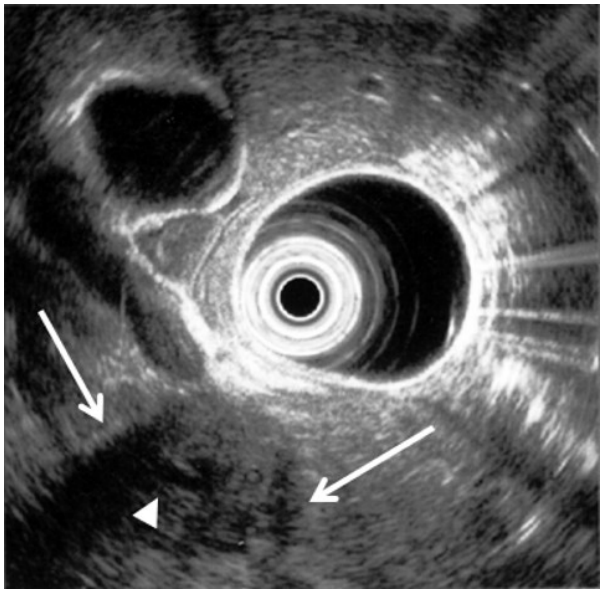

Figure 3. Endoscopic ultrasonography showing a low echoic mass (arrows) with penetrating duct sign (arrow head) 30 mm in diameter in the pancreatic head.

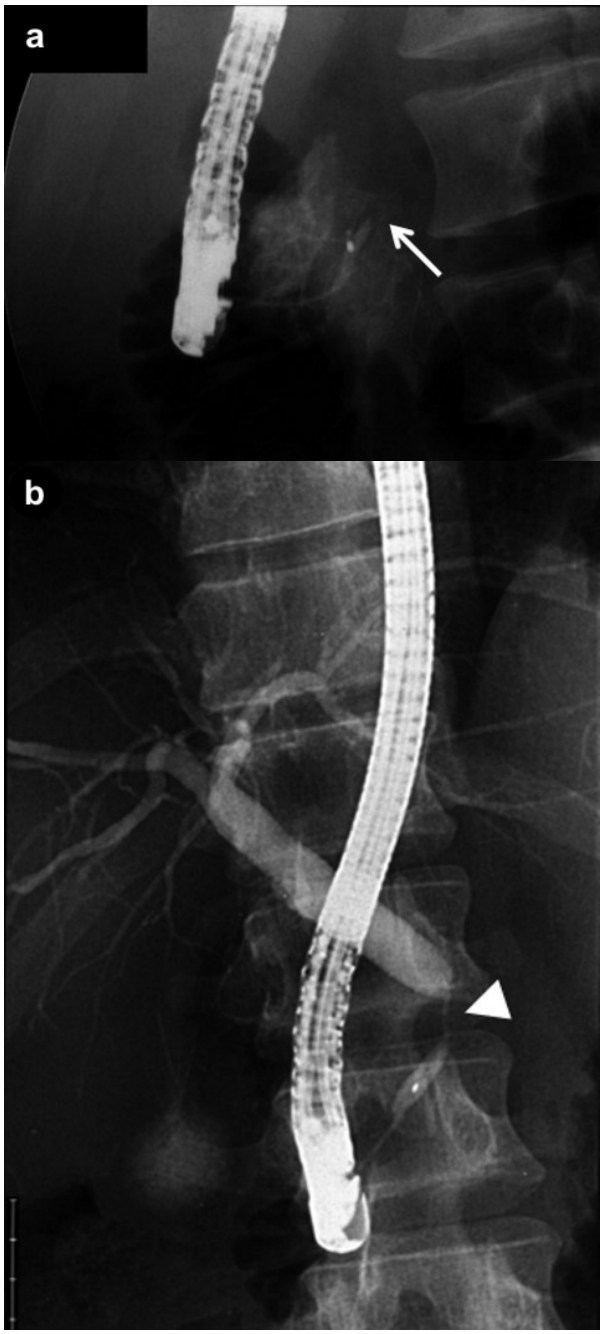

Figure 4. Endoscopic retrograde cholangiopancreatography showing obstruction of the main pancreatic duct in the pancreatic head (**a.** arrow) and marked stenosis of the lower common bile duct (**b.** arrowhead).

findings. Although both mild fibrosis and neutrophil infiltration were observed, we observed no evidence of dense fibrous tissue, lymphocyte or plasma cell infiltration, or malignant cells.

