Új izotópdiagnosztikai és manometriás módszerek kifejlesztése és klinikai alkalmazása az epehólyag és az Oddi sphincter funkcionális megbetegedéseinek kimutatására

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Development and clinical application of new scintigraphic and manometric methods for the diagnosis of functional disorders of the gallbladder and the sphincter of Oddi

Egyetemi doktori (PhD) értekezés

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The studies described in this thesis were carried out in part at Hvidovre Hospital, University of Copenhagen, and at Århus Kommunehospital, University of Århus, in Denmark, under the guidance and supervision of Peter Funch-Jensen MD, DMSc.
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Preface and acknowledgements

At the beginning of my research career, some twelve years ago while I was a young resident in the Nuclear Medicine Department, Professor János Lonovics provided with an opportunity to study as a member of his scientific team in the First Department of Medicine. I deeply appreciate the valuable knowledge that I was able to learn from him on the functional biliary diseases included both basic science and clinical aspects. The economical support, which covered the development of our motility lab and permitted the clinical investigations are gratefully acknowledged. My research was focused on gastrointestinal motility and especially the non-invasive diagnosis of functional biliary disorders. Functional diseases of the biliary tract are not life-threatening, but they may cause severe pain syndromes and significant morbidity. The diagnosis of functional abnormalities at any site in the gastrointestinal tract is quite difficult, and this is particularly true of the biliary tract. The present studies were carried out during the years from 1990 to 2000, and were aimed at the development of new scintigraphic and manometric methods with a view to improving the diagnostic efficacy in patients with functional biliary disorders.

I am greatly indebted to Professor László Csernay, my teacher in the field of nuclear medicine. He introduced me to scientific research based on nuclear medicine, and later furnished a possibility for close cooperation. After I left the Nuclear Medicine Department to become an internist, he continued to regard me as an honorary member of his scientific staff. I am extremely grateful for his never-failing support and valuable criticism. After his retirement, his successor, Professor László Pávics helped with the continuation of these studies, and our scientific cooperation is still ongoing.

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impressed I was about his open mind, his obvious skills and his kind personality. Afterwards we remained in contact and he then gave me the possibility to do scientific work with him in Denmark from 1996 to 1997. Thanks to him, during this period I was a recipient of the Eötvös Fellowship of the Hungarian Ministry of Education and a Scientific Fellowship of Hvidovre Hospital, University of Copenhagen, Denmark. With this financial support, I could spend an unforgettable year in his motility laboratory in Copenhagen. Since then, I have visited Denmark repeatedly for short periods and our scientific cooperation is still promising. Meanwhile I have become his friend and family. I will never forget the enthusiastic way in which he strived to improve my skills in the practice of gastrointestinal endoscopy and manometry, which was always accompanied by respect and a sense of humour. His assistance was greatly conducive to my accomplishing these studies.

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THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS TO WHICH ARE CITED IN THE TEXT USING ROMAN NUMERALS I-VI:

I. Madácsy, L., Toftdahl, D., Middelfart, H.V., Hojgaard, L., Funch-Jensen, P. Comparison of the dynamics of bile emptying by quantitative hepatobiliary scintigraphy before and after cholecystectomy in patients with uncomplicated gallstone disease. Clinical Nuclear Medicine, 24(9): 649-654, 1999 (IF: 0.283)

II. Madácsy, L., Velősy, B., Szepes, A., Szilvássy, Z., Pávics, L., Csernay, L., and Lonovics, J. Effect of nitric oxide on the gallbladder motility in patients with acalculous biliary pain - a cholescintigraphic study. Digestive Diseases and Sciences (accepted 1997) (IF: 1.972)

III. Madácsy, L., Middelfart, H.V., Matzen, P., Hojgaard, L., Funch-Jensen, P. Quantitative hepatobiliary scintigraphy and endoscopic sphincter of Oddi manometry in patients with suspected sphincter of Oddi dysfunction: assessment of flow-pressure relationship in the biliary tract. European Journal of Gastroenterology and Hepatology, 12: 777-786, 2000 (IF: 1.589)

IV. Madácsy, L., Middelfart, H.V., Matzen, P., Funch-Jensen, P. Video manometry of the sphincter of Oddi: a new aid for interpreting manometric tracings and excluding manometric artefacts. Endoscopy, 32(1): 22-26, 2000 (IF: 1.634)

V. Madácsy, L., Velősy, B., Lonovics, J., Csernay, L.: Differentiation between organic stenosis and functional dyskinesia of the sphincter of Oddi with amyl nitrite -augmented quantitative hepatobiliary scintigraphy. European Journal of Nuclear Medicine, 21: 203-208, 1994 (IF: 2.690)

VI. Madácsy, L., Velősy, B., Lonovics, J., Csernay, L.: Evaluation of results of the prostigmine-morphine test with quantitative hepatobiliary scintigraphy: a new method for the diagnosis of sphincter of Oddi dyskinesia. European Journal of Nuclear Medicine, 22: 227-232, 1995 (IF: 2.930)

CITATIONS OF THE BASE REFERENCES

1. Stotland, B.R., Kochman, M.L.: Biliary motility. Current Opinion in Gastroenterology, 12: 482-490, 1996, Number of Cited Refs: 75.
2. Behr, T., Gratz, S., Becker, W.: Sphincter of Oddi dysfunction in idiopathic recurrent pancreatitis. Deutsche Medizinische Wochenschrift, 121: 1312-1313, 1996, Number of Cited Refs: 8.
3. Sauerbruch, T., Stoschus, B., Willkomm, P., Neubrand, M.: Diagnosis and treatment of dyskinesias of the sphincter of Oddi. Deutsche Medizinische Wochenschrift, 122: 547-550, 1997, Number of Cited Refs: 33.
4. Krishnamurthy, S., Krishnamurthy, G.T.: Biliary dyskinesia – Role of the Sphincter of Oddi, gallbladder and chlolecystokinin. Journal of Nuclear Medicine, 38: 1825-1830, 1997, Number of Cited Refs: 45.
5. Tzovaras, G., Rowlands, B.J.: Diagnosis and treatment of sphincter of Oddi dysfunction. British Journal of Surgery, 85: 588-595, 1998, Number of Cited Refs: 81.
6. Allescher, H.D.: Clinical impact of sphincter of Oddi manometry, Endoscopy, 30: 231-236, 1998, Number of Cited Refs: 57.
7. Schöfl, R.: Diagnostic Endoscopic Retrograde Cholangiopancreatography. Endoscopy, 33: 147-157, 2001, Number of Cited Refs: 4.

LIST OF PUBLICATIONS RELATED TO THE SUBJECT AND CITED IN THE TEXT USING ROMAN NUMERALS (full papers and book chapters are in bold letters)

VII. **Lonovics, J., Velősy, B., Madácsy, L.: (Motility disorders of the biliary tract: pathogenesis and medical treatment) Epeút-motilitási zavarok pathomechanizmusa és gyógyszeres kezelése. Magyar Belorvosi Arcivum, 2: 85-95, 1993**

VIII. **Lonovics, J., Velősy, B., Madácsy, L.: Sphincter of Oddi dyskinesia, in Zagoni T (ed): The papilla of Vater. Budapest, Melania Publishing Ltd., pp 125-163, 1995**

IX. **Velősy, B., Madácsy, L.: (Diagnostic methods for investigation of gallbladder and sphincter of Oddi motility) Az epehólyag motilitás vizsgáló módszerei, Az Oddi-sphincter motilitás vizsgáló módszerei In: Lonovics, J., Simon, L., Forgács, A., Wittmann, T., Bálint, A. (eds): Gastrointestinális motilitás. Budapest, Medicom, pp 215-223, 1996**

X. **Madácsy, L.: (Diagnosis of diseases of the gallbladder and the biliary tract - Scintigraphic methods) Az epehólyag és epeutak megbetegedéseinek diagnosztikus eljárásárai – Izotópdiagnosztikai eljárások In: Varró V (ed): Gastroenterologia. Budapest, Medicina, pp 543-545, 1997**

XI. **Lonovics, J., Madácsy, L., Szepes, A., Szilvássy, Z., Velősy, B., Varró, V.: Humoral mechanisms and clinical aspects of biliary tract motility. Scand J Gastroenterol, 33: 73-89, 1998 (IF: 2.360)**

XII. **Madácsy, L., Szepes, A., Lonovics, J.: (Functional disorders of the biliary tract) Funkcionális epeúti megbetegedések In: Lonovics, J., Simon, L., Tulassay, Zs., Újszászi, L., Wittmann, T. (eds): Funkcionális gastroenterologiai kórképek. Budapest, Medicom, pp: 129-140, 1999**

XIII. Szepes, A., Madácsy, L., Lázár, M., Velősy, B., Lonovics, J., Pávics, L.: Differentiation between intrahepatic and extrahepatic cholestasis by means of quantitative hepatobiliary scintigraphy (QHBS). Eur J Nucl Med, 25: A578 (Abstract), 1998 (IF: 2.564)

XIV. Madácsy, L., Velősy, B., Takács, T., Szepes, A., Lázár, M., Csernay, L., Lonovics, J.: Comparison of the results of endoscopic sphincter of Oddi manometry and quantitative hepatobiliary scintigraphy in patients with acalculous biliary pain and intact gallbladder. Eur J Nucl Med, 23: A119 (Abstract), 1996 (IF: 3.097)

XV. Madácsy, L., Szepes, A., Bertalan, V., Lazar, M., Pávics, L., Lonovics, J.: Enhancement of gallbladder filling and caerulein-induced emptying after glyceryl trinitrate administration in patients with gallbladder dyskinesia. Gastroenterology, 118(4): A1253 (Abstract), 2000 (IF: 10.330)

XVI. Várkonyi, T.T., Róka, R., Lengyel, C., Légrády, P., Madácsy, L., Velősy, B., Kempler, P., Pávics, L., Lonovics, J.: Impairment of gallbladder and stomach emptying in patients with

diabetic autonomic and peripheral sensory neuropathy. Gastroenterology, 116: G4759 (Abstract), 1999 (IF: 10.330)

XVII. **Várkonyi, T.T., Lengyel, C., Madácsy, L., Velősy, B., Boda, K., Kempler, P., Fazekas, T., Csernay, L., Lonovics, J.: [Gallbladder hypomotility in diabetic polyneuropathy] Epehólyag-hypomotilitás diabeteses polyneuropathiában. Orv Hetil, 138: 1177-1182, 1997**

XVIII. Madácsy, L., Middelfart, H.V., Matzen, P., Lonovics, J., Funch-Jensen, P.: Endoscopic retrograde cholangiographic videomanometry of the sphincter of Oddi: some physiological observations. Gastroenterology, 116: G0084 (Abstract), 1999 (IF: 10.330)

XIX. Madácsy, L., Middelfart, H.V., Matzen, P., Funch-Jensen, P.: The effect of duodenal motility on the sphincter of Oddi (SO) pressure profile, studied by endoscopic sphincter of Oddi manometry (ESOM) combined with real-time endoscopic picture (RTEP) analysis. Endoscopy, 29: P543 (Abstract), 1997 (IF: 1.380)

XX. Bertalan, V., Madácsy, L., Szepes, A., Lázár, M., Pávics, L.: Efficacy of amyl nitrite-augmented quantitative hepatobiliary scintigraphy (QHBS) in the assessment of clinical staging of sphincter of Oddi dysfunction. Z Gastroenterol, 38: A20 (Abstract), 2000 (IF: 0.890)

XXI. Madácsy, L., Middelfart, H.V., Matzen, P., Funch-Jensen, P., Hojgaard, L.: Comparison of the results of cholecystokinin-augmented quantitative hepatobiliary scintigraphy and endoscopic sphincter of Oddi manomery. Scand J Gastroenterol, 32: A72 (Abstract), 1997 (IF: 1.641)

XXII. Madácsy, L., Middelfart, H.V., Matzen, P., Funch-Jensen, P., Hojgaard, L.: Cholecystokinin-augmented quantitative hepatobiliary scintigraphy and sphincter of Oddi manometry in patients with suspected sphincter of Oddi dyskinesia - is it a real provocation test? Eur J Nucl Med, 24: A363 (Abstract), 1997 (IF: 2.692)

XXIII. Szepes, A., Madácsy, L., Bertalan, V., Lázár, M., Pávics, L., Middelfart, H.V., Funch-Jensen, P., Lonovics, J.: Effect of caerulein on the sphincter of Oddi and duodenal motility in patients with suspected sphincter of Oddi dysfunction. Neurologastroenterol Mot, 11: A293 (Abstract), 1999 (IF: 1.692)

XXIV. Szepes, A., Madácsy, L., Velősy, B., Szilvássy, Z., Nagy, I., Pávics, L., Lonovics, J.: Impaired nitric oxide-mediated relaxation of the sphincter of Oddi (SO) in patients with hyperlipidaemia and suspected SO dysfunction. Gastroenterology, 116: G4730 (Abstract), 1999 (IF: 10.330)

XXV. **Szilvássy, Z., Nagy, I., Madácsy, L., Hajnal, F., Velősy, B., Takács, T., Lonovics, J.: Beneficial effect of lovastatin on sphincter of Oddi dyskinesia in hypercholesterolemia and hypertriglyceridemia. Am J Gastroenterol, 92: 900-902, 1997 (IF: 2.344)**

XXVI. Madácsy, L., Middelfart, H.V., Szepes, A., Velősy, B., Hojgaard, L., Pávics, L., Lonovics, J., Funch-Jensen, P.: Comparison of the results of antroduodenal manometry and quantitative hepatobiliary scintigraphy in patients with biliary type pain and suspected sphincter of Oddi dysfunction. Z Gastroenterol, 36: A74 (Abstract), 1998 (IF: 0.890)

XXVII. Madácsy, L., Middelfart, H.V., Lonovics, J., Funch-Jensen, P.: Altered antroduodenal motility in patients with sphincter of Oddi dysfunction. Neurologastroenterol Mot, 10: 455(A95) (Abstract), 1998 (IF: 1.692)

XXVIII. Velősy, B., Jakab, I., Madácsy, L., Hajnal, F., Pap, Á., Lonovics, J.: Comparison of the effect of nitroglycerin, aminophyllin and papaverin on the prostigmine-morphine-induced sphincter of Oddi's spasm. Z Gastroenterol, 30: A106(Abstract), 1992 (IF: 0.68)

XXIX. Velősy, B., Madácsy, L., Lonovics, J., Csernay, L.: Effect of glyceryl trinitrate on the sphincter of Oddi spasm evoked by prostigmine-morphine administration. Eur J Gastroenterol Hepatol, 9: 1109-1112, 1997 (IF: 1.453)

XXX. Velősy, B., Madácsy, L., Nagy, I., Takács, T., Máté, E., Lonovics, J., Csernay, L.: Prevention of octreotide-induced sphincter of Oddi spasm by glyceryl trinitrate administration in humans measured by quantitative hepatobiliary scintigraphy. Gut, 37: A224 (Abstract), 1995 (IF: 3.023)

XXXI. Velősy, B., Madácsy, L., Szepes, A., Pávics, L., Csernay, L., Lonovics, J.: The effects of somatostatin and octreotide on the human sphincter of Oddi. Eur J Gastroenterol Hepatol, 11(8): 897-901, 1999 (IF: 1.589)

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Abbreviations

ABP	**acalculous biliary pain**
AN	**amyl nitrite**
AR	**accumulation ratio**
AST	**aspartate aminotransferase**
BP	**basal pressure**
CBD	**common bile duct**
CCK	**cholecystokinin**
CCK_{10}	**caerulein**
DAT	**duodenum appearance time**
DISIDA	**99mTc-N-2,6-diisopropyliminodiacetic acid**
EF	**ejection fraction**
EHIDA	**99mTc-N-2, 6-dimethylphenylcarbamoylmethyliminodiacetic acid**
ERCP	**endoscopic retrograde cholangio-pancreatography**
ESOM	**endoscopic sphincter of Oddi manometry**
EST	**endoscopic sphincterotomy**
GB	**gallbladder**
GI	**gastrointestinal**
GTN	**glyceryl trinitrate**
HDTT	**hilum-to-duodenum transit time**
HH	**hepatic hilum**
LP	**liver parenchyma**
MS	**morphine sulphate**
NANC	**non-adrenergic, non-cholinergic**
NO	**nitric oxide**
PC	**phasic contraction**
QHBS	**quantitative hepatobiliary scintigraphy**
ROI	**region of interest**
RTEPA	**real time endoscopic picture analysis**
RUQ	**right upper quadrant**
SO	**sphincter of Oddi**
SOD	**sphincter of Oddi dysfunction**
TAC	**time-activity curve**
$T_{1/2}$	**half-time of excretion**
T_{max}	**time to peak activity**
VIP	**vasoactive intestinal polypeptide**

Contents

1. BACKGROUND, HYPOTHESIS AND AIMS

Functional gastrointestinal (GI) disorders can be defined as a variable combination of chronic or recurrent GI symptoms not explained by any structural alterations [1]. Although such functional disorders are not life-threatening, they affect a significant proportion of the population, and account for a large number of those patients seeking either primary care or the gastroenterology specialist. The estimated frequency of functional biliary diseases is much lower. A recent large survey, based only on symptomatic criteria, suggested that the frequency of functional biliary pain in the general population was 1.5%. Biliary pain was associated with complaints which were more persistent and gave rise to a significantly higher rate of physician visits than for patients with other functional GI disorders. Functional biliary pain presented with a female predominance, the female/male ratio being approximately 4:1, and with an increased prevalence in the age group over 45 years [2]. Functional biliary pain can be defined as repetitive episodes of moderate to severe abdominal pain recurring over a minimum of 3 months, located in the epigastrium and/or right upper quadrant (RUQ), and persisting for a minimum of 20 min [VII, VIII]. Biliary pain may be associated with nausea or vomiting, the onset coming after meals with typical radiation into the back and/or right subscapular region. Theoretically, biliary pain can be evoked by the biliary tract distension caused by an impaired bile outflow, ischaemia arising from spastic smooth muscle contractions and hypersensitivity of pain sensory nerve endings around the gallbladder (GB), the sphincter of Oddi (SO) or the common bile duct (CBD) (3). Oddi initially suggested that biliary pain and jaundice could be caused by a functional spasm of the SO [4]. Later, Zollinger and Cutler documented that distension of the GB or the CBD triggered deep epigastric and/or RUQ pain [5]. Interestingly, biliary pain was more frequently located in the epigastrium and, as compared to the GB, distension of the CBD was accompanied by more

severe pain, nausea and vomiting [5]. It is important to emphasize the problem of distinguishing hepatobiliary pain from that originating from organs such as the oesophagus, the stomach, the small and large bowel, the pancreas and the coronary arteries [6]. Organic and functional extrabiliary disorders may also evoke upper abdominal pain with a similar location and characteristics. Furthermore, balloon distension at different points of the gut, such as rectal and duodenal distension, may induce abdominal pain at unpredictable sites throughout the abdomen, as demonstrated in patients with irritable bowel syndrome [7]. It is therefore obvious that, if differentiation is based only on the patient symptoms, a precise distinction between abdominal pain of biliary and non-biliary origin is hardly possible. Quite clearly, objective diagnostic methods are needed for the diagnosis of functional disorders of the biliary tract [IX, X].

A short description of the physiological background of biliary motility is essential for an understanding of functional biliary pain syndromes [XI]. The biliary tract plays an essential role in the discharge of bile into the duodenum. The two major compartments that actively control the bile flow are the GB and the SO. Hepatic secretion and GB contraction are the main driving forces of bile flow. The direction of the bile flow and the partition of the secreted bile within the biliary tract are determined by the relative resistances of the cystic duct and the SO. The SO represents a zone with a higher basal (resting) pressure (BP) than the duodenal or the CBD pressure with superimposed phasic activity. Filling of the GB occurs when a negative pressure gradient develops across the cystic duct due to simultaneous SO contraction and GB relaxation. During fasting, the GB stores and concentrates the secreted hepatic bile. Food intake simultaneously inhibits the SO basal tone and phasic activity, and facilitates GB contraction [3]. This motility pattern of the GB and the SO is controlled by complex humoral and neural mechanisms [XI]. The physiological effects of cholecystokinin (CCK) are postprandial contraction of the GB and relaxation of the SO. CCK acts directly on

CCK-A receptors in the muscle coat of the GB and evokes a dose-dependent contraction on the GB; in contrast CCK is a potent inhibitor of the SO basal and phasic activity. The release of CCK after food intake is the most important factor in the inhibition of the SO in the postprandial period, allowing an increased bile flow in consequence of the simultaneous GB contraction across the SO with decreased resistance. The CCK-induced GB contraction can be partially inhibited by atropine, suggesting that CCK-stimulated acetylcholine release may also contribute to the direct GB-contracting effect of CCK. Theoretically, either hypersensitivity of the GB or hyposensitivity of the SO to CCK might cause an acalculous biliary colic when the GB is contracting against a partially closed SO. The inhibitory effect on the SO of CCK after intravenous administration lasts for 2-6 min, and the SO activity subsequently returns to normal [8]. The mechanism of this dominant inhibitory action of CCK on the human SO appears to be mediated by stimulation of the CCK receptors on the non-adrenergic non-cholinergic (NANC) inhibitory neurones in the intramural ganglions. This NANC receptor stimulation involves the release of direct smooth muscle relaxants, such as nitric oxide (NO) and/or vasoactive intestinal polypeptide (VIP). The latter effect overrides the direct smooth muscle-stimulatory effect of CCK on the SO [9]. Theoretically, an imbalance between the dominant inhibitory effect and the direct smooth muscle-excitatory action of CCK on the SO (called the denervation pattern, since it may be due to the loss of inhibitory neurones) could lead to an inappropriate spasm, referred to as the paradoxical response of the SO after CCK administration [10]. Although this paradoxical response of the SO (including the reproduction of biliary pain) evoked by high-dose CCK administration has been demonstrated by endoscopic SO manometry (ESOM) in a few patients with a SO dysfunction (SOD) [10], there are no convincing human immunohistochemical data relating to the loss of inhibitory neurones in patients with functional biliary disorders [11].

In addition to CCK and VIP, gastrin, secretin, glucagon, methionine enkephalin and substance P are also able to relax the SO, but, with the exception of substance P, they are less potent than CCK [XI]. Other GI peptides and their analogues, such as pentagastrin, motilin, morphine, somatostatin and pancreatic polypeptide can stimulate the SO and inhibit bile flow across the sphincter. Motilin is believed to be a major humoral component influencing the interdigestive GB motility, resulting in periodic GB contraction in conjunction with the migrating myoelectrical activity of the duodenum. Cholinergic and muscarinergic stimulation increases the resistance of the SO, while in contrast nicotinic agonists induce SO relaxation. Specific pharmacological stimulation of adrenergic alpha-receptors increases the SO tone while beta-adrenergic stimulants diminish it. It is currently believed that the autonomic nervous system do not exert a major influence on the SO contractile activity, but appear to set the background tone, which is then modulated by the enteric nervous system, GI hormones and peptides. Furthermore, studies on humans and experimental animals have demonstrated neuronal connections between the proximal bile ducts, the GB and the SO. These connections take part in a reflex relaxation of the SO following distension of the GB and proximal bile ducts. Interruption of the reflex inhibitory pathways between the SO and the GB as a result of cholecystectomy might also contribute to the frequent generation of functional disorders after cholecystectomy [12, 13].

Functional disorders of the biliary tract can be classified as a GB dysfunction and a SOD [XII]. In clinical practice, it is often difficult to localize the anatomical site of the abnormality and to differentiate purely functional conditions from imperceptible structural changes. This is particularly true for the biliary tract, where the implementation of any direct measuring device is difficult, and the interpretation of minor histological abnormalities is not well standardized, so that in many instances any functional and structural abnormalities may

therefore merge [14]. Consequently, the development of objective diagnostic methods for differentiation between functional and structural disorders is of great importance.

The characteristic features of a GB dysfunction are acalculous biliary pain (ABP) and abnormal GB motility [XI]. Decreased contraction (GB hypokinesia), excessive contraction (GB hyperkinesia) and dyscoordinated contraction (GB dyskinesia) have been postulated to cause biliary pain. The mechanism of GB hypokinesia due to underlying primary disorders is well described, but the pathogenesis of GB dyskinesia is often confused with cystic duct syndrome, and is poorly understood. GB hypokinesia, which is a primary defect of the GB contraction, may be caused by either an impaired smooth muscle function and/or defective neuroendocrine mechanisms. GB hypokinesia may be induced by a number of organic disorders, such as chronic cholecystitis, cirrhosis, diabetes or somatostatinoma, and can be secondary to impaired endogenous CCK release due to Billroth II gastric resection, vagotomy and celiac disease. However, it is problematic to speculate on how defective smooth muscle contractility could induce biliary pain. Hence, attempts have been made to explain the mechanism of ABP by an exaggerated GB contraction, which was intended to overcome a partial cystic duct obstruction. The term "cystic duct syndrome" was originally proposed by Cozzolino et al. to describe this clinical condition [15], in which the cystic duct obstruction is interpreted to be due to a stone, inflammation or stricture. Nevertheless, it has been speculated that the clinical syndrome of GB dyskinesia might be due to a pure motility disorder, caused by a lack of coordination between the GB and the cystic duct, i.e. a cystic duct spasm, which can produce an increased resistance at the level of the cystic duct at the time of GB contraction [XI, XII]. The concomitant occurrence of biliary pain and impaired GB emptying is more easily understood, as the GB is attempting to empty its contents against an increased cystic duct resistance. The biliary pain might originate from the pressure rise due to the GB contraction against the increased cystic duct resistance. This logical pathogenic mechanism of

GB dyskinesia originally suggested by Toouli ten years ago, however, there are no human data as to support this hypothesis yet [16].

