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Repeated Negative Urine Trypsinogen-2 Dipstick Test Rules out Diagnosis of Post-ERCP Pancreatitis

Short head: Urine Trypsinogen-2 in Post-ERCP Pancreatitis

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Abstract

Background A dipstick test for urine trypsinogen-2 has been used in diagnosis of acute pancreatitis, but there are only a few studies exploring the effectiveness of this test for early diagnose of PEP.

Goals We explore if the rapid point of care urine trypsinogen-2 dipstick test can replace assay of amylase in diagnosing PEP.

Study For this prospective study, we enrolled from Helsinki University Hospital 400 ERCP patients in whom we analyzed plasma amylase or pancreas-specific amylase, bilirubin, and urine trypsinogen-2, and urine trypsinogen-2 with dipstick before, 4 and 24 h after ERCP.

Results PEP developed in 15 (3.8%) patients. Urine trypsinogen-2 concentrations were significantly higher in PEP than in non-PEP patients 24 h after ERCP (p=0.001, Mann-Whitney U test) but not 4 h after ERCP (p=0.094). When combined with abdominal pain symptoms at 4 h the dipstick test had a sensitivity of 60%, specificity of 99%, positive predictive value of 64%, and negative predictive value 98%. At 24 h, sensitivity was 100%, specificity 98%, positive predictive value 71%, and negative predictive value 100%.

Conclusions A positive dipstick seem to identify PEP cases and negative test excludes PEP with high accuracy.

Key words: trypsinogen-2, post-ERCP pancreatitis, ERCP, urine dipstick test, diagnosis

Introduction

Acute pancreatitis is the most common and feared complication after endoscopic retrograde cholangiopancreatography (ERCP). Its incidence varies between 1.6 and 10% depending on the procedure and risk factors.[1-4] In pancreatitis the inflammatory process causes leakage of pancreatic enzymes into the circulation leading to increased trypsinogen-2 concentrations in serum and urine.[5,6] Symptoms and biomarkers for post ERCP pancreatitis (PEP) become positive shortly after the procedure, and patients at risk of developing PEP can mostly be identified within a few hours of observation. Patients without adverse symptoms after ERCP can usually be discharged from the hospital on the same day. Traditionally assays of serum or plasma amylase are routinely used tests for PEP. Analysis of trypsinogen-2 concentration in urine have been shown to be more sensitive than amylase but is not in common use as no automatic tests are available. A rapid point of care dipstick test for urine trypsinogen-2 with a nominal detection limit of 50 µg/L has been used in diagnosis of acute pancreatitis,[7-9] but so far, there are only a few studies exploring the effectiveness of trypsinogen-2 dipstick test for early diagnoses of PEP.[10-12] Results have been encouraging showing equal or better accuracy than serum amylase in PEP diagnosis. However, previous studies have been small and the study design has been variable. We routinely measure plasma amylase or plasma pancreas-specific amylase 4 h after ERCP, and if patients stay overnight at hospital, we check amylase also 24 h after procedure. Elevated amylase guides our discharge policy since nurses usually are discharging patients after office hours, and it would be a sign to be even more alert with abdominal pain symptoms.

The aim of our study was to explore if the urine trypsinogen-2 dipstick test can replace assay of amylase in the follow up of ERCP patients. The dipstick test is usable without laboratory facilities and urine sampling is less invasive than drawing of blood.

Patients and Methods

Between September 2011 and January 2018, we enrolled 400 ERCP patients referred to Helsinki University Hospital for this prospective study. Inclusion criteria were a native papilla and normal pre-ERCP plasma amylase (<120 U/l) or pancreas-specific amylase (<65 U/l), while exclusion criteria were acute or chronic pancreatitis, and age under 18 years. After inclusion, we recorded demographic and clinical characteristics of the patients (Table 1). The most common indication for ERCP was stones in biliary duct (275 patients, 69%) followed by different malignancies associated with biliary stricture (118 patients, 30%). The Ethics committee of Helsinki University Hospital approved the study. All patients gave their informed consent before participation in the study.

