Importance of Quantitative Analysis in the Generation of Insulin-Expressing Cells From Human Embryonic Stem Cells

To the Editor:

Human embryonic stem (hES) cells have the capacity for proliferation and differentiation into virtually any cell type in the body, making them excellent candidates for therapeutic application in regenerative medicine, such as cell replacement therapy for type 1 diabetes mellitus. Embryonic stem cells cultured as monolayers or embryoid bodies differentiate into cells from all 3 germ layers, including endoderm, which eventually gives rise to the cells of the pancreas. Early reports demonstrated that hES cells could differentiate spontaneously into an insulin-expressing phenotype, albeit at a low frequency with only 1% to 3% of the cells expressing insulin.[1] Later studies reported differentiation into insulin-expressing cells by the overexpression of key developmental or pancreatic genes or by lineage selection using pancreas-specific promoters.[2] However, to date, the most successful and clinically applicable differentiation strategies are those that recapitulate in vitro the sequence of developmental cues that are known to be important during pancreas development in vivo.[3–5] The critical stage in the efficient generation of insulin-expressing cells is the transition through definitive endoderm into pancreatic endocrine progenitor cells. There have been some attempts to engineer fully differentiated insulin-secreting cells from these lineage-specific progenitors by a fully in vitro protocol,[3] but to date, the most successful strategies have involved the transplantation of hES cell–derived pancreatic progenitors into mice where the in vivo environment facilitates a maturation of the cells into glucose-responsive insulin-producing cells.[5] As a result, it is likely that for a clinical application in cell replacement therapy, there will be a requirement for a large number of pancreatic endocrine progenitor cells primed for in vivo maturation and/or improved in vitro differentiation protocols.

There are a number of critical requirements when evaluating differentiation protocols aimed at the generation of definitive endoderm and endocrine progenitors. Because there is a significant overlap in the expression patterns of many of the markers of differentiated cells, the formation of specific cell phenotypes must be confirmed by the expression of more than one marker. In addition, these measurements should be quantitative at the level of RNA and/or protein and should be compared with a control population of hES cells, which are not subjected to the differentiation protocol. The importance of these requirements can be illustrated in our experiments in a well-characterized hES cell line (Shef-3), available from the Medical Research Council UK stem cell bank (http://www.ukstemcellbank.org.uk/), which we have exposed to an established differentiation protocol based on in vivo pancreas development.[4] This 17-day differentiation protocol generates, in a sequential manner, definitive endoderm (day 3), primitive gut tube (day 7), posterior foregut (day 11), pancreatic endocrine precursors (day 14), and finally, hormone-expressing cells (day 17). In a parallel (control) population of cells, allowed to spontaneously differentiate as hES cell colonies without passage, the resultant cell phenotype was assessed at equivalent time points. Figure 1A demonstrates gel electrophoresis analysis of reverse transcriptase polymerase chain reaction (RT-PCR) identifying the expression of recognized markers of definitive endoderm in these 2 populations of differentiating hES cells. As expected, cells exposed to the differentiation protocol (directed) expressed the genes for these markers (sex determining region Y-box 17 [SOX17], Cerberus [CER], Forkhead box protein A2 [FOXA2]) at the appropriate time point (3 days) representing the generation of definitive endoderm. However, spontaneously differentiated cells (spontaneous) also expressed the genes for these markers at all stages of the 17-day time course. These data may reflect, at least in part, the high sensitivity of RT-PCR and the nonquantitative nature of electrophoresis as end point analyses of the products of PCR. However, immunofluorescence staining with an anti-SOX17 antibody was consistent with the expression of SOX17 in the directed population after 3 days and also demonstrated SOX17 expression in small localized regions of cells in the spontaneous population (Fig. 1B). From these data alone, it is difficult to evaluate the efficiency of the differentiation protocol for the generation of definitive endoderm from hES cells because some cells seem to spontaneously adopt this phenotype in the absence of any external differentiation stimuli. To further investigate the formation of definitive endoderm and subsequent cell types, we performed a quantitative assessment of the temporal expression of a range of markers of cellular phenotype in both populations of cells during their differentiation. We first measured the temporal expression of the pluripotency gene *OCT4* using quantitative RT-PCR (qRT-PCR) in both spontaneous and directed cells. Figure 1C demonstrates mean OCT4 messenger RNA (mRNA) expression in cells exposed to either spontaneous or directed differentiation (4 separate cell populations in each treatment). These data demonstrate reproducible decreases in the expression of OCT4 mRNA in cells undergoing directed differentiation (Fig. 1C, right panel), consistent with previous observations suggesting the down-regulation of this gene in hES cells undergoing endodermal specialization.[6] In cells undergoing spontaneous differentiation, we observed changes in morphology from day 3 onward, with a loss of most undifferentiated hES cells by day 17 (data not shown), but this was not accompanied by a progressive reduction in OCT4 expression (Fig. 1C, left panel), suggesting the absence of efficient differentiation. We next extended these quantitative measurements to investigate the temporal expression patterns of multiple markers for each of the cellular phenotypes in the later stages of pancreatic development. Thus, Figure 1D demonstrates qRT-PCR data showing the time course of mRNA expression for some examples of these markers including (i) mean SOX17 expression (a marker of definitive endoderm; expected at day 3), (ii) mean Hepatocyte nuclear factor 1 beta (HNF1β) expression (a marker of primitive gut tube; expected at day 7), and (iii) mean Pancreatic and duodenal homeobox 1 (PDX1) expression (posterior foregut; day 11). Cells undergoing directed differentiation exhibited a peak of SOX17 expression at day 3, which subsequently reduced over the time course of differentiation consistent with the formation of definitive endoderm (similar expression patterns were observed with the definitive endoderm markers CER and FOXA2; data not shown). These cells then sequentially exhibited peaks of HNF1β and PDX1 expressions at day 7 and day 11 of the differentiation protocol, respectively, indicating the directed formation of