SOD can be defined as a functional disturbance of the SO with clinical signs of biliary or pancreatic disorders. Episodes of moderate to severe steady pain located in the epigastrium or the RUQ, recurring more often than every 3 months, are the most common presenting symptoms in patients subsequently shown to have SOD. Pain is more commonly manifested in cholecystectomized patients and leads to the development of postcholecystectomy syndrome [XI, XII]. It has been estimated that SOD occurs in around 1% of patients undergoing cholecystectomy, and in about 14% to 28% of those having postcholecystectomy pain syndrome [17, 18]. The clinical suspicion is supported by a transient elevation of the levels of liver enzymes such as alkaline phosphatase or aspartate aminotransferase (AST) and/or a dilated CBD in an absence of any obvious structural alterations. Endoscopic retrograde cholangiopancreatography (ERCP) plays a major role in the diagnostic evaluation of patients with SOD by the exclusion of structural disorders. The diagnostic cholangiographic criteria for SOD include a dilated CBD (> 12 mm) and a delayed contrast drainage time (> 45 min). It has been suggested that these patients with SOD should be subdivided into three groups on the basis of the results of laboratory tests and the ERCP findings. SOD biliary group I patients present with abdominal pain, abnormally elevated liver enzymes, a dilated CBD and a delayed contrast drainage time. SOD biliary group II patients have abdominal pain and at least one of the previously mentioned criteria. In the patients in SOD biliary group III, the abdominal pain is not accompanied by other objective criteria [19]. Toouli et al. applied the pathogenic mechanism for a simplified categorization of SOD patients into two subgroups: with SO stenosis or with SO dyskinesia [16]. SO stenosis is a structural narrowing of the SO, which may be caused by inflammation, fibrosis, smooth muscle hypertrophy or adenomyomatosis, while SO dyskinesia is a purely functional

disturbance of the SO [20, 21]. It has been demonstrated that most patients with SO dyskinesia (i.e. the functional group) display no objective diagnostic abnormality on ERCP and most of them can therefore be categorized into biliary groups II and III of SOD [20, 21]. The introduction of ESOM into clinical practice can be regarded as a major step both in the exploration of the physiologic motor function of the human SO and in the diagnosis of motility disorders in patients with SOD [22, 23]. In patients with SOD, several motility abnormalities have been described, such as a constantly elevated BP, short periods of raised BP (spasm or dys-coordination), increased amplitude of phasic contractions (PCs), the frequent occurrence of retrograde propagation of PCs, a higher frequency of PCs (tachyoddia), and a paradoxical response to CCK administration [24, 25].

Interestingly, GB dysfunction and SOD may co-exist in the same patient, and their pathogenic mechanism may therefore be related to each other, as suggested by follow-up studies [26]. Manometric abnormalities of the SO were demonstrated not only in cholecystectomized patients, but also in those with an intact GB. This finding strongly supports the evidence that SOD may exist before cholecystectomy [26]. Removal of the GB in these patients results in a high probability of the development of biliary pain through loss of the reservoir and pressure equalizing functions of the GB, the previously compensated abnormality thereby being unmasked [27].

The reported frequencies of abnormal ESOM in postcholecystectomy patients in SOD biliary groups I, II and III were 85.7%, 55.1% and 28.1% respectively [19]. These findings strongly suggest that, in a significant number of postcholecystectomy patients with SOD III, either the motility of the SO is normal or the motility abnormalities are unrelated to the symptoms [28]. Thus, as in other fields of gastroenterology, the hypothesis of abnormal responses to physiological stimuli (i.e. hyperreactivity) or abnormal perception of normal events (i.e. hypersensitivity) must be emphasized in connection with the possible pathogenic

mechanism of functional biliary pain [29]. This seemingly new concept was already mentioned in a review by Varró and Lonovics in 1988 [30]. They suggested that, at an early stage of the disease, the hypersensitive SO responds to various physiological stimuli with spasm (hyperreactivity) and/or typical biliary pain (hypersensitivity), whereas an elevated SO pressure and signs of biliary outflow obstruction (dysmotility) mainly occurred during provocation periods (pure hypertonic SO dyskinesia). In a later stage, a partial SO stenosis may develop, with an intermittently elevated SO BP, but the SO is still able to relax after the administration of smooth muscle relaxants such as amyl nitrite (AN) and glyceryl trinitrate (GTN), or to contract during provocation tests (mixed hypertonic SO dyskinesia). Eventually, organic stenosis and biliary obstruction with a constantly elevated SO pressure unequivocally develop (stenosis of the SO). Several indirect lines of evidence suggest a possible involvement of hypersensitivity or hyperalgesia in the pathogenesis of functional biliary pain, but this concept has still not been generally accepted [29]. Central sensitization i.e. hyperactivity of the central nervous system due to long-term nociceptive peripheral stimuli, might have a pathogenic role in the development of postcholecystectomy pain syndrome. The latter may be supported by the fact that the duration of the symptomatic history before cholecystectomy has been strongly correlated with the poor symptomatic outcome after cholecystectomy, as demonstrated 30 years ago in the excellent study by Bodvall and Overgaard [31]. Central sensitization leads to site-selective visceral hypersensitivity, which means a lower threshold to symptomatic recognition and pain perception as well. These mechanisms are also modulated by spinal and cortical stimuli, consequently, psychic vulnerability might be able to influence the development of biliary pain [32]. Lasson et al. demonstrated that up to 34% of patients with postcholecystectomy pain and normal ERCP experienced severe pain at the time of contrast injection [33]. In some patients with SO dyskinesia, cannulation of the papilla of Vater itself was painful, this condition was therefore

called "tender papilla". However, these studies were criticized for the lack of a correlation between the occurrence of biliary hypersensitivity during ERCP and the presence of SO motility disturbances with ESOM [34]. Hyperreactivity of the SO is another possible pathogenic mechanism of postcholecystectomy biliary pain and SO dyskinesia. Provocation tests have traditionally been used in the diagnosis of SO dyskinesia. Most of these provocation tests are based on morphine sulphate (MS) administration [35]. MS has a physiological stimulatory effect on the human SO. Very low doses of MS (2.5 μg/kg) increase the frequency and the amplitude of PCs, whereas higher doses can cause an SO spasm with a substantial elevation of the SO BP [36]. The effect of MS can be antagonized by naloxone, but not by atropine [37]. This phenomenon suggests a direct smooth muscle-stimulatory effect of MS by facilitating the Ca^{2+} influx through the voltage-dependent or receptor-operated calcium channels. In patients with SO dyskinesia, a marked rise in CBD pressure was demonstrated by means of ESOM during MS administration, which was associated with biliary pain [38]. Provocation tests based on MS administration, such as the Debray and the Nardi test, have been suggested in the diagnostic evaluation of patients with SOD [39, 40], but the usefulness of these tests has been criticized because of their relatively low specificity and sensitivity as compared with the results of ESOM [41]. In fact, biliary pain and the CBD pressure elevation after MS administration could be demonstrated by means of intraoperative manometry in a large percentage of patients with SO dyskinesia [42]. The frequencies of SO dysmotility demonstrated by ESOM were significantly higher in patients who gave a positive Nardi provocation test [43, 44]. The usefulness of these provocation tests was strongly supported by the fact that clinical and symptomatic improvements occurred after endoscopic sphincterotomy (EST) in a high percentage of those patients in whom the provocative tests were positive [45]. These findings make it probable that, in a number of patients with SO

dyskinesia, hypersensitivity and hyperreactivity of the SO might be an essential pathogenic mechanism [45], as initially suggested by Varró and Lonovics [30].

Despite many advances in the management of biliary tract diseases, the diagnosis of functional disorders remains a challenge. Recent advances in radiology, such as computed tomography, endoscopic ultrasonography and magnetic resonance imaging, have not resulted in real progress in this field, because of a lack of morphological alterations in this group of patients. The diagnosis must therefore be based on functional testing, such as provocation tests, hepatobiliary scintigraphy and ESOM. However, the ideal method has not yet emerged, and a number of controversies are still unsolved.

ESOM is considered to be the best method currently available in the diagnosis of SOD [46], but several problems can arise in the performance and interpretation of biliary manometry. The procedure itself is expensive, with special requirements as concerns the technical background and medical knowledge. Therefore, it can be performed only in a few specialized centres. ESOM is an invasive procedure that allows only minimal premedication and consequently is difficult to carry out and is often inconvenient to the patient. Even an experienced endoscopist could perform an adequate and complete examination in only 54-87% of the patients [47]. There are inherent technical problems with ESOM itself, since a relatively large tube is placed into a small orifice of a narrow channel, which is then perfused with water. Conclusively, after the mechanical insult to the papilla of Vater due to the cannulation, there is an obstruction of the biliary system during the measurement, making this procedure non-physiological. Furthermore, the frequent occurrence of several manometric artefacts often makes the interpretation problematic. The difficulties in the correct performance and interpretation of ESOM studies may influence intra- and interobserver variability [48]. Methodological variations in catheter and infusion systems, as well as variations in the condition of the patient, may also exert critical effects on the results of

ESOM. The SO pressure profile may also depend on the duodenal contractions, and the cyclic phases of the duodenal migrating motor complex at the time of ESOM [49, 50]. Furthermore, possible misinterpretation of the tracings due to the manometric artefacts, such as initial hyperactivity, catheter movement and hyperventilation, can be a real problem [3]. Unfortunately, with the progress in the clinical applications of ESOM, it has become obvious that it is associated with a higher incidence of procedure-related pancreatitis, as compared to simple diagnostic ERCP [51, 52]. The incidence of post-ESOM pancreatitis is reported to vary from 8% to 31%, depending on the length of the investigation, the rate of manometric catheter perfusion and whether the biliary or pancreatic segment of the SO is evaluated. Although a reduction of post-ESOM pancreatitis from 31% to 4% was reported when one lumen of the manometric catheter was used for continuous aspiration, it is still a significant risk for those patients having only functional GI disorders [53]. These problems may facilitate the clinical strategy by not troubling with ESOM at all, but by going ahead and performing EST in all patients with suspected SOD. Unfortunately, this is not a favourable approach either, because the risk of EST-induced complications, such as pancreatitis, bleeding and perforation, is significantly higher in patients with SOD and non-dilated bile ducts (functional group, biliary type III) than in patients with other indications [54, 55]. It is unacceptable to induce a serious complication in a subject who perhaps has no biliary disease. Furthermore, it is estimated that if ESOM is applied to screen patients with postcholecystectomy syndrome, more than four examinations are required for all cases of SOD with elevated BP to be detected. This efficacy ratio is intolerable for an invasive procedure that is both difficult to perform and associated with potentially dangerous complications in patients with a functional disease.

A widely available, non-invasive screening method is therefore required for the diagnosis of SOD. From this respect, hepatobiliary scintigraphy is an ideal substitute for

ESOM: it can be performed with a low radiation exposure and without any procedure-related complications. The radiation dose during hepatobiliary scintigraphy is approximately 3 mSv in effective dose equivalent, which is equal to an annual background radiation dose. Although hepatobiliary scintigraphy was originally suggested as a promising method for the diagnosis of SOD, several controversies have led to its acceptance as an alternative diagnostic test in patients with suspected SOD that is progressing rather slowly. It has been claimed that, although hepatobiliary scintigraphy may be used as a method for the detection of a low-grade biliary obstruction in patients with SO stenosis [56, 57], it is not sensitive enough to be a definitive screening test in patients with SO dyskinesia. It has been documented that a significant overlap may exist between the results of hepatobiliary scintigraphy on the normal population, asymptomatic cholecystectomized patients, patients with cholestasis due to hepatocellular disease, and patients with SOD [58, 59]. Another criticism of hepatobiliary scintigraphy is that it is not able to differentiate between patients with organic (SO stenosis) and functional (SO dyskinesia) disorders [60].

To summarize the current status, on the one hand we are dealing with a number of patients who have a biliary pain syndrome without any obvious explanation, while on the other hand the available functional diagnostic methods with which these patients might be investigated have obvious shortcomings. The present work has the aims of further elaboration of these problems and the provision of new prospects in the diagnosis of functional biliary disorders. The main goals were to make the available tests more objective; to select the most sensitive scintigraphic parameters with quantification; to validate hepatobiliary scintigraphy as a possible alternative for invasive manometry; to reinforce the value of provocation or pharmacological tests in the diagnosis of functional biliary disorders; to develop new methods that can be applied in the differentiation of organic and functional abnormalities; and to improve the diagnostic efficacy in patients with functional biliary disorders.

2. METHODS

2.1. QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY

Hepatobiliary scintigraphy is a simple, widely available, non-invasive isotope diagnostic method with which to investigate the function of the hepatobiliary system. It can be accomplished with a low radiation exposure with the use of 99mTc agents. Radiopharmaceuticals such as 99mTc-N-2,6-dimethylphenylcarbamoylmethyl-iminodiacetic acid (99mTc-EHIDA) and a variety of its analogues, such as 99mTc-N-2,6-diisopropyl-iminodiacetic acid (DISIDA) and 99mTc-2,4,6-trimethyl-3-bromo-iminodiacetic acid (mebrofenin) can be used for hepatobiliary imaging [61]. With the clinical application of hepatobiliary scintigraphy, it is possible not only to detect a biliary obstruction, but also to determine the hepatic cell function via the rate of bile secretion and to investigate the GB and SO motility by following the course of bile emptying [62, 63]. After their i.v. injection, these 99mTc agents bind loosely to serum proteins; subsequently, about 98% of the injected dose is taken up by the hepatocytes and rapidly secreted unaltered into the bile without further metabolism. This uptake mechanism proceeds in parallel to that of bilirubin, and hyperbilirubinaemia therefore has a competitive inhibitory effect. After excretion by the hepatocytes, the bile continues to flow from the bile canaliculi through the canals of Hering to the major intrahepatic bile ducts and the CBD, and then empties into the GB and the duodenum. Normally, almost 70% of the radioactivity secreted by the liver enters the GB during fasting, giving excellent conditions for imaging and quantification. After the hepatic excretion, the bile flow is predominantly a passive process, determined by the least resistance and pressure. Pressure changes inside the extrahepatic bile ducts and the GB therefore exert a critical influence on the isotope emptying.

The methodology of hepatobiliary scintigraphy is simple, but should be followed precisely in every patient to achieve optimal results and comparability [IX, X]. Patients should be fasted for a minimum of 4 hours prior to the investigation. The gamma-camera is positioned over the right upper abdomen of the supine patient. After an i.v. bolus injection of 5 mCi (140 MBq) 99mTc-EHIDA, gamma-camera images are acquired. Analogue images should be obtained every 10 min for the first hour, with delayed images if required (first image acquisition for 500 000 counts, and subsequent images for the same length of time). The analogue pictures should be examined for liver radiotracer uptake, size, shape and homogeneity. The liver uptake of 99mTc-EHIDA is rapid, with scarcely any tracer left in the blood by the 5^{th} min. In most normal fasting subjects, radioactivity appears in the GB and in the small bowel by 1 hour after its administration. The extent of visualization of the hepatic ducts is variable, but the left duct tends to be more prominent than the right. Excretion of radioactivity into the duodenum occurs by 60 min in 81% of patients with an intact GB. In the remaining 19% of patients without bowel activity at 60 min, duodenal visualization may be achieved after exogenous CCK administration. This failure to visualize the small bowel by 1 hour in a small proportion of normal fasting subjects is thought to be attributable to a physiologically tight SO during the GB filling period [64]. After cholecystectomy, the bile flow from the bile ducts into the duodenum can be described by a simple two-compartment model, and it is therefore less variable. In cholecystectomized patients without extra-hepatic bile duct obstruction, the level of radioactivity of the CBD reaches its maximum before 45 min, and the amount of radioactivity in the bile ducts afterwards decreases, with rapid emptying into the duodenum (*Figure 1*).

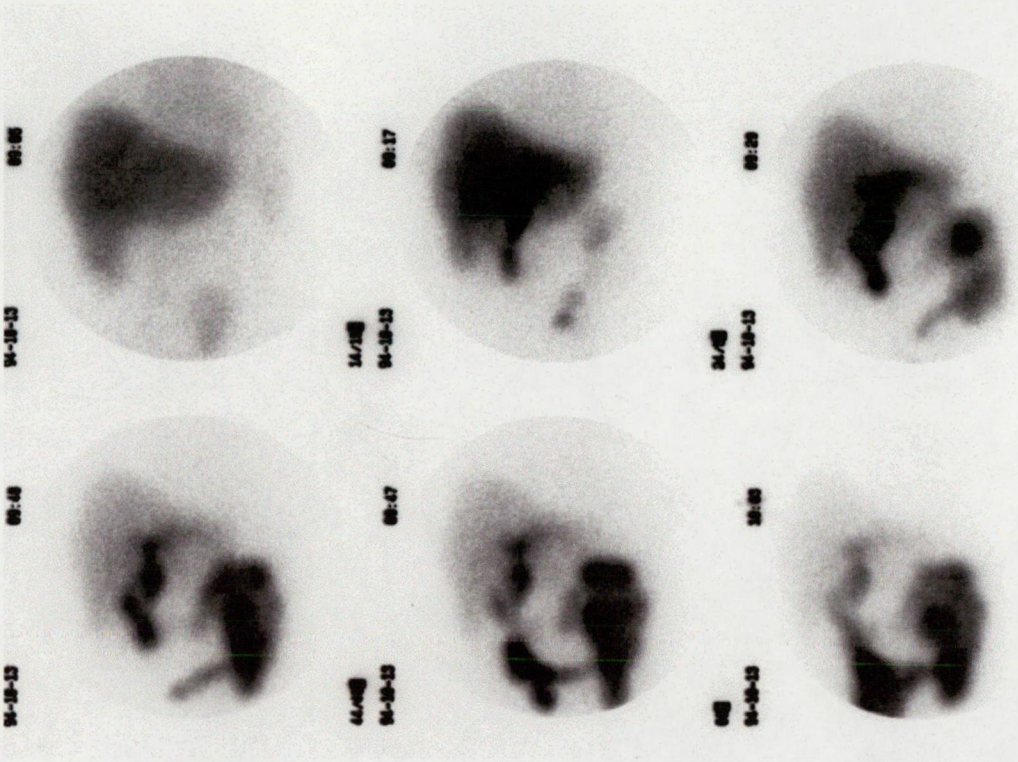


Figure 1. Representative hepatobiliary scintigraphy recording in a cholecystectomized patient with a normal liver size, a normal hepatic uptake of the radioactivity, with rapid excretion into the bile ducts, and obviously free transpapillary bile flow, without signs of extrahepatic biliary obstruction.

Analysis of the hepatic bile secretion and biliary flow characteristics on the basis of planar images alone involves the risk of an interpretation bias. To exclude this shortcoming of the original method, therefore, quantitative hepatobiliary scintigraphy (QHBS) was introduced into clinical practice [XIII, XIV]. Computer-assisted quantitative analysis of the time-activity curves (TACs) constructed from regions of interests (ROIs) led to an improvement in the diagnostic accuracy of hepatobiliary scintigraphy [63, 64]. After administration of the tracer, digital images should be obtained consecutively at one frame/min for 90 min, and computer-recorded in a 64x64 matrix. During the analysis, ROIs should first be selected over the right peripheral liver parenchyma (LP), hepatic hilum (HH), CBD and duodenum. TACs are then generated by the computer. In our practice, we routinely calculate several numerical

parameters, such as the time to peak activity (T_{max}), the half-time of excretion ($T_{1/2}$), the hilum-to-duodenum transit time (HDTT), the duodenum appearance time (DAT) and the accumulation ratio (AR). AR is a quotient, which can be calculated as $T_{1/2}$ for the CBD divided by $T_{1/2}$ for of the LP (*Figure 2*).

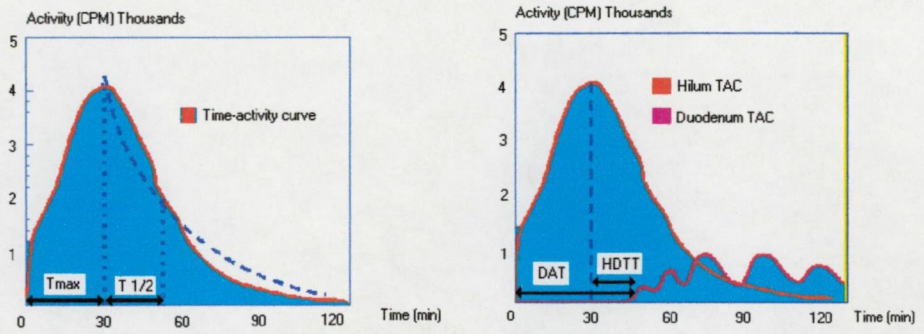


Figure 2. Schematic presentation of the method of calculation of QHBS parameters from the time-activity curves (T_{max}: maximal activity, $T_{1/2}$: half-time of excretion, DAT: duodenal appearance time, HDTT: hilum-duodenum transit time).

The applicability of hepatobiliary scintigraphy for quantification is extremely useful because it allows numerical measurement of the bile secretion and flow. These values can be utilized to compare different groups of patients, to perform follow-up studies in a given patient group and to monitor the efficacy of specific therapeutic interventions. In patients with normal bile secretion and free trans-papillary bile flow, the TACs have a typical exponential shape during the emptying phase (*Figure 3*). Quantification of hepatobiliary scintigraphy allows the detection of minor bile flow disturbances, which frequently occur in patients with functional SO dyskinesia.

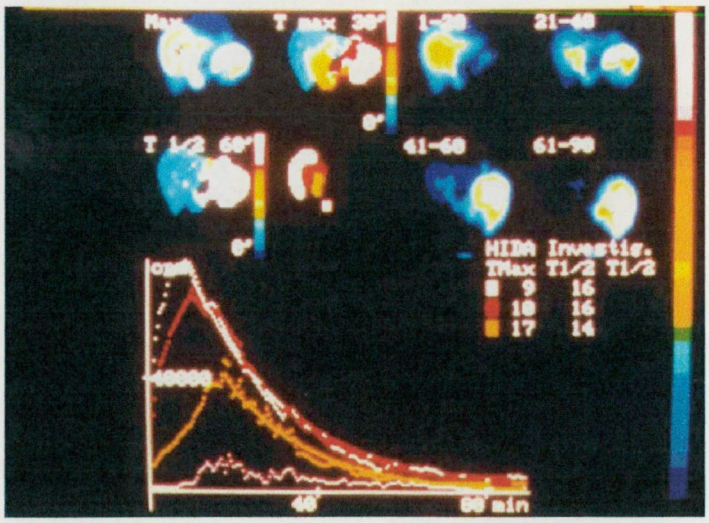


Figure 3. Representative recording of the final results of QHBS in a cholecystectomized patient with normal bile secretion and emptying. The upper panel depicts parametric and cumulative pictures with ROI selection, while the lower panel demonstrates TACs of the LP (white), HH (red), CBD (yellow) and duodenum (pink).

QHBS is a valuable method for differentiation between primary cholestatic liver diseases and extrahepatic biliary obstruction. In patients with intrahepatic cholestasis, there is a primary increase in $T_{1/2}$ over the LP (slower hepatic secretion), which then determines the slower emptying ($T_{1/2}$) of the HH and CBD. However, in patients with an extrahepatic biliary obstruction, $T_{1/2}$ over the LP is usually normal, but becomes abnormal over the HH, and reaches its maximum over the CBD [XIII].

The GB function can be optimally determined by the application of QHBS during the GB phase imaging [65]. These data can preferably be acquired in a separate 30-min study between 60 to 90 min after the administration of 99mTc-EHIDA. If there was any overlap of the CBD and GB radioactivity during the hepatic phase imaging, an attempt should be made

to separate them by changing the angle of the gamma-camera. Superimposed duodenal activity can be cleared if the patient drinks a glass of water. The GB emptying after consumption of a test meal or administration of exogenous CCK may be measured numerically by means of QHBS, which is a reliable test for assessment of the GB function. QHBS combined with administration of a fatty meal or an exogenous CCK analogue is a validated and accurate method for measurement of the ejection fraction (EF) of the GB, with satisfactory reproducibility of both parametric variables and emptying patterns [66]. The intra- and interobserver variability of endogenous CCK release after administration of a fatty meal made this stimulation test somewhat unfavourable for GB EF determination. This variability is not only due to the different CCK receptor count and activity in the upper GI tract, but is also induced by the high interindividual variability of gastric emptying after a caloric meal. Most of the investigators therefore apply exogenous CCK administration to evoke GB contraction. These data can be acquired into the computer separately at one frame/min, 60 min after the initial isotope administration, as a GB phase imaging study. The ROIs should first be placed over the GB and over the liver parenchyma adjacent to the GB. After a background correction, a GB TAC is generated, and the GB EF is then calculated by using the equation $EF\%=(GB_{max}-GB_{min})/GB_{max} \times 100$. The degree of GB emptying is CCK dose-dependent, and the manner of CCK administration therefore has a critical effect on the overall GB contraction. The GB EF increases linearly as the dose of CCK is increased from 0.5 to 3.3 ng/bwkg/min, with a subsequent decrease when larger doses are infused, especially when CCK is given as a rapid i.v. bolus [51]. The effects of small infused doses of CCK (under 0.5 ng/bwkg/min) on GB contraction are quite variable. Additionally, a supraphysiological dose of CCK produces a low GB EF too in association with abdominal pain, even in normal subjects. This is probably due to a cystic duct contraction, and should therefore be avoided. The normal GB EF value is 35% or higher, based on 3.3 ng/body weight

kg/min CCK infusion administered for a minimum of 3 min, and can be regarded as an indicator of a normal GB motor function [65]. Abnormal GB emptying can be diagnosed in patients with a GB EF less than 35%, which may be caused by GB hypokinesia, GB dyskinesia, chronic cholecystitis and partial cystic duct obstruction due to gallstone disease (*Figure 4*).