Prior to ERCP, patients were fasting at least for 6 h. They received prophylactic antibiotics, levoflocxacin 500 mg orally or kefuroxime 1.5 g intravenously before ERCP. All patients without contraindications received 100 mg of diclophenac per rectum to prevent PEP. During the ERCP procedure intravenous administration of glucagon or buscopan restrained bowel motility. All the ERCPs were therapeutic and we performed endoscopic sphincterotomy, biliary stone extraction, brush cytology, or stent placement if indicated.

Table 1

Patient Characteristics, n = 400	
Age, years*	71 (18 - 100)
Female	228 (56.9)
Indications for ERCP:	
Stones in biliary ducts	275 (69)
Malignancy causing biliary stricture	118 (30)
Sphincter of Oddi dysfunction	1 (0.3)
Leakage after cholecystectomy	2(0.5)
Other	4 (1)
Co-morbidities:	
Diabetes	77 (19)
Heart and vascular disease	251 (63)
Pulmonary disease	43 (11)
Elevated pre-ERCP p-bilirubin µmol/l	302 (76)
Post- ERCP hyperamylasemia ≥ 3 x URL	50 (13)
Adverse events:	
Pancreatitis	16 (3.8)
Bleeding	10 (3)
Cholangitis	4 (1)
Perforation	0 (0)
Hospitalization days after ERCP*	0 (0-26)
Positive dipstick test before ERCP	121 (33)
Data presented as median (range)* or number of patients (%). ERCP endoscopic retrograde cholangiopancreatography; URL upper reference limit	

Our criteria for PEP were new onset of abdominal pain which lasted for at least 24 h accompanied by increase of amylase concentrations more than 3 times the upper reference limit (plasma amylase >360 U/l or pancreas-specific amylase >185 U/l) or diagnostic alterations in radiologic examinations [magnetic resonance imaging (MRI) or computed tomography (CT)]. The severity of PEP was graded according to the revised Atlanta classification as mild (no systemic or local complications), moderately severe (local and/or systemic complications without persistent organ dysfunction) and severe (persistent organ dysfunction).[13] We also graded pancreatitis according to Cotton's classification into mild if hospitalization was prolonged by 2-3 days, moderate by 4-10 days, and severe by more than 10 days or if pancreatic necrosis, abscess, or pseudocysts developed.[14]

We analyzed plasma amylase, or pancreas-specific amylase, bilirubin, and urine trypsinogen-2 and urine trypsinogen-2 with the dipstick test in all patients before ERCP, 4 and 24 h after ERCP. We tested urine samples immediately with the urine trypsinogen-2 dipstick test (Actim Pancreatitis; Medix Biochemica, Kauniainen, Finland). The test strip was dipped into urine and the result was read after 5 min. The detection limit of the test is 50 µg/l, and it is positive if a clear blue line appeared in addition to the reference line within 5 min. Urine trypsinogen-2 was also measured by a quantitative time-resolved immunofluorometric assay (IFMA). We transported urine within 2h after sampling to the laboratory for storage at +4 ^{o}C for IFMA-analysis, and if samples were analyzed after 8h they were further stored at -20 ^{o}C and analyzed by IFMA within 7 days. The reference range for trypsinogen-2 concentration in urine is 0.3-11.0µg/l while the median concentration in acute pancreatitis is 1100µg/l (range 15-190,000µg/l).[7,16] Plasma bilirubin, amylase, and pancreas specific amylase were analyzed on Abbott Architect analyzer.

After ERCP, patients were followed in the surgical ward, in the emergency room, or the day care unit to detect possible complications and to perform follow up tests. On the day of ERCP 85 patients (21 %) were discharged and thus only the first 4 h follow up test was performed. We included all patients from whom we obtained urine and blood samples before ERCP and at least either one of the 4 h or 24 h post ERCP urine tests. We followed patients one month after discharge to recognize PEP and other ERCP complications e.g. bleeding, perforation, or cholangitis.