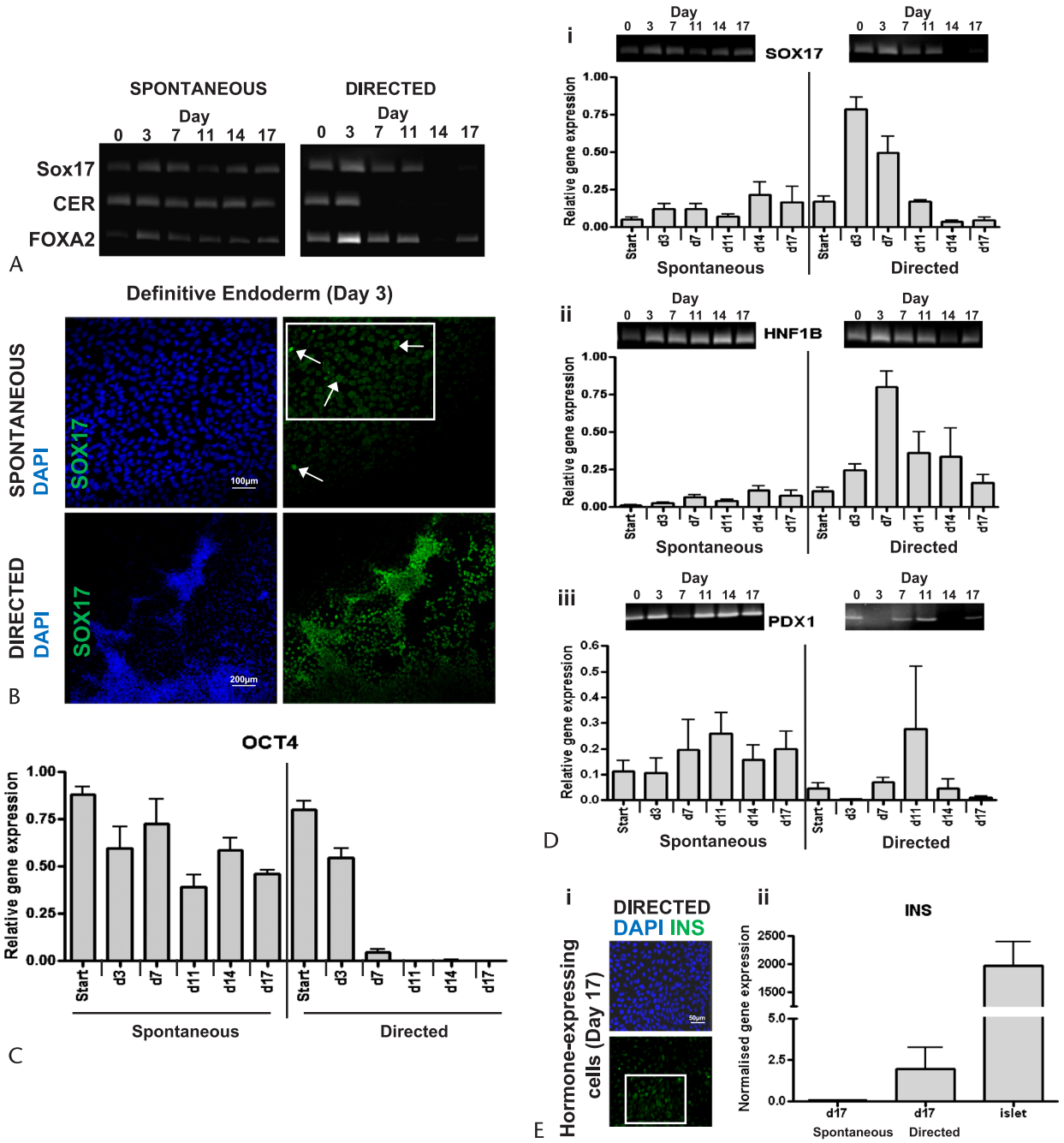

FIGURE 1. Expression of markers of pancreatic development in differentiating hES cells. Shef3 hES cells were either allowed to spontaneously differentiate (spontaneous) or were exposed to a 17-day in vitro differentiation protocol based on in vivo pancreatic development (directed). A, Agarose (1% wt/vol) gel electrophoresis of the products of RT-PCR to identify the expression of markers of definitive endoderm (SOX17, CER, and FOXA2) in spontaneous and directed hES cells. B, SOX17 immunoreactivity showing nuclear expression after 3 days of differentiation in large areas of the directed cultures and in much smaller focused areas of the spontaneous cultures (white box and arrows). C and D, qRT-PCR data showing the mean OCT4 (C), SOX17, HNF1β, and PDX1 (D, i–iii) expressions throughout the time course of differentiation, in spontaneous and directed cells, expressed as relative gene expression (relative to the highest observed expression, n = 4). These data demonstrate reproducible decreases in the expression of OCT4 in cells undergoing directed differentiation but no such decreases in cells that were spontaneously differentiating. This was coupled to peaks of the expression of SOX17 (day 3), HNF1β (day 7), and PDX1 (day 11) in the directed cell population. No such reproducible pattern of expression of these genes was observed in the spontaneously differentiating cells. Also shown are agarose (1% wt/vol) gel electrophoresis analyses of the products from a single representative nonquantitative RT-PCR for these genes. E (i), confocal images of insulin immunofluorescence revealed small areas (white box) of localized positive immunoreactivity in the cells in the directed population, with no detectable immunoreactivity in the spontaneous population (data not shown). This correlated with barely detectable levels of insulin mRNA in the spontaneously differentiated cells assessed by qRT-PCR (E, ii), which were up-regulated 46-fold (n = 4) by the directed differentiation protocol. The levels of insulin mRNA in a human islet are included for comparative purposes only.

primitive gut tube and then posterior foregut. Cells permitted to differentiate spontaneously expressed low levels of SOX17 and HNF1β, however, they did not demonstrate the same expression dynamics as those in the directed population most likely because of the lack of formation of definitive endoderm. This was supported by flow cytometric measurements demonstrating that in the directed population of cells, 42% were SOX17 positive, whereas the spontaneous population contained only 5% SOX17-positive cells (data not shown). The spontaneous population of cells expressed PDX1 mRNA at levels similar to the peak of expression in the directed population, which is consistent with its role in the development of other tissues, and this is supported by high levels of SOX7 mRNA expression, a marker of extraembryonic endoderm,[7] at days 11 to 17 in the spontaneously differentiating cells (data not shown). Figure 1D also shows gel electrophoresis analysis of the products of RT-PCR for SOX17, HNF1β, and PDX1 in representative populations of spontaneous and directed cells throughout the experimental time course. These nonquantitative analyses demonstrate the expression of these markers in both populations of cells at the time points expected but provide no information on the quantitative or temporal changes in the patterns of their expression. The critical importance of the formation of definitive endoderm as the first stage in pancreatic development is only revealed from quantitative measurements of marker expression in both a directed population and a spontaneously differentiating control population of cells. This is further evidenced from qRT-PCR data in experiments where cells were allowed to spontaneously differentiate up to day 3 of the protocol and were then exposed to the remaining inductive signals of the directed differentiation protocol. These cells exhibited an identical pattern of expression for SOX17, HNF1β, and PDX1 (data not shown) to that observed in the spontaneous population of cells (Fig. 1D). The expression of insulin was assessed after 17 days of differentiation in both spontaneous and directed populations of differentiated cells as a measure of the success of these protocols in the generation of pancreatic hormone–expressing cells. Insulin mRNA was detectable at very low levels in the spontaneously differentiated cells but was up-regulated up to 70-fold (mean, 46-fold from 4 separate cell populations) by the directed differentiation protocol (Fig. 1E, ii). This was consistent with small areas of positive insulin immunofluorescence assessed by confocal microscopy (Fig. 1E, i) in the directed cell population, which was accompanied by the presence of positive C-peptide immunofluorescence (data not shown). Insulin and C-peptide immunoreactivity were undetectable in the spontaneously differentiated cell populations.

Many previous studies in this area have relied on measurements that are nonquantitative or semiquantitative at best, such as RT-PCR and immunocytochemistry. We demonstrate here the utility of quantitative parallel analyses in populations of cells exposed to a directed differentiation protocol and in a control population of cells allowed to spontaneously differentiate, revealing the consequences of the failure to form definitive endoderm on the subsequent formation of cellular phenotypes important for pancreatic development. The successful generation of clinically acceptable endocrine progenitors from hES cells will require evaluation of both the starting material and of a range of differentiation protocols, and these fully quantitative assessments will provide essential information as to the most appropriate starting populations of cells and on the functional phenotype of the final graft material. This is particularly important with the recent development of human induced pluripotent stem (iPS) cells,[8,9] which may prove to be a more ethically acceptable source of patient-specific cell types for transplantation therapy. Although reports to date suggest that iPS cells share many characteristics with hES cells, they may exhibit different potentials for differentiation into particular cell types, and as a result, differentiation protocols and strategies may have to be adapted or optimized. These cells can be generated by reprogramming any of a number of distinct adult somatic cell types, using a range of different reprogramming strategies, which means the resulting iPS cells are likely to show distinct genetic and epigenetic variation. In the absence of a full understanding of these differences between iPS cell lines and of how these may affect their differentiation potential, it will be especially important to quantitatively compare a spontaneously differentiating population of iPS cells with the population of cells exposed to the differentiation protocol. These comparisons will provide the necessary information to further inform the development of differentiation protocols.

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REFERENCES

1. Assady S, Maor G, Amit M, et al. Insulin production by human embryonic stem cells. *Diabetes*. 2001;50(8):1691–1697.
2. Raikwar SP, Zavazava NJ. Insulin producing cells derived from embryonic stem cells: are we there yet? *J Cell Physiol*. 2009; 218(2):256–263.
3. Jiang W, Deng H, Li D, et al. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. *Cell Res*. 2007;17:333–344.
4. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol*. 2006;24(11): 1392–1401.
5. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose responsive insulin-secreting cells in vivo. *Nat Biotechnol*. 2008;26(4):443–452.
6. Guo Y, Costa R, Ramsey H, et al. The embryonic stem cell transcription factors Oct-4 and FoxD3 interact to regulate endodermal-specific promoter expression. *Proc Natl Acad Sci U S A*. 2002;99(6): 3663–3667.
7. Seguin CA, Draper JS, Nagy A, et al. Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. *Cell Stem Cell*. 2008; 3:182–195.
8. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
9. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920.

Serum and Urine Trypsinogen Activation Peptide in Assessing Post–Endoscopic Retrograde Cholangiopancreatography Pancreatitis

To the Editor:

Trypsinogen activation peptide (TAP) is a small peptide of 7 to 10 amino acids capable of activating trypsinogen into trypsin,[1] and it reflects the amount of activated trypsinogen. Serum TAP concentrations determined before and 6 hours after the endoscopic retrograde cholangiopancreatography (ERCP) did not differ probably because the half-life of TAP is approximately 8 minutes,[2] and this time interval is too long to detect its alteration. Therefore, the primary end point of the study was to evaluate the hourly TAP concentration elevation after ERCP and to establish its role in the early diagnosis of postprocedural acute pancreatitis. The secondary end point was to explore whether the administration of gabexate mesylate (GM) may reduce the levels of TAP in both serum and urine.