Despite having stopped drinking, the pancreatic head mass did not disappear. In addition to tumor markers within normal limits, negative cytology and mutations in pure pancreatic juice, the concentrations of many auto-antibodies including the ANA had increased, suggesting autoimmune pancreatitis rather than alcoholic mass-forming pancreatitis or pancreatic cancer. He was treated with 40 mg/day of prednisolone, but the levels of the pancreatic enzymes and the size of the pancreatic head mass did not decrease. Six weeks later, the pancreatic head mass became enlarged to 35 mm in diameter,

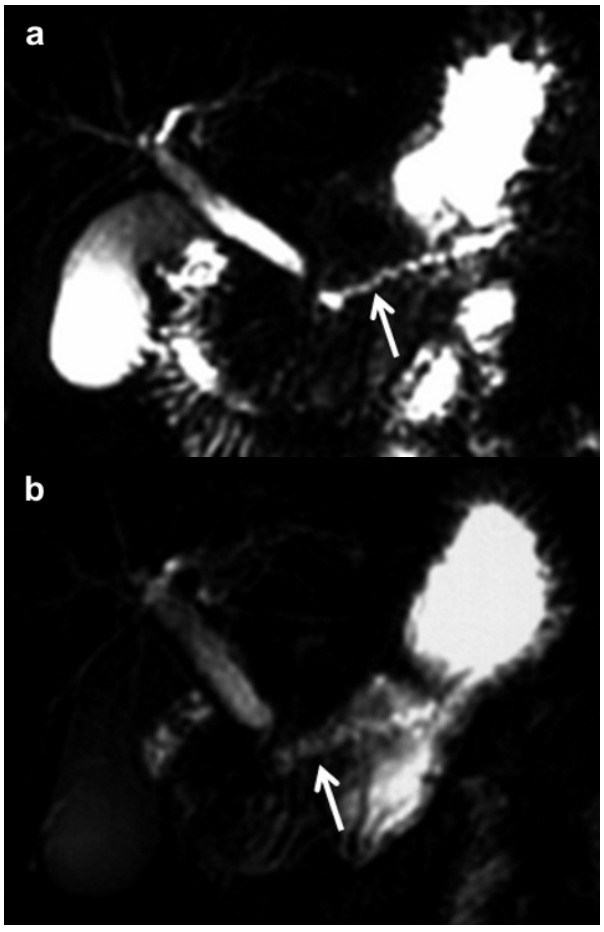

Figure 5. Magnetic resonance cholangiopancreatography showing a mildly dilated main pancreatic duct upstream from the stricture before steroid therapy (**a.** arrow) and dilatation of the main pancreatic duct which had worsened after steroid therapy (**b.** arrow).

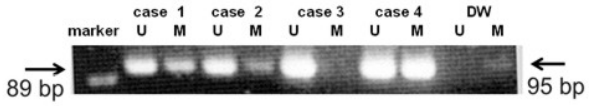

Figure 6. Methylation-specific PCR analysis of *TFPI2* in pure pancreatic juice from patients with pancreatic cancer. The PCR products in the lanes U and M indicate the presence of unmethylated (89 bp) and methylated (95 bp) amplicons, respectively. Hypermethylation of *TFPI2* was detected in cases 1 (this case), 2, and 4.
DW: distilled water

and the main pancreatic duct dilatation deteriorated as seen by MRCP (Figure 5b). His total bilirubin became markedly elevated to 18.2 mg/dL (reference range: 0.3-1.2 mg/dL), and percutaneous transhepatic cholangiodrainage was performed. Subsequently, the pancreatic head mass was resected by pancreaticoduodenectomy. Pathological examination of the mass showed that it was a moderately differentiated tubular adenocarcinoma 40x30 mm in diameter. The resection was not curative due to retroperitoneal invasion. The patient was treated with gemcitabine 1,000 mg/m^2/week for 3 weeks, followed by a 1-week rest. Nevertheless, he died 5 months after surgery.