Statistical analysis

We performed statistical analysis using IBM SPSS* Statistic version 22.0 (SPSS, Chicago, Illinois, USA) statistical software. The results are expressed as median and range or inter quartile range (IQR), or number and percentage. We made comparison between two groups with Chi-square test or the Mann-Whiney U test. *P* values less than 0.05 were considered significant. We defined diagnostic performance of each test by calculating sensitivity, specificity, positive predictive value

(PPV) and negative predictive value (NPV) for cut off values. We evaluated agreement between the quantitative urine trypsinogen-2 test and the dipstick test with kappa statistics ($\kappa < 0.20$ indicates poor agreement and $\kappa > 0.81$ very good agreement).[17]

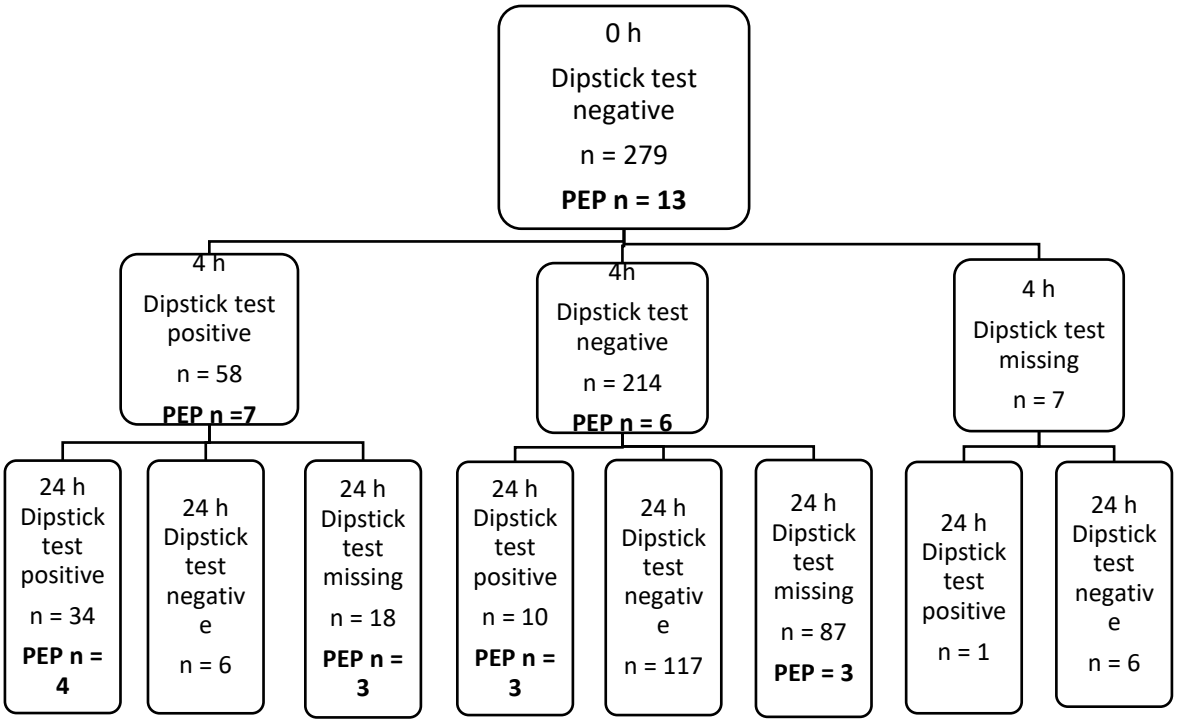
Results

The study comprised 228 women and 172 men with a mean age of 71 years (range 18 - 100). Table 1 shows patient characteristics. PEP developed in 15 (3.8%) patients. All PEP cases were diagnosed with increased amylase with abdominal pain symptoms, but 6 cases had additional CT done due to severe pain and suspicion of other complications. Only findings in these CT scans were acute pancreatitis. 4 patients without elevated amylase had CT scan done due to prolonged abdominal pain; none of them had pancreatitis. 17 patients had positive post-ERCP dipstick test with abdominal pain: 12 of them recovered rapidly during the follow up period of 24 h, but 5 patient had severe or prolonged abdominal pain and had CT done. Of these, 4 had oedematous pancreatitis in the scan and one had minor perforation and no pancreatitis.