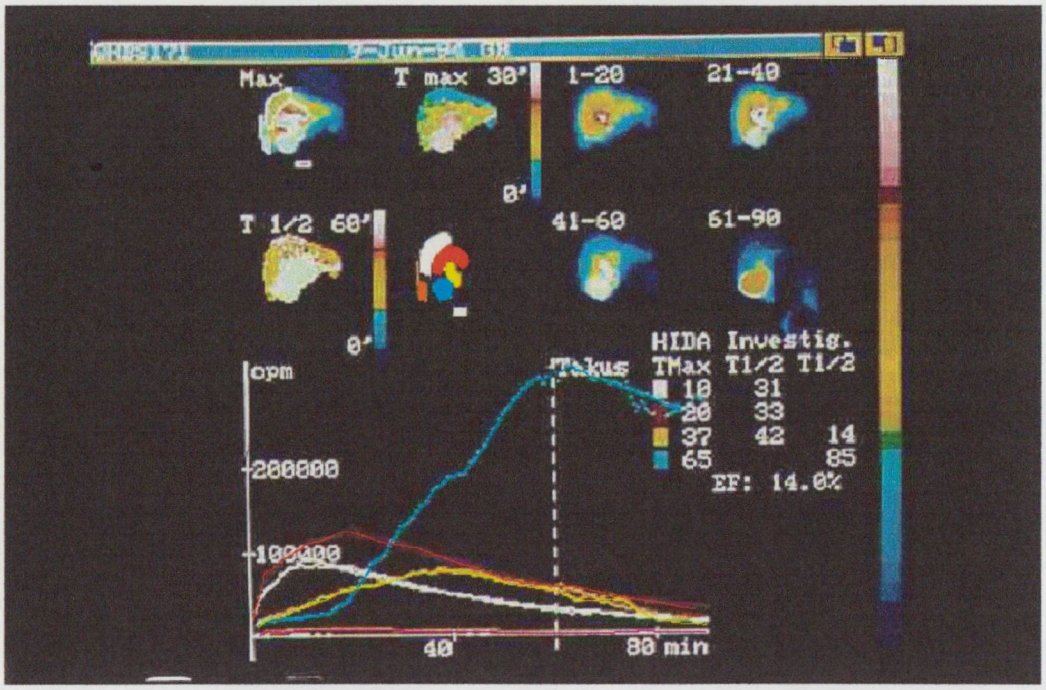


Figure 4. Representative QHBS recording in a diabetic patient with GB hypokinesia and impaired GB contraction (GB EF: 14%) after caerulein (CCK_{10}) administration (1 ng/bwkg/min for 10 min) during the GB phase imaging. TACs were selected as follows: LP (white), HH (red), CBD (yellow), GB (blue) and background (pink).

Augmentation during QHBS, such as CCK or MS administration, has long been suggested as a provocation test to enhance the diagnostic aid of scintigraphy [66]. CCK augmentation during QHBS may have two different rationales. One is to evoke a paradoxical spasm of the SO in a subgroup of patients with SOD and to detect the consequent bile outflow obstruction. The other is to facilitate bile flow into the duodenum in patients with GB and late duodenal appearance of the radioactivity. CCK increases the hepatic bile secretion and relaxes the SO; it therefore washes out the bile stasis when there is no organic obstruction. This design is very similar to that of the Lasix test in isotope renography to rule out renal obstruction. MS augmentation has been suggested to induce GB filling due to the enhanced SO pressure in patients with suspected chronic cholecystitis and lack of GB activity at 60 min [66]. However, the applicability of this stress test is limited, due to some potential serious complications, particularly in patients with acute cholecystitis, such as the aggravation of biliary colic, the progression of biliary obstruction and the occurrence of GB perforation. Although CCK augmentation during QHBS is a rational prospect in the diagnosis of functional biliary pain and SOD too, the precise indications and the diagnostic consequences of this possible provocative test have not yet been determined.

2.2. ENDOSCOPIC SPHINCTER OF ODDI MANOMETRY

The only diagnostic method that allows direct assessment of the motor function of the SO is manometry. Accurate pressure measurements from the SO through the endoscope has become possible following the miniaturization of manometry catheters and the development of low-compliance, fluid-perfusion, pressure-recording systems [22, 23]. Pressure recording can be accomplished with a triple-lumen, water-perfused Teflon catheter that is attached to

external transducers, and connected to the computer with a multi-channel Polygraf interface. For adequate water perfusion and overpressure, a dual-chamber, high-pressure perfusion pump may be applied (*Figure 5*).

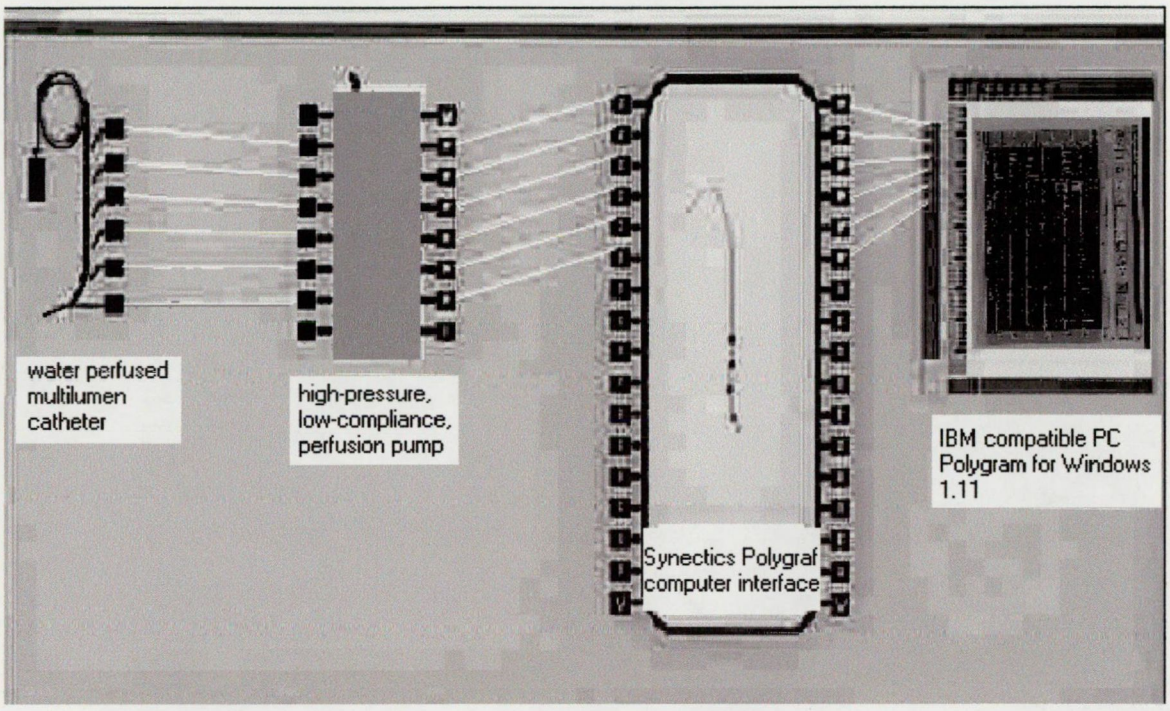


Figure 5. Schematic presentation of the water-perfusion manometric system.

After selective deep cannulation of the CBD with a standard ERCP catheter, a guide wire is placed high up into the CBD. The duct is then entered with the manometric catheter over the wire. The direction can be identified by gently aspirating on the proximal channel. The appearance of yellow fluid in the endoscopic view indicates entry into the bile duct. Clear aspirate indicates the pancreatic duct. In an uncertain case, a small amount of dilute contrast media can also be injected. Two different manometric techniques can be applied to determine the SO pressure profile: a stationary pull-through and a rapid pull-through technique. During the stationary pull-through measurement of SO pressures, the manometric catheter is withdrawn from the CBD into the SO zone at 1 mm intervals, and is then stationed in this position for a minimum of 4-5 min The SO zone can be identified during ESOM as a zone of

a stepwise pressure elevation (baseline pressure) relative to the duodenal and the CBD pressure with superimposed PCs [67]. SO BP abnormalities should ideally be observed for at least 30 sec and should be reproducibly observed in each lead. PC characteristics such as amplitude, frequency and propagation can also be determined with this stationary pull-through technique (*Figure 6*).

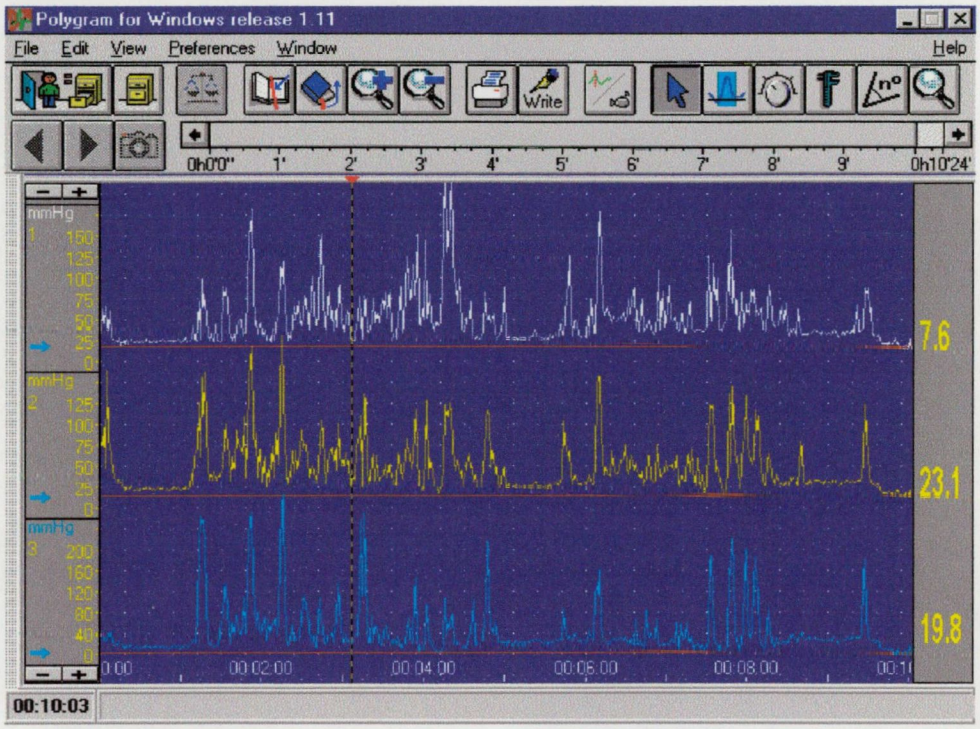


Figure 6. Representative recording of an original stationary pull-through ESOM, made in a control patient with normal SO motility and normal SO basal pressure.

In contrast, the rapid pull-through technique means that the manometric catheter is rapidly withdrawn from the CBD into the duodenum at a speed of 1 mm/sec. This manoeuvre should be repeated at least 5 times during a single study in order to calculate the average SO BP. With the latter technique, the PC characteristics cannot be determined, and artificially high pressure values may occur due to movement artefacts and reflex contractions of the SO at the time of catheter movement. However, this is the method to measure the length of the SO [68].

ESOM is a troublesome procedure both for the endoscopist and for the patient, since premedication should be minimized. Moreover, the difficulty of performing an acceptable manometric study necessitates close cooperation between an experienced endoscopist and a motility expert. Although the intra- and interobserver reproducibility of ESOM have been demonstrated to be reasonably good [69, 70], in a particular patient the correct analysis and interpretation of the SO pressure profile can be rather difficult; this is particularly true in patients with borderline abnormalities. Some of these problems with the analysis are related to the sphincter characteristics, such as the relatively short and narrow SO segment with a significant longitudinal and transversal pressure profile asymmetry, while others are related to the manometric technique itself, such as spontaneous in-and-out catheter movement during the stationary pull-through technique, and reflex sphincter contractions during the rapid pull-through technique. Artefacts occur quite frequently during ESOM, but their importance is considerably underestimated by the literature. Due to these shortcomings, interpretation of the tracings is often subjective (*Figure 7*). The development of new methods, in order to recognize and exclude periods of manometric artefacts, is therefore highly necessary.

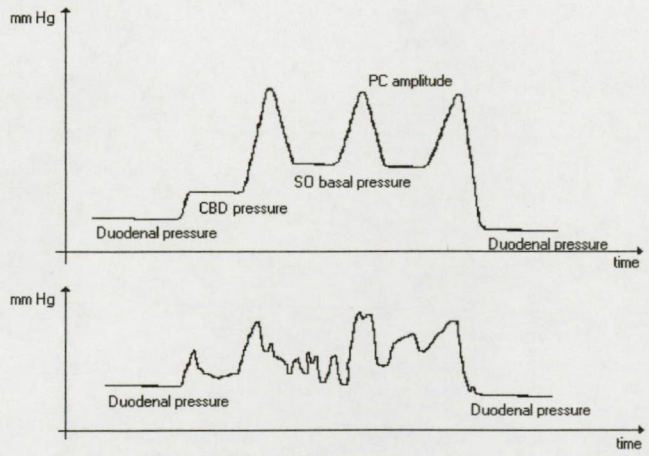


Figure 7. The upper panel depicts a stylized idealistic manometric tracing, while the lower panel demonstrates a stylized actual tracing from a patient with artefacts, which illustrates the potential difficulties in interpretation.

We prefer to use the stationary pull-through technique for SO BP determination, since it has the advantage of allowing a precise longitudinal pressure mapping of the SO with a

longer measurement period (approximately 8-10 min). The application of this method allowed us to detect short periods of SO spasm and/or dyscoordination, together with the detailed analysis of the PCs [71]. However, this method is applied, it is extremely important to monitor the catheter position and to keep the manometric catheter in exactly the same place for a certain period of time, which is sometimes practically impossible, or necessitates continuous small corrections by the endoscopist. In our experiments, we performed ESOM by applying the stationary pull-through method validated by Funch-Jensen et al. [72]. Following an overnight fasting, sedation was induced with 2.5-7.5 mg i.v. midazolam (Dormicum, Roche). No other drugs were given. Duodenoscopy was then performed with a video-duodenoscope JF-130 (Olympus). For SO pressure measurements, a standard triple-lumen manometric catheter was used (Arndorfer Med. Spec., USA, and Synectic, Sweden), with an external diameter of 1.7 mm and side-holes spaced 2 mm apart. All capillary channels were constantly perfused with sterile water at a rate of 0.33 ml/min from a dual-chamber pressure pump (MUI Scientific Pump Perfusion System, Mississauqa, Ontario, Canada) with a 14-PSI overpressure in the water reservoir. The pressures transmitted by the external transducers were recorded simultaneously on a Synectics Medical computer system with a time-correlated basis, using Synectics Medical Polygram Windows 1.1 software. Selective deep cannulation of the CBD was achieved with a standard ERCP catheter, and a flexible-tip guide wire (Wilson Cook HSF 25/400) was then introduced high up into the bile tract. Next, the ERCP catheter was changed for the ESOM catheter over the guide wire, which was kept in place. The duodenal pressure was recorded before and immediately after the SO measurements in order to establish the average zero reference pressure. After the ESOM catheter had been placed into the CBD, the guide wire was removed and the CBD pressure was recorded for a minimum of 2 min to exclude periods of initial hyperactivity of the SO. A stationary pull-through measurement of the SO segment was next made. During the stationary pull-through

recording, the ESOM catheter was withdrawn into the SO zone and retained there for a minimum of 5 min. The SO BP, PC amplitude, frequency and peristalsis were then calculated. The amplitude, frequency, duration, and propagation of the PCs were measured in each channel, and mean pressure values were calculated. To calculate the maximum SO BP during the stationary pull-through technique, we used the manometric channel with the highest sustained SO BP occurring for a minimum of 30 sec., after which this high BP pressure can be located reproducibly in each of the other channels by the catheter pull-through (*Figure 8*).

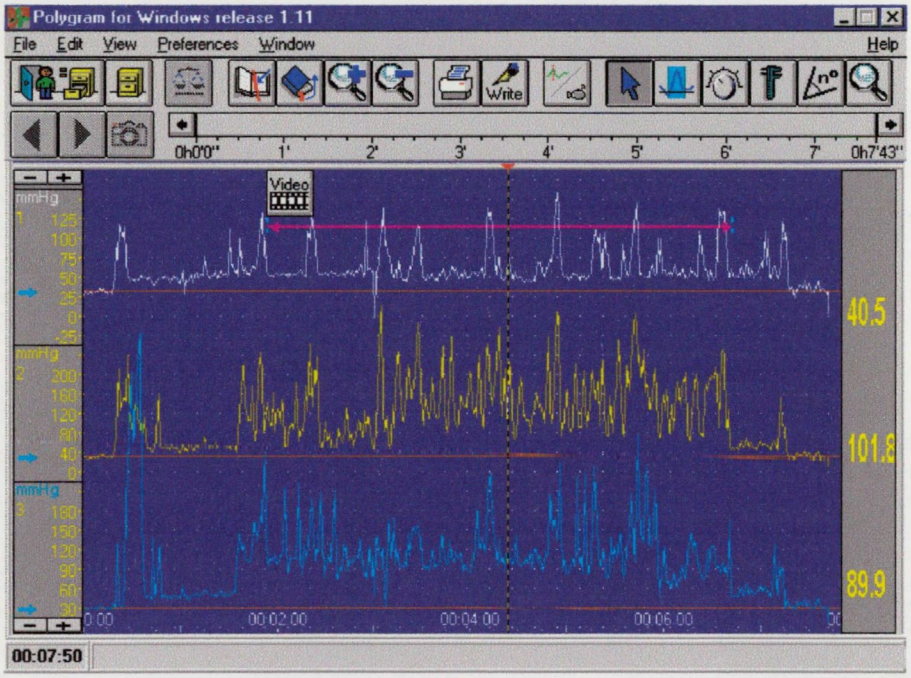


Figure 8. Representative recording of an original ESOM procedure in a patient with SO stenosis and constantly an elevated SO basal pressure.

During this station pull-through measurement, we determined the average SO BP in all channels between the PCs, relative to the duodenal pressure, after careful exclusion of all periods with obvious manometric artefacts and periods of duodenal hyperactivity. The mean amplitude of the PCs in each channel relative to the SO BP was also determined. The frequency of PCs was calculated for the total recording time. The propagation of PCs was

analysed for each single PC wave, and the percentages of anterograde, simultaneous and retrograde peristalsis were calculated relative to the total activity. With the reference SO pressure values obtained by Funch-Jensen in healthy controls, SO BP values exceeding 35 mm Hg (*Figure 9*), mean SO PC amplitudes over 200 mm Hg, PC frequencies over 10/min (*Figure 10*), and retrograde PC peristalsis over 50% were considered abnormal (*Figure 11*) [73].

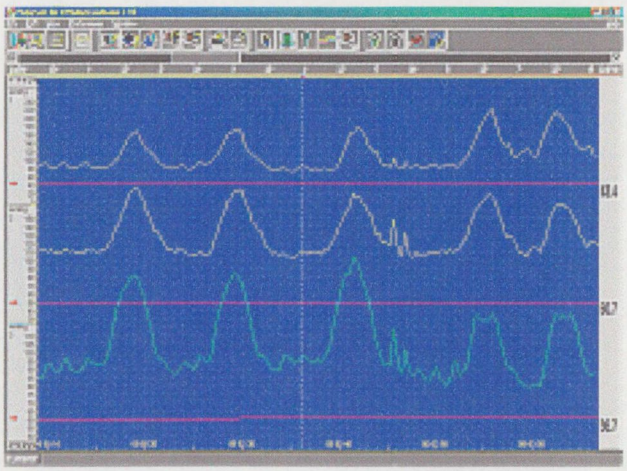


Figure 9. Representative period of elevated SO BP that exceeds 60 mm Hg in the mid channel in a patient with SO dyskinesia and periods of SO spasm during ESOM.

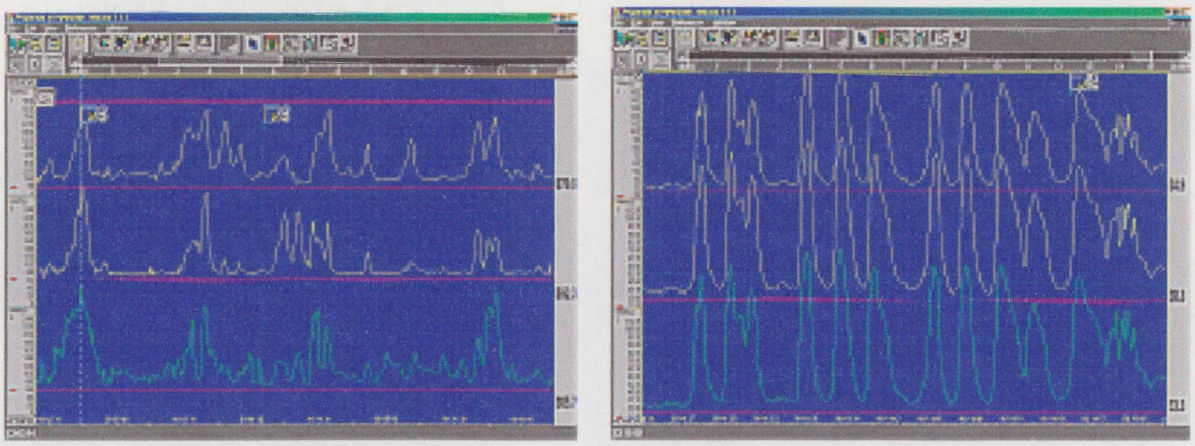


Figure 10. Giant SO contractions with PC amplitudes that exceed 300 mm Hg demonstrated on the left, and tachyoddia (12 PCs/min) on the right.

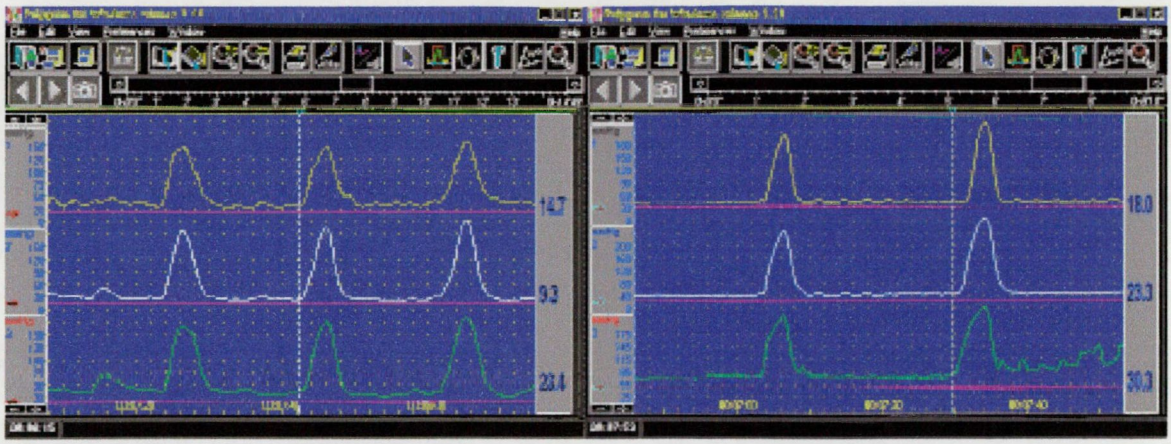


Figure 11. Selected short periods of original ESOM tracings with normal SO BP. The left side demonstrates normal propagation of the PCs, while the right side depicts typical retrograde peristaltic activity.

2.3. STATISTICS AND ETHICAL CONSIDERATIONS

For statistical evaluation, the Student t-tests (paired and unpaired) were used. Correlations were analysed by the Spearman rank correlation test, and the correlation coefficient was calculated. Significance was achieved at $p<0.05$. All results are given as means with standard deviation and error of the mean, except for the control values, which are given as means plus two times the standard deviation (mean + 2SD). Each of the following studies was approved by the local ethics committee and was conducted in accordance with the Helsinki II Declaration. Informed consent was obtained from each subject before inclusion in the study.

3. RESULTS

3.1. DETERMINATION OF NORMAL PARAMETERS AND DYNAMICS OF BILE EMPTYING BY QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY BEFORE AND AFTER CHOLECYSTECTOMY

QHBS has been validated in the diagnosis of cystic duct occlusion due to acute cholecystitis and of extrahepatic biliary obstruction caused by stone or stricture. However, the diagnosis of minor bile flow abnormalities, frequently occurring in patients with SOD, requires a full knowledge of the normal scintigraphic pattern of bile emptying in patients with and without the GB. We therefore conducted a prospective study to determine the dynamics and normal variations of transpapillary bile flow by QHBS before and after cholecystectomy and to calculate reference values for parameters of QHBS in asymptomatic cholecystectomized patients [I]. Twenty patients with uncomplicated GB stones were investigated (19 women, 1 man, with a median age of 51 years, range 29-70) 1 month before and 3 months after laparoscopic cholecystectomy. QHBS was performed with the standard method described above. At the time of the study, all of the patients were completely free of symptoms. None of them had CBD stones or extrahepatic biliary obstruction. On the basis of the results of QHBS, the transpapillary bile flow was judged to be normal in every patient. An assessment of the quantitative parameters before and after the operation in the same patient group revealed that there were significant differences in the dynamics of CBD emptying following cholecystectomy. After cholecystectomy, the isotope emptying from the bile ducts was accelerated as compared to the preoperative status (*Figure 12*).

