CLINICAL EVALUATION OF PRIMARY AND SECONDARY OESOPHAGEAL MOTILITY DISORDERS: NEW DATA ON THE PATHOGENESIS OF CARDIAC AND RESPIRATORY MANIFESTATIONS OF GASTRO-OESOPHAGEAL REFLUX DISEASE (