We classified 14 cases as mild PEP with rapid recovery and one as moderately severe PEP according to the Atlanta classification. According to Cotton's classification 7 PEP cases were mild and 8 were moderate.

Figure 1

A



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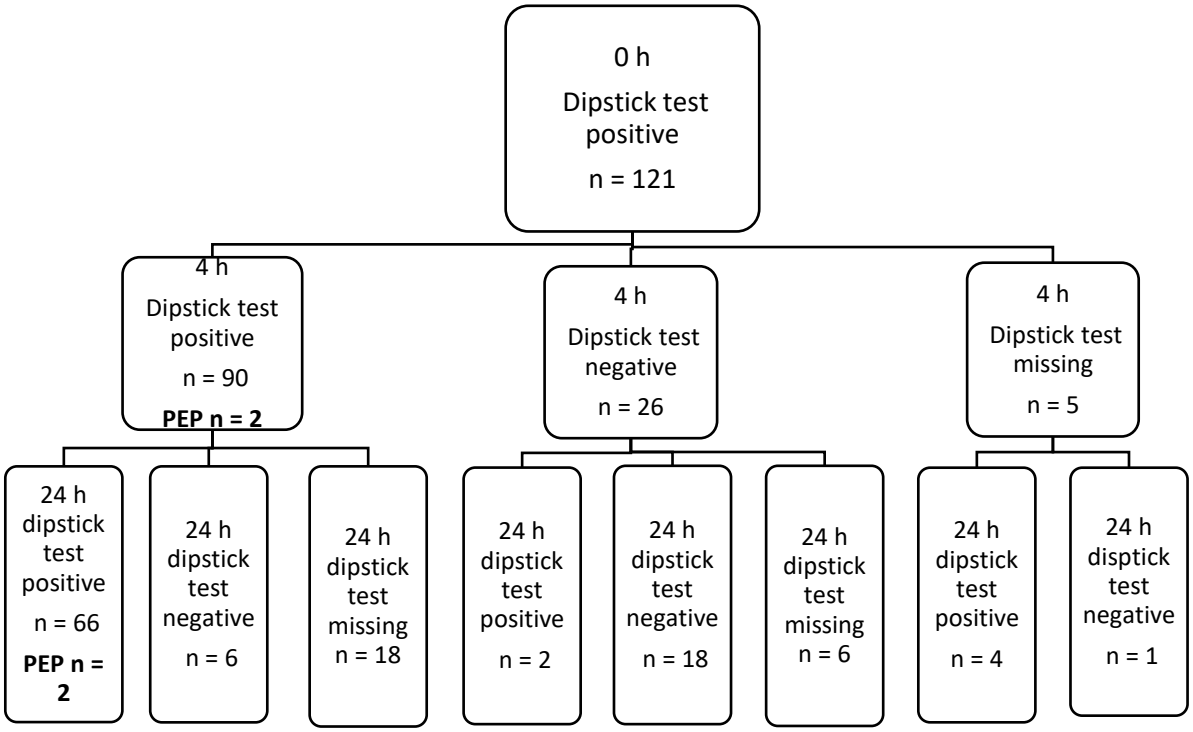

Figure 1.

Flow chart of the negative (A) and positive (B) pre-ERCP dipstick test results and number of patients with post-ercp pancreatitis (PEP)

We performed a baseline dipstick test before ERCP in all 400 patients and it was negative in 279 patients and positive in 121 (30%) cases (Figure 1 and Table 2). Among those with a negative dipstick test at 0 h, 58 had a positive test at 4 h and 7 of these developed PEP. Among 214 cases with a negative test at 4 h, 6 developed PEP, of these 3 were positive at 24 h while the test was missing in 87, 3 of which developed PEP (Fig 1A). At 4 h the test was missing in 7 cases, none of whom developed PEP. Thus, a negative dipstick test at both 4 and 24 h seem to exclude PEP.