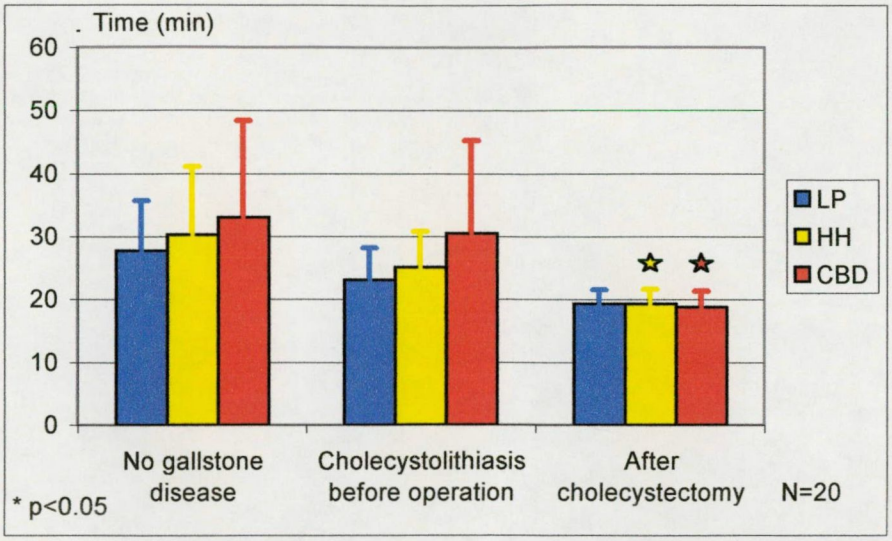


Figure 12. Comparison of transpapillary bile flow parameters (half-time of excretion) in patients without gallstone disease and with the intact GB, and in patients with uncomplicated cholecystolithiasis before and after laparoscopic cholecystectomy.

The parameters of QHBS in cholecystectomized patients and in patients with the intact GB are therefore not comparable. In fact, in patients with the intact GB, the bile emptying rates vary widely and the CBD emptying can be moderately slow without obstruction. This phenomenon is not related to the presence of gallstone disease, since a similar variation in the quantitative parameters can be found in patients without gallstones and with an intact GB (*Figure 12*) [XIV]. This wide variation may be explained by the presumption that, during the GB filling period with a higher SO resistance, the CBD emptying towards the GB is expected to be considerably slower process than during the periods of spontaneous CBD and GB emptying through an opened SO into the duodenum. After cholecystectomy, however, the bile emptying from the CBD into the duodenum occurs continuously at a higher and constant rate. We concluded that in asymptomatic, cholecystectomized patients, only minor variations in the

dynamics of bile emptying can be detected by QHBS, thereby increasing the probability of identification of abnormalities in patients with SOD.

The determination of control values is the first step in the assessment of the diagnostic value of QHBS in patients with SOD. After quantitative analysis, the T_{max} and $T_{1/2}$ values of the LP, HH and CBD, the DAT, the HDTT and the AR were calculated, and the upper limits of the normal values (mean + 2SD) were determined in asymptomatic patients after cholecystectomy (*Table 1*).

	Mean	***SD***	***Upper limit of normal range***
LP T_{max}	9.7 min	1.8 min	13.3 min
LP $T_{1/2}$	19.3 min	2.3 min	23.9 min
HH T_{max}	14.3 min	2.6 min	19.5 min
HH $T_{1/2}$	19.3 min	2.4 min	24.1 min
CBD T_{max}	21.1 min	4.6 min	30.3 min
CBD $T_{1/2}$	18.8 min	2.6 min	24.0 min
DAT	16.6 min	3.0 min	22.6 min
HDTT	2.3 min	1.3 min	4.9 min
AR	0.98	0.12	1.22

Table 1. Calculation of the upper limit of the normal range of QHBS in asymptomatic patients after cholecystectomy.

3.2 NEW METHODS FOR THE DIAGNOSIS OF FUNCTIONAL DISORDERS OF THE GALLBLADDER AND THE SPHINCTER OF ODDI:

3.2.1. DIAGNOSIS OF GALLBLADDER DYSKINESIA BY QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY

Recurrent RUQ abdominal pain induced by a fatty meal in patients without demonstrable gallstones frequently appears to be a diagnostic and therapeutic challenge. A subgroup of ABP patients exhibits an impaired GB emptying and the reproduction of biliary pain after CCK administration. Although GB dyskinesia, i.e. an uncoordinated GB contraction with a spasm of the GB neck or cystic duct due to CCK administration, may be responsible for this clinical condition, no diagnostic test is yet available to prove this pathogenic mechanism.

Accordingly, we initiated a study to develop a new scintigraphic method in patients with ABP and suspected GB dyskinesia [II, XV]. Thirty-eight patients with ABP were investigated with our standard QHBS method. In 5 patients without sufficient GB filling at 60 min, only GTN was administered. In 33 patients with sufficient GB filling, at 60 min after the administration of EHIDA, CCK_{10} (Takus, FarmItalia) was infused i.v. in a dose of 1 ng/kg/min for 10 min The GB EF was then calculated for the period from 60 to 90 min. The CCK_{10} infusion was next repeated from 90 min in the presence of sublingually administered GTN (0.5 mg) (21 patients) or placebo (12 patients), and the GB EF was once more calculated from 90 to 120 min. After the first dose of CCK_{10}, the GB EF was less than 35% in 23 of the 33 ABP patients, indicating a reduced GB emptying (non-responder (NR) group), while the remaining 10 patients had a normal GB EF (responder (R) group). In 16 NR patients, GTN and CCK_{10} coadministration induced a significant improvement of the previously impaired

GB EF as compared to CCK_{10} alone. In 5 of the 10 patients who exhibited a normal GB EF during the initial assessment (group R), GTN and CCK_{10} coadministration caused a further increase in GB EF (*Figures 13 and 14*).

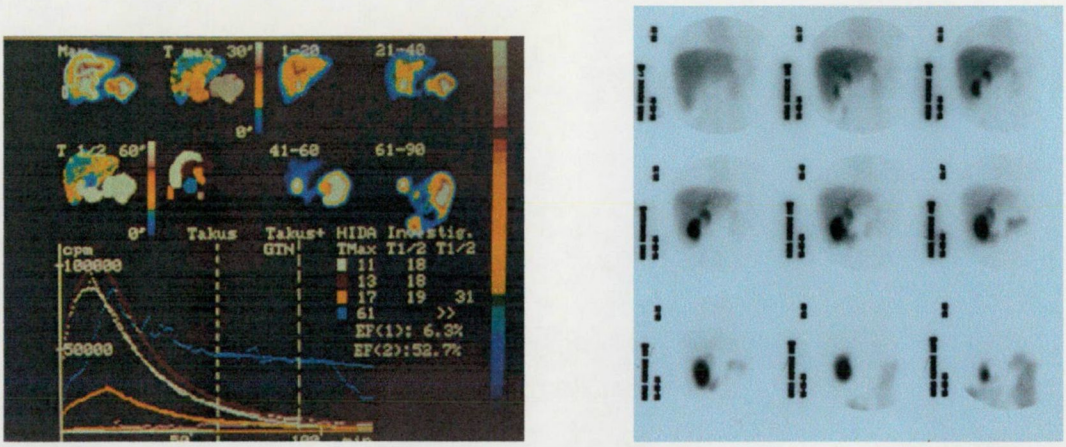


Figure 13. Representative hepatobiliary scintigraphy (TACs on the left and analogue pictures on the right) on an ABP patient with an obvious improvement of the GB contraction due to GTN and CCK_{10} coadministration as compared to CCK_{10} alone.

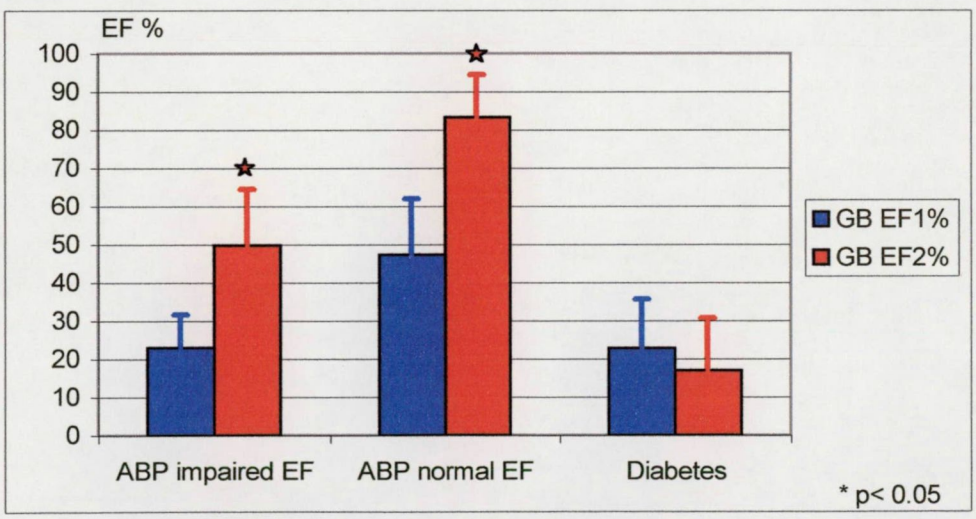


Figure 14. Effect of GTN on CCK_{10}-evoked GB contraction (GB EF2%) as compared to CCK_{10} alone (GB EF1%) in ABP patients with an impaired versus a normal GB EF and in patients with diabetic neuropathy.

In contrast, in the remaining 7 NR and 5 R patients, placebo and CCK_{10} coadministration did not affect the GB EF.

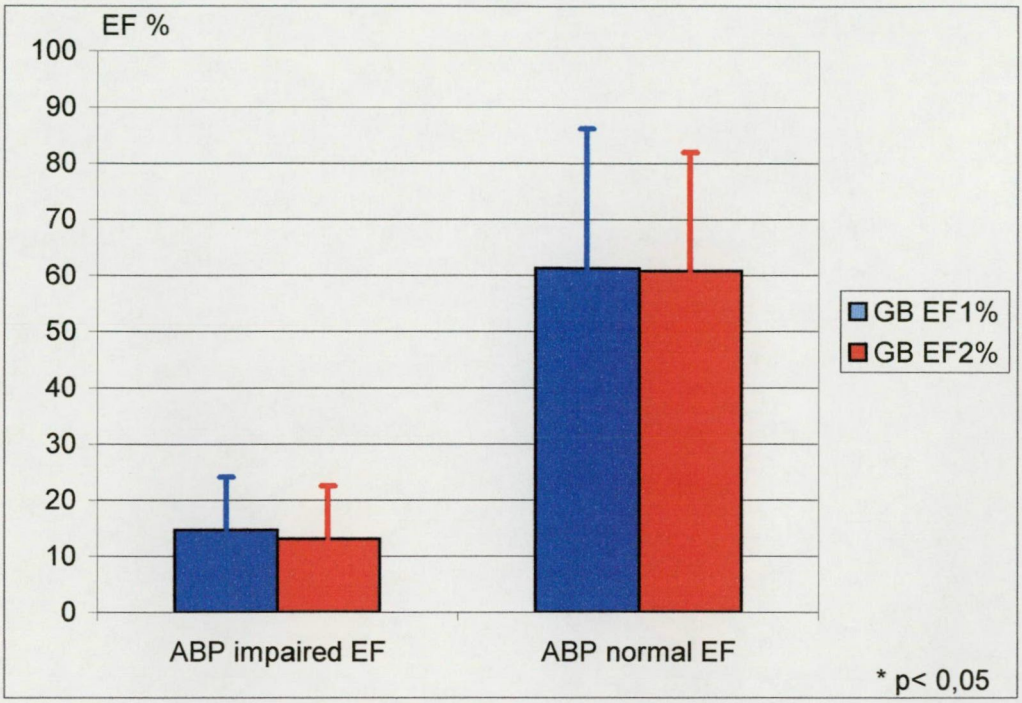


Figure 15. Effect of placebo on CCK_{10}-evoked GB contraction (GB EF 2%) as compared to CCK_{10} alone (GB EF 1%) in ABP patients with an impaired vs. a normal GB EF.

In 16 NR patients where the GB EF was improved following GTN and CCK_{10} coadministration, static hepatobiliary scans frequently revealed a scintigraphic sign which we have termed GB remodelling. This means that during the first CCK_{10} infusion the GB configuration appeared to be spherical in shape and there was no obvious visual evidence of GB emptying, with a lack of any activity in the CBD and the duodenum. However, during GTN and CCK_{10} coadministration, the GB configuration became oval or pear-shaped, with a simultaneous reduction in the amount of the isotope in the GB area, and appearance of the isotope in the CBD and the duodenum. Uncoordinated (ineffective emptying) vs coordinated

(effective emptying) GB motor responses due to CCK_{10} were always associated with typical GB configurations, i.e. spherical vs pear-shaped GB (*Figure 16*).

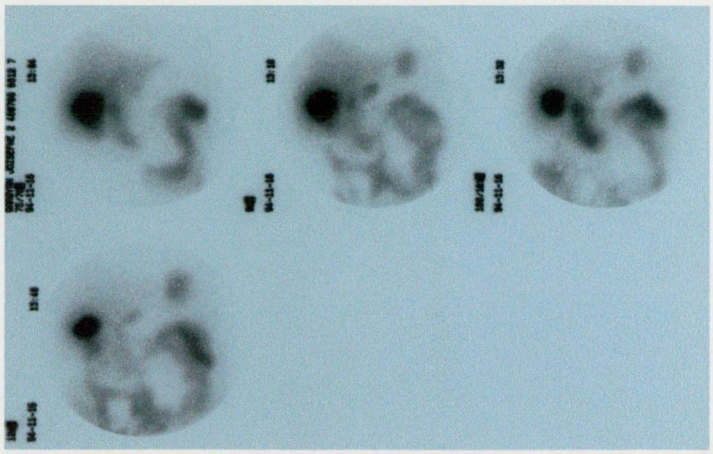


Figure 16. Note the GB remodelling at the time of effective GB contraction during CCK_{10} and GTN coadministration in the lower panel.

Finally, in patients with incomplete or no GB filling at 60 min, GTN administration induced a rapid enhancement of the activity over the GB area, indicating a significant GB filling, which was probably due to a cystic duct and/or GB relaxation (*Figure 17*).

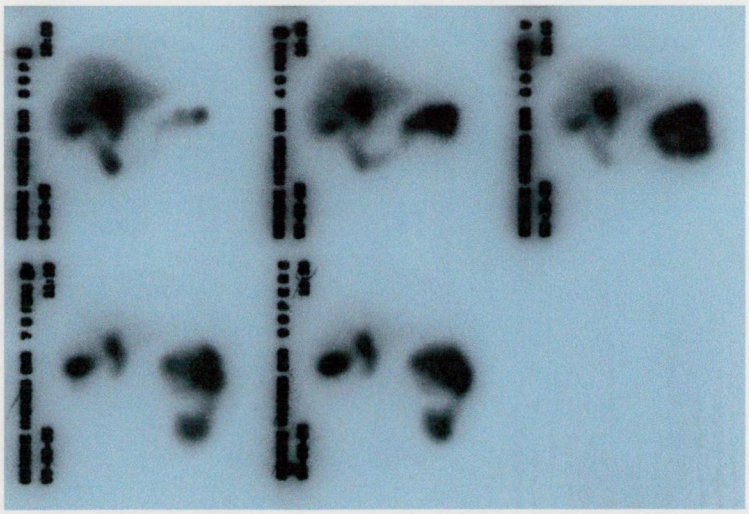


Figure 17. Representative hepatobiliary scintigraphy on a patient with insufficient GB filling at 60 min, which was rapidly enhanced after GTN administration.

QHBS was performed with a similar protocol in 8 patients with insulin-dependent diabetes mellitus and diabetic neuropathy, demonstrated by cardiovascular reflex tests [XVI, XVII]. In this small group of patients GTN coadministration did not improve the CCK_{10}-induced GB EF. Lack of any effect of GTN on the GB contraction in these patients was probably explained by a definite GB hypokinesia due to the diabetic neuropathy (Figure 14).

Our results provided the first evidence that GTN causes a significant improvement in the CCK_{10}-induced GB emptying in patients with ABP. Normalization of the GB EF in the majority of ABP patients following GTN and CCK_{10} coadministration suggests a functional disorder rather than an organic biliary abnormality. We have also demonstrated a new scintigraphic sign of GB remodelling, which may be regarded as visual evidence of the functional spasm of the cystic duct at the time of ineffective GB emptying. In patients with no or late GB filling, we proved that GTN administration induced a rapid and complete GB filling. In patients with diabetic neuropathy, the GB EF was unaffected by GTN augmentation, indicating that the application of this method permitted the separation of those patients with definite GB hypokinesia. From these results, the following conclusions could be drawn. First, GTN administration alone facilitates GB filling, which may be explained by a primary relaxing effect of GTN on the cystic duct and/or the GB. Secondly, GTN and CCK_{10} coadministration induces a significant improvement of the GB EF in patients with ABP and GB dyskinesia, which is probably induced by a NO-mediated cystic duct relaxation at the time of CCK_{10}-evoked GB contraction. Finally, QHBS combined with simultaneous GTN and CCK_{10} administration may be regarded as a prospective new method in the diagnosis of functional spasm of the cystic duct in patients with ABP. Furthermore, the differentiation of patients with GB dyskinesia from those with primary abnormalities of GB contraction, such as GB hypokinesia due to diabetic neuropathy, can also achieved with GTN and CCK_{10} augmentation during QHBS.

3.2.2. DIAGNOSIS OF BILE FLOW ABNORMALITIES IN PATIENTS WITH SPHINCTER OF ODDI DYSKINESIA BY QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY

QHBS is a useful method to assess disturbances of bile flow, and it was therefore suggested as a simple screening test in patients having postcholecystectomy biliary pain and suspected SOD. However, the clinical acceptance of QHBS has progressed only gradually, since it has been demonstrated that overlaps may exist between normal populations and patients with SOD. Furthermore, there is still uncertainty as to which is the most sensitive scintigraphic parameter for the selection of patients with SOD, and how to measure this parameter.

Accordingly, to investigate the diagnostic value of QHBS in patients with suspected SOD, we performed a prospective scintigraphic investigation in a group of cholecystectomized patients with suspected SOD and biliary pain similar to that before the operation [III]. In this study, we compared the results with those obtained in our cholecystectomized control group mentioned above [III]. During this study, we investigated 20 cholecystectomized patients (17 women and 3 men, with a median age of 50 years, range 27 - 68 years) with suspected SOD by means of QHBS. All 20 patients were referred to the Hvidovre Hospital for postcholecystectomy pain syndrome, indicated by RUQ or epigastrial pain being similar to that before the operation, but without any organic explanation. Twelve of the 20 patients (57 %) had concomitant dyspeptic symptoms (i.e. nausea, vomiting, early satiety and bloating) too. The questionnaires revealed that the median time of the onset of symptoms after cholecystectomy was approximately 1.5 years. Apart from the diagnostic tests necessary to exclude extrabiliary organic diseases (laboratory tests, upper GI endoscopy and abdominal ultrasonography), diagnostic ERCP was performed in all patients before their

inclusion in the present study. The ERCP and liver function tests were normal in the majority of the patients, 2 displayed a moderate CBD dilation without an obvious cause, and 3 had a moderately elevated AST on admission. On Geenen's classification, these patients belonged in the SOD groups of biliary types II and III.

QHBS was performed with the standard method described above, and the quantitative parameters were then and compared with those for the control group described above. In 7 of the 20 patients, the static hepatobiliary scintigram revealed a significant accumulation of the isotope in the biliary tree (*Figure 18*).

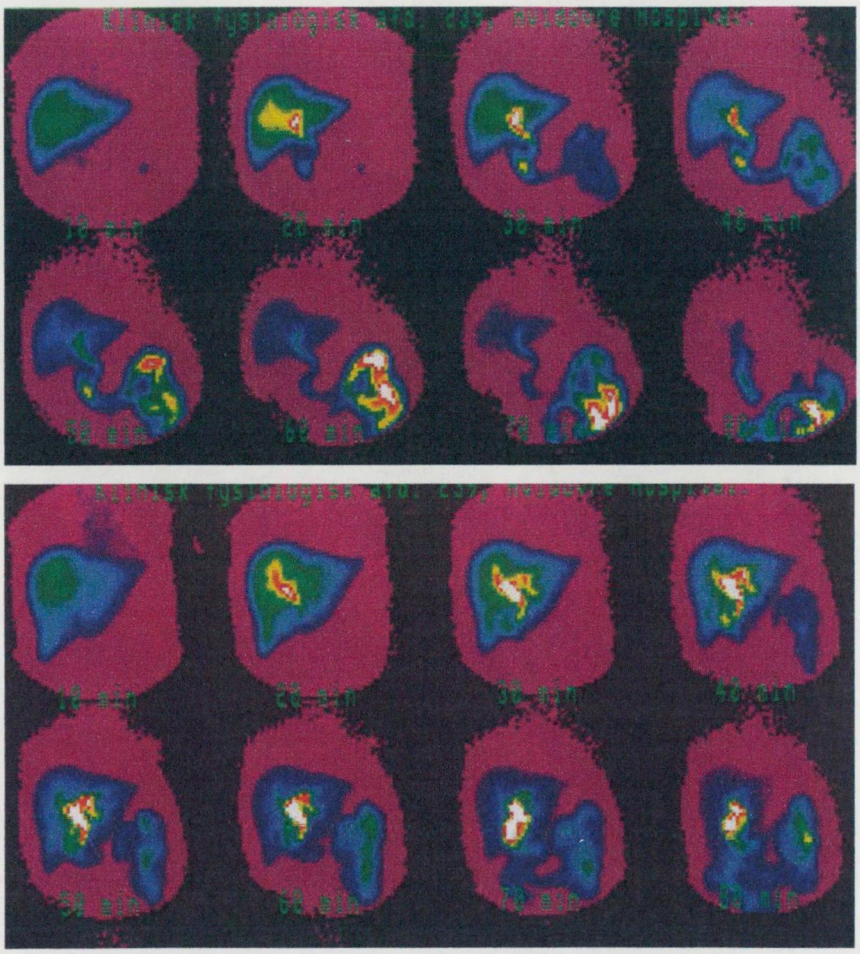


Figure 18. The upper panel depicts a static hepatobiliary scintigram of a control patient with normal bile flow, and the lower panel that of a patient with SOD and accumulation of the isotope in the biliary tree.

The transpapillary bile flow differences between the two groups were more obvious after the quantitative analysis. The TACs over the HH and CBD tended to show an accumulation pattern in most of the patients with suspected SOD (*Figure 19*).

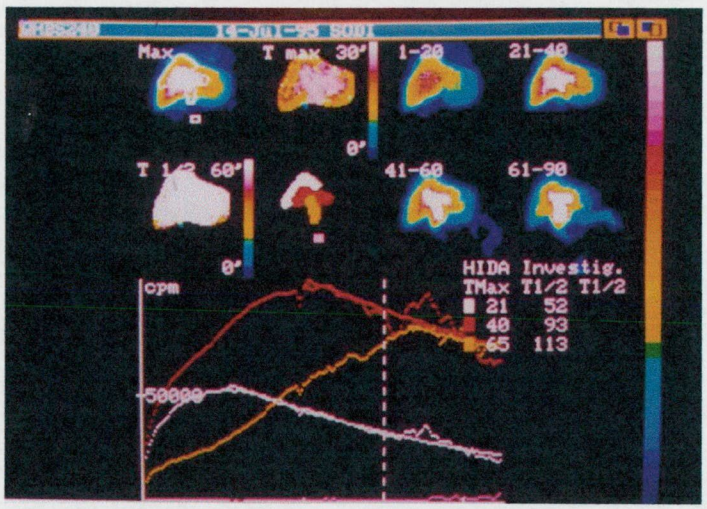


Figure 19. Representative QHBS result in a patient with SO dyskinesia, demonstrating the typical accumulation pattern of the TAC over the CBD.

In patients with suspected SOD, $T_{1/2}$ calculated from the ROI over the CBD, and also the DAT, HDTT and AR values were significantly higher than those in the controls. These results called attention to the parameters HDTT and AR, which proved to be highly sensitive with only a moderate overlap between the controls and the SOD patients, despite the inclusion of patients with SOD of biliary types II and III, i.e. mainly the patients with SO dyskinesia. When the individual values of the patients were compared with the upper limit of the normal values, 14 of the 20 patients with suspected SOD had at least one abnormal quantitative parameter. These results indicated that HDTT and AR should be applied for the detection of bile flow abnormalities in patients with SO dyskinesia (*Figure 20*).