METHODS

All consecutive patients who underwent interventional ERCP from June 2005 to December 2007 were studied. Patients who were younger than 18 years, were pregnant, or had had a recent attack of acute pancreatitis and with a known allergy to GM were excluded. Blood sample was taken before the endoscopic examination and 1, 2, 3, 4, and 6 hours after ERCP; 2 mL of urine was also collected before ERCP and 2, 4, and 6 hours after the completion of the ERCP. The patients voided completely each time, and each subsequent urine was newly excreted. Serum and urine TAPs were assayed using a technique previously described.[3] Serum trypsinogen concentrations were determined (reference value, 10–57 ng/mL) using commercially available radioimmunoassay kits (Trypsik; Sorin Biomedica, Saluggia, Italy). The use of GM was decided by the endoscopist, and it was administered at a dose of 500 mg beginning 30 minutes before endoscopy and continuing 6 hours after ERCP.[4]

Post-ERCP acute pancreatitis was defined as the appearance of typical abdominal pain associated with an increase in serum amylase activity greater than 3 times the upper reference limit,[5] and its severity was assessed by the Atlanta criteria.[6]

The following data were also recorded: the drugs used for patient sedation, the duration of the ERCP, difficult cannulation, Wirsung injection, the possibility of mechanic lithotripsy, biliary and/or pancreatic sphincterotomy, the insertion of endoscopic biliary or pancreatic drainage, the presence of postprocedural pain at 1, 2, 3, 4, and 6 hours, and the use of analgesics.

The study protocol was approved by the ethical committees of both San Raffaele Hospital of Milan and Campus Biomedico of Rome, and all patients signed informed consent.

Elevation of serum TAP concentration was estimated in 30% of patients who developed postprocedural acute pancreatitis.[3] Taking into account an estimated frequency of acute pancreatitis in high-risk patients of approximately 15%,[5] we chose a ratio of the number of controls to the number of patients studied equal to 5. The size of the sample (beta error, 0.80; alpha error, 0.05) was equal to 12 patients with acute pancreatitis and 60 controls; therefore, 72 patients, those who develop and those who do not develop acute pancreatitis, have been selected as the minimum allowable number of subjects. This number was also acceptable for the secondary end point.

Data are reported as mean, SD, range, and frequencies. The Fisher exact, the Pearson χ^2, the McNemar, the Mann-Whitney *U*, the Kruskal-Wallis, and the Wilcoxon matched-pairs tests were used. Two-tailed $P < 0.05$ were considered significant.

RESULTS

We enrolled 75 patients (38 men and 37 women; mean age, 57.6 ± 18.5 years; range, 18–92 years). The reasons for which they underwent operative ERCP were cholangitis or jaundice in 63 patients (84.0%), the need to insert a biliary stent in 4 (5.3%), persistent pain or jaundice in 6 patients (8.0%) with recurrent pancreatitis, and the need to insert a pancreatic stent in 2 (2.7%). The final diagnoses were as follows: lithiasis of the common bile duct in 52 patients (69.3%), benign stenosis of the common bile duct in 5 (6.7%), chronic pancreatitis in 7 (9.3%), neoplasms of the common bile duct in 5 (6.7%), and pancreatic neoplasms in 6 (8.0%). Fourteen patients (18.7%) received propofol; 33 (44.0%), midazolam; and the remaining 28 (37.3%), midazolam plus propofol. The mean endoscopic procedure lasted for 44.5 ± 15.1 minutes (range, 15–95 minutes). There was difficult cannulation in 29 patients (38.7%); Wirsung injection in 40 (53.3%); mechanic lithotripsy in 2 (2.7%); biliary sphincterotomy in 44 (58.7%); major papilla pancreatic sphincterotomy in 6 (8.0%); minor papilla sphincterotomy in 2 (2.7%); and insertion of endoscopic biliary drainage in 23 (30.7%), of pancreatic drainage in 6 (8.0%), of a biliary stent in 17 (22.7%), and of a pancreatic stent in 3 (4.0%). Sixty-one patients (81.3%) received intravenous GM as a prophylactic treatment to prevent postprocedural ERCP. Postprocedural abdominal pain was recorded at 1 hour in 35 patients (46.7%), 2 hours in 34 (45.3%), 3 hours in 14 (18.7%), 4 hours in 8 (10.7%), and 6 hours in another 8 (10.7%); 6 patients (8.0%) had pain for more than 6 hours. Analgesics were required by 20 patients (26.7%), and all of them received nonsteroidal anti-inflammatory drugs.

Postprocedural acute pancreatitis developed in 13 patients (17.3%), and it was in all of them clinically mild. The frequency of patients with acute pancreatitis who had pain after 2 hours (11/13; 84.6%) was significantly higher when compared with patients without acute pancreatitis (23/62; 37.1%; $P = 0.002$). Postprocedural acute pancreatitis developed in 19.7% (12/61) of patients who received GM and 7.1% (1/14) of those who did not ($P = 0.440$).

Ten patients (13.3%) refused to continue the study. The characteristics of this group were similar to those of the 65 patients who continued except for the sedation drugs administered and the time of the pain appearance. None of the 33 patients who received midazolan alone refused to participate versus 2 (14.3%) of the 14 patients who received propofol and 8 (28.6%) of the 28 patients who received propofol associated with midazolam ($P = 0.005$). Fewer of them (2/10, 20.0%) had no pain 1 hour after the ERCP as compared with 38 (58.5%) of the 65 patients who continued to participate ($P = 0.038$).

In the 65 patients who completed the study, serum and urine TAP concentrations and that of serum trypsinogen are shown in Table 1. At basal examination (before ERCP), serum TAP was detectable in all patients. One- and 2-hour post-ERCP serum TAP concentrations remained elevated ($P = 0.097$ and $P = 0.034$, respectively, vs pre-ERCP), whereas these concentrations significantly declined at 4 hours ($P = 0.006$ vs pre-ERCP). Urine TAP showed the same behavior as serum TAP; detectable urine concentrations were present in 6 (9.2%) of the 65 patients before ERCP and after 2 hours, whereas at 4 and 6 hours, all patients had no detectable urinary TAP concentrations

TABLE 1. Serum and Urinary TAP Concentrations and Serum Trypsinogen Concentration in the 65 Patients Who Completed the Study

	Serum TAP, ng/mL		Urine TAP, ng/mL		Serum Trypsinogen, ng/mL	
	Mean ± SD	***P***	**Mean ± SD**	***P***	**Mean ± SD**	***P***
Pre-ERCP	7.18 ± 9.02		0.41 ± 1.44		54.6 ± 64.1	
1 h after ERCP examination	7.85 ± 8.27	0.097	—	—	135.8 ± 160.3	<0.001
2 h after ERCP examination	8.05 ± 10.82	0.034	0.26 ± 0.87	0.646	134.1 ± 170.6	<0.001
3 h after ERCP examination	6.18 ± 3.30	0.427	—	—	122.9 ± 160.2	<0.001
4 h after ERCP examination	7.08 ± 5.31	0.006	0.00 ± 0.00	0.028	114.9 ± 155.0	0.001
6 h after ERCP examination	6.45 ± 4.67	0.491	0.00 ± 0.00	0.028	117.1 ± 175.2	0.019

P values are calculated by using the Wilcoxon matched-pairs test versus pre-ERCP values.

($P = 0.031$ vs pre-ERCP). Urine TAP concentrations significantly declined 4 and 6 hours after ERCP ($P = 0.028$ for both). Mean serum trypsinogen concentrations were slightly below the upper reference limit (57 ng/mL) before ERCP examination, and then they were significantly increased thereafter. Before ERCP, there were no significant differences in the serum and urinary levels of the enzymes studied among the different final diagnoses.

Serum and urine TAP levels and serum trypsinogen concentration showed no significant differences between patients who developed acute pancreatitis after ERCP and those who did not in any of the time intervals studied. The same behavior was present between patients who were treated prophylactically with GM and those who did not receive the drug. Within the group of the 53 patients who received GM, serum TAP concentrations at 1 and 2 hours after ERCP were significantly lower in the 10 patients who developed acute pancreatitis (mean ± SD, 4.88 ± 2.55 and 4.68 ± 1.90, respectively) as compared with the 43 who did not (mean ± SD, 8.54 ± 9.13 and 7.22 ± 4.16, respectively; $P = 0.033$ and $P = 0.041$, respectively).

DISCUSSION

In this study, the percentage of post-procedural pancreatitis was higher than that reported in other studies coming from Italy,[4,7] but only patients who underwent operative ERCP were enrolled, and acute pancreatitis had a mild course in all cases.