Table 2.

Urine Trypsinogen-2 Dipstick Results					
	Positive (%)	False positive (%)	Negative (%)	False negative (%)	Missing (%)
All patients					
Before ERCP	121 (30)	121 (30)	279 (70)	0	0
4 h after ERCP	148 (37)	139 (35)	240 (60)	6	12 (3)
24 h after ERCP	115 (29)	109 (27)	154 (39)	0	131 (33)
PEP patients					
Before ERCP	2 (13)	2 (13)	13 (87)	0	0
4 h after ERCP	9 (60)	0	6 (40)	6 (40)	0
24 h after ERCP	9 (100)	0	0	0 (0)	6 (40)
Patients with abdominal pain after ERCP					
Before ERCP	4 (17)	4 (17)	20 (83)	0	
4 h after ERCP	14 (58)	5 (21)	10 (41)	6 (25)	0
24 h after ERCP	14 (58)	5 (21)	4 (17)	0	6 (25)
ERCP endoscopic retrograde cholangiopancreatography; False positive test = positive test without diagnosed PEP; False negative test = negative test in patient with diagnosed PEP					

We also studied how the dipstick test performed at 4 and 24 h in 121 cases who had positive pre-ERCP dipstick test (Fig 1B). These patients had more comorbidities than those with a negative test

(Table 3). At 4 h, the dipstick test was negative in 26 cases, of which 2 turned positive at 24 h. None of these 26 developed PEP. In 90 cases, the dipstick test remained positive at 4 h and 2 cases developed PEP. PEP did not develop in 6 cases that turned negative at 24 h. In 18 patients 24 h dipstick was not tested. The baseline test is not routinely performed but the results show that a dipstick test that turns negative during follow-up excludes PEP. The 24 h test was not performed on 82 patients (21%), who were discharged within a few hours after ERCP. The 4 h test was missing in 12 cases (Fig. 1).

Table 3.

Patients' Comorbidities and Pre-ERCP Dipstick Performance			
	Positive test n (%)	Negative test n (%)	***P****
Cancer	48 (39)	74 (26)	0.009
Renal failure	33 (27)	25 (9)	0.000
Diabetes	35 (29)	42 (15)	0.001
Heart and vascular disease	88 (72)	168 (58)	0.007
Elevated bilirubin	107 (88)	195 (70)	0.000
Pulmonary disease	10 (8)	33 (12)	0.291
ERCP endoscopic retrograde cholangiopancreatography; *Chi-square test			

We also studied the utility of the dipstick in all cases without information of 0h results. Four hours after ERCP the dipstick test was negative in 240 of 388 patients and after 24 h in 154 of 269 patients. 4 h after ERCP the dipstick test was positive in 139 (35%) patients without PEP. However, none of the patients who had negative dipstick test both 4 and 24 h after ERCP developed PEP (Figure 1 A and B).

One of the diagnostic criteria for PEP is abdominal pain and therefore we evaluated the diagnostic accuracy of a positive dipstick test together with abdominal pain. This increased accuracy in diagnostics of PEP. 4 h after ERCP dipstick tests sensitivity was 60%, specificity 99%, positive predictive value 64% and negative predictive value 98%. 24 h after ERCP the sensitivity was 100%, specificity 98%, positive predictive value 71% and negative predictive value 100%. The kappa

value between urine dipstick results and quantitative analysis 4 h after ERCP was 0.828 and 0.773 24 h after ERCP (Table 4).