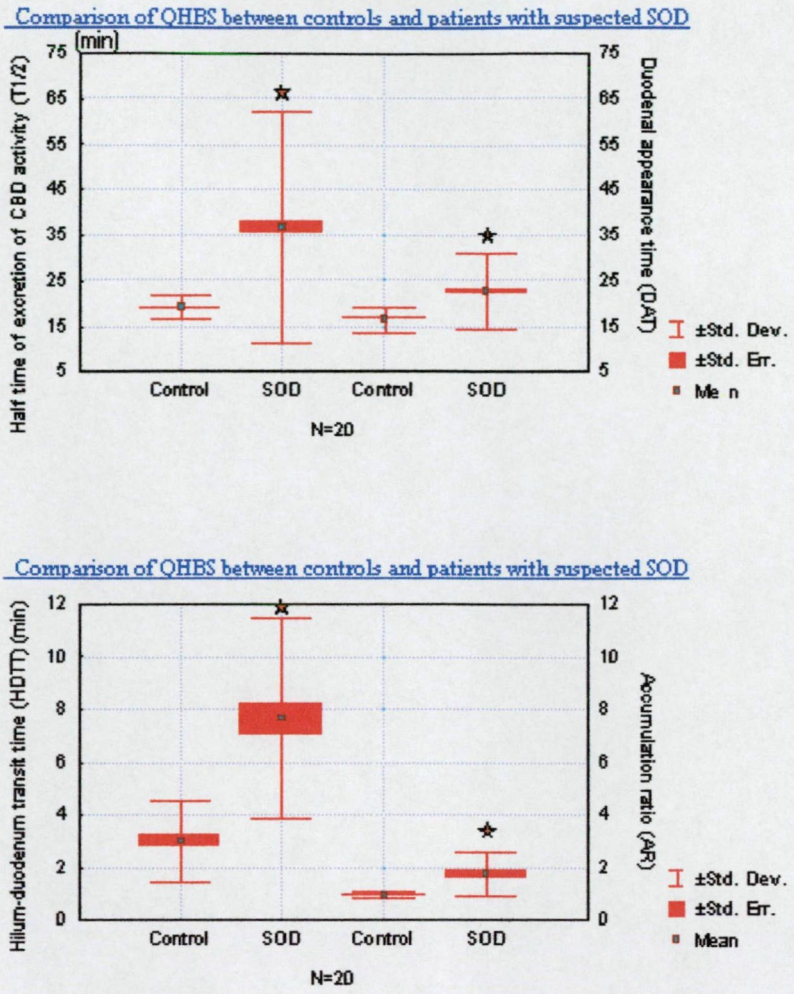


Figure 20. Comparison of the QHBS results on the controls and on the patients with SOD of biliary types II and III.

This study allowed the conclusion that QHBS is a sensitive method for the detection of bile flow abnormalities in patients with SO dyskinesia. In this respect, AR and HDTT proved to be the most sensitive parameters, with only moderate overlap between the SO dyskinesia patients and the controls. Accordingly, we can suggest the application of QHBS as a simple screening test in the diagnostic strategy of patients with postcholecystectomy symptoms and suspected SO dyskinesia before the application of risky and invasive investigations such as ESOM.

3.2.3. DIAGNOSIS OF SPHINCTER OF ODDI MOTILITY DISORDERS IN PATIENTS WITH SPHINCTER OF ODDI DYSKINESIA BY VIDEOMANOMETRY

Videomanometry of the SO is a fundamentally new technique of manometric analysis, which was originally proposed for the comparison of fluoroscopic pictures of ERCP and pressure recordings of the SO. This technique became possible only after the introduction of the new Windows version of the Polygram analysis software developed by Synectics Co, whereby we are able to record video movies and pressure recordings simultaneously on an exact time-correlated basis. We initially developed this new method to compare 3-channel ESOM pressure recording and real-time fluoroscopic pictures of ERCP. The recording speed of the fluoroscopic movie was 15 pictures/sec. Separate and independent qualitative analyses of the SO peristaltic activity on fluoroscopy, and the SO phasic activity on manometry, allowed some important physiological observations [XVIII]. Bile emptying from the CBD into the duodenum always occurred at the time of concomitant PCs on ESOM. Furthermore, periods of intra- and inter-PC activity on ESOM corresponded exactly to the systolic and diastolic phases of the SO on fluoroscopy (*Figure 21*).

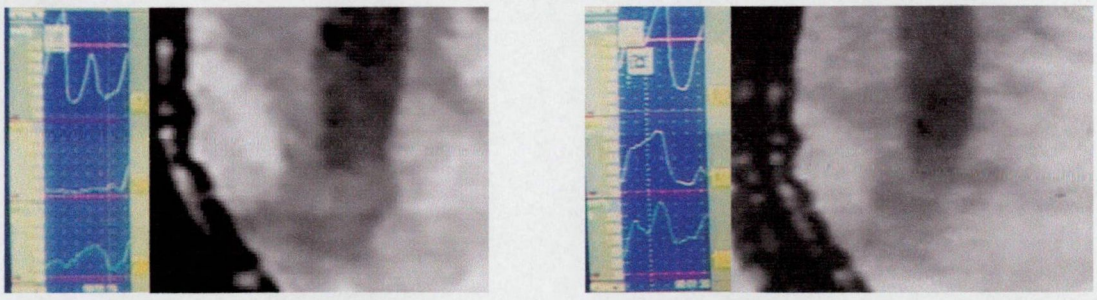
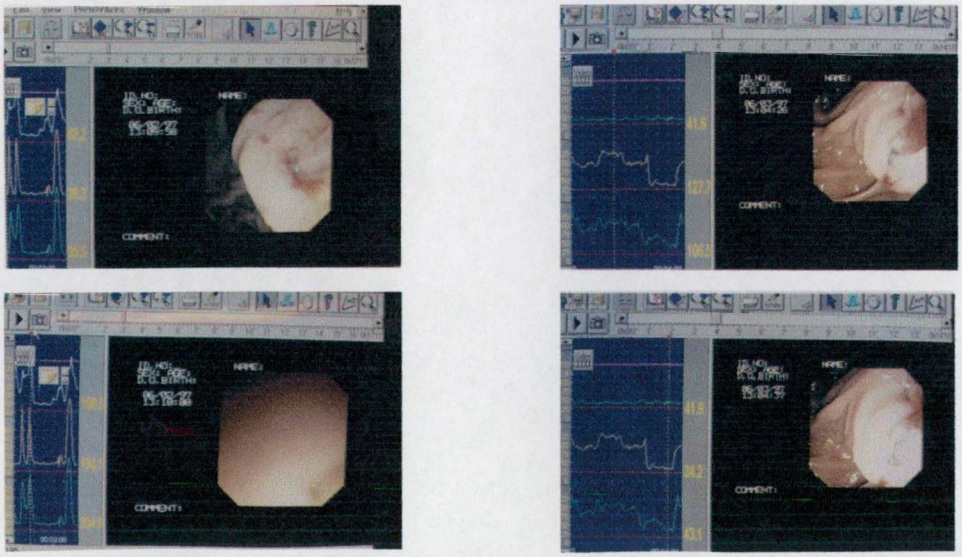


Figure 21. Relationship between peristaltic activity of the SO on fluoroscopy and PC on ESOM. The left side demonstrates a SO systole on fluoroscopy and the lack of PC on ESOM, while on the right side is a SO diastole at the time of a propagating PC on ESOM with a transient pressure drop at this moment of bile emptying.

Analysis of ESOM can be difficult because of the occurrence of manometric artefacts, as discussed previously, which might influence the reproducibility too. However, there was earlier no objective method to recognize and exclude these periods from the manometric tracings. It is obvious that personal attendance and participation at the manometric investigation makes the tracing analysis easier afterwards. This led to the proposal that availability of the real-time recorded duodenoscopic movie during the manometric tracing analysis could be extremely useful to exclude artefacts. Accordingly, we conducted a prospective study to develop a new method, videomanometry of the SO, based on simultaneous manometric pressure and real-time endoscopic picture analysis during the ESOM procedure [IV]. The aims of this study were to describe and document the most frequent manometric artefacts, to investigate the usefulness of videomanometry for the detection of manometric artefacts during ESOM; and to analyse the effects of duodenal contractions on the SO manometric pressure profile by means of videomanometry. In 7 consecutive patients, both computer and analogue recording of the video endoscopic movie was performed at a frame rate of 15 pictures/sec during the ESOM investigation. The ESOM and real-time endoscopic picture analysis results were evaluated separately and compared on a time-correlated basis. The most frequently recognized artefacts that occurred during the ESOM procedures were retching, catheter movement-induced pseudo-contractions, duodenal contractions and ventilation artefacts (*Figure 22*).



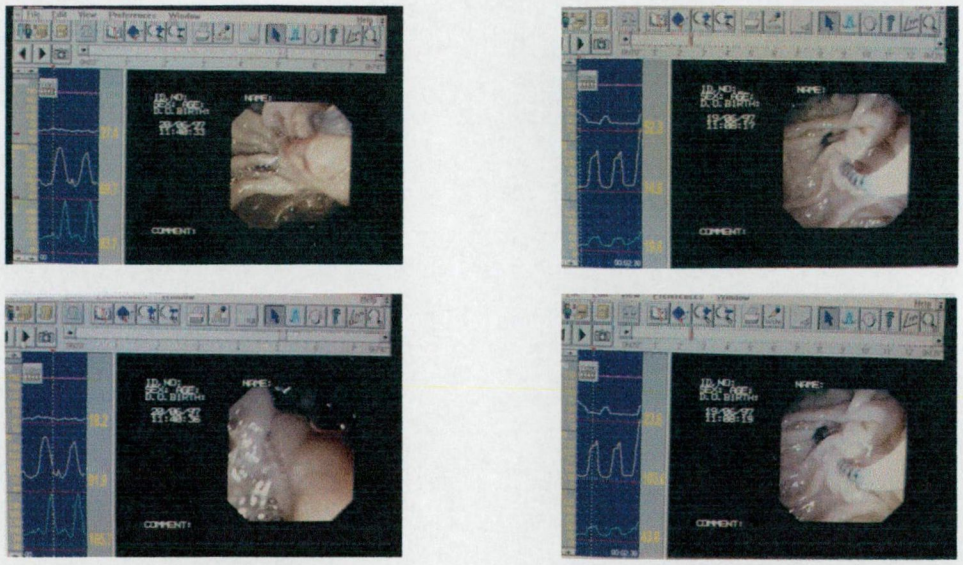


Figure 22. Videomanometric documentation of the most frequent manometric artefacts. Demonstrative pictures of artefacts such as retching (upper left), major catheter movement-induced pseudo-contractions (upper right), duodenal contractions (lower left) and hyperventilation-induced pseudo-contractions (lower right).

Retching caused a sudden, simultaneous, high and narrow pressure rise in all 3 channels, with an amplitude up to 200 mm Hg and a duration of less than 2 sec. Major movements of the manometric catheter into and out of the SO gave rise to the phenomenon of pseudo-contractions. These in-and-out movements of the catheter may also be due to the transmitted effect of the hyperventillation with an amplitude and duration quite similar to those of real SO or duodenal contractions. However, we found that pseudo-contractions were always simultaneous in all 3 channels and they usually exhibited a reciprocal or mirror phenomenon in channels I and III. Duodenal contractions could readily be differentiated from the real SO contractions by having a lower amplitude, a shorter duration and a slower but definite propagation. Duodenal contractions can also be easily recognized on

videomanometry. In fact, we found that the availability of the RTEPA is extremely useful at the time of ESOM analysis. With the aid of videomanometry, we could continuously monitor the duodenal activity and the catheter position as well, and therefore we could detect the exact positions of the measurement periods and explain most of the unexpected baseline undulations. The frequencies of the previously-described artefacts were scored during the ESOM tracing analysis and then compared. The most frequent artefacts were due to duodenal contractions, followed by pseudo-contractions, and then by retching. When the results of real-time endoscopic picture analysis were compared with the ESOM tracing, we found that in 78% of all duodenal contractions a corresponding pressure wave could be detected in the ESOM tracing, with an average amplitude of 71.9 $\pm$ 16.7 mm Hg and a duration of 2.8 $\pm$ 0.4 sec. However, the manometric characteristics (amplitude, duration and frequency) of the PCs originating from the SO and from the duodenum were different, since true SO PCs had a significantly higher amplitude and longer duration, with an average amplitude of 153.9 $\pm$ 85.0 mm Hg and a duration of 7.9 $\pm$ 1.2 sec (*Figure 23*).

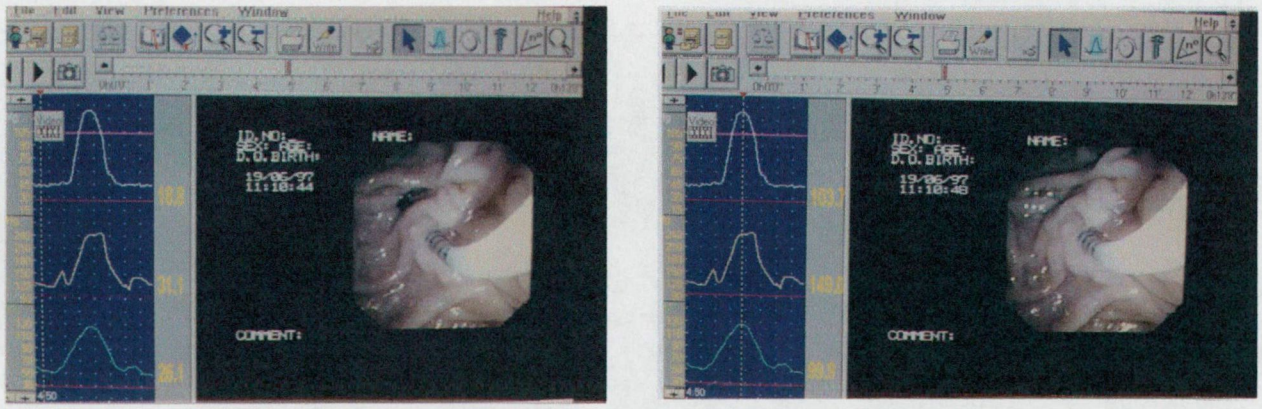


Figure 23. Peristaltic SO PC on ESOM without any visible duodenal activity or other artefact on the videomanometry pictures.

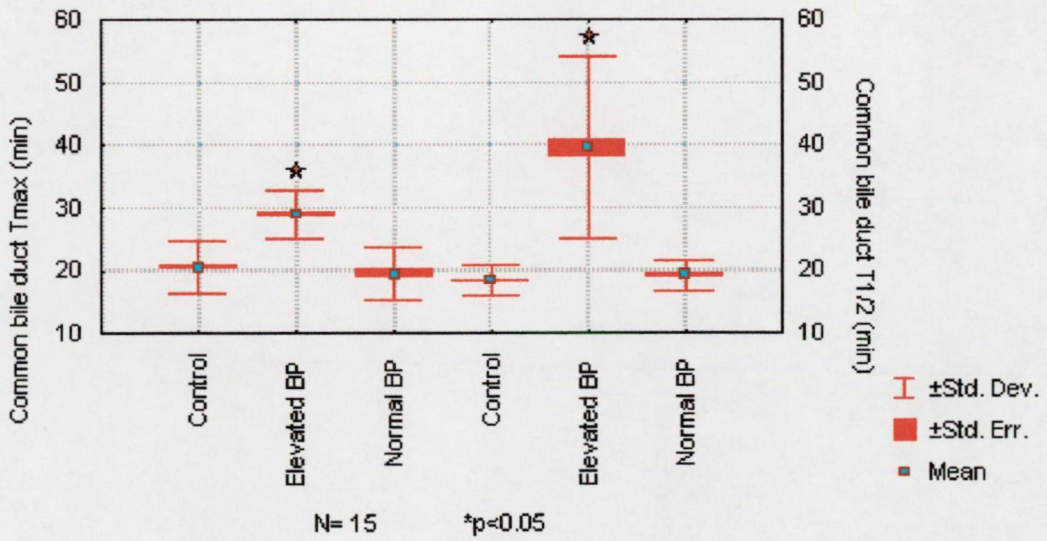
From these results, it became obvious that duodenal contractions were not only visible in the ESOM tracing, but could also induce significant pressure rises in the SO zone and they might therefore influence the bile outflow even without simultaneous genuine SO motor activity [XIX]. We proved that, apart from duodenal contractions, the catheter movement is the most problematic issue, since it can cause instability of the BP and lead to the formation of pseudo-contractions. With the help of videomanometry, the position of the manometric catheter can be continuously monitored with the ring marks outside the papilla. After the exclusion of all periods of manometric artefacts, the diagnosis of SO motility abnormalities can be made more effectively. In conclusion, videomanometry of the SO is a promising new method, which can be used as an aid for correct tracing analysis, for the exclusion of manometric artefacts and to improve tracing documentation. Application of videomanometry of the SO in clinical practice is recommended.

3.2.4. ASSESSMENT OF FLOW PRESSURE RELATIONSHIP BY COMPARISON OF THE RESULTS OF QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY AND ENDOSCOPIC SPHINCTER OF ODDI MANOMETRY IN PATIENTS WITH SPHINCTER OF ODDI DYSKINESIA

Since ESOM is regarded as the gold standard for the diagnosis of motility disorders of the SO, an assessment of the exact diagnostic value of QHBS is not possible without a comparison of the results of these methods in the same group of cholecystectomized patients with suspected SOD. Therefore, we conducted a prospective study to investigate the same group of patients by means of QHBS and ESOM [III]. Both QHBS and ESOM were performed by applying our standard methods, as described previously. Twenty cholecystectomized patients with suspected SOD were investigated (17 females and 3 males, mean age 50 years) by means of QHBS and then ESOM. All patients had biliary pain similar to that before the operation, and they belonged in the SOD groups of biliary types II and III. QHBS was performed in all 20 patients, but ESOM could be completed only in 15 patients. Dropouts were due to poor patient cooperation in 3 cases, and to technical difficulties, such as an inability to introduce the manometric catheter into the CBD in 2 cases. No complications occurred after QHBS. However, after manometry, mild pancreatitis occurred in 5 of the 15 patients (33%), manifested by abdominal discomfort and a transient serum amylase elevation on the day of the investigation. All patients could be discharged one day after the ESOM procedure without long-term complications. Nine of the 15 symptomatic patients exhibited an elevated SO BP (over 40 mm Hg) on ESOM, while in the remaining 6 patients the SO BP was normal. A predominantly retrograde PC peristalsis was found in 4 patients, all but one accompanied by an elevated SO BP. In this series of 15 patients, no tachyoddia or increased PC amplitudes were observed. We diagnosed SO motility abnormalities in 10 of the 15

patients (67%) with postcholecystectomy pain and suspected SOD, most of them having an elevated SO BP, which always normalized in response to CCK_{10} administration.

In those 15 patients with successful ESOM and QHBS, we were able to compare the pressure values and the bile flow parameters. On the basis of the ESOM results, we divided the patients into two groups: 9 patients with an elevated SO BP (over 40 mm Hg) and 6 patients with a normal SO BP (under 40 mm Hg). The QHBS parameters in the two patient groups with a normal or an elevated SO BP were then compared with the results on our 20 asymptomatic cholecystectomized controls (described in section 3.1.). In the 9 patients with an elevated SO BP, the $T_{max,}$ and $T_{1/2}$ values calculated from the ROIs over the HH and CBD, HDTT, DAT and AR were significantly increased as compared to the values obtained in the controls or in the patients with a normal BP. In the remaining 6 patients with a normal SO BP, the QHBS parameters did not differ significantly as compared with those for the control group. Moreover, no overlap of QHBS parameters was observed in the SOD patients with elevated BP and the controls (*Figure 24*).



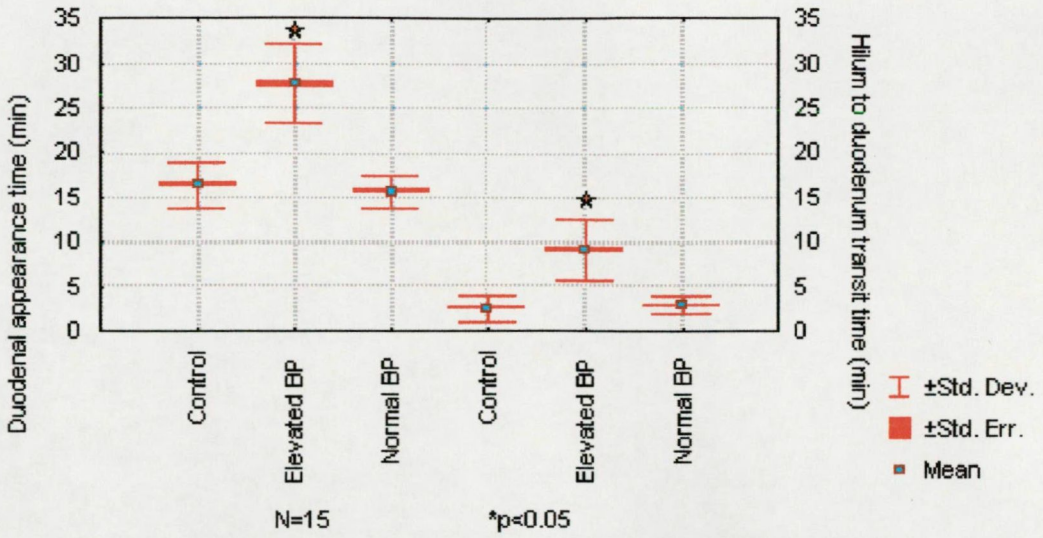


Figure 24. Comparison of the results of QHBS in patients with biliary pain and normal versus elevated SO BP and in asymptomatic controls. The upper panel depicts the T_{max} and $T_{1/2}$ parameters of CBD emptying, while the lower panel relates to DAT and HDTT.

Finally, in order to establish a possible relation between the flow parameters derived from QHBS and the pressure values calculated from ESOM, we systematically compared our quantitative parameters by the Spearman rank correlation test. For the pooled data on 15 symptomatic patients with suspected SOD, a statistically significant linear correlation was found between the SO BP and all of the QHBS parameters: SO BP vs. LP T_{max} ($R = 0.69$, $p = 0.005$); SO BP vs. LP $T_{1/2}$ ($R = 0.84$, $p = 0.00009$); SO BP vs. HH T_{max} ($R = 0.82$, $p = 0.0002$); SO BP vs. HH $T_{1/2}$ ($R = 0.79$, $p = 0.0005$); SO BP vs. CBD T_{max} ($R = 0.65$, $p = 0.008$); SO BP vs. CBD $T_{1/2}$ ($R = 0.68$, $p = 0.005$); SO BP vs. HDTT ($R = 0.72$, $p = 0.003$); and SO BP vs. DAT ($R = 0.89$, $p = 0.00001$) (*Figure 25*). However, no statistical relationship was detected between the QHBS parameters and the amplitude, frequency and propagation characteristics of the SO PCs.