Regarding the primary end point, all patients had detectable concentrations of serum and urine TAP before ERCP, and no differences in basal TAP values were observed among patients with lithiasis of the common bile duct, those with benign stenosis of the common bile duct, those with chronic pancreatitis, and those with common bile duct pancreatic neoplasms. On the other hand, serum trypsinogen concentrations at basal examination were slightly lower than the upper reference limit; however, patients with lithiasis and neoplasms of the common bile duct have serum concentrations of this enzyme slightly higher than the upper reference limit. It is possible that in humans, obstruction of the common bile duct acts in the same manner as that which happens in animals after ligation of the common bile duct.[8]

The serum concentrations of TAP remained elevated for the subsequent observation period (at 1 and 2 hours) and then progressively declined at 3, 4, and 6 hours. This observation confirms our previous data on the low sensitivity of serum TAP in diagnosing acute pancreatitis[3] because TAP is rapidly eliminated from the circulation. Urine TAP gave the same results, whereas serum trypsinogen concentration progressively increased at 1 and 2 hours after ERCP and then slowly declined thereafter.

Regarding the other aim of our study, we found no significant differences in serum and urine TAP levels and in serum trypsinogen concentration between patients who developed acute pancreatitis after ERCP and those who did not, confirming that the clinical and imaging assessment of acute pancreatitis is the cornerstone in assessing this complication of ERCP.[9] Finally, regarding GM, we found no significant differences in serum and urine TAP levels and in serum trypsinogen concentration between patients who received GM and those who did not, even if the number of patients who did not received GM was quite small. However, within the group of patients who received GM, the decrease of serum TAP concentration was significantly more rapid in those who developed acute pancreatitis as compared with those who did not; these data seem to confirm that GM is a potent inhibitor of trypsin activation.[10]

In conclusion, TAP determination is of limited value in diagnosing post-ERCP acute pancreatitis, and more studies need to precisely establish the role of TAP determination in patients treated prophylactically with antiproteasic drugs.

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REFERENCES

1. Frossard JL. Trypsin activation peptide (TAP) in acute pancreatitis: from pathophysiology to clinical usefulness. *JOP.* 2001;2:69–77.
2. Simpson KW, Beechey-Newman N, Lamb CR, et al. Cholecystokinin-8 induces edematous pancreatitis in dogs associated

with short burst of trypsinogen activation. *Dig Dis Sci*. 1995;40:2152–2161.
3. Pezzilli R, Venturi M, Morselli-Labate AM, et al. Serum trypsinogen activation peptide in the assessment of the diagnosis and severity of acute pancreatic damage: a pilot study using a new determination technique. *Pancreas*. 2004; 29:298–305.
4. Masci E, Cavallini G, Mariani A, et al. Gabexate in Digestive Endoscopy—Italian Group II. Comparison of two dosing regimens of gabexate in the prophylaxis of post-ERCP pancreatitis. *Am J Gastroenterol*. 2003;98:2182–2186.
5. Freeman ML, Nelson DB, Sherman S, et al. Complications of endoscopic biliary sphincterotomy. *N Engl J Med*. 1996;335: 909–918.
6. Bradley EL. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, GA, September 11 through 13, 1992. *Arch Surg*. 1993;128: 586–590.
7. Masci E, Toti G, Mariani A, et al. Complications of diagnostic and therapeutic ERCP: a prospective multicenter study. *Am J Gastroenterol*. 2001;96:417–423.
8. Karanjia ND, Widdison AL, Jehanli A, et al. Assay of trypsinogen activation in the cat experimental model of acute pancreatitis. *Pancreas*. 1993;8:189–195.
9. Pezzilli R, Romboli E, Campana D, et al. Mechanisms involved in the onset of post-ERCP pancreatitis. *JOP*. 2002;3: 162–168.
10. Chen CC, Wang SS, Lee FY. Action of antiproteases on the inflammatory response in acute pancreatitis. *JOP*. 2007; 8(suppl 4):488–494.

First Case of 2 Intraductal Papillary Mucinous Tumors of Both Ventral and Dorsal Ducts in Pancreas Divisum

To the Editor:

Pancreas divisum (PD) is a congenital anomaly of the pancreatic system, consisting in the defect of fusion between ventral and dorsal buds and ducts. Intraductal papillary mucinous tumor (IPMT) is a rare neoplasm of the exocrine pancreas characterized by mucin production, intraductal papillary growth, and cystic dilation of the pancreatic ducts. The diagnosis of benign tumors, including the IPMT, complicating PD is very rare. Here, we present what we believe is the first case of 2 simultaneous IPMTs arising from the dorsal and ventral ducts in PD.

A 74-year-old woman was admitted to our hospital for recurrent episodes of mild rectal bleeding in the past 2 to 3 months. Her medical history included hypertension, type 2 diabetes mellitus, and chronic obstructive pulmonary disease. The patient was asymptomatic, but laboratory tests showed severe sideropenic anemia, requiring transfusion of 3 units of red packed blood cell. In the following days, the patient did not present bleeding and remained hemodynamically stable. An upper endoscopy showed no lesion of the esophagus, stomach, and duodenum, whereas a colonoscopy revealed a cecal polyp that was removed and diagnosed as a low-grade tubulovillous adenoma at histologic examination.

During hospitalization, a routinary abdominal ultrasound discovered a hypoechoic lesion of the pancreas. An abdominal computerized tomography confirmed a fluid lesion in the presence of normal pancreatic volume without calcifications. Furthermore, amylase, cholestatic enzymes, liver transaminases, and oncomarkers (cancer antigen 19-9 and carcinoembryonic antigen) were normal. To better characterize the pancreatic fluid lesion, the patient underwent a magnetic resonance cholangiopancreatography (MRCP) with secretin. As shown in Figure 1, a picture of complete PD was documented by the absence of communication between the MPD and the Wirsung duct. Furthermore, MRCP revealed the presence of 2 multicystic grapelike dilatations of the branch ducts: the first in the dorsal pancreas arising from the MPD and the second in the ventral pancreas arising from the Wirsung duct. Both lesions have been diagnosed as branch duct–type IPMTs.

Because of the recent progress in imaging techniques, an increasing number of anomalies and tumors developing in the pancreatic ductal system is diagnosed. To the best of our knowledge, 18 cases of IPMT in PD have been described, and all developed in the MPD,[1–9] except for 1 case arising from the Wirsung duct.[10]

Our case is the first diagnosis of 2 IPMTs arising one from MPD and the other one from the Wirsung duct. Another peculiarity of this report is represented by the fact that the diagnosis of IPMTs was incidental, as our patient did not present any symptom or sign of pancreatic disease, whereas the reported cases of IPMT in PD were symptomatic for recurrent acute or chronic pancreatitis.

This case suggests that IPMTs in PD can occur simultaneously in both ducts and

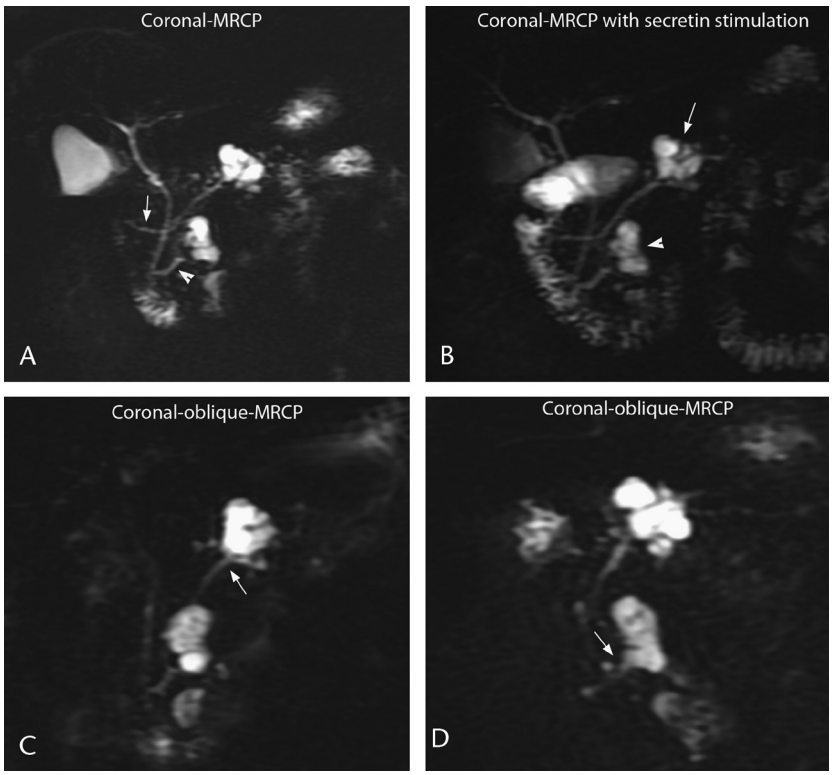

FIGURE 1. Magnetic resonance cholangiopancreatography. A, PD: pancreatic drainage through the Santorini duct (arrow) and the main pancreatic duct (MPD) not fused with the Wirsung duct (arrowhead). B, Dorsal (arrow) and ventral (arrowhead) IPMTs. C and D, Two branch duct–type IPMTs arising from the MPD and the Wirsung duct (arrow).

may remain asymptomatic. With the progress of the radiological techniques, the incidence of this finding and the knowledge on the pathogenetic relation between IPMT and PD will probably increase.