Table 4

Diagnostic Performance of Urine Trypsinogen-2 dipstick Test With Abdominal Pain in Detecting PEP				
	Sensitivity (95% CI) %	Specificity (95% CI) %	PPV %	NPV %
4h dipstick positive and abdominal pain (12 tests missing)	60 (35-80)	99 (97-99)	64	98
24h dipstick positive and abdominal pain (129 tests missing)	100 (70-100)	98 (96-99)	71	100
PPV positive predictive value; NPV negative predictive value; CI confidential interval				

We further analyzed the characteristics of patients developing PEP. The 4 h dipstick test was performed in all patients with PEP, and it was positive in 9 of 15. The test was positive either at 4 or 24 h after ERCP in 12 of 15 PEP patients. In the remaining 3 patients with PEP the 24 h test was missing, all 3 pancreatitis were classified as mild PEP according to the Atlanta classification while 2 were moderately severe and one mild according to Cotton's classification. Five patients with a negative 4 h post ERCP dipstick test had mild and one moderately severe PEP according to the Atlanta classification, while 4 were moderately severe according to Cotton's classification. At 24 h, the dipstick test was performed on 9 of 15 PEP patients and it was positive in all cases. The concentration of urine trypsinogen-2 of cases developing PEP at different time points are shown in Figure 2.

Figure 2.

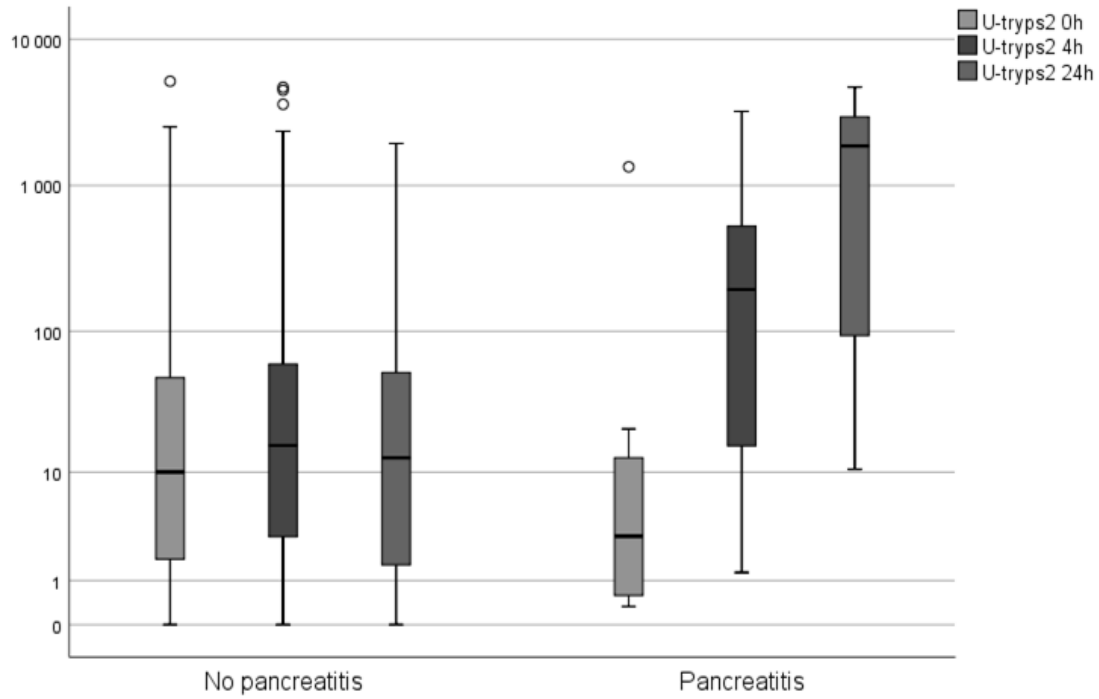

Figure 2.

Box plot of urinary trypsinogen concentration before, 4 h, and 24 h after ERCP in patients with and without ERCP-induced pancreatitis. The boxes show 25 and 27 percentiles and the whiskers 5 and 95 percentiles.

Discussion

A negative urine trypsinogen-2 dipstick test excludes PEP. Especially if both 4 and 24h tests are negative, PEP did not develop after ERCP. A positive test alone was not a strong indicator of PEP because 31% of the cases had a positive test due to various comorbidities. However, together with abdominal pain symptoms the urinary trypsinogen-2 dipstick test is very specific and sensitive for PEP 24 h after ERCP. Sensitivity increased with time reaching 100% 24 h after ERCP.

