

Original article

Automated spectrophotometric bicarbonate analysis in duodenal juice compared to the back titration method[☆]

Friedemann Erchinger^{a, b, *}, Trond Engjom^{b, c, 1}, Oddrun Anita Gudbrandsen^{b, 1}, Erling Tjora^{d, e}, Odd H. Gilja^{b, f}, Georg Dimcevski^{b, c}

^a Department of Medicine, Voss Hospital, Haukeland University Hospital, Bergen, Norway

^b Department of Clinical Medicine, University of Bergen, Bergen, Norway

^c Department of Medicine, Haukeland University Hospital, Bergen, Norway

^d Pediatric Department, Haukeland University Hospital, Bergen, Norway

^e Department of Clinical Science, University of Bergen, Bergen, Norway

^f National Centre for Ultrasound in Gastroenterology, Haukeland University Hospital, Bergen, Norway

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ABSTRACT

Objectives: We have recently evaluated a short endoscopic secretin test for exocrine pancreatic function. Bicarbonate concentration in duodenal juice is an important parameter in this test. Measurement of bicarbonate by back titration as the gold standard method is time consuming, expensive and technically difficult, thus a simplified method is warranted. We aimed to evaluate an automated spectrophotometric method in samples spanning the effective range of bicarbonate concentrations in duodenal juice. We also evaluated if freezing of samples before analyses would affect its results.

Methods: Patients routinely examined with short endoscopic secretin test suspected to have decreased pancreatic function of various reasons were included. Bicarbonate in duodenal juice was quantified by back titration and automatic spectrophotometry. Both fresh and thawed samples were analysed spectrophotometrically.

Results: 177 samples from 71 patients were analysed. Correlation coefficient of all measurements was $r = 0.98$ ($p < 0.001$). Correlation coefficient of fresh versus frozen samples conducted with automatic spectrophotometry ($n = 25$): $r = 0.96$ ($p < 0.001$)

Conclusions: The measurement of bicarbonate in fresh and thawed samples by automatic spectrophotometric analysis correlates excellent with the back titration gold standard. This is a major simplification of direct pancreas function testing, and allows a wider distribution of bicarbonate testing in duodenal juice. Extreme values for Bicarbonate concentration achieved by the autoanalyser method have to be interpreted with caution.

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Introduction

Direct pancreas function testing (DPFT) is invasive and thereby challenging. Used on correct indication it adds useful information

in exocrine pancreatic function testing. The test probably serves best as a second line test in situations where primary tests, as faecal elastase 1, are insufficient. DPFT can discriminate primary from secondary pancreatic dysfunction [1,2]. Furthermore, direct tests may prove useful in detecting early exocrine dysfunction, before development of clinical obvious pancreatic exocrine insufficiency. In our short endoscopic secretin test (short EST), duodenal juice aspiration is performed in the period from 30 to 45 min after secretin stimulation, in the plateau phase of duodenal bicarbonate concentration. The whole endoscopic procedure, including a diagnostic gastroscopy, lasts normally not longer than 20 min [1–3], hence overcoming some of the disadvantages of the time-

Abbreviations: DPFT, direct pancreas function testing; MDH, malatdehydrogenase; PEPC, phosphoenolpyruvate carboxylase.

^{*} The work was conducted at Haukeland University Hospital in Bergen, Norway.

^{*} Corresponding author. Voss Hospital, Medical Department, 5700 Voss, Norway. Tel.: +47 56533500; fax: +47 56513072.

E-mail address: friedemann.erchinger@helse-bergen.no (F. Erchinger).

¹ Engjom and Gudbrandsen have contributed equally to this work.

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consuming and cumbersome tube based tests [3–7]. To distinguish pancreatic exocrine failure from sufficient pancreas function a cut off of 80 mmol/L bicarbonate concentration in duodenal juice is generally accepted. Beneficial information about pancreatic exocrine insufficiency (PEI) can be obtained in a variety of patients with chronic pancreatitis, diabetes, cystic fibrosis, celiac disease. We have used our short EST to evaluate exocrine pancreatic function in patients with chronic pancreatitis, cystic fibrosis and diabetes [3,8–13].

Back titration has long been considered the gold standard for duodenal bicarbonate measurements [14,15]. This analysis is time consuming (minimum 2 h) and technically difficult, hence being expensive and vulnerable in routine diagnostics. As a consequence, only a few specialised or research centres perform this test today. Furthermore, back titration requires minimum 0.5 mL of duodenal juice. Such volumes are sometimes difficult to obtain from patients with severe ductal failure. In contrast, the auto-analyser used in this study requires only a few microlitres of duodenal juice and a short analysing time of 7 min. At present, autoanalysers are certified to quantify ingredients in blood or urine but not in duodenal juice. However, some earlier small studies have demonstrated a good correlation between auto-analysers and the back titration method [16,17]. Automation of bicarbonate analyses is required to simplify short EST, but the method still needs further validation to replace back titration. Daily routine in a busy medical institution makes immediate analyses of duodenal juice to a challenge, and instant freezing of samples could be an option for institutions sending samples for analyses elsewhere.