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REFERENCES

1. Akizuki E, Kimura Y, Mukaiya M, et al. A case of intraductal papillary mucinous tumor associated with pancreas divisum pancreas. *Pancreas*. 2006;32:117–118.
2. Scatton O, Sauvanet A, Cazals-Hatem D, et al. Dorsal pancreatectomy: an embryology-based resection. *J Gastrointest Surg*. 2006;10:434–438.
3. Nishino T, Toki F, Oi I, et al. Prevalence of pancreatic and biliary tract tumors in pancreas divisum. *J Gastroenterol*. 2006; 41:1088–1093.
4. Kamisawa T, Yoshiike M, Egawa N, et al. Pancreatic tumor associated with pancreas divisum. *J Gastroenterol Hepatol*. 2005;20: 915–918.
5. Talbot ML, Foulis AK, Imrie CW. Total dorsal pancreatectomy for intraductal papillary mucinous neoplasm in a patient with pancreas divisum. *Pancreatology*. 2005;5:285–288.
6. Sterling MJ, Giordano SN, Sedarat A, et al. Intraductal papillary mucinous neoplasm associated with incomplete pancreas divisum as a cause of acute recurrent pancreatitis. *Dig Dis Sci*. 2007;52:262–266.
7. Sakurai Y, Matsubara T, Imazu H, et al. Intraductal papillary-mucinous tumor of the pancreas head with the complete absence of the ventral pancreatic duct of Wirsung. *J Hepatobiliary Pancreat Surg*. 2004;11: 293–298.
8. Yarze JC, Chase MP, Herlihy KJ, et al. Pancreas divisum and intraductal papillary mucinous tumor occurring simultaneously in a patient presenting with recurrent acute pancreatitis. *Dig Dis Sci*. 2003; 48:915.
9. Thayer SP, Fernandez-del Castillo C, Balcom JH, et al. Complete dorsal pancreatectomy with preservation of the ventral pancreas: a new surgical technique. *Surgery*. 2002; 131:577–580.
10. Sakate Y, Ohira M, Maeda K, et al. Intraductal papillary-mucinous adenoma developed in the ventral pancreas in a patient with pancreas divisum. *J Hepatobiliary Pancreat Surg*. 2004; 11:366–370.

Total Pancreatectomy for Intractable Pain in Chronic Pancreatitis?

To the Editor:

Pain is a cardinal feature of chronic pancreatitis (CP), and according to the recent (mainly surgical) literature, pain in CP is often chronic and resistant to conservative management so that a surgical intervention, that is, drainage or resectional procedure, is required.[1,2] A total pancreatectomy for pain relief in CP has been proposed as ultima ratio, but the rationale for such a mutilating option has not been defined, and the discussion on indication, results, and outcome of this procedure remains contradictory. The recent publication of Garcea et al[3] based on a series of 85 CP patients documents that total pancreatectomy (combined with islet cell transplantation in 50 cases) can be performed technically with a rather low postoperative mortality (3.5%), but a couple of relevant questions regarding the strategy of such a procedure are not answered, and the conclusions of the authors are therefore hardly generalizable.

There are several major problems why series of CP from different centers regarding success of therapeutic interventions are not comparable. In particular, the pathomechanism of pain in CP is poorly understood; there is no generally accepted pain score system, and in most series, nature and cause of pain are not adequately documented. Moreover, reliable data on the long-term pain profile of CP should be based on prospective studies of mixed medical-surgical series of CP of various etiology, primarily because data of surgical series are biased excluding approximately 50% of CP patients who never required surgical intervention for pain relief.[4,5]

The publication of Garcea et al[3] is essentially a retrospective study based on a questionnaire sent to the patients postoperatively asking about their feeling regarding their operation and improvements in their pain control and quality of life.

Patients were especially asked to complete a visual analog scale of the severity and frequency of their pain before and after pancreatectomy. The patient's median 24-hour requirements for opiate analgesia were also recorded. A total of 50 of the surviving 82 patients returned their questionnaire (approximately 60%), and these data form the basis of the statistical analysis.

There are essentially 2 unsolved problems that need consideration before the conclusions of the article of Garcea et al can be accepted, that is, the definition of CP and the relationship between CP and chronic intractable pain. Over 90% of the 85 patients regularly used opiate analgesia preoperatively, which points to the well-known dilemma of differentiating between opiate addiction versus pancreatitis-related pain.[4] The fact that a large proportion of patients continued with opiate analgesia for 3 to 5 years postoperatively is supporting evidence for opiate addiction, and it questions a direct causative relationship between CP and intractable pain. In addition, the diagnostic criteria of CP are rather vague because the term "minimal-change CP," as generally used by the authors, is not adequately defined and is prone to increase uncertainty about the clinical relevance of the article. Moreover, the results of the article are in contrast to the experience of many experts who observed a general tendency of spontaneous pain relief in medical and surgical series in the long-term course of CP (except in patients with opiate addiction).[4–6]

A study in progress of our group (unpublished) analyzes incidence, nature, and cause of late pain (>10 years from onset) in a prospective long-term study of alcoholic and nonalcoholic CPs with and without surgery that, for instance, emphasizes that, virtually, all patients eventually got permanent pain relief except patients with narcotic addiction.

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REFERENCES

1. Warshaw AL, Banks PA, Fernandez-Del Castillo C. Treatment of pain in chronic pancreatitis. *Gastroenterology.* 1998;115: 763–776 (AGA medical position statement).
2. Büchler MW, Warshaw AL. Resection versus drainage in treatment of chronic pancreatitis. *Gastroenterology.* 2008;134: 1605–1607 (editorial).
3. Garcea G, Weaver J, Phillips J, et al. Total pancreatectomy with and without islet cell transplantation for chronic pancreatitis. *Pancreas*. 2009;38:1–7.
4. Ammann RW, Muellhaupt B. The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology.* 1999;116: 1132–1140.
5. DiMagno EP. Toward understanding (and management) of painful chronic pancreatitis. *Gastroenterology.* 1999;116: 1252–1257.
6. Ammann RW. Diagnosis and management of chronic pancreatitis: current knowledge. *Swiss Med Wkly.* 2006;136:166–174.

Invading and Long-Lasting Enormous Pancreatic Head Tumor

To the Editor:

Malignant tumors affecting the head of the pancreas are mostly identified as adenocarcinomas, especially the larger ones.[1] Pancreatic cancer grows aggressively but gives us limited time to view the tumor because the patients usually die shortly after its discovery. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract.[2] Gastrointestinal stromal tumor represents a morphologically heterogeneous group of tumors and has emerged in recent years as a distinct entity with specific histogenesis.[3] This tumor was considered rare and reported arising in the close proximity of the pancreatic head[4] but not directly originating here as an enormous mass.

In this document, we report an extremely rare case of a very large GIST, which arose in the pancreatic head with long-time aggressive growth and had characteristic image features as detected in magnetic resonance imaging (MRI) and digital subtraction angiography.

CASE REPORT

A 67-year-old man was found having a mass of 5 cm in diameter in the pancreatic head region 3 years ago by MRI in a local hospital. Pancreatic head carcinoma was diagnosed and stereotactic conformal radiotherapy prescribed. Two months ago, the patient began to have fever, nausea, and vomiting, and MRI revealed a 10 × 9 × 14-cm mass in the pancreatic head, which was lobulated with central necrotic liquefaction and infiltrating the small omentum and the posterior wall of the gastric antrum. There was a nodule in the left lobe of the liver suggesting metastasis (Figs. 1A, B). On admission, physical examination results revealed a normal soft abdomen. No positive findings were obtained in laboratory studies such as blood routine, liver function, and so on. Another MRI showed that the large mass increased in size, protruded into the gastrohepatic ligament. Compared with the previous MRI, the necrotic region was filled with some newly developed solid contents (Figs. 1C, D). Superior mesenteric angiogram demonstrated jejunal and ileal tributary supply, without contrast staining of the pancreatic head region or collateral to pancreatic artery (Figs. 1E, F).