Three earlier studies have explored PEP diagnostics with the trypsinogen-2 dipstick test: Kemppainen et al (1997), Sankaralingam et al (2007), and Tseng et al (2011).[10-12] In these studies

the sample size has been smaller than in the present one (n = 106, 29, and 150 cases, of whom PEP developed in 11, 4, and 13, respectively). Our sample size was larger (n = 400) but we had only slightly more PEP cases (15) compared to them, although we only included cases with native papilla. Sankaralingam et al and Tseng et al excluded all patients with positive pre ERCP dipstick test (3.3% and 6.8% of the patients) from their studies. Kemppainen et al did not test dipstick before ERCP, but instead measured pre-ERCP urine trypsinogen-2 concentrations. They also excluded 6 (6%) patients with high pre-ERCP urine trypsinogen-2 levels. We tested dipstick before ERCP and the proportion of positive tests was surprisingly high (33%). We did not exclude patients with positive pre ERCP dipstick test from our study, but in addition, we analyzed also subgroups with positive and negative pre-ERCP separately.

Serum and urine trypsinogen-2 concentrations increase in many conditions such as pancreatic and biliary tract malignancies, biliary stones, as well as in biliary and hepatic inflammations.[18,19] The patients in this study with a positive pre-ERCP dipstick test had more comorbidities than patients testing negatively. Among the positive patients there were remarkably more cases with cancer, heart and vascular diseases, and diabetes as well as higher bilirubin levels. When we excluded all patients with a positive pre-ERCP dipstick results, our results turned out similar to those in previous studies.[8,10-12]

In our study, all 6 PEP patients with negative 4 h dipstick test had urine tyrpsinogen-2 concentrations below 50 µg/l. The dipstick test turned positive in all three patients who had follow-up tests done 24 h after ERCP although their urine trypsinogen-2 concentrations did not exceed 20 µg/l. This indicates that urine trypsinogen-2 concentrations increase slowly in some PEP cases and the test is quite sensitive. Urine trypsinogen-2 is a sensitive marker for acute pancreatitis with reference range for heathy individuals 0.3-11 µg/l [16] and for acute pancreatitis patients the urine typsinogen-2 concentrations exceeded 11-18 µg/l.[7,16] The higher sensitivity of the dipstick test than

of the quantitative IFMA can be explained by partial loss of trypsinogen-2 during storage. We have earlier shown that the dipstick test with a detection limit of 50 µg/l is a better test for acute pancreatitis than the corresponding IFMA. Urine trypsinogen-2 concentration rises 10-fold within the first 2 h of onset of the disease and the concentration stays elevated for weeks. In some PEP patients the concentration increases slowly indicating that pancreatitis develops gradually. Thus, all patients tested at 24 h were positive giving 100 % sensitivity.

The urine T-2 dipstick test is a cheap method that can be analyzed without laboratory facilities, it is easier to perform and faster to analyze compared to blood test, and therefore it could be used more widely in endoscopic units. Since the result of dipstick test is readable within 5 minutes but amylase result can easily take one hour, led to earlier PEP diagnosis in 9 of 15 PEP patients with trypsinogen dipstick test in this study.

There are some limitations in this study. We had only 15 PEP cases, which is, however, more than in previous dipstick studies due to our larger study size. The PEP rate here was equal to our previous studies in patients with native papillae 3.8%.[20,21] We did not obtain urine at 24 h from all patients, mainly because 21% were discharged within a few hours after ERCP. The lack of some 24 h samples hampers this test reliability, since we do not know what the test results would have been in these cases. If the 24 h test would have been negative in all 3 PEP cases with missing 24 h tests, the overall sensitivity and false negative rate would have been worse than we calculated; 80% and 20%, respectively.

A negative dipstick test for trypsinogen-2 in urine can be used to exclude PEP with high accuracy. A positive test results detects PEP with high sensitivity but specificity is low because about 30 % of the patients have a positive test before ERCP. However, the combination of a positive dipstick test and abdominal pain detects PEP with high sensitivity and specificity.

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