We retrospectively assessed DNA hypermethylation in this patient's pure pancreatic juice using the methylation-specific PCR method [19]. The 5 markers selected were *preproenkephalin (ppENK), secreted frizzled-related protein 1* (*SFRP1*, also known as *secreted apoptosis related protein 2, SARP2), neuronal pentraxin II (NPTX2), claudin 5 (transmembrane protein deleted in velocardiofacial syndrome, CLDN5)* and *tissue factor pathway inhibitor 2 (TFPI2)*, for all of which hypermethylation has been documented in most pancreatic cancers, but not in normal pancreatic tissue [20, 21, 22, 23, 24, 25, 26]. We observed hypermethylation of *SFRP1, NPTX2* and *TFPI2*, but not of *ppENK, CLDN5*, in this patient's pure pancreatic juice (Figure 6); these findings were suggestive of pancreatic cancer.

DISCUSSION

Mass-forming pancreatitis can be divided into two distinct types, each with its own distinct

mechanism [1]. In alcoholic pancreatitis, mass formation is thought to be due to a reparative process for centroductal acute inflammation with a background of chronic pancreatitis whereas, in autoimmune pancreatitis, mass formation is thought to be due to lymphoplasmacytic infiltration with lymphoid and fibrous proliferation in normal pancreatic tissue [1]. The significant factors that differentiate alcoholic mass-forming pancreatitis from pancreatic cancer are age of onset, alcoholism, history of pancreatitis, lower serum levels of CA 19-9 and hypervascularity [13]. Autoimmune pancreatitis is character-ized by a diffuse enlargement of the pancreas, diffusely irregular narrowing of the main pancreatic duct, increased levels of serum gammaglobulin, IgG or IgG4, the presence of auto-antibodies, and severe lympho-plasmacytic infiltration and fibrosis of the pancreas [2, 3, 4, 5, 6, 7, 8]. Most patients with autoimmune pancreatitis display diffuse enlargement of the pancreas whereas others show the formation of a mass. Some cases of autoimmune pancreatitis with mass formation have been misdiagnosed as being pancreatic cancer [9, 10, 11, 12]. The significant factors differentiating focal type autoimmune pancreatitis from pancreatic cancer are lower serum levels of CA 19-9, homogeneous delayed enhancement on dynamic CT, and ERCP showing a longer stenosed main pancreatic duct and a thinner main pancreatic duct upstream from the stricture [14]. In addition, contrast-enhanced US and magnetic resonance imaging have been shown to be useful in the differential diagnosis of pancreatic tumors [27, 28, 29].

This patient was a young man and a heavy drinker, suggesting alcoholic mass-forming pancreatitis while the increased levels of many auto-antibodies, including ANA, suggested autoimmune pancreatitis. Moreover, his tumor markers were normal, and his pure pancreatic juice was negative for cytology and for K-*ras* and *p53* mutations [15, 16, 17, 18]. Taken together, these findings suggested a diagnosis of alcoholic mass-forming pancreatitis or autoimmune pancreatitis.

Patients with autoimmune pancreatitis have significantly higher levels of serum IgG4 than do patients with pancreatic cancer [30], and most autoimmune pancreatitis patients show serum IgG4 elevation [30, 31, 32, 33], making IgG4 a useful marker for distinguishing autoimmune pancreatitis from other diseases of the pancreas, such as pancreatic cancer. In our patient, however, both serum IgG and IgG4 levels were normal. Although CT with enhancement in the delayed phase showed a pancreatic head mass [13, 14], the degree of enhancement was low. In addition, ERCP did not show diffuse narrowing of the main pancreatic duct but its obstruction while obstruction of the main pancreatic duct has been seen in patients with autoimmune pancreatitis [9, 34]. Therefore, these findings were suggestive of pancreatic cancer rather than of alcoholic mass-forming pancreatitis or autoimmune pancreatitis.

A US-guided percutaneous pancreatic biopsy is useful in diagnosing a pancreatic mass, whether malignant or not [35, 36]. Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) has been shown to be useful in diagnosing pancreatic diseases, including pancreatic cancer and autoimmune pancreatitis [37, 38, 39]. Although we performed a US-guided percutaneous pancreatic biopsy, the specimen showed no evidence of alcoholic mass-forming pancreatitis, autoimmune pancreatitis or pancreatic cancer, which may have been due to sampling error.