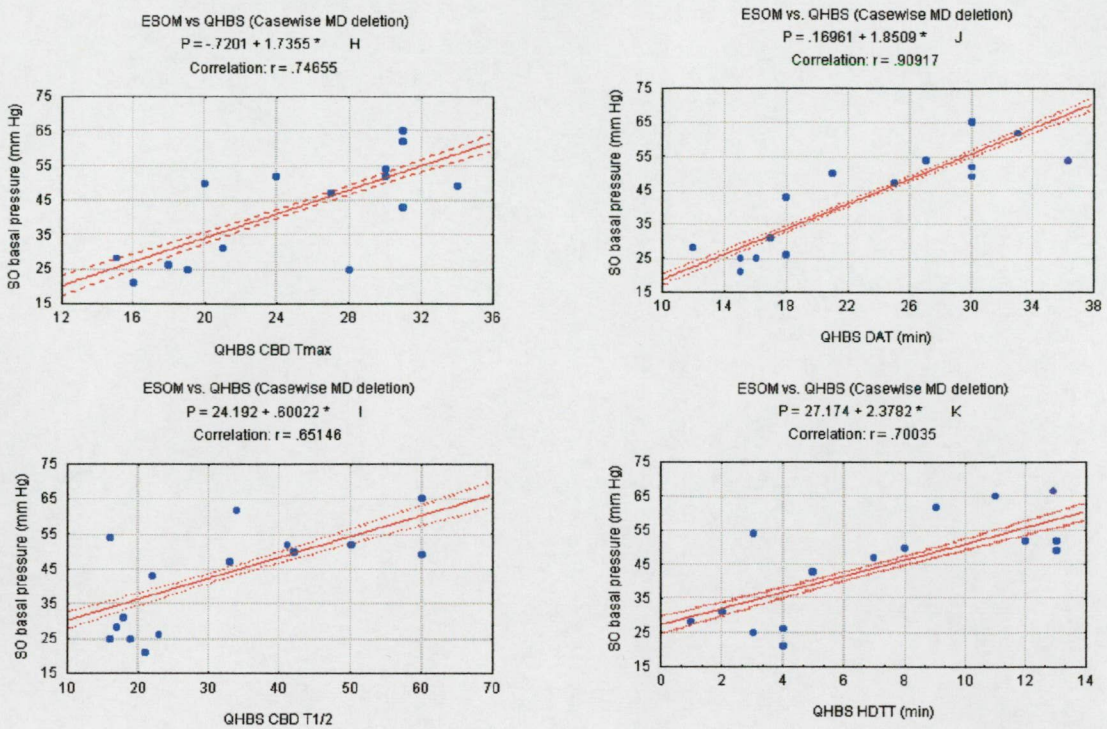


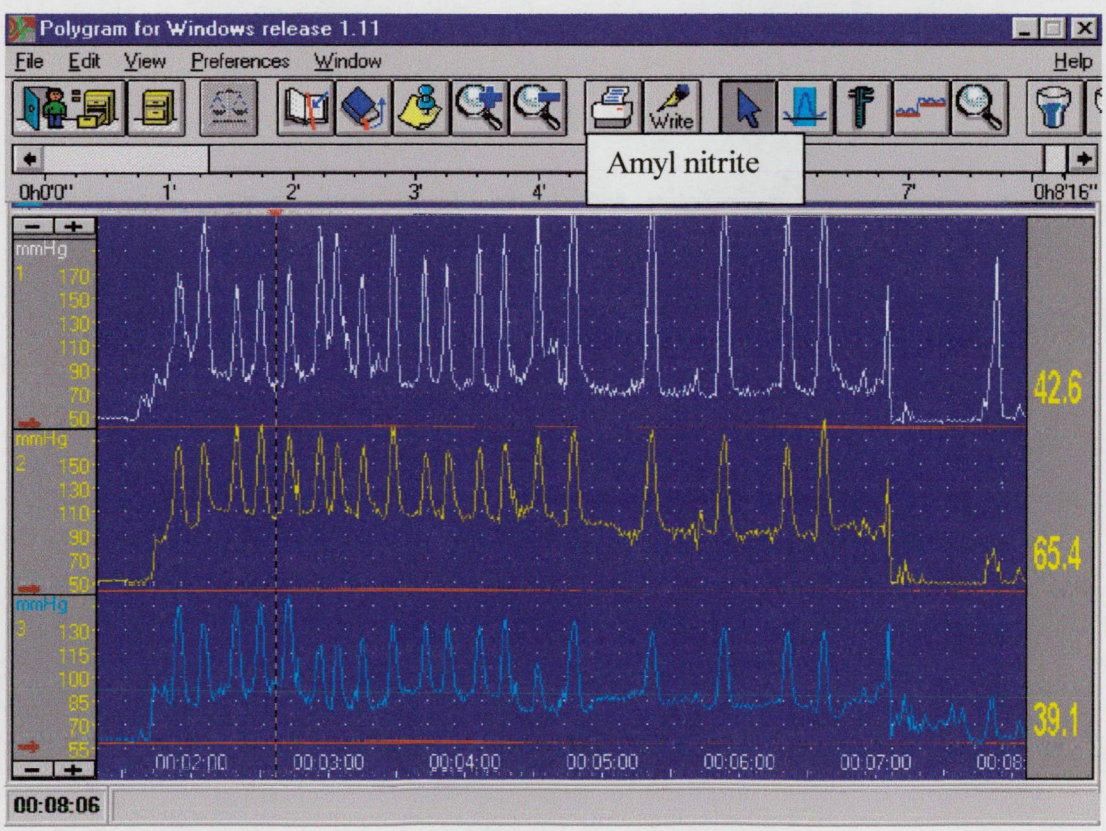
Figure 25. A significant linear correlation was established between the SO BP values determined by ESOM and the transpapillary bile flow parameters measured by QHBS.

Calculation of the upper confidence limits of the normal values (mean + 2 SD) of each QHBS parameter in the control group allowed us to determine the sensitivity and specificity of QHBS in the diagnosis of SOD with an elevated SO BP. Although HDTT was the most sensitive scintigraphic parameter for the detection of an elevated SO BP (89%), DAT, $T_{1/2}$ of the CBD, and AR proved to have excellent sensitivities of 78%, 78% and 67%, respectively. In fact, the combined sensitivity of T_{max} and $T_{1/2}$ of the CBD reached 100%. Hence by a simple quantification of the CBD TACs, we were able to identify all patients who had an elevated SO BP on ESOM. The specificity turned out to be 100% for all QHBS parameters, which was obviously due to the highly preselected patient cohorts, and should also be evaluated with regard to the limited number of patients.

These results permitted the following conclusions. A significant correlation has been established between the bile flow parameters measured by QHBS and the SO BP determined by ESOM. The SO BP has a critical influence on the transpapillary bile flow in cholecystectomized patients. QHBS proved to be a sensitive and specific method in the differentiation between patients with a normal or an elevated SO BP. Therefore, QHBS is a useful non-invasive method for the diagnosis of SOD patients with an elevated SO BP.

3.2.5. DIAGNOSTIC VALUE OF AMYL NITRITE AND CAERULEIN AUGMENTATION DURING QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY AND ENDOSCOPIC SPHINCTER OF ODDI MANOMETRY IN PATIENTS WITH SUSPECTED SPHINCTER OF ODDI DYSKINESIA

SO dyskinesia is a primary functional abnormality of the SO, where the SO responds with exaggerated spasm to various physiological stimuli. During these periods of dysmotility, transient elevation of the SO BP (also described as dyscoordination) and abnormal transpapillary bile flow can be detected by means of ESOM and QHBS. The diagnostic characteristic of SO dyskinesia is that these transient elevations of the SO BP can be relieved after by CCK_{10} or AN administration. Normalization of the elevated SO BP following the administration of CCK_{10} or AN during ESOM suggests dyskinesia rather than organic stenosis of the SO (*Figure 26*).



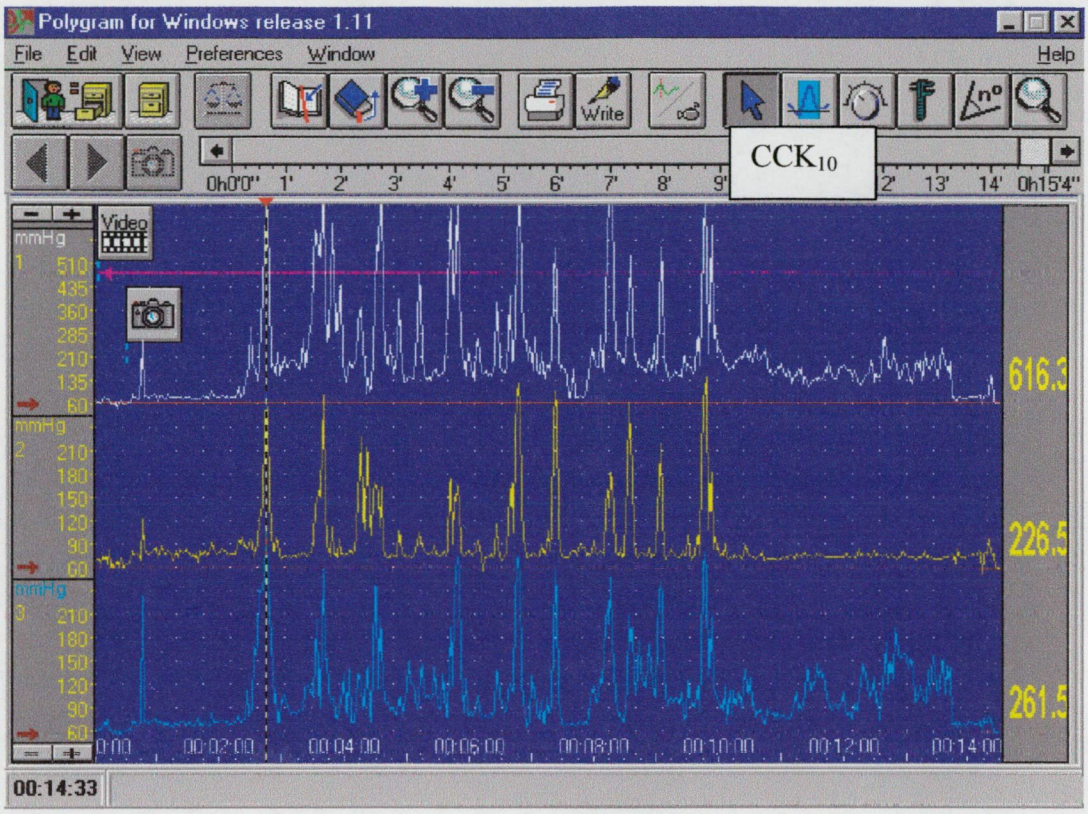


Figure 26. The upper panel demonstrates the effect of AN, and the lower panel that of CCK_{10} on the SO pressure profile in patients with SO dyskinesia. Note the obvious drop in SO BP after the administration of either SO relaxants. AN had only a minor influence on the PCs, whereas CCK_{10} completely abolished them.

Non-invasive differentiation between SO dyskinesia and SO stenosis would be extremely valuable during QHBS with augmentation of the bile flow with SO relaxants. Therefore, we developed a new scintigraphic method, referred to as AN-augmented QHBS, to differentiate patients with organic and functional obstruction at the level of the SO [V]. The rationale of this study was based upon the assumption that AN administration would cause relaxation in all functional obstructions of the SO, and the bile flow changes due to the action of AN could be detected by QHBS. QHBS was performed with our standard method as

described above, except that from the 60th min. until the end of the examination, AN (dosage: 1 ml of 45 mg/ml solution per patient) inhalation was applied. During the quantitative analysis of the scintigraphy, apart from the regular parameters, the $T_{1/2}$ of the CBD was calculated for both pre- and post-AN periods ($T_{1/2}$ and $T_{1/2a}$). CBD EF (%) was then calculated as a percentage of the CBD activity change after AN administration as compared to the CBD activity before the pharmacological stimulus. With these quantifications, the effect of AN administration on the transpapillary bile flow could be expressed numerically, and patients with SO stenosis and SO dyskinesia could be readily differentiated [V] (*Figure 27*).

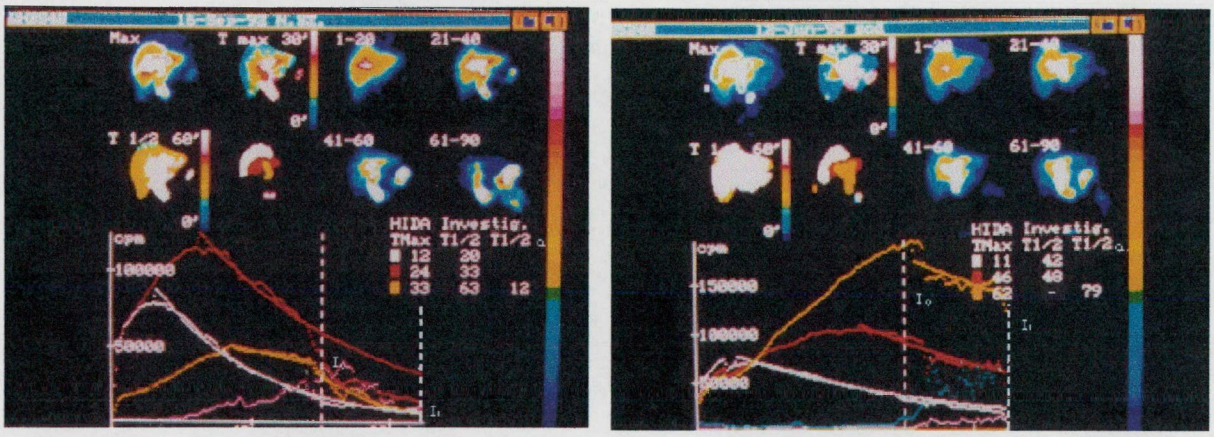
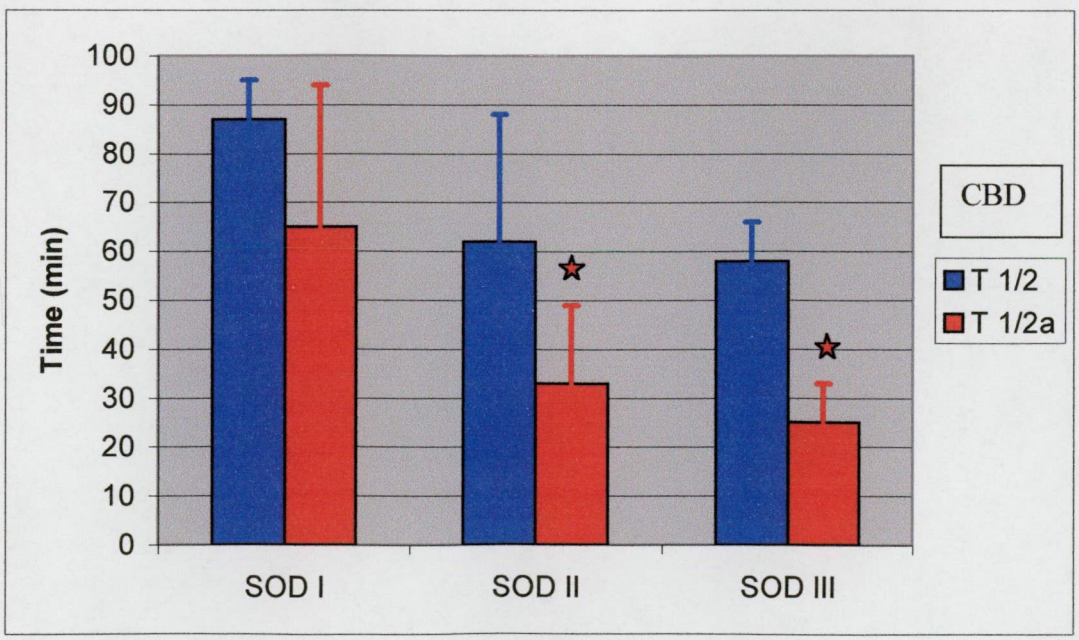


Figure 27. Pictures summarizing the results of AN-augmented QHBS investigation in a patient with SO dyskinesia (CBD $T_{1/2}$: 63 min, CBD $T_{1/2a}$: 12 min, CBD EF%: 87%) on the left, and in a patient with SO stenosis (CBD $T_{1/2}$: > 90 min, CBD $T_{1/2a}$: 79 min, CBD EF%: 23%) on the right. The upper panel presents parametric and cumulative pictures, and the lower panel TACs (LP: white, HH: red, CBF: yellow). CBD EF was calculated by applying the formula CBD EF (%) = $(I_0-I_1)/I_0$ x 100.

We recently conducted a retrospective study aimed at evaluating the usefulness of AN augmentation in patients with suspected SOD for the differentiation of patients with structural stenosis (SOD of biliary type I) and functional dyskinesia (SOD of biliary type II or III) [XX].

AN-augmented QHBS was performed on 66 patients with suspected SOD (mean age: 60 years; 61 female and 5 male). Of these 66 patients, 9 belonged in the SOD group of biliary type I, 38 in the SOD group of biliary type II, and 19 in the SOD group of biliary type III. All patients had been cholecystectomized (an average of 10 years before the investigation) and had RUQ abdominal pain similar to that before the operation. QHBS revealed a marked obstructive pattern in all 9 SOD patients of biliary type I, and AN administration did not induce significant changes in the parameters of CBD EF% and $T_{1/2a}$ for the CBD. In the 38 patients with SOD of biliary type II and the 19 patients with SOD of biliary type III, QHBS revealed a partial obstructive pattern of the transpapillary bile flow, which was significantly improved by AN administration, indicating a functional obstruction at the level of the SO. However, in those patients in the SOD group of biliary type III (i.e. purely functional group), the acceleration of the rate of CBD excretion was so prominent that $T_{1/2a}$ for the CBD not only decreased significantly, but also normalized during AN administration. Moreover, we found significant differences between the results for the SOD I, II and III patients as regards both the $T_{1/2a}$ for the CBD and the CBD EF% values (*Figure 28*).



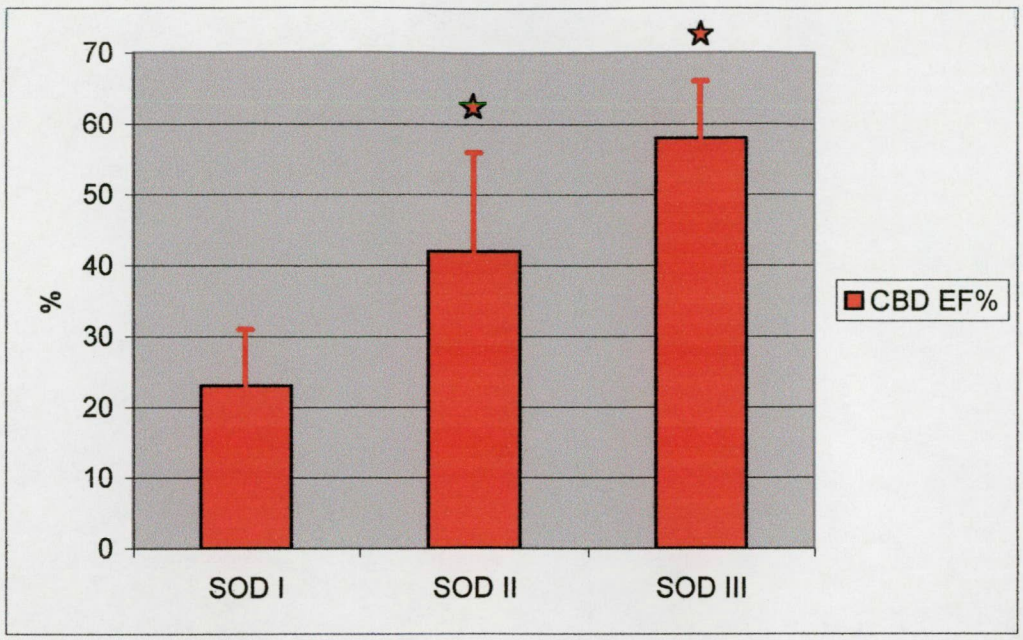
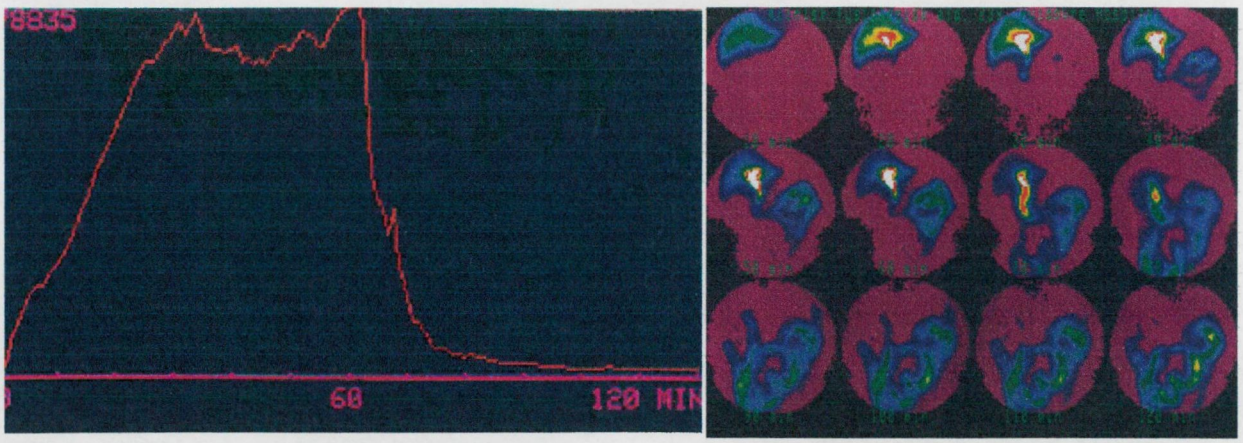


Figure 28. The upper diagram compares $T_{1/2}$ and $T_{1/2a}$ for the CBD in the SOD patients in groups of biliary types I, II and III, while the lower diagram compares the CBD EF% values. $T_{1/2}$ for the CBD before AN augmentation is not useful for separation of these groups, but both $T_{1/2a}$ and CBD EF are significantly different, with only a moderate overlap.

Another innovative approach could be the use of CCK or its analogue CCK_{10} in an attempt to enhance the transpapillary bile flow in patients with partial biliary obstruction on QHBS. In order to investigate the effect of CCK on the bile flow in cholecystectomized patients, we conducted prospective studies in patients with suspected SO dyskinesia [III, XXI, XXII, XXIII]. During QHBS at the 60th min, we administered 5 ng/bwkg/min CCK_{10} (Takus) i.v. for 10 min. $T_{1/2}$ for the CBD was then calculated before and after CCK_{10} administration ($T_{1/2}$ and $T_{1/2c}$). After the administration of CCK_{10} during QHBS, the rate of bile excretion from the CBD was accelerated and $T_{1/2}$ for the CBD was decreased significantly (*Figure 29*).



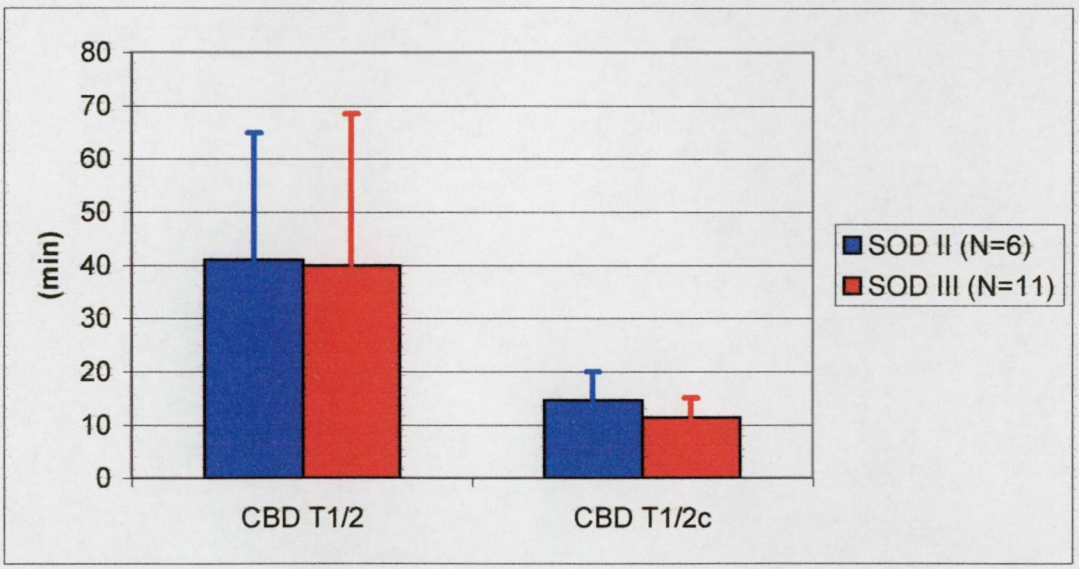


Figure 29. The upper panel depicts a representative CCK_{10}-augmented QHBS in a patient with partial biliary obstruction due to SOD that was completely relieved after CCK_{10} administration at the 60^{th} min. The lower diagram presents the results of CCK_{10}-augmented QHBS in patients with SOD of biliary types II and III. CCK_{10} augmentation significantly accelerated the transpapillary bile flow in both groups. However, the CBD $T_{1/2}$ did not differ significantly in the two groups of patients, regardless of the effect of CCK_{10} administration.

This effect of CCK_{10} was uniform, regardless of the SOD staging, or the previous scintigraphic or manometric result. No scintigraphic or manometric signs of a paradoxical response to CCK (i.e. accumulation of the isotope due to SO spasm) were detected. It must be emphasized therefore, that in contrast with AN, CCK_{10} administration diminished the previous differences in the transpapillary bile flow.

These studies permitted the following conclusions. AN-augmented QHBS allows a non-invasive differentiation between organic stenosis and functional motor abnormalities of the SO. The results of AN-augmented QHBS closely correlated with the SOD staging, based on morphologic criteria. The clinical staging of SOD could therefore be assessed by the application of AN augmentation during QHBS. In contrast, CCK augmentation uniformly accelerated the transpapillary bile flow in every patient, therefore masking the previous differences. No paradoxical response of the SO was detected after CCK augmentation, making this test inadequate as an evocative method.

3.2.6. DIAGNOSTIC VALUE OF PROSTIGMINE AND MORPHINE AUGMENTATION DURING QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY IN PATIENTS WITH SUSPECTED SPHINCTER OF ODDI DYSKINESIA

It is assumed that in patients with SO dyskinesia, the SO responds with a functional spasm (periods of elevated SO BP) to various exogenous stimuli, such as emotional, physical or pharmacological stress [30]. As concerns the subjective complaints of these patients, these symptomatic periods are completely unpredictable; they recur randomly and sometimes only occasionally after a certain period. Most of the attacks resolve spontaneously within a few hours, i.e. sometimes without medical attention. It is also likely that, even with detailed investigations in these SO dyskinesia patients neither morphological nor functional disturbances can be detected during the dominant asymptomatic periods of the disease. Accordingly, evocative tests have long been utilized as the only possibility suggested to demonstrate functional abnormalities in these patients. Provocation tests involving CCK augmentation have been proposed, but their diagnostic usefulness is questionable, since the paradoxical response of the SO is believed to be a rare phenomenon [74]. Evocative tests utilizing MS administration, such as the Debray and Nardi tests, were intended to identify this subgroup of patients with SO dyskinesia through the possible hyperreactivity of the SO due to MS, but the diagnostic value of these tests has been criticized in consequence of their poor reproducibility and low specificity. This was mainly due to the fact, that their diagnostic scoring system was based only on an assessment of subjective complaints, which can be completely unreliable in a patient with functional abdominal pain [41]. Moreover, MS may induce segmental spasm of the small bowel without SO spasm, which can be painful in patients with irritable bowel syndrome. Therefore, Varró et al. suggested that the diagnostic

accuracy of these provocation tests might improve through a combination of liver enzyme determination and hepatobiliary scintigraphy, in order to prove that the SO spasm really existed at the time of the subjective complaints [75]. However, without the quantification of hepatobiliary scintigraphy, this method was not sensitive enough to make a real distinction between controls with physiological responses and patients with exaggerated responses and SO spasms after MS administration. In order to improve the diagnostic value of the original method with the advent of QHBS, we conducted a prospective study in 22 patients with suspected SO dyskinesia [VI]. All patients belonged in the group with SOD of biliary type III. After a basic scintigraphic study a few days earlier, QHBS was combined with a prostigmine-morphine test in all patients as follows. After an overnight fast, 10 mg MS was administered s.c. Thirty min. later, 0.5 mg prostigmine was injected i.m., and 140 MBq EHIDA was administered i.v. Subjective complaints were recorded, and the serum levels of AST and amylase were determined at the beginning and 2, 4 and 6 hours following MS administration. In 12 of the 22 patients (55%) (Nardi-positive group), QHBS revealed a biliary obstruction after MS administration, and T_{max} over the CBD and the DAT were significantly increased as compared to the corresponding data obtained without provocation (*Figures 30 and 32*).

