Urine Trypsinogen 2 Dipstick for the Early Detection of Post-ERCP Pancreatitis

Principal Investigator: Jeffrey Easler, MD

Assistant Professor of Medicine
IU School of Medicine

Table of Contents:

1.0 Background

Post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) is the most common, serious complication of ERCP and accounts for a significant proportion of morbidity, mortality, and health care expenditures accrued in the post procedure setting.[3-5] Large studies have shown that PEP occurs approximately 4-10% procedures with a mortality rate up to 0.7%.[6-8]

ERCP is most often performed in the outpatient setting with the expectation for patients to be discharged home the same day of the procedure. Patients are often observed for symptoms of complications after procedures. Data suggests prolonged observation (4-6 hours) captures symptoms indicative of a complication in 80% of these patients and consequently prevents un-intended discharge with subsequent hospitalizations.[9] However, a standardized observation protocol for this interval of time is not practical at ERCP centers. Given 11% of patients require admission after ERCP and fewer patients ultimately are verified as having complications; the majority of centers do not have a 4-6 hour observation protocol for post-ERCP care. Moreover, such a protocol for all ERCP outpatients would create unnecessary costs, strain on recovery room resources (staff, nursing and space), and significant inconvenience for patients.[10] While several factors including peri-procedural pain, history of pancreatitis, and performance of sphincterotomy predict need for hospital admission after ERCP, no single clinical factor has proven accuracy. Complex models for predicting admission are cumbersome and struggle to achieve a AUC ROC beyond 0.90 for predicting need for admission, much less post ERCP pancreatitis.[11] However, while same-day discharge after ERCP is widely utilized and relatively safe, it has been associated with readmissions.[9] We have reported rates of inadvertent discharge and readmission of outpatients with post ERCP pancreatitis to occur in up 25%. [12] A recent review of patients enrolled in a prospective study, at risk for post ERCP pancreatitis demonstrated that rate of inappropriate discharge may occur in over 50% of patients. This delay in recognition may be clinically important as this represents a missed opportunity for timely interventions. For instance, early intravenous fluid hydration after the development of pancreatitis can reduce the morbidity and mortality at least 3-fold.[12,13]

By definition, a confirmatory diagnosis of post ERCP pancreatitis requires an assessment well after the ERCP procedure. Post ERCP pancreatitis is defined by the presence of two of three criteria: characteristic abdominal pain, elevation in serum lipase or amylase three times greater than the upper limit of normal assessed 24 hours following ERCP, and characteristic findings of acute pancreatitis on cross sectional imaging.[14].[15] Consequently, PEP is confirmed for the majority of patients with PEP symptoms in the inpatient setting a day or more after the ERCP procedure. This delay in confirmation requires empiric admission and treatment for PEP for many patients that develop suspicious symptoms after. Early measurement of serum amylase and lipase levels have been studied; however offer limited accuracy and are by no means practical as a rapid, bedside

assessment is not possible given time need for serum collection, transport and processing.[16-19] Also, elevated serum pancreatic enzymes are common in asymptomatic patients after ERCP (up to 75% of patients) with confirmed clinical pancreatitis being much less common.[20] Therefore, an early diagnostic tool is yet to be identified.

Trypsinogen is the main proteolytic proenzyme in pancreatic secretions. Activation of trypsinogen and other proenzymes is part of the initial pathobiology of acute pancreatitis. Trypsinogen-2 is an isoenzyme that peaks at 6 hours with a median level of 1790 ug/L in patients with pancreatitis, compared to 3.6ug/L in patients without.[21,22] The isoenzyme trypsinogen-2 is both preferentially elevated in pancreatitis[23] and poorly reabsorbed in the renal tubules[24], making this a potentially useful early diagnostic marker for acute pancreatitis. In contrast, serum amylase peaks at 12 hours and lipase 24 hours after pancreatitis. While amylase and lipase are currently the established diagnostic tests for the diagnosis of PEP; the delay in physiologic peak is likely the reason their utility as an early marker for PEP is limited. A urine trypsinogen-2 dipstick (UTDT) is commercially available for purchase ($3.18/unit) with promising studies for accuracy of its cut point for pancreatitis. Trypsinogen-2 urine testing for PEP potentially offers several advantages including an earlier peak concentration, ease of deployment (at the bedside), interpretation (positive/negative test strip) and a low cost.