Half a month later, the patient died of massive digestive hemorrhage. Postmortem histopathologic result showed GIST of the pancreas invading the neighborhood. Hematoxylin-eosin stain revealed that the tumor tissue consisted mainly of darkly stained spindle cells and occasional epithelioid cells. Immunohistochemistry analysis showed CD117(+), CD34(+), Des(−), S-100(−), and PCK(−).

DISCUSSION

Gastrointestinal stromal tumor represents a subset of gastrointestinal mesenchymal tumors; their clinical manifestations include abdominal uncomfortableness, digestive ulcer, bleeding, and palpable but vague abdominal mass in more than half of the patients.[2–4] Our patient had a soft abdomen probably because the mass was cystic, which changed after radiation, and easily to be confused with hollow organs such as intestine. Its clinical diagnosis requires a high index of suspicion. Pancreatic adenocarcinoma has a very short clinical course. Median overall survival of unresectable pancreatic cancer has a maximum of 8 months,[1] whereas our patient had his tumor discovered more than 3 years until its rupture into the intestinal lumen. Severe and persistent epigastric or back pain and weight loss are characteristics of pancreatic cancer,[1] but this patient had none of these symptoms.

Most patients with pancreatic cancer, especially those with large tumors, present with obstructive jaundice caused by compression of the bile duct in the head of the pancreas, which is helpful for differentiating GIST. However, signs of invasiveness and metastasis are not so useful for differentiation because more than one half of pancreatic cancer cases have distant metastasis at diagnosis and approximately 10% of patients with GIST present with metastatic disease at first diagnosis, usually to the liver,[5] that is in our case.

Imaging plays an important role in establishing diagnosis of GIST, demonstrating a circumscribed mass and its size, shape, internal architecture, and relation to the neighborhood.[4,5] Computed tomography is believed to be sensitive and accurate for detecting pancreatic cancer, and MRI is considered helpful for ruling out pitfalls[1] in establishing diagnosis of pancreatic carcinoma. In the present patient, the lobulated mass with central necrotic liquefaction is not the usual sign of pancreatic cancer, and the newly developed solid contents in the next MRI suggested homogeneous and compact mesenchymal tissue with the tone of toughness not loose acinous tissue with the tone of fragility.

Angiography helps define size, range, and location of GIST, showing enlarged arteries and early developed veins, and is especially valuable to patients with melena with unknown reasons.[6] In our case, angiogram showed abundant tumor blood supply by fine vessels in just half of the tumor, without contrast staining of the pancreatic head region or collateral to pancreatic artery (Figs. 1E, F), probably because of the previous radiotherapy, which destructed blood vessels in that area. The part with abundant vessels implies new growth of the tumor with infiltration. In our experience, pancreatic cancers have particularly poor contrast staining of tumor tissue on angiogram, readily distinguished from GISTs that usually have rich blood supply.

Positive CD117/KIT and CD34 immediately establish the diagnosis of GIST. Expression of KIT is seen in almost all GISTs, regardless of the site of origin, histologic appearance, or biologic behavior, and is therefore regarded as one of the key diagnostic markers.[2] In a study of 35 GIST cases,[7] the positivity rates of CD117 and CD34 in the tumors were 94.3% and 91.4%, respectively. Status of Des, S-100, and PCK is useful for differentiation of other gastrointestinal tumors. On the contrary, pancreatic adenocarcinoma does not

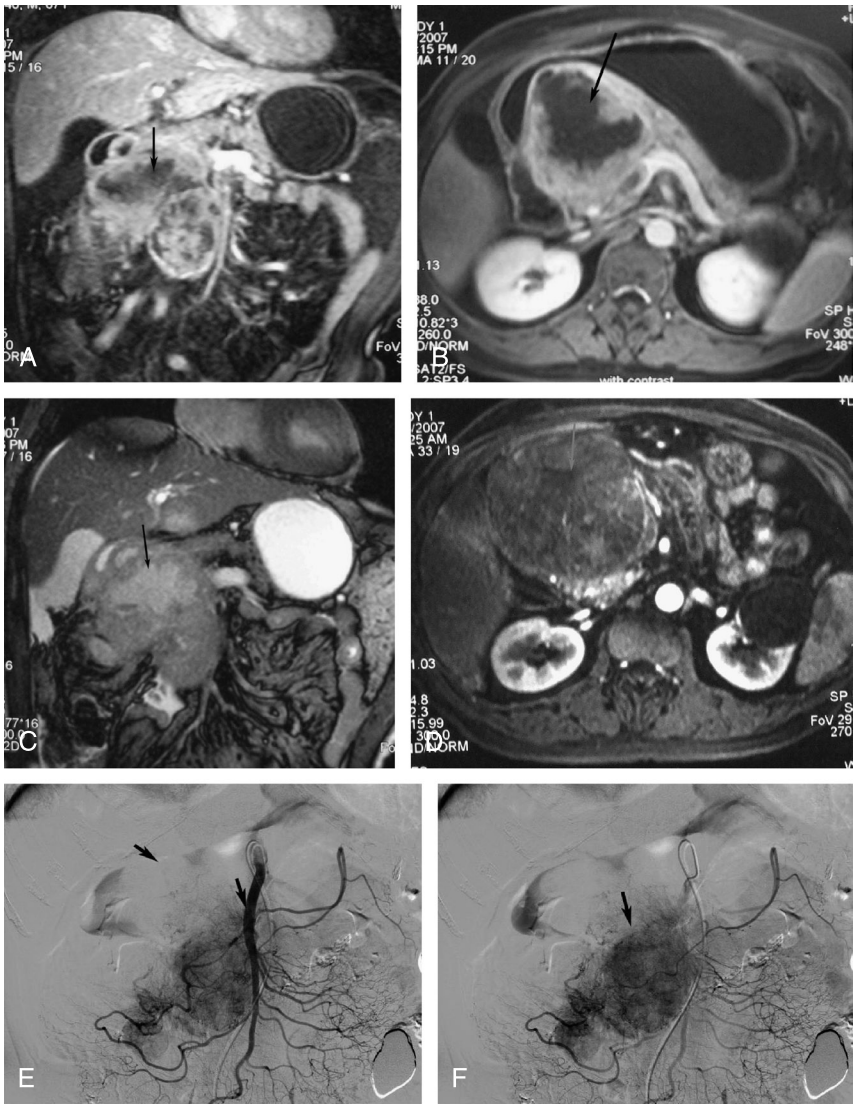

FIGURE 1. A, Magnetic resonance imaging of the coronal section demonstrated a mass in the pancreatic head. The tumor spread anterosuperiorly to the right, invaded the small omentum and the posterior wall of the gastric antrum, across the front of the descending portion of the duodenum. Dark areas in the tumor implied necrosis (arrow). B, Cross-section exhibited cystic change of the pancreatic head (arrow). C, Two months later, an MRI of the coronal section demonstrated that the large mass increased in size, protruded into the gastrohepatic ligament, and the necrotic region was filled with newly developed solid contents with the tone of toughness (arrow). D, Cystic change of the pancreatic head was missing (arrow). E, Arterial phase of superior mesenteric angiography demonstrated that the tumor consisted markedly of both avascular and hypervascular parts (arrows). F, Venous phase angiogram showed that contrast staining of the hypervascular part was even denser (arrow).

express CD117/KIT in immunohistochemical studies.[8]

Gastrointestinal stromal tumor has various organ distribution. In a local study in Pakistan,[3] GIST was reported involving the pancreas in 8.1% of the 37 cases studied, without description of clinical and image features. For many years, cases of spindle cell and epithelioid cell neoplasms were taken as smooth muscle tumors, classified previously as leiomyomas, schwannomas, leiomyoblastomas, or leiomyosarcomas, lacking evidence of smooth muscle origin.[5] There are articles reporting such tumors as pancreatic leiomyosarcoma,[9] pancreatic carcinosarcoma, pancreatic schwannoma, and pancreatic epithelioid leiomyoma, among which pancreatic GISTs really should exist. Many of these cases represent a cystic mass resembling our case, resulting in the incorrect diagnosis of pancreatic pseudocyst at times.[10]

In summary, our case illustrates that a rare case of a very large GIST in the pancreatic head, its clinical manifestations, image features, and pitfalls led to misdiagnosis of pancreatic cancer. Gastrointestinal stromal tumor of the pancreas might be not as rare as it is considered currently, which raises a question worthy of our attention.