Epigenetic alterations have been found to induce the transcriptional silencing of tumor suppressor genes, leading to carcinogenesis [40, 41]. In pancreatic cancer tissue, hypermethylation has been observed in several tumor suppressor genes, including *p16* [42, 43, 44], *mutL homolog 1, colon cancer, nonpolyposis type 2* (*E. coli*) (*MLH1*) [45], and *Ras association (RalGDS/AF-6) domain family 1 (RASSF1)* [46]. In addition, hypermethylation of *ppENK*, *SFRP1*, *NPTX2*, *CLDN5* and *TFPI2* has been documented in most pancreatic cancer tissues, but not in normal pancreatic tissues [20, 21, 22, 23, 24, 25, 26]. Using the methylation-specific PCR

method, we therefore retrospectively assessed DNA hypermethylation of these 5 markers in pure pancreatic juice obtained on admission [19]. The methylation-specific PCR method is sufficiently sensitive to detect hypermethylation of DNA from a single cell out of 1,000 normal cells [19]. We found that the *SFRP1*, *NPTX2* and *TFPI2* genes were hypermethylated in pure pancreatic juice of our patient, results which suggested pancreatic cancer. In particular, hypermethylation of *TFPI2* in pure pancreatic juice has been found to be cancer-specific and, therefore, a useful marker in the diagnosis of pancreatic cancer [23, 26]. On the other hand, the CEA levels in pure pancreatic juice were significantly higher in patients with pancreatic cancer than in those with pancreatitis [47, 48, 49]. Furthermore, measurement of sialylated stage-specific embryonic antigen-1 (SSEA-1) and NCC-ST-439 in pure pancreatic juice were useful for the diagnosis of pancreatic cancer [50, 51]. However, the CEA, SSEA-1 and NCC-ST-439 levels in pure pancreatic juice were not measured in this case. The method of analyses of hypermethylation is very different from that of tumor markers in pure pancreatic juice. Therefore, analyses of hypermethylation or tumor markers in addition to quantitative K-*ras* or *p53* mutation in pure pancreatic juice would be promising for the diagnosis of pancreatic cancer.

The algorithm of the diagnosis of pancreatic cancer proposed by the Japan Pancreas Society is as follows: clinical symptoms, pancreatic enzymes, tumor markers, and US in the first step; CT and magnetic resonance imaging (or MRCP) in the second; EUS, ERCP and positron emission tomography (PET) in the third; if a diagnosis is not determined, cytology or biopsy (by ERCP or US/EUS guided) in the fourth. If pancreatic cancer cannot be diagnosed by cytology or biopsy, tumor markers, genetic and epigenetic alterations in pure pancreatic juice are recommended to differentiate pancreatic diseases as mentioned above. In this patient, it was difficult to differentiate between pancreatic cancer and alcoholic mass-forming pancreatitis or autoimmune pancreatitis. Retrospectively, the significant findings were: 1) Although the patient's serum IgG and IgG4 concentrations and his CT and ERCP results were not inconsistent with alcoholic mass-forming pancreatitis or autoimmune pancreatitis, they were more suggestive of pancreatic cancer. These findings indicate that, if a histological diagnosis cannot be determined by US-guided percutaneous pancreatic biopsy or EUS-FNA, an open biopsy should be performed [52]. 2) Although autoimmune pancreatitis is readily responsive to steroid therapy [8], we found that steroid therapy had no effect on this patient. These findings indicate that, if a response to steroid therapy is not observed after 2 weeks, the diagnosis should be re-considered. 3) Hypermethylation analyses in pure pancreatic juice supported the diagnosis. Therefore, if this assay had been performed before the initiation of steroid therapy, the latter could have been avoided.

Although we observed many serum autoantibodies in this patient, he did not have the symptoms related to these markers. The reason these markers were elevated remains unclear. These findings, however, led us to a diagnosis of autoimmune pancreatitis and the initiation of prednisolone treatment. Treatment of mice with glucocorticoids has been shown to almost completely inhibit the immune response to tumor cells [53]. Steroid therapy may therefore cause immunosuppression in the patients with malignancy, leading to tumor enlargement. Therefore, empirical steroid therapy should not be administered as a tool for diagnosing autoimmune pancreatitis.

In conclusion, we have reported the case of pancreatic cancer in a young man, in whom it was difficult to discriminate between pancreatic cancer and alcoholic mass-forming pancreatitis or autoimmune pancreatitis.

Received October 13th, 2007 - Accepted October 18th, 2007

Keywords Autoimmune Diseases; Methylation; Pancreatic Juice; Pancreatic Neoplasms; Pancreatitis, Alcoholic

Conflict of interest The authors have no potential conflicts of interest

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Document URL: http://www.joplink.net/prev/200801/03.html

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