Actim Pancreatitis (Medix Biochemica) is a urine trypsinogen-2 dipstick test (UTDT) that uses trypsinogen-2 as a biomarker for acute pancreatitis. At a cutpoint of 50 ug/L it has been assessed in 3 small prospective studies for the diagnosis of PEP.[1,25,26] Kemppainen et al.[25] demonstrated an adequate sensitivity (81%) and specificity (97%) with the UTDT for diagnosing PEP at 6 hours after ERCP in 106 patients. Limitations include the impracticality of a 6 hour testing interval after ERCP and unclear inclusion/exclusion criteria. Sankaralingam et al.[26] demonstrated a very high sensitivity (100%) and specificity (96%) with the UTDT for diagnosing PEP at 4 hours after ERCP, but was limited by the small sample size of 29 patients. Tseng et al.[1] demonstrated a sensitivity of 85%, specificity of 97%, and a superior accuracy of 96% compared to serum amylase/lipase for the diagnosis of PEP at 3 hours after ERCP in 150 patients, but limitations include a high number (100) of excluded patients. A meta-analysis of these available studies calculated an overall sensitivity of 86%, specificity of 94% and area under the operator curve of 0.92. [27] However, as mentioned above, the published studies suffer from variation in timing of UTDT, inclusion/exclusion criteria, and lack of rigorous study design with adequately powered sampled size. Consequently, further study is needed to assess its accuracy of UTDT in the post ERCP setting.

The primary study objective is to determine the test characteristics of UTDT for the diagnosis of PEP 2 hours post ERCP.

2.0 Rationale and Specific Aims

Post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) is the most common, serious complication of ERCP. More than 500,000 ERCP procedures are performed from which 25,000 cases of PEP occur in the U.S.A. annually. PEP accounts for significant morbidity and health care expenditures. While symptoms of PEP arise immediately after ERCP, they are non-specific. Consequently, unnecessary admissions of outpatients without PEP and inadvertent discharge of outpatients with PEP from ERCP recovery are common. An accurate, confirmatory test for diagnosis of PEP immediately after ERCP is lacking. Urine Trypisinogen-2 Dipstick test (UTDT) is a simple, inexpensive test with promising preliminary data for accuracy for immediate diagnosis of PEP. Prior studies of ITDT test characteristics lack rigorous scientific design.

Proposed Study and Methods:
We will enroll 1825 ERCP outpatients at our institution in a prospective cohort study. A pre-ERCP UTDT test and diagnostic UTDT 2 hours after the ERCP will be performed. Patients with a positive baseline UTDT will be followed clinically as part of this study without 2 hour testing. Care providers and study primary investigators will be blinded to the 2 hour UTDT results. Baseline, intra-procedure and recovery room clinical data will be recorded. Diagnosis of PEP will be made blinded to the UTDT result. Admission status for PEP will be assessed by review of records and phone/e-mail contact 5 days and 30 days after ERCP procedure. Sensitivity and specificity of 2 hour post ERCP UTDT for the diagnosis of PEP will be calculated.

Aims: 1) To determine the test characteristics of UTDT for the diagnosis of PEP 2 hours after completion of ERCP. 2) To identify and describe patients with baseline UTDT positivity, in whom this test offers limited utility.

Specific Aims:

Primary Aim

1.1 To determine the test characteristics (sensitivity and specificity) of the UTDT for the diagnosis of PEP:

Hypotheses:

1) UTDT is a sensitive and specific test for the diagnosis of PEP for patients undergoing ERCP.
2) At a cutpoint for a negative test of <50ug/L, UTDT will be accurate at 2 hours post ERCP in patients whose baseline test is negative.