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REFERENCES

1. Wilkowski R, Wolf M, Heinemann V. Primary advanced unresectable pancreatic cancer. *Recent Results Cancer Res*. 2008;177: 79–93.
2. Koh JS, Trent J, Chen L, et al. Gastrointestinal stromal tumors: overview of pathologic features, molecular biology, and therapy with imatinib mesylate. *Histol Histopathol*. 2004;19:565–574.
3. Rauf F, Bhurgri Y, Pervez S. Gastrointestinal stromal tumors: a demographic, morphologic and immunohistochemical study. *Indian J Gastroenterol*. 2007;26:214–216.
4. Van den Abbeele AD. The lessons of GIST-PET and PET/CT: a new paradigm for imaging. *Oncologist*. 2008;13(suppl 2): 8–13.
5. Bucher P, Villiger P, Egger JF, et al. Management of gastrointestinal stromal tumors: from diagnosis to treatment. *Swiss Med Wkly*. 2004;134:145–153.
6. Fang SH, Meng L, Dong DJ, et al. Angiographic diagnosis of gastrointestinal stromal tumors. *Zhonghua Zhong Liu Za Zhi*. 2005;27:496–498.
7. Liu J, Liu YH. Expression of CD117, CD34, SMA, S-100 protein, Vim and desmin in patients with gastrointestinal stromal tumors. *Nan Fang Yi Ke Da Xue Xue Bao*. 2008;28:438–440.
8. Bateman AC, Judd M, Radenkovic D, et al. CD117/KIT expression in pancreatic adenocarcinoma. *Pancreas*. 2008;36: 76–79.
9. Deveaux P, Aranha G, Yong S. Leiomyosarcoma of the pancreas. *HPB (Oxford)*. 2001;3: 175–177. PMID: 18332921.
10. Gillatt DA, Cooper MJ, Williamson RC. Pseudo-pseudocysts of the pancreas. *Pancreas*. 1986;1:460–463.

Early Predictive Factors of Inhospital Mortality in Patients With Severe Acute Pancreatitis

To the Editor:

Acute pancreatitis (AP) is a common and sometimes life-threatening inflammatory condition of the pancreas. Several studies indicated that the mortality rate of patients with AP is currently approximately 3.8% to 7%[1,2]; in severe AP (SAP), it varies from 7% to 42%.[2–5]

In previous studies, several biological markers and clinical events had been used to predict the mortality of AP. However, to the best of our knowledge, few studies have addressed the role of factors as independent predictors that can early predict fatal outcome in hospitalized medical patients. The primary aim of this study was therefore to analyze the conventional clinical data and parameters within 24 hours after admission of 338 patients with SAP, particularly the effect of C-reactive protein (CRP), albumin (ALB), and total cholesterol (TC).

RESULTS

The mean age of the 338 patients with SAP was 53.9 years. The overall inhospital mortality rate was 8.28% (28/338). The mean time from hospital admission to death was 29.11 days (range, 9–47 days).

The Results of Multivariate Analysis for Factors Within 24 Hours on Admission

Multivariate analysis indicated that the inhospital mortality increased significantly more than 7-fold higher in patients with severe hypoalbuminemia (ALB $\leq$ 30 g/L). The CRP levels exceeding 170 mg/L were significantly associated with a 7-fold inhospital death. A serum TC level between 4.37 to 5.23 mmol/L had significant protective effect. Total cholesterol levels exceeding 5.23 mmol/L were risk factors to predict inhospital mortality with no significant difference. The strongest prognostic factor was serum ALB.

The Relationship Between Serum TC and CRP Levels

Subanalysis indicated that CRP was negatively linearly associated with TC ($r = -0.681$, $P < 0.001$). Quartile 3 of TC (4.37–5.23 mmol/L), the quartile with the greatest likelihood of survival, had the lowest mean and median CRP levels, and the highest mean and median CRP level measurements were found to be in the lowest quartile of the TC (3.67 mmol/L).

DISCUSSION

In this study, we demonstrate the factors within 24 hours on admission that early predict the inhospital mortality in SAP.

Serum levels of CRP, an acute-phase protein, was a sensitive marker of systematic inflammation.[6] According to this record, a CRP level of 170 mg/L or higher indicate a more than 7-fold odds ratio for mortality. As we know, CRP is an acute-phase protein that increases its level after 48 to 78 hours. In our study, patients hospitalized for SAP reflected an increase in inflammatory marker serum levels. Furthermore, many retrospective studies have argued that serious infections account for high serum CRP levels at hospital admission.[7] Both may explain why the CRP levels were considerably high at the first 24 hours of admission.

During the early phase of SAP, hypoalbuminemia was a predictor for severe prognosis. Our analysis indicated that the mortality significantly increased approximately 7 times when the serum ALB level was lower than 30 g/L. This analysis also identified that the odds ratio of hypoalbuminemia for mortality was much higher than that of high CRP levels.

A variety of studies have evaluated this cholesterol mortality relation and found low TC level to be associated with increased mortality and increased levels to be associated with survival.[8–10]

In our study, a serum TC level between 4.37 to 5.23 mmol/L had protective effect, and the effect was significant. The moderate elevation of the serum TC level could reduce mortality if it exceeds 5.23 mmol/L and result in an increase in mortality but with no significant difference. The ability of elevated serum TC level to predict survival in SAP seems unintuitive because the primary cause of AP is hypercholesteremia, which is a strong risk factor to SAP.

The Relationship Between Serum CRP and TC

In our study, the highest mean (median) CRP level was found in the lowest quartile of TC, which indicated a possible cholesterol-inflammatory association. These results indicate that a low cholesterol level may serve as a clinically useful prognostic parameter of the severity of inflammatory. Moderately elevated TC levels can increase resistance to inflammatory reaction and reduce the severity of SAP and the rate of mortality. The mechanism has to be further investigated.

In conclusion to this study, when the CRP level is 170 mg/L or higher ($P < 0.05$), the ALB level is lower than 30 g/L, the TC level is 5.23 mmol/L or higher or 3.67 mmol/L or lower, the patient should be treated in a highly specialized institution because of the high risk for mortality.

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REFERENCES

1. Cavallini G, Frulloni L, Bassi C, et al. Prospective multicentre survey on acute pancreatitis in Italy (ProInf-AISP): results on 1005 patients. *Dig Liver Dis*. 2004;36:205–211.
2. Fu CY, Yeh CN, Hsu JT, et al. Timing of mortality in severe acute pancreatitis: experience from 643 patients. *World J Gastroenterol*. 2007;13:1966–1969.
3. Banks PA, Freeman ML, Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. *Am J Gastroenterol*. 2006;101:2379–2400.
4. Heinrich S, Schafer M, Rousson V, et al. Evidence based treatment of acute pancreatitis: a look at established paradigms. *Ann Surg*. 2006;243:154–168.
5. Bai Y, Jia L, Wang B, et al. Acute pancreatitis in the Guangdong province, China. *Digestion*. 2007;75:74–79.
6. Mansfield CS, James FE, Robertson ID, et al. Development of a clinical severity index for dogs with acute pancreatitis. *J Am Vet Med Assoc*. 2008; 233:936–944.
7. Vanderschueren S, Deeren D, Knockaert DC, et al. Extremely elevated C-reactive protein. *Eur J Intern Med*. 2006;17: 430–433.
8. Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med*. 1998;339:1349–1357.
9. Gui D, Spada PL, De Gaetano A, et al. Hypocholesterolemia and risk of death in the critically ill surgical patient. *Intensive Care Med*. 1996;22: 790–794.
10. Rauchhaus M, Clark AL, Doehner W, et al. The relationship between cholesterol and survival in patients with chronic heart failure. *J Am Coll Cardiol*. 2003;42: 1933–1940.

Successful Single–Balloon Enteroscopic Dilation of Late Anastomotic Pancreaticojejunostomy Stricture Following Whipple Procedure

To the Editor:

Endoscopic access to the pancreaticobiliary system remains challenging in patients who have undergone surgical procedures such as gastric bypass and Roux-en-Y gastroenterostomies. Balloon enteroscopy is a new procedure that allows deep access to the small bowel and has been used to access the biliary system in patients with surgically altered anatomy. We describe the successful use of single-balloon enteroscopy to access the pancreatic duct after pancreaticoduodenectomy.