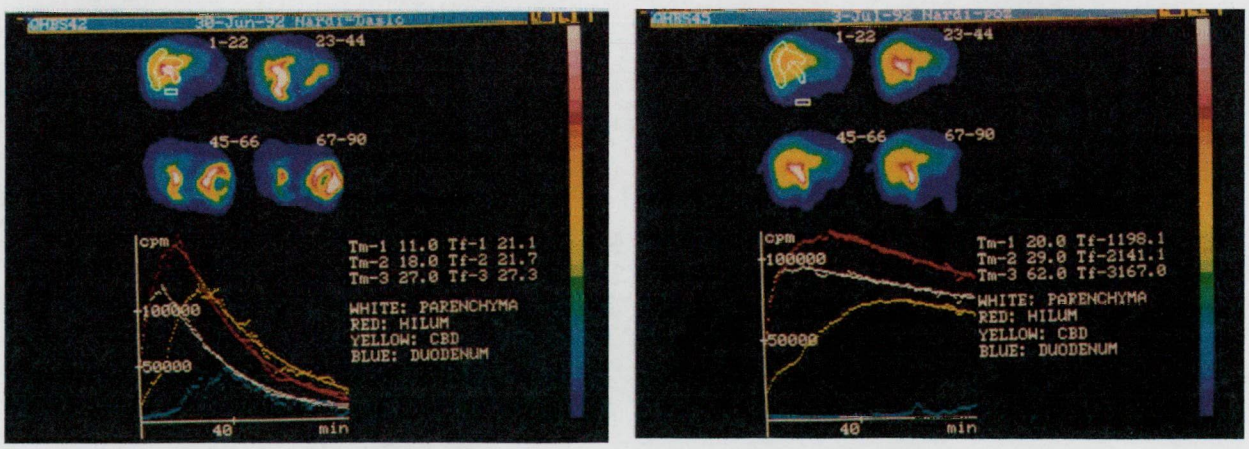


Figure 30. Complete functional obstruction at the level of the SO, due to MS administration, in a patient with a positive Nardi test on the right, and normal bile flow during the baseline QHBS study without provocation on the left, in the same patient.

In the remaining 10 patients (45%) (Nardi-negative group), no obstructive pattern was detected in the hepatobiliary scintigram, and QHBS revealed the free transpapillary flow of the tracer. In this Nardi-negative group, T_{max} over the CBD and the DAT were not changed significantly as compared with the baseline QHBS study (*Figures 31 and 32*).

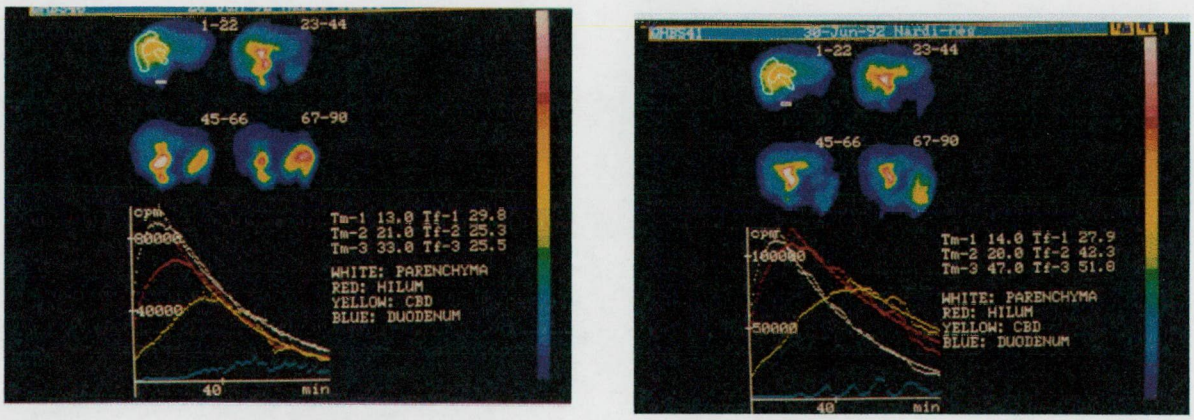


Figure 31. MS has only a subtle effect on bile flow in a Nardi-negative patient on the right as compared with the baseline QHBS study on the left.

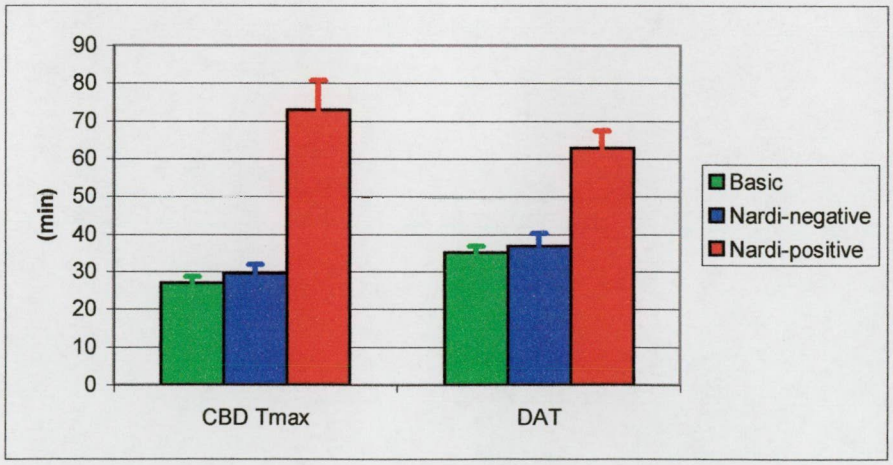


Figure 32. The QHBS parameters are markedly increased in Nardi-positive patients as compared with those in the Nardi-negative group or in the basic study.

The combination of QHBS and Nardi test has led to the non-invasive visualization of the MS-induced exaggerated SO spasm in SO dyskinesia patients, which is probably due to a hyperreactive response of the SO to MS administration. Moreover, there was an excellent correlation between the MS-induced AST rises and the QHBS parameters. All 12 patients, who were judged to be Nardi positive on the basis of the results of QHBS had a significant AST elevation. In fact, in 4 of these 12 Nardi-positive patients, the AST elevation was less than twofold, so it did not reach the diagnostic level. However, none of the 10 patients who gave negative QHBS results exhibited significant AST changes.

In contrast, no correlation was observed between the QHBS results and the subjective complaints of these patients during the Nardi test. 16 patients complained of upper abdominal pain during prostigmine-morphine provocation, whereas only 12 had a SO spasm according to the QHBS results, providing evidence that abdominal pain alone is not a specific sign, and should not be used as a diagnostic criterion.

These studies furnished the following conclusions. QHBS is an excellent method for visualization of the prolonged SO spasm during the prostigmine-morphine provocation test. In patients with SO dyskinesia and a positive Nardi test, QHBS revealed a characteristic obstructive pattern over the CBD, indicating that a sphincter spasm really does exist at the time of the evoked biliary pain. This method allows a clear distinction of patients who give a physiological response to morphine administration from those who respond with an exaggerated sphincter spasm due to hyperreactivity of the SO. QHBS combined with the prostigmine-morphine test should therefore be regarded as a useful method with which to improve the diagnostic accuracy of the conventional Nardi test. Clinical application of this test might be a real prospect in the diagnosis of SO dyskinesia in the future.

4. DISCUSSION

Despite the recent advances in the fields of functional GI disorders and GI motility, the diagnosis of biliary dyskinesia is still difficult, as many of the available methods are not sufficiently sensitive or specific. ESOM has been growing in acceptance in the diagnosis of SO dyskinesia, although it is an imperfect standard, being invasive with a significant procedure-related morbidity. Non-invasive examinations have therefore been introduced and suggested as a possible alternative to ESOM. QHBS originally showed promise as a widely available non-invasive method that is easy to perform. Nevertheless, as a definite diagnostic test in patients with SOD, it must meet the following requirements. First of all, significant bile flow differences should be documented by QHBS between normal controls, patients with hepatocellular diseases, and patients with SOD. Next, it should be noted how cholecystectomy itself alters the transpapillary bile flow pattern. Furthermore, it must be established which is the most sensitive scintigraphic parameter to differentiate between controls and SOD patients, and how to measure it. The sensitivity and specificity of the QHBS parameters should then be reasonably good as compared with those of ESOM. Finally, a differentiation between organic and functional causes of bile flow abnormalities is necessary in order to determine disease severity and optimal therapy.

In the present work, our main proposals were to deal in detail with these problems, and to establish the diagnostic role of QHBS in patients with biliary dyskinesia. First, we determined the control values in a group of patients before and after cholecystectomy. These results can be utilized to determine whether the transpapillary bile flow is accelerated after cholecystectomy and occurs more constantly as compared with the preoperative status, therefore increasing the probability of the identification of abnormalities in patients with SOD [I]. It is well known that there are variations in the normal pattern of bile emptying [62], but

the effect of cholecystectomy on the bile flow had not been investigated previously. Our results made it obvious that there are wide variations in bile emptying from the CBD into the duodenum preoperatively, even in the absence of a biliary obstruction, a phenomenon which may lead to interpretation errors during the analysis of QHBS in patients with suspected SOD an GB. In patients with an intact GB and suspected SOD, therefore ESOM must be performed to establish the diagnosis [I].

The next step was to investigate whether QHBS could be suggested as an initial screening test in patients with suspected SOD. To test this theory, we compared the results of QHBS in patients with postcholecystectomy pain and suspected SOD and in asymptomatic cholecystectomized controls [III]. We demonstrated, that when the patient selection was adequate and the optimal quantitative parameters were selected and applied, there were significant differences and only moderate overlaps between patients with suspected SOD and cholecystectomized controls. Our findings revealed that T_{max} and $T_{1/2}$ of the CBD, DAT, HDTT and AR were the most sensitive parameters for the detection of transpapillary bile flow abnormalities in patients with suspected SOD. Although these results had been postulated in the literature, no previous investigators had been able to demonstrate such a clear distinction [58–60].

As concerns the diagnostic value of QHBS in cholecystectomized patients with suspected SOD, a crucial question is whether QHBS is an accurate method for the selection of SOD patients when the results are compared with those of ESOM. For the pooled data on the symptomatic patients with suspected SOD, we found a statistically significant linear correlation between the SO basal pressure and the QHBS parameters [III]. Our results are in accordance with those reported by Corazziari et al. [76]. Previous investigations had revealed that QHBS has a high sensitivity (83-100%) and specificity (100%) for the diagnosis of SOD as compared with the results of ESOM [77-80]. The main criticism of those studies is that

they were performed in a group of patients mainly with SOD of biliary type I, with a clinically obvious biliary obstruction involving CBD dilatation [77, 78]. However, a dilated CBD may be associated with a slow transpapillary bile transit even without a SO motility disorder [75]. In contrast, we were able to demonstrate bile flow disturbances not only in patients with SO stenosis (biliary group I), but also in patients with SO dyskinesia (SOD biliary groups II and III). The second questionable point is the uniformly 100% specificity with high sensitivity, which at first sight appears incredible [79, 80]. The reason for this finding could be that the selection of these patients was based on the exclusion of other organic GI disorders. In clinical practice, the diagnosis of functional GI disorders is exclusive, and this high specificity is therefore a natural consequence of a correct diagnostic strategy. Moreover, QHBS and ESOM should be applied as functional methods and not to investigate morphology or to differentiate between organic biliary disorders. Our results proved an excellent correlation between the QHBS parameters and the SO BP determined by ESOM, which suggests that from this aspect these methods are comparable. After the separation of patients with SOD on the basis of an elevated SO BP determined by ESOM, the quantitative parameters of HDTT, DAT, AR and CBD emptying were significantly different from those of cases with a normal SO BP, and also from those of the controls. In fact, the combined sensitivity of T_{max} and $T_{1/2}$ of the CBD reached 100%, i.e. quantification of the CBD TAC allowed us to select all patients who had an elevated SO BP on ESOM. The specificity turned out to be 100%, which was obviously due to the strong preselection of the patient population, and which is certainly not the case if QHBS is applied as an initial screening test. Therefore, our results indicate that, after the exclusion of organic disorders, QHBS is a reliable method for the identification of SOD patients with an elevated BP [III].

Further improvement of the diagnostic value of QHBS may be achived by the differentiation of SO dyskinesia patients from those with an organic biliary obstruction, such

as strictures, CBD stones or SO stenosis, which might furnish identical findings on QHBS. Such a diagnostic application of QHBS may be possible following the administration of SO relaxants to document the functional spasm in patients with SO dyskinesia. Accordingly, we developed and introduced a new scintigraphic method in which the QHBS procedure was combined with the concomitant administration of AN, which is a potent smooth muscle relaxant [V]. This new method can be applied to measure the AN-evoked acceleration of the bile flow due to SO relaxation in patients with SOD. With this augmentation, QHBS has become a real functional test. We later demonstrated that untreated hyperlipidaemia might alter the relaxatory effect of NO on the SO [XXIV, XXV], but this phenomenon does not exert a major influence on the diagnostic efficacy [XX]. AN augmentation is a highly effective method for the induction of SO relaxation in all patients who have a functional obstruction of the SO due to SO dyskinesia. In this way, functional and organic causes of biliary obstruction can be differentiated and the accuracy of QHBS has been further increased [V].

The intermittent nature of the complaints of patients with SO dyskinesia is well known, though it is rarely mentioned in clinical practice. This periodicity of the symptoms in these patients suggests that SO motility abnormalities can also occur intermittently. The intermittent occurrence of SO dysmotility might be due to simultaneous alterations of duodenal motility [XXVI, XXVII]. However, an assessment of the SO function with ESOM or QHBS is possible only over short periods. One potential solution of this difficulty is the use of provocation tests during ESOM or QHBS; these might unmask abnormalities which might be missed by the standard methods. The pharmacological provocative tests suggested for the diagnosis of functional biliary pain are based on the administration of MS and CCK [40, 81, 82]. The reproduction of biliary pain is required for a positive result in these provocation tests, in consequence of either the hyperreactivity and/or the hypersensitivity of

the SO following the pharmacological stimulus. The clinical value of the diagnosis of hyperreactivity of the SO after MS administration is supported by the fact that a sustained symptomatic improvement was observed after EST in the patients who gave an exaggerated response [83]. However, a subjective complaint such as biliary pain is difficult to assess adequately and obviously has a low specificity for SO hyperreactivity when considered alone. Therefore, the diagnostic value of these evocative tests is still a matter of debate, since they may furnish a certain number of false-positive results and uncertain reproducibility [41]. To overcome these problems, it was first suggested that these tests should be combined with estimations of the serum amylase and transaminase levels, since transient rises in the levels of these enzymes might be suggestive of a SO spasm [84]. Changes in the plasma levels of liver enzymes are clearly related to a MS-induced spasm of the SO, since an AST elevation is not observed in patients with an intact GB and is abolished by EST [85]. The combination of the Nardi test and hepatobiliary scintigraphy originally suggested by Varró and Csernay has the aims of a further improvement of the diagnostic accuracy and visualization of the SO spasm [86, 87]. As an improvement of the method originally described, we developed a new technique referred to as QHBS combined with prostigmine and morphine provocation, whereby the MS-induced SO spasm can be assessed both qualitatively and quantitatively. Our results clearly demonstrated that, in a subgroup of patients with suspected SOD, prostigmine and MS administration could induce biliary pain and a prolonged spasm of the SO with the blockage of the bile flow, revealed by means of QHBS. Furthermore, there was a good correlation between the AST rise and the results of QHBS during the Nardi test. QHBS combined with the Nardi test can therefore be regarded as an extremely useful method in the diagnosis of biliary dyskinesia induced by hyperreactivity of the SO [VI]. By applying this method, we were recently able to assess the effectiveness of different drugs on the prevention

of MS-induced SO spasm in order to explore new therapeutic possibilities in patients with SO dyskinesia [XXVIII - XXXI].

After the description of the inhibitory and excitatory receptors for CCK on the SO, a paradoxical response after CCK administration was suggested as a possible pathogenic mechanism of SO dyskinesia, which would be another obvious manifestation of SO hyperreactivity [21]. It was also supposed that cholecystectomy itself alters the neural pathways that mediate the inhibitory response of the SO to CCK [88]. Later a paradoxical effect of CCK on the SO was demonstrated in 5-16% of patients with suspected SOD by means of ESOM [89, 23]. It is noteworthy, however, that all of these studies involved the administration of a bolus of CCK in relatively high doses (20 to 300 ng/bwkg). This paradoxical effect of CCK on the SO might therefore be regarded as a consequence of the non-physiological CCK administration. Interestingly, 74% of these patients with postcholecystectomy pain syndrome experienced abdominal pain during CCK administration, regardless of the manometric findings [23]. A paradoxical response to CCK, i.e. a SO spasm, would presumably delay CBD emptying, but in contrast with the manometric findings, no such paradoxical effect could be demonstrated in previous scintigraphic studies [90, 91]. To investigate this field further, we also administered CCK_{10} during ESOM and QHBS in cholecystectomized patients with suspected SO dyskinesia. We found no paradoxical effect of CCK_{10} by QHBS. Interestingly, 9 of 20 patients (45%) experienced biliary pain during CCK administration that was unrelated to the QHBS results (unpublished data). In contrast, QHBS demonstrated that the transpapillary bile flow was highly accelerated in each patient after CCK_{10} administration; this was probably induced by a prompt SO relaxation accompanied by a simultaneous increase in the hepatic bile secretion [92]. Biliary pain after CCK administration might identify a subgroup of patients with SOD and biliary hypersensitivity at the time of increased bile secretion and bile flow induced by CCK. Our results confirmed that

CCK_{10} administration did not increase the sensitivity of QHBS in the diagnosis of SOD, but instead diminished the previous bile flow differences [III].

ABP is also a diagnostic problem in patients with an intact GB, since there are no morphologic abnormalities and it is difficult to assess the site of the functional abnormality. As mentioned above, physiological bile flow variations in these patients with an intact GB made the efficacy of QHBS questionable in the diagnosis of SOD. It has recently been demonstrated by ESOM that SOD with an elevated SO BP may be present in patients with the intact GB, some of them having a well-functioning GB [24, 93]. More importantly, GB and SO dyskinesia might occur concomitantly in the same patients, since most of those patients with ABP and an elevated SO BP who did not despond to sphincterotomy were cured after subsequent cholecystectomy [24]. The lack of a therapeutic response after sphincterotomy, but with a symptomatic cure after cholecystectomy, in patients with ABP provides obvious indirect evidence in support of the existence of GB dyskinesia [94]. Initially, the diagnosis of GB dyskinesia was based upon the reproduction of biliary pain during CCK administration; however, this provocation method was also based on the subjective complaints and had a low specificity [81]. Therefore, the CCK provocation tests were combined with simultaneous determination of the GB EF. With this method, it was demonstrated that a subgroup of patients with ABP exhibits an impaired GB emptying with the reproduction of biliary pain after CCK administration; and the symptoms in this group of patients their symptoms can be alleviated by means of cholecystectomy [95]. However, a low GB EF is obviously not a specific sign for the diagnosis of GB dyskinesia, since it may be due to various organic disorders, such as chronic cholecystitis, cystic duct obstruction by stone or stricture, etc. [96]. It was later proposed that an asymmetric and ineffective contraction of the GB in the hepatobiliary scintigram in response to a CCK stimulus may be an important indicator of GB dyskinesia [97]. These facts and our previous experience in patients with biliary dyskinesia

led us recently to develop a new QHBS method, in which CCK_{10} and GTN augmentation were combined for the diagnosis of GB dyskinesia. Our results provided the first evidence that the NO donor GTN causes a significant improvement in the CCK-induced GB emptying in patients with ABP. Normalization of the GB EF in the majority of ABP patients following GTN administration strongly suggests that the impairment of GB emptying, frequently associated with a biliary pain response after CCK administration, is caused by a functional spasm of the cystic duct or the GB neck area, rather than any organic GB disease. In these patients, GTN has been shown to abolish biliary pain and to prevent the CCK-induced functional spasm of the cystic duct area, with normalization of the GB EF. The influence of SO relaxation on this phenomenon may be ignored, in consequence of the fact that, in these patients with GB dyskinesia, there was no apparent accumulation of the isotope over the CBD when the initial CCK administration was associated with an impaired GB emptying. We have also demonstrated a change in the GB shape in the hepatobiliary scintigram due to GTN coadministration. The latter phenomenon, termed GB remodelling, strongly supports the pathogenic mechanism of GB dyskinesia and may be visual evidence of the functional spasm of the cystic duct and the GB neck area. QHBS combined with CCK and GTN coadministration may be regarded as a useful method in the diagnosis of GB dyskinesia [II].

To summarize our results, an optimal diagnostic and therapeutic approach may be suggested in patients with functional biliary disorders. It is worth mentioning that the diagnostic strategy should be modified individually in accord with the clinical status of the patient. Consequently, the clinical value and diagnostic role of QHBS must be evaluated in relation to the degree of biliary obstruction. ERCP must be performed in all cases when the obstruction is a high-grade one, when gallstone disease is suspected by the ultrasound, or when AN-augmented QHBS suggests an organic biliary obstruction. If no clinical sign of biliary obstruction is evident in a patient with functional biliary pain, it is reasonable to start

with AN-augmented QHBS to determine bile flow abnormalities. After the exclusion of organic causes, the probability of SO dyskinesia or GB dyskinesia is high if a typical clinical picture is accompanied by a suggestive QHBS result. The clinician should then decide wheter ESOM is indicated to establish the diagnosis. The latter also depends on the therapeutic plan, since the QHBS results might not be sufficient to plan an operative endoscopic procedure such as EST, which is not without risk. In contrast, if drug therapy is planned, positive scintigraphy should be sufficient for the initiation of medical treatment. It is generally accepted and proved by long-term follow-up studies that in patients with SO stenosis (SOD of biliary group I) EST is the treatment of choice (98-100). As these patients with SOD of biliary type I invariably benefit from EST, ESOM is not necessary (100). In contrast, in patients with SO dyskinesia (biliary groups II and III) ESOM is needed to perform in order to prove the elevated SO BP as an indication of EST, since in patients with SO dyskinesia and a normal SO BP EST did not prove to be more beneficial than the sham procedure (101). Moreover, in another follow-up study, a sustained symptomatic improvement was detected after EST in only 8% of the patients with SOD of biliary type III (functional group) (102). Therefore, with regard to the high incidence of complications following EST in patients with non-dilated ducts, it should be considered only after a failure of conservative therapy in the subgroup of patients with an elevated SO BP (103). If all these tests are negative, then a provocation test may be considered, such as a prostigmine-morphine test combined with QHBS. Provocation tests might unmask subtle abnormalities, such as hyperreactivity of the SO, which explain the patient`s complaints. In patients with ABP, and an intact GB with normal SO motility, QHBS combined with CCK and GTN coadministration should be performed to establish the diagnosis of GB dyskinesia.

To summarize the present work, we proved that QHBS is a useful method in the diagnosis of functional disorders of the biliary tract. We established a close correlation

between the bile flow determined by QHBS and the SO pressure measured by ESOM. We applied AN and prostigmine-morphine augmentations, QHBS thereby becoming a real functional test in the diagnosis of SOD. We combined QHBS with CCK and GTN coadministration in patients with intact GB and ABP, which could be a reliable method in the diagnosis of GB dyskinesia. We hope that, in the future, these methods will gain general acceptance as a first line diagnostic test in patients with suspected biliary dyskinesia.

5. CONCLUSIONS AND NEW FINDINGS

- **In asymptomatic cholecystectomized patients, there are only minor patient-to-patient variations in the dynamics of bile emptying, thereby increasing the chance that abnormalities in patients with SOD would be exposed.**
- **After cholecystectomy, the CBD emptying is accelerated as compared to the preoperative status. The parameters of QHBS in cholecystectomized patients and in patients with intact GB are therefore not comparable.**
- **QHBS combined with simultaneous CCK_{10} and GTN coadministration is a useful method with which to diagnose GB dyskinesia, and to differentiate this from other functional abnormalities of the GB.**
- **There are significant differences, with only moderate overlaps in the results of QHBS when the optimal quantitative parameters (i.e. DAT, HDTT and AR) are applied to compare patients suffering from postcholecystectomy pain and suspected SOD with asymptomatic cholecystectomized controls.**
- **Videomanometry of the SO is a promising new method to improve ESOM tracing analysis and documentation. It has several advantages over the conventional technique, such as an enhancement of the recognition of manometric artefacts and the possibility to monitor the catheter position continuously.**
- **A statistically significant linear correlation was established between the SO BP measured by ESOM and the bile flow parameters determined by QHBS in patients with suspected SOD.**
- **HDTT is the most sensitive scintigraphic parameter for detecting an elevated SO BP (89%), while the combined sensitivity of T_{max} and $T_{1/2}$ of the CBD reached**

100%. Hence, a simple quantification of the CBD TAC during QHBS allows the identification of all patients with elevated SO BP on ESOM.