Secondary AIM:

1.1 To identify and describe patients with baseline UTDT positivity.

Hypothesis:

1) A subgroup of patients will demonstrate baseline positivity for UTDT. Based on previous studies, the subgroup is likely to include patients with chronic inflammatory or obstructive conditions of the pancreatic duct (pancreatic ductal adenocarcinoma, obstructive chronic pancreatitis)[1] This subgroup will represent a minority (<6%) of outpatients presenting for ERCP. [1]

3.0 Inclusion/Exclusion Criteria

Inclusion Criteria	18 years of Age Undergoing Outpatient ERCP
Exclusion Criteria	Unwillingness or inability to consent for the study Acute pancreatitis on presentation or within 1 month Recent ERCP (i.e. within 2 Weeks) Known stage 3B or higher renal disease (GRF less than 45) and/or oliguria per Pre-_ERCP labs or outside hospital labs within 7 days prior to the 2-hour Post- ERCP urine collection procedure. History of renal transplant Consumed supplemental Biotin within 7 days prior to enrollment Total Pancreatectomy MRCP with secretin within 48 hours prior to ERCP Inability to access the ampulla at ERCP attempt (unable to attempt cannulation of the ampulla or minor papilla, e.g. gastric outlet obstruction) Unable to collect Baseline or Post -ERCP urine sample.

4.0 Enrollment/Randomization

Patients will be recruited in the pre-procedure area prior to ERCP. Only patients who would be offered ERCP as part of their previously outlined care plan will be included in this study. Patients will be screened according to inclusion/exclusion criteria described above.

5.0 Study Procedures

Informed Consent: Eligible patients will sign an IRB-approved, written informed consent to verify their willingness to participate in this study. Informed consent will be obtained on the day of their scheduled ERCP. Consent for the study will be obtained by one of the participating endoscopists and/or a research assistant. Patients will receive a copy of the signed and dated informed consent document. Original informed consent documents will be maintained on-file. A note may be made in the subject's medical record regarding participation in the research study. Once consented and enrolled into the trial, patients will be issued a unique identifier for the purposes of data entry.

Pre-Procedure data and sample collection: After obtaining informed consent, patients will be asked health questions prior to ERCP to assess and quantify current and prior alcohol use, current and prior tobacco use, quantification of narcotic use and quantification of non-steroidal anti-inflammatory drug (NSAID) use. Narcotic and NSAID use in the 7 days prior to ERCP will be recorded. Abdominal pain scores will be collected at baseline and post ERCP. Subjects will be shown the study pain scale (appendix 1) and asked to pick a number on the scale to rate their abdomial pain at baseline and post ERCP. Amount and type of IV fluids given Pre-ERCP may be collected. Post- ERCP Pain scores will be attempted 2 -3 hours Post –ERCP, but if necessary may be collected prior to the 2 hours Post –ERCP time point up to the time of patient discharge by the study research technician.

Data will be collected on the indication of ERCP, history of acute pancreatitis, recurrent acute pancreatitis, or chronic pancreatitis, history of pancreatic surgery, presence of peri-pancreatic fluid collection and prior sphincterotomy. Data will also be collected to note if subjects underwent an endoscopic ultrasound with fine needle aspirate during the same visit as baseline ERCP. Prior imaging may be reviewed for the presence of pancreatic mass and/or pancreatic duct obstruction.

Baseline tests may be obtained prior to undergoing ERCP including serum pancreatic enzymes (amylase, lipase), serum liver enzymes, and coagulation profile, as is the standard for our endoscopy unit. Available outpatient laboratory data within 48-72 hours of ERCP will be reviewed. If Pre- ERCP baseline lab

results are not yet available at the time of the 2 hour Post- ERCP urine test this will not be a criterion for exclusion from the study. If the GFR is less than 45 after the 2 hour Post ERCP urine collection has been completed, these patients will not be contacted for the 5 day and 30 day follow-up call and their data will not be included in the final analysis.