A 67-year-old man was referred for the management of recurrent episodes of acute pancreatitis in 2007, after a Whipple procedure for pancreatic adenocarcinoma in 1999. Magnetic resonance imaging revealed an atrophic pancreas and a dilated pancreatic duct. No pancreatic masses were seen. Endoscopic retrograde cholangiopancreatography with a duodenoscope and a pediatric colonoscope was attempted. Because of postoperative changes in the stomach and marked looping of the endoscopes, the pancreaticojejunostomy could not be accessed. Therefore, a single–balloon enteroscope was used to access the pancreaticojejunostomy through the gastrojejunostomy. After advancement of the single–balloon overtube into the gastroenterostomy, the balloon was inflated and the enteroscope was advanced into the afferent limb until the pancreaticojejunostomy was seen. The orifice was stenosed to a small pinhole 1 to 2 mm in diameter (Fig. 1). Contrast injection revealed an irregular and dilated main pancreatic duct. The opening was dilated to 4 mm using a balloon. A 5F pancreatic stent was placed in the pancreatic duct. Six weeks later, a second dilation was performed with a 6-mm dilating balloon with good effect (Fig. 2). After the procedure, the patient remained free of pain and attacks of acute pancreatitis.

Endoscopic access to the pancreaticobiliary system remains challenging in patients who have undergone procedures such as gastric bypass procedures and Roux-en-Y gastroenterostomies. Success rates of cholangiography in these patients have ranged from 73% to 84%[1,2] However, access to the pancreatic duct is considerably more difficult and has a reported success rate of 8% to 40%.[1,2] This may be due to the location and small size of the anastomosis and transparency of the pancreatic juice. Failure of an endoscopic approach in this situation requires more invasive procedures such as percutaneous transhepatic cholangiography or combined laparoscopic and endoscopic approaches through the gastric lumen. Balloon enteroscopy is a new procedure that allows deep access to the small bowel and has been used to

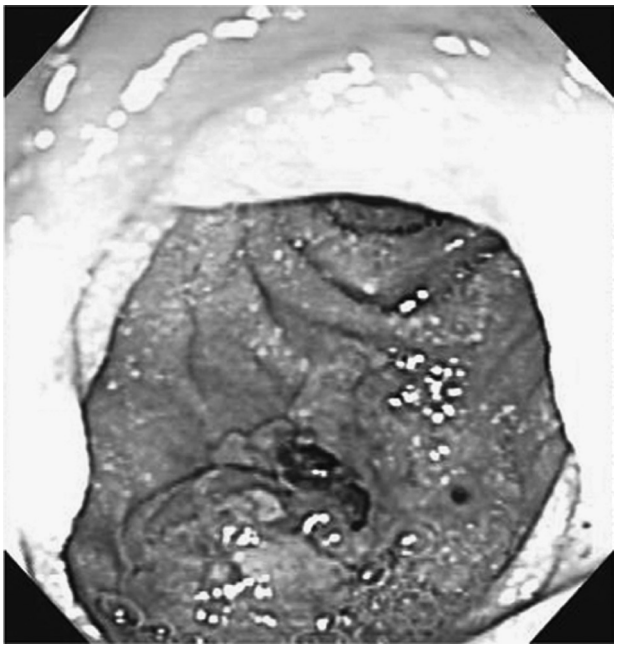

FIGURE 1. Strictured pancreaticojejunostomy as a pinhole.

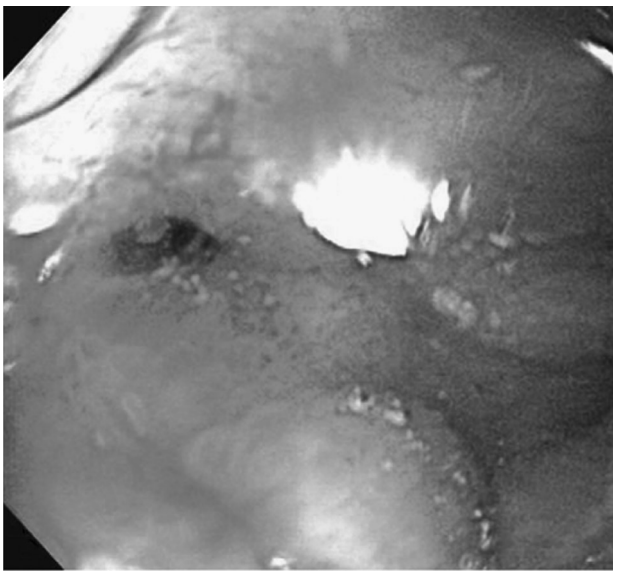

FIGURE 2. After dilation of pancreaticojejunostomy.

access the biliary system in patients with surgically altered anatomy.[3,4] We were able to use a single–balloon enteroscope to access the pancreatic duct after pancreaticoduodenectomy. Use of a regular duodenoscope or colonoscope often leads to looping and inability to intubate the necessary limbs as illustrated in our patient. We believe that the use of the balloon overtube stabilized the endoscope in the small bowel in this situation and helped overcome the problem of looping.

Double–balloon enteroscopy has been used to access the pancreaticobiliary system in patients with surgically altered anatomy.[3,4] However, we feel that a single-balloon overtube may be all that is necessary in this situation. Advantages of the single–balloon technique include that it is easier to assemble and set up with significant time savings and is less expensive. This approach is considerably more successful than routine endoscopy and provides a useful, less invasive, alternate approach in these patients.

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REFERENCES

1. Chahal P, Baron TH, Topazian M, et al. Endoscopic retrograde cholangiopancreaticography in post-Whipple patients. *Endoscopy*. 2006;38:1241–1245.
2. Farrell J, Carr-Locke D, Garrido T, et al. Endoscopic retrograde cholangiopancreaticography after pancreaticoduodenectomy for benign and malignant disease: indications and technical outcomes. *Endoscopy*. 2006;38:1246–1249.
3. Monkemuller K, Bellutti M, Neumann H, et al. Therapeutic ERCP with double–balloon enteroscope in patients with Roux-en-Y anastomosis. *Gastrointest Endosc*. 2008;6: 992–996.
4. Haruta H, Yamamoto H, Mizuta K, et al. A case of successful enteroscopic balloon dilatation for late anastomotic stricture of cholodochojejunostomy after living donor liver transplantation. *Liver Transpl*. 2005;11: 1608–1610.

Nicotine Gum Causing Pancreatitis
A Case Report

To the Editor:

Recently, we encountered a case of acute pancreatitis in an otherwise healthy 57-year-old man who attributed his condition to Nicorette gum (London, UK).

RD, a 57-year-old white man, attended the emergency department with severe epigastric pain, nausea and vomiting, and a raised serum amylase level of 800 μg/L. His condition was diagnosed as acute pancreatitis, and he revealed that he had experienced a similar bout 2 years previously, also necessitating admission to hospital. He was commenced on intravenous fluids and kept nil by mouth. Twenty-four hours after admission, he was scored using the Modified Glasgow Score – 3 (raised leukocytes, lactate dehydrogenase, and serum glucose levels).

His symptoms resolved quickly, and an abdominal ultrasound scan revealed an unremarkable biliary tree with no gallstones. He drank minimal amounts of alcohol at approximately 7 units per week, and his γ-glutamyl transferase level was normal (24 IU/L). He had not been involved in any trauma to his abdomen and was not taking any regular medications or over-the-counter analgesia. His serum calcium, glucose, and triglyceride levels were all within reference range. He had no significant medical history other than mild chronic obstructive pulmonary disease for which he used as required salbutamol but no inhaled steroids.

The patient reported that 1 week before this and his previous admission with acute pancreatitis, he had begun to use nicotine gum in an effort to quit smoking. He used roughly 10 pieces of 4-mg nicotine gum per day for 7 days (40 mg of nicotine per day) with no cigarettes and, on both occasions, developed acute epigastric pain with nausea and vomiting requiring admission, and upon admission, his condition was diagnosed as acute pancreatitis.

On each admission, his symptoms have resolved with cessation of the gum and simple conservative measures.

A thorough search of the literature has revealed another reported case of acute pancreatitis as a result of nicotine patches but never gum.[1] An experimental article by Chowdhury et al[1] confirms a link etween nicotine and pancreatic injury as seen in laboratory animals.[2] The mechanism by which nicotine induces such effects is perhaps mediated via signal transduction pathways in the pancreatic acinar cell, leading to enhanced levels of intracellular calcium release, resulting in cytotoxicity and eventual cell death.

We wonder if pancreatitis should be regarded as a rare but potentially serious adverse effect of nicotine replacement therapy. In addition, we invite correspondence from others who may have experienced a similar case.

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REFERENCES

1. Chowdhury P, MacLeod S, Udupa KB, et al. Pathophysiological effects of nicotine on the pancreas: an update. *Exp Biol Med (Maywood)*. 2002;227(7):445–454.
2. Berthier S, Michiels C, Sgro C, et al. Acute nonalcoholic nonbiliary pancreatitis. Difficulties in diagnosis and possibility of nicotine toxicity. *Presse Med*. 2005;34(11):795–796.