In this study, we aimed to demonstrate the accuracy of an automated spectrophotometric method compared to back titration when analysing bicarbonate in duodenal juice. Additionally, we studied if freezing of samples affected bicarbonate concentrations.

Materials and methods

Patients

The use of samples from short EST in the following projects was approved by the local ethical committee: Chronic pancreatitis or other causes of abdominal pain (approval no. 3.2008.2516), cystic fibrosis (approval no.: 2010/2857-7) and celiac disease (approval no. 2011/1592). Short EST of these patients was performed between September 2012 and October 2014. Samples were chosen at random for comparison of back titration and automated spectrometry. Three consecutive aliquots of duodenal juice with different bicarbonate concentrations are collected in 15 min during the short EST, hence 1 to 3 samples per patient could be analysed.

Short endoscopic secretin test

Secretin was administered intravenous at a dose of 1 CU per kg bodyweight, maximum 70 CU. Gastroscopy started 25 min after secretin administration. A diagnostic gastroscopy was initially performed to identify or exclude other pathological findings. All gastric juice was aspirated and discharged. After 30 min the tip of the endoscope was placed distal to the papilla Vateri. Duodenal juice was aspirated in three 5 min sequences. The procedure is illustrated in Fig. 1 and described in detail elsewhere [3].

Handling of duodenal juice before analysis

The pH and volume of each sample was measured. Duodenal juice with pH < 6 was discarded due to probable pollution from gastric juice. One aliquot of duodenal juice from each sampling period was immediately placed on ice and bicarbonate concentration was immediately analysed using back titration and automated analysis. Otherwise samples were frozen to $-196\text{ }^{\circ}\text{C}$. In the experiment

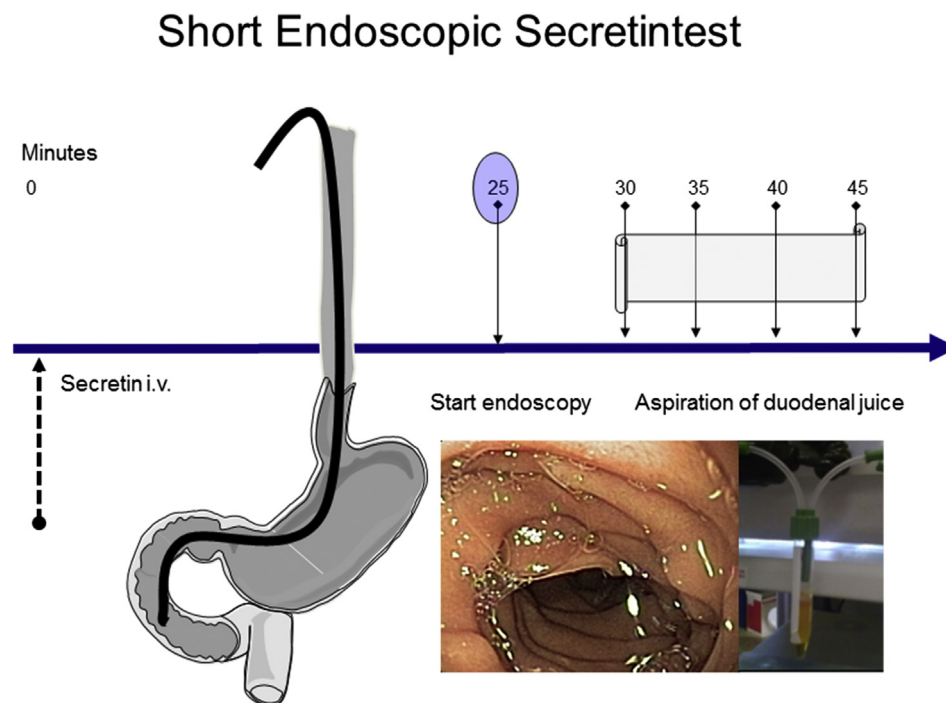


Fig. 1. Short endoscopic pancreas function testing (EST): 25 min after injection of secretin (1 CU/kg bodyweight, max 70 CU) an upper endoscopy was started. During the first 5 min a diagnostic gastroscopy was carried out. All juice from stomach and duodenum was discharged. Thereafter the tip of the endoscope was placed below the papilla; duodenal juice was collected in three aliquots of 5 min. The intervention requires only 20 min.

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