Patients with baseline, pre-procedure positivity for UTDT (> or = 50ug/L) will undergo all study procedures as outlined below with the exception of 2 hour UTDT level.

Patients who are unable to provide a urine sample at baseline or for Post- ERCP testing will be considered a study screen failure. Subjects who provided a baseline urine sample will still be included in the final analysis of baseline UTDT level. Subjects who are excluded due to the inability to access the ampulla at ERCP will also still be Included in the final analysis of baseline UTDT level.

Intra-procedural Data Collection: Patients will undergo ERCP as intended. All clinical decisions and endoscopic interventions prior to, during and after ERCP will be performed at the discretion of the treating physician. Data on all ERCP procedure findings and interventions including, duration of procedure, placement of pancreatic or biliary duct stents, ERCP maneuvers deployed for cannulation (e.g. needle knife), findings associated with chronic pancreatitis (e.g pancreatic duct stones), pancreatic duct leak, peri-procedural NSAIDs administration and any immediate complications including perforation, bleeding, or hemodynamic instability will be recorded. Amount and type of intraoperative IV fluids may be collected.

Immediate Postoperative Care, Data and Sample collection: At our institution, patients typically remain in the recovery area for 2-3 hours after completion of the ERCP. This time interval in part is related to two-phase recovery after general anesthesia, which is the standard sedation approach for ERCP outpatients. Patients may remain for recovery after ERCP for two hours for a post procedure observation period and collection of UTDT result. During this time, urine sample collection for UTDT testing will be attempted 2 -3 hours Post –ERCP, but if necessary may be collected prior to the 2 hours Post –ERCP time point up to the time of patient discharge by the study research technician. Patients, study investigators, ERCP care team, nursing staff will be blinded to the results. The statistician will be blinded to the urine trypsinogen-2 hour test during statistical analysis.

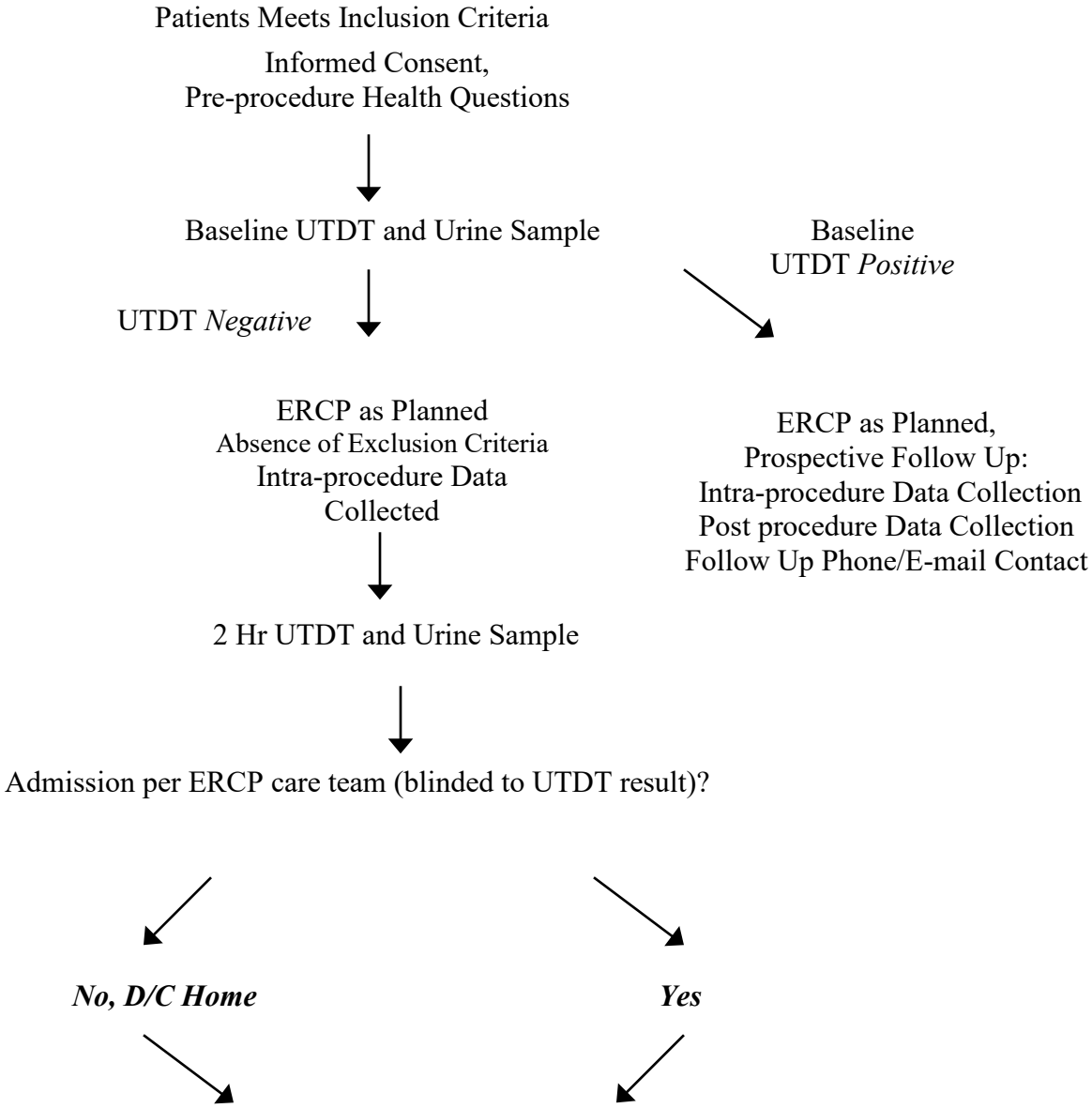
The decision to admit/monitor the patient will be left to the discretion of the endoscopist / ERCP care team and will occur blinded to the UTDT results. Data regarding symptoms (e.g. nausea/vomiting, abdominal pain scores), anti –emetic use and narcotics administered for pain symptoms, duration in recovery and decision to admit or discharge will be recorded. Volume and type of IV fluids administered in recovery may also be collected. Patients will receive a risk assessment for post-ERCP pancreatitis. This will calculated and tracked for the purposes of this study.