- **AN-augmented QHBS is a valuable non-invasive method in the diagnosis of SOD; it permits a non-invasive differentiation between organic stenosis and functional dyskinesia of the SO.**
- **QHBS combined with prostigmine-morphine administration is an effective method for visualization of the SO spasm at the time of the evoked pain and it can therefore be used to improve the diagnostic accuracy of the conventional Nardi test.**

6. REFERENCES

1. Corazziari, E., Funch-Jensen, P., Hogan, W.J., Tanaka, M., Toouli, J.: Functional disorders of the biliary tract (working team report). *Gastroenterology International*, 6(3): 129-144, 1993.

2. Drossman, D.A., Li, Z., Andruzzi, E., Temple, R.D., Talley, N.J., Thompson, W.G., Whitehead, W.E., Janssens, J., Funch-Jensen, P., Corazziari, E.: U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. *Dig Dis Sci*, 38(9): 1569-1580, 1993.

3. Funch-Jensen, P.: Sphincter of Oddi motility. *Acta Chir Scand Suppl*, 553: 1-35, 1990.

4. Oddi, R.: D'une disposition a'sphicter speciale de l'ouverture due canal choledohogue. *Arch Ital Biol*, 8: 317-322, 1887.

5. Zollinger, R., Cutler, E.C.: Observations following distension of the gallbladder and common bile duct in man. *Proc Soc Exper Biol Med*, 30: 1260-1261, 1933.

6. Hogan, W.J., Geenen, J.E., Dodds, W.J.: Dysmotility disturbances of the biliary tract: classification, diagnosis, and treatment. *Semin Liver Dis*, 7(4): 302-310, 1987.

7. Abrahamsson, H.: Functional gut disorders and biliary-like pain. *Ital J Gastroenterol*, 19: 168-175, 1987.

8. Toouli, J., Hogan, W.J., Geenen, J.E., Dodds, W.J., Arndorfer, R.C.: Action of cholecystokinin-octapeptide on sphincter of Oddi basal pressure and phasic wave activity in humans. *Surgery*, 92(3): 497-503, 1982.

9. Behar, J., Biancani, P.: Effect of cholecystokinin and the octapeptide of cholecystokinin on the feline sphincter of Oddi and gallbladder. *J Clin Invest*, 66: 1231-1239, 1980.

10. Rolny, P., Arleback, A., Funch-Jensen, P., Kruse, A., Ravnsbaeck, J., Jarnerot, G.: Paradoxical response of sphincter of Oddi to intravenous injection of cholecystokinin or ceruletide. Manometric findings and results of treatment in biliary dyskinesia. *Gut*, 27(12): 1507-1511, 1986.

11. Sand, J., Tainio, H., Nordback, I.: Peptidergic innervation of human sphincter of Oddi. *Dig Dis Sci*, 39(2): 293-300, 1994.

12. Funch-Jensen, P., Sorensen, S.S.: Influence of graded distension of the gallbladder on the sphincter of Oddi activity in dogs. *Dig Dis*, 9(6): 408-413, 1991.

13. Thune, A., Saccone, G.T., Scicchitano, J.P., Toouli, J.: Distension of the gall bladder inhibits sphincter of Oddi motility in humans. *Gut*, 32(6): 690-693, 1991.

14. Funch-Jensen, P.: Biliary motility. *Scand J Gastroenterol Suppl*, 128: 70-78, 1987.

15. Cozzolino, H., Goldstein, J., Greening, R.R., Wirst, C.W.: The cystic duct syndrome. *JAMA*, 185: 100-104, 1963.

16. Toouli, J.: Biliary tract motor dysfunction. *Bailliere's Clinical Gastroenterology*, 5(2): 409-431, 1991.

17. Bar Meir, S., Halpern, Z., Bardan, E., Gilat, T.: Frequency of papillary dysfunction among cholecystectomized patients. *Hepatology*, 4(2): 328-330, 1984.

18. Meshkinpour, H., Mollot, M.: Sphincter of Oddi dysfunction and unexplained abdominal pain: clinical and manometric study. *Dig Dis Sci*, 37(2): 257-261, 1992.

19. Sherman, S., Troiano, F.P., Hawes, R.H., O'Connor, K.W., Lehman, G.A.: Frequency of abnormal sphincter of Oddi manometry compared with the clinical suspicion of sphincter of Oddi dysfunction. *Am J Gastroenterol*, 86(5): 586-590, 1991.

20. Hogan, W.J., Geenen, J.E.: Biliary dyskinesia. *Endoscopy*, 20(Suppl.1.): 179-183, 1988.

21. Toouli, J.: Clinical relevance of sphincter of Oddi dysfunction. *Br J Surg*, 77: 723-724, 1990.

22. Csendes, A., Kruse, A., Funch-Jensen, P., Oster, M.J., Ornsholt, J., Amdrup, E.: Pressure measurements in the biliary and pancreatic duct systems in controls and in patients with gallstones, previous cholecystectomy, or common bile duct stones. *Gastroenterology*, 77(6): 1203-1210, 1979.

23. Geenen, J.E., Hogan, W.J., Dodds, W.J., Stewart, E.T., Arndorfer, R.C.: Intraluminal pressure recording from the human sphincter of Oddi. *Gastroenterology*, 78(2): 317-324, 1980.

24. Funch-Jensen, P., Kruse, A., Csendes, A., Oster, M.J., Amdrup, E.: Biliary manometry in patients with post-cholecystectomy syndrome. *Acta Chir Scand*, 148: 267-268, 1982.

25. Toouli, J., Roberts Thomson, I., Dent, J., Lee, J.: Manometric disorders in patients with suspected sphincter of Oddi dysfunction. *Gastroenterology*, 88(5): 1243-1250, 1985.

26. Choudhry, U., Ruffolo, T., Jamidar, P., Hawes, R.., Lehman, G.: Sphincter of Oddi dysfunction in patients with intact gallbladder: therapeutic response to endoscopic sphincterotomy. *Gastrointest Endosc*, 39(4): 492-495, 1993.

27. Tanaka, M., Ikeda, S., Nakayama, F.: Change in bile duct pressure responses after cholecystectomy: loss of gallbladder as a pressure reservoir. *Gastroenterology*, 87: 1154-1159, 1984.

28. Desautels, S.G., Slivka, A., Hutson, W.R., Chun, A., Mitrani, C., DiLorenzo, C., Wald, A.: Postcholecystectomy pain syndrome: pathophysiology of abdominal pain in spincter of Oddi type III. *Gastroenterology*, 116: 900-905, 1999.

29. Kellow J.E.: Sphincter of Oddi dysfunction type III: Another manifestation of visceral hyperalgesia? Editorial. *Gastroenterology*, 116: 996-1000, 2000.

30. Varró, V., Lonovics, J.: Sphincter of Oddi dyskinesia: pathology and clinical aspects. In: *Bianchi P, editor. Topics in digestive disease 2. Cortina International Verona. New York: Raven Press,* p: 357-383, 1988.

31. Bodvall, B., Overgaard, B.: Computer analysis of postcholecystectomy biliary tract symptoms. *Surg Gynecol Obstet,* 124(4): 723-732, 1967.

32. Jorgensen, T., Teglbjerg, J.S., Wille Jorgensen, P., Bille, T., Thorvaldsen, P.: Persisting pain after cholecystectomy. A prospective investigation. *Scand J Gastroenterol,* 26(1): 124-128, 1991.

33. Lasson, A., Fork, F.T., Tragardh, B., Zederfeldt, B.: The postcholecystectomy syndrome: bile ducts as pain trigger zone. *Scand J Gastroenterol,* 23(3): 265-271, 1988.

34. Schmalz, M.J., Geenen, J.E., Hogan, W.J., Dodds, W.J., Venu, R.P., Johnson, G.K.: Pain on common bile duct injection during ERCP: does it indicate sphincter of Oddi dysfunction? *Gastrointest Endosc,* 36(5): 458-461, 1990.

35. Madura J.A., McCammon R.L., Paris, J.M.: The Nardi test and biliary manometry in the diagnosis of pancreatobiliary sphincter dysfunction. *Surgery,* 90(4): 588-594, 1981.

36. Venu R., Toouli J., Geenen J.E.: Effect of morphine on motor activity of the human sphincter of Oddi. *Gastroenterology,* 84: 1342, 1983. (abs).

37. Toouli J., Collinson T., Bushell M.: Effect of morphine on sphincter of Oddi motility. *Gastroenterology,* 86: 1282, 1985. (abs.)

38. Tanaka M., Ikeda S., Nakayama F.: Continous measurement of common bile duct pressure with an indwelling microtransducer catheter introduced by duodenoscopy: new diagnostic aid for postcholecystectomy dyskinesia – a preliminary report. *Gastrointest Endosc,* 29: 83-88, 1983.

39. Debray P.C., Hardouin J.P., Fablet J.: Le test choleretique-morphine. Son enteret dans les affections des voies biliaries et dans les migraines. *Gastroenterologia,* 97: 137-148, 1962.

40. Nardi G.L., Acosta J.M.: Papillitis as a cause of pancreatitis and abdominal pain: role of evocative test, operative pancreatography and histologic evaluation. *Ann Surg,* 164: 611-621, 1966.

41. Steinberg W.M., Salvato R.F., Toskes P.P.: The morphine-prostigmine provocative test - is it useful for making clinical decisions? *Gastroenterology,* 78: 728-731, 1980.

42. Tanaka, M., Ikeda, S., Matsumoto, S., Yoshimoto, H., Nakayama, F.: Manometric diagnosis of sphincter of Oddi spasm as a cause of postcholecystectomy pain and the treatment by endoscopic sphincterotomy. *Ann Surg,* 202(6): 712-719, 1985.

43. Roberts-Thomson I.C., Toouli, J.: Abnormal responses to morphine-neostigmine in patients with undefined biliary type pain. *Gut,* 26: 1367-1372, 1985.

44. Roberts-Thomson I.C., Pannal P.R., Toouli, J.: Relationship between morphine responses and sphincter of Oddi motility in undefined biliary pain after cholecystectomy. *J Gastroenterol Hepatol*, 4(4): 317-324, 1989.

45. LoGiudice, J.A., Geenen, J.E., Hogan, W.J., Dodds, W.J.: Efficacy of the morphine-prostigmin test for evaluating patients with suspected papillary stenosis. *Dig Dis Sci*, 24(6): 455-458, 1979.

46. Juhász, L., Orosz, P., Foldváry, G., Mátai, E., Juhász, A.: [Endoscopic manometry of Oddi's sphincter] Az Oddi-sphincter endoscopos manometriája. *Orv Hetil*, 127(29): 1753-1756, 1986.

47. Lans, J.L., Parikh, N.P., Geenen, J.E.: Application of sphincter of Oddi manometry in routine clinical investigations. *Endoscopy*, 23: 139-143, 1991.

48. Deschner, W.K., Johnson, D., Maher, K., Benjamin, S.B., Cattau, E.L.: Interobserver variability in the interpretation of sphincter of Oddi pressures. *Gastrointestinal Endoscopy*, 35: 163-164, 1989.

49. Torsoli, A., Corazziari, E., Habib, F.I., De Masi, E., Biliotti, D., Mazzarella, R., Giubilei, D., Fegiz, G.: Frequencies and cyclical pattern of the human sphincter of Oddi phasic activity. *Gut*, 27(4): 363-369, 1986.

50. Lee, S.K., Kim, M.H., Seo, D.W., Yoo, B.M., Lee, M.H., Myung, S.J., Min, Y.I.: Frequency of phasic wave contraction is variable during long-term sphincter of Oddi manometry. *Am J Gastroenterol*, 91(11): 2395-2398, 1996.

51. Rolny, P., Anderberg, B., Ihse, I., Lindstrom, E., Olaison, G., Arvill, A.: Pancreatitis after sphincter of Oddi manometry. *Gut*, 31(7): 821-824, 1990.

52. Scicchitano, J., Saccone, G.T., Baker, R.A., Roberts Thomson, I.C., Toouli, J.: How safe is endoscopic sphincter of Oddi manometry? *J Gastroenterol Hepatol*, 10(3): 334-336, 1995.

53. Sherman, S., Troiano, F.P., Hawes, R.H., Lehman, G.A.: Sphincter of Oddi manometry: decreased risk of clinical pancreatitis with use of a modified aspirating catheter. *Gastrointest Endosc*,, 36(5): 462-466, 1990.

54. Sherman, S., Ruffolo, T.A., Hawes, R.H., Lehman, G.A.: Complications of endoscopic sphincterotomy. A prospective series with emphasis on the increased risk associated with sphincter of Oddi dysfunction and nondilated bile ducts. *Gastroenterology*, 101(4): 1068-1075, 1991.

55. Simon, L., Dobronte, Z., Patai, A.: [Management of hypertensive dyskinesia of Oddi's sphincter. Correlation between the frequency of complications from endoscopic sphincterotomy and the diameter of the common bile duct]. *Orv Hetil*, 136(31): 1659-1662, 1995.

56. Lee, R.G.L., Gregg, J.A., Koroshetz, A.M., Hill, T.C., Clouse, M.E.: Sphincter of Oddi stenosis: diagnosis using hepatobiliary scintigraphy and endoscopic manometry. *Radiology*, 156: 793-796, 1985.

57. Zeman, R.K., Burrell, M.I., Dobbins, J., Jaffe, M.K., Choyke, P.L.: Postcholecystectomy syndrome: evaluation using biliary scintigraphy and endoscopic retrograde cholangiography. *Radiology*, 156: 787-792, 1985.

58. Grimon, G., Buffet, C., and André, L., Etienne, J.P., Desgrez, A.: Biliary pain in postcholecystectomy patients without biliary obstruction. A prospective radionuclide study. *Dig Dis Sci*, 36(3): 317-320, 1991.

59. Darweesh, R.M., Dodds, W.J., Hogan, W.J., Geenen, J.E., Collier, B.D., Shaker, R.: Efficacy of quantitative hepatobiliary scintigraphy and fatty-meal sonography for evaluating patients with suspected partial common duct obstruction. *Gastroenterology*, 94: 779-786,1988.

60. Roberts Thomson, I.C, Toouli, J., Blanchett, W., Lichtenstein, M., Andrews, J.T.: Assessment of bile flow by radioscintigraphy in patients with biliary-type pain after cholecystectomy. *Aust NZ J Med*, 16: 788-793, 1986.

61. Loberg, M.D., Cooper, M., Harvey, E., Callery, P., Faith, W.: Development of new radiopharmaceuticals based on N-substitution of iminodiacetic acid. *J Nucl Med*, 17(7): 633-638, 1976.

62. Williams, W., Krishnamurthy, G.T., Brar, H.S., Bobba, V.R.: Scintigraphic variations of normal biliary physiology. *J Nucl Med*, 25: 160-165, 1984.

63. Doo, E., Krishnamurthy, G.T., Eklem, M.J., Gilbert, S., Brown, P.H.: Quantification of hepatobiliary function as an integral part of imaging with Technetium-99m-mebrofenin in health and disease. *J Nucl Med*, 32(1): 48-57, 1991.

64. Krishnamurthy, G.T., Bobba, V.R., McConnell, D., Turner, F., Mesgarzadeh, M., Kingston, E.: Quantitative biliary dynamics: introduction of a new noninvasive scintigraphic technique. *J Nucl Med*, 24(3): 217-223, 1983.

65. Krishnamurthy, G.T., Bobba, V.R., Kingston, E.: Radionuclide ejection fraction: a technique for quantitative analysis of motor function of the human gallbladder. *Gastroenterology*, 80: 482-490, 1981.

66. Krishnamurthy, S., Krishnamurthy, G.T. Cholecystokinin and morphine pharmacological intervention during 99mTc-HIDA cholescintigraphy: a rational approach. *Semin Nucl Med*, 26(1):16-24, 1996.

67. Juhász, L., Orosz, P., Foldvary, G., Matai, E., Juhász, A. [Pharmacodynamic examination of Oddi's sphincter using endoscopic manometry] Az Oddi sphincter pharmacodynamias vizsgalata endoscopos manometriaval. *Orv.Hetil.* 128(37):1933-1936, 1987.

68. Gilbert, D.A., DiMarino, A.J., Jensen, D.M., Katon, R., Kimmey, M.B., Laine, L.A., MacFayden, B.V., Michaletz Onody, P.A., Zuckerman, G.: Status evaluation: sphincter of Oddi manometry. American Society for Gastrointestinal Endoscopy. Technology Assessment Committee. *Gastrointest Endosc*, 38(6): 757-759, 1992.

69. Smithline, A., Hawes, R., Lehman, G.: Sphincter of Oddi manometry: interobserver variability. *Gastrointest Endosc*, 39(4): 486-491, 1993.

70. Thune, A., Scicchitano, J., Roberts Thomson, I., Toouli, J.: Reproducibility of endoscopic sphincter of Oddi manometry. *Dig Dis Sci*, 36(10): 1401-1405, 1991.

71. Funch-Jensen, P., Ebbehoj, N.: Sphincter of Oddi motility. *Scand J Gastroenterol*, 31(Suppl 216): 46-51, 1996.

72. Funch-Jensen, P. Kruse, A., Ravnsbaek, J.: Reproducibility and estimation of minimal recording duration in endoscopic sphincter of Oddi manometry. *Ital J Gastroenterol*, 18: 37-38, 1986.

73. Funch-Jensen, P., Kruse, A., Ravnsbaek, J.: Endoscopic sphincter of Oddi manometry in healthy volunteers. *Scand J Gastroenterol*, 22(2): 243-249, 1987.

74. Drane, W.E., Johnson, D.A.: Sincalide-augmented quantitative hepatobiliary scintigraphy (QHBS): definition of normal parameters and preliminary relationship between QHBS and sphincter of Oddi (SO) manometry in patients suspected of having SO dysfunction. *J Nucl Med*, 31(9): 1462-1468, 1990.

75. Varró, V., Döbrönte, Z., Hajnal, F., Csernay, L., Nemessányi, Z., Láng, J., Nárai, G., Szabó, E.: The diagnosis of hypertonic Oddi sphincter dyskinesia. *Am J Gastroenterol*, 78(11): 736-739, 1983.

76. Cicala, M., Scopinaro, F., Corazziari, E., Vignoni, A., Viscardi, A., Habib, F.I., Torsoli, A.: Quantitative cholescintigraphy in the assessment of choledochoduodenal bile flow. *Gastroenterology*, 100(4): 1106-1113, 1991.

77. Fullarton, G.M., Allan, A., Hilditch, T., Murray, W.R.: Quantitative 99mTc-DISIDA scanning and endoscopic biliary manometry in sphincter of Oddi dysfunction. *Gut*, 29: 1397-1401,1988.

78. Corazziari, E., Cicala, M., Habib, F.I., Scopinaro, F., Fiocca, F., Pallotta, N.: Hepatoduodenal bile transit in cholecystectomized subjects. Relationship with sphincter of Oddi function and diagnostic value. *Dig Dis Sci*, 39: 1985-1993, 1994.

79. Sostre, S., Kalloo, A.N., Spiegler, E.J., Camargo, E.E., Wagner H.N. Jr.: A noninvasive test of sphincter of Oddi dysfunction in postcholecystectomy patients: the scintigraphic score. *J Nucl Med*, 33: 1216-1222, 1992.

80. Peng, N.J., Lai, K.H., Tsay, D.G., Liu, R.S., Su, K.L., Yeh, S.H.: Efficacy of quantitative cholescintigraphy in the diagnosis of sphincter of Oddi dysfunction. *Nucl Med Commun*, 15(11): 899-904, 1994.

81. Sunderland, G.T. Carter, D.C.: Clinical application of the cholecystokinin provocation test. *Br J Surg*, 75(5): 444-449, 1988.

82. Evans, P.R.., Dowsett, J.F., Bak, Y.T., Chan, Y.K., Kellow, J.E. Abnormal sphincter of Oddi response to cholecystokinin in postcholecystectomy syndrome patients with irritable bowel syndrome. The irritable sphincter. *Dig Dis Sci*, 40(5):1149-1156, 1995.

83. Bozkurt, T., Orth, K.H., Butsch, B., Lux, G. Long-term clinical outcome of post-cholecystectomy patients with biliary-type pain: results of manometry, non-invasive techniques and endoscopic sphincterotomy. *Eur.J.Gastroenterol.Hepatol.* 8(3):245-249, 1996.

84. Holtzer, J.D., Hulst, S.G.: Confirmation of postcholecystectomy biliary dyskinesia by elevation of serum transaminases (GOT and GPT) after injection of morphine? *Acta Med Scand*, 194: 221-224, 1973.

85. Gowen, G.F.: A second look at the neostigmine-morphine test. *Am Surg*, 55: 640-644, 1989.

86. Nemessányi, Z., Csernay, L., Láng, J., Döbrönte, Z., Varró, V.: Objektivierung der Dyskinesia nach Cholezystectomie durch dynamische HIDA-Untersuchung. In: *Nuklearmedizin, edited by Schmidt, H.E.A., Wolf, F., and Mahlstedt, J.Stuttgart:Schattauer*, p. 710-716., 1981

87. Varró, V.: Dysfunktion des sphincter Oddi, eine Verlegenheitsdiagnose? *Fortsch Med*, 99(18): 683-688, 1981.

88. Grace, P.A., Pitt, H.A.: Cholecystectomy alters the hormonal response of the sphincter of Oddi. *Surgery*, 102(2): 186-194, 1987.

89. Hogan, W.J., Geenen, J.E., Dodds, W.J., Toouli, J., Venu, R.P., Helm, J.: Paradoxical motor response to cholecystokinin (CCK-OP) in patients with suspected sphincter of Oddi dysfunction (abs). *Gastroenterology*, 82(5): 1085, 1982.

90. Krishnamurthy, S., Krishnamurthy, G.T.: Biliary dyskinesia: role of the sphincter of Oddi, gallbladder and cholecystokinin. *J Nucl Med*, 38(11): 1824-1830, 1997.

91. Shaffer, E.A., Hershfield, N.B., Logan, K., Kloiber, R.: Cholescintigraphic detection of functional obstruction of the sphincter of Oddi. Effect of papillotomy. *Gastroenterology*, 90: 728-733,1986.

92. Gardiner, B.N., Small, D.M.: Simultaneous measurement of the pancreatic and biliary response to CCK and secretin. *Gastroenterology*, 70: 403-407, 1976.

93. Ruffolo, T.A., Sherman, S., Lehman, G.A., Hawes, R.H.: Gallbladder ejection fraction and its relationship to sphincter of Oddi dysfunction. *Dig Dis Sci*, 39(2): 289-292, 1994.

94. Elta, G.H.: Pain and an intact gallbladder: is the sphincter of Oddi to blame? *Gastroenterology*, 106(6): 1716-1717, 1994.

95. Yap, L., Wycherley, A.G., Morphett, A.D., Toouli, J.: Acalculous biliary pain: cholecystectomy alleviates symptoms in patients with abnormal cholescintigraphy [see comments]. *Gastroenterology*, 101(3): 786-793, 1991.

96. Shaffer, E.A.: Cholescintigraphy in acalculous biliary pain: if abnormal, should cholecystectomy follow? *Hepatology*, 15(4): 737-739, 1992.

97. Atkins, H.L., Oster, Z.H.: Asymmetric gallbladder contraction following cholecystokinin hepatobiliary imaging. *Clin Nucl Med*, 14(2): 82-86, 1989.

98. Fullarton, G.M., Hilditch, T., Campbell, A., Murray, W.R.: Clinical and scintigraphic assessment of the role of endoscopic sphincterotomy in the treatment of sphincter of Oddi dysfunction. *Gut*, 31: 231-235, 1990.

99. Neoptolemos, J.P., Bailey, I.S., Carr Locke, D.L. Sphincter of Oddi dysfunction: results of treatment by endoscopic sphincterotomy. *Br J Surg*, 75(5): 454-459, 1988.

100. Rolny, P., Geenen, J.E., Hogan, W.J.: Post-cholecystectomy patients with "objective signs" of partial bile outflow obstruction: clinical characteristics, sphincter of Oddi manometry findings, and results of therapy. *Gastrointest Endosc*, 39(6): 778-781, 1993.

101. Geenen, J.E., Hogan, W.J., Dodds, W.J., Toouli, J., Venu, R.P.: The efficacy of endoscopic sphincterotomy after cholecystectomy in patients with sphincter-of-Oddi dysfunction. *N Engl J Med*, 320(2): 82-87, 1989.

102. Wehrmann, T., Wiemer, K., Lembcke, B., Caspary, W.F., Jung, M.: Do patients with sphincter of Oddi dysfunction benefit from endoscopic sphincterotomy? A 5-year prospective trial. *Eur J Gastroenterol Hepatol*, 8(3): 251-256, 1996.

103. Döbrönte, Z., Simon, L., Patai, A.: [Management of Oddi sphincter dyskinesis. Results of drug therapy and sphincterotomy]. *Orv Hetil*, 136(40): 2165-2167, 1995.

7. ANNEX (References I-VI)