Follow Up Data Collection: Patients who are hospitalized will have serum amylase and lipase drawn after the ERCP procedure per standard clinical protocol timelines. Patients who are discharged to home/other after ERCP will be contacted by telephone and/or email 5 days (+ 4 days or – 2 days) and 30 days (+ or – 10 days) after the ERCP by a study team member to determine whether PEP occurred and was managed outside of our institution. Patients will be asked about ERCP procedure complications such as infection, bleeding, perforation and death. This contact may be made by phone, email or text message. Subjects will be asked for their preferred method of contact at study enrollment. Records confirming post ERCP pancreatitis will be requested if the patient is admitted and/or initially managed at an outside institution for PEP. Patients without hospitalization for symptoms attributable to post ERCP pancreatitis will be classified as not having developed PEP. Records pertaining to any ERCP procedure complications will also be requested.

PEP will be defined by the primary ERCP team. The ERCP team will be blinded to the UTDT result when determining PEP.

Clinical data regarding volume and type of IV fluids administered at 24, 48 hours of admission and severity of pancreatitis (based on Cotton criteria, Modified Marshall Score and graded presence of necrosis) will be assessed recorded for all patients admitted with PEP.

Figure 2: Study Flow Diagram for Outpatient ERCP Subjects



Follow Up Procedures:

1) Clinical Follow Up and Data collection for Hospital Course if Admitted
2) Day 5 phone/email contact if outpatient
3) Day 30 phone/email if outpatient

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

An adverse event will be considered a complication that occurred due to the collection of urine for testing or administration of health questions. Associated adverse events will be reported to the IRB per the IRB reporting criteria.

Study Withdrawal/Discontinuation

Patients may withdraw from the study at any time upon their request. Data collected prior to their withdrawal may still be used in the final analysis.

7.0 Statistical Considerations

Descriptive statistics will be performed for all continuous variables (mean ± standard deviation for normally distributed variables and median ± interquartile range for non-normal variables) and categorical variables (count and proportion). Categorical outcomes will be analyzed using X^2 test or Fisher's test for small samples. Comparison of group outcomes will be analyzed using Student's t test for normally distributed data and nonparametric Wilcoxon-Mann-Whitney test for data that violate the normality assumption. Univariate logistic regression analysis will be used to identify predictors of interest and multiple predictor analysis will estimate effects adjusted for covariates.

Diagnostic test characteristics including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy will be assessed for the UTDT in diagnosing PEP and for different severity levels of PEP. In addition, the UTDT will be assessed in predicting additional outcomes as outlined above.

Specific pre-procedure, intra-procedure, or post-procedure characteristics as noted above will be assessed in univariate and multivariable regression models to assess whether they contribute to the inaccurate diagnosis of PEP based on the UTDT. Similar multivariable regression analysis will be done to assess for additional risk factors of PEP.

A cost analysis will also be performed to determine the number of UTDT strips needed to prevent one inadvertent discharge of a patient with PEP.

8.0 Privacy/Confidentiality Issues

Each subject will be assigned a unique study number. Study material will be kept on a limited number of password protected computer stations, and paper records will be kept in locked cabinets/offices in areas with limited public access. Study material will be retained until seven years after study closure, at which point records will be destroyed, and hard drives containing study data will be erased.

9.0 Follow-up and Record Retention

We anticipate a 30-month enrollment period to achieve our estimated sample size. We expect a 30- 40 day period from the onset of patient enrollment to completion of follow-up. This does not include final statistical analysis and interpretation. Data collected for research purposes will be retained for seven years after the study is closed. At that time, all paper and electronic records pertaining to this study will be destroyed.

Literature Cited

1. Tseng CW, Chen CC, Lin SZ, Chang FY, Lin HC, Lee SD. Rapid urinary trypsinogen-2 test strip in the diagnosis of pancreatitis after endoscopic retrograde cholangiopancreatography. Pancreas 2011;40:1211-4.
2. Cotton PB, Lehman G, Vennes J, et al. Endoscopic sphincterotomy complications and their management: an attempt at consensus. Gastrointest Endosc 1991;37:383-93.
3. Fagenholz PJ, Fernandez-del Castillo C, Harris NS, Pelletier AJ, Camargo CA, Jr. Direct medical costs of acute pancreatitis hospitalizations in the United States. Pancreas 2007;35:302-7.
4. Freeman ML, Nelson DB, Sherman S, et al. Complications of endoscopic biliary sphincterotomy. N Engl J Med 1996;335:909-18.
5. Gottlieb K, Sherman S. ERCP and biliary endoscopic sphincterotomy-induced pancreatitis. Gastrointest Endosc Clin N Am 1998;8:87-114.
6. Andriulli A, Loperfido S, Napolitano G, et al. Incidence rates of post-ERCP complications: a systematic survey of prospective studies. Am J Gastroenterol 2007;102:1781-8.
7. Kochar B, Akshintala VS, Afghani E, et al. Incidence, severity, and mortality of post-ERCP pancreatitis: a systematic review by using randomized, controlled trials. Gastrointest Endosc 2015;81:143-9 e9.
8. Masci E, Mariani A, Curioni S, Testoni PA. Risk factors for pancreatitis following endoscopic retrograde cholangiopancreatography: a meta-analysis. Endoscopy 2003;35:830-4.
9. Freeman ML, Nelson DB, Sherman S, et al. Same-day discharge after endoscopic biliary sphincterotomy: observations from a prospective multicenter complication study. The Multicenter Endoscopic Sphincterotomy (MESH) Study Group. Gastrointest Endosc 1999;49:580-6.
10. Cote GA, Lynch S, Easler JJ, et al. Development and Validation of a Prediction Model for Admission After Endoscopic Retrograde Cholangiopancreatography. Clin Gastroenterol Hepatol 2015;13:2323-32 e1-9.
11. Ho KY, Montes H, Sossenheimer MJ, et al. Features that may predict hospital admission following outpatient therapeutic ERCP. Gastrointest Endosc 1999;49:587-92.
12. Sagi SV, Schmidt S, Fogel E, et al. Association of greater intravenous volume infusion with shorter hospitalization for patients with post-ERCP pancreatitis. J Gastroenterol Hepatol 2014;29:1316-20.
13. Wall I, Badalov N, Baradarian R, Iswara K, Li JJ, Tenner S. Decreased mortality in acute pancreatitis related to early aggressive hydration. Pancreas 2011;40:547-50.
14. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013;62:102-11.

15. Testoni PA, Bagnolo F. Pain at 24 hours associated with amylase levels greater than 5 times the upper normal limit as the most reliable indicator of post-ERCP pancreatitis. Gastrointest Endosc 2001;53:33-9.
16. Gottlieb K, Sherman S, Pezzi J, Esber E, Lehman GA. Early recognition of post-ERCP pancreatitis by clinical assessment and serum pancreatic enzymes. Am J Gastroenterol 1996;91:1553-7.
17. Sutton VR, Hong MK, Thomas PR. Using the 4-hour Post-ERCP amylase level to predict post-ERCP pancreatitis. JOP 2011;12:372-6.
18. Testoni PA, Bagnolo F, Caporuscio S, Lella F. Serum amylase measured four hours after endoscopic sphincterotomy is a reliable predictor of postprocedure pancreatitis. Am J Gastroenterol 1999;94:1235-41.
19. Thomas PR, Sengupta S. Prediction of pancreatitis following endoscopic retrograde cholangiopancreatography by the 4-h post procedure amylase level. J Gastroenterol Hepatol 2001;16:923-6.
20. Pieper-Bigelow C, Strocchi A, Levitt MD. Where does serum amylase come from and where does it go? Gastroenterol Clin North Am 1990;19:793-810.
21. Rinderknecht H, Geokas M. Anionic and cationic trypsinogens (trypsins) in mammalian pancreas. Enzyme 1972;73:116-30.
22. Yadav D, Agarwal N, Pitchumoni CS. A critical evaluation of laboratory tests in acute pancreatitis. Am J Gastroenterol 2002;97:1309-18.
23. Itkonen O, Koivunen E, Hurme M, Alfthan H, Schroder T, Stenman UH. Time-resolved immunofluorometric assays for trypsinogen-1 and 2 in serum reveal preferential elevation of trypsinogen-2 in pancreatitis. J Lab Clin Med 1990;115:712-8.
24. Kemppainen EA, Hedstrom JI, Puolakkainen PA, et al. Rapid measurement of urinary trypsinogen-2 as a screening test for acute pancreatitis. N Engl J Med 1997;336:1788-93.
25. Kemppainen E, Hedstrom J, Puolakkainen P, et al. Urinary trypsinogen-2 test strip in detecting ERCP-induced pancreatitis. Endoscopy 1997;29:247-51.
26. Sankaralingam S, Wesen C, Barawi M, Galera R, Lloyd L. Use of the urinary trypsinogen-2 dip stick test in early diagnosis of pancreatitis after endoscopic retrograde cholangiopancreatography. Surg Endosc 2007;21:1312-5.
27. Jin T, Huang W, Jiang K, et al. Urinary trypsinogen-2 for diagnosing acute pancreatitis: a meta-analysis. Hepatobiliary Pancreat Dis Int 2013;12:355-62.
28. Coelho-Prabhu N, Shah ND, Van Houten H, Kamath PS, Baron TH. Endoscopic retrograde cholangiopancreatography: utilisation and outcomes in a 10-year population-based cohort. BMJ Open 2013;3.
29. Freeman ML, Guda NM. Prevention of post-ERCP pancreatitis: a comprehensive review. Gastrointest Endosc 2004;59:845-64.

Appendix 1

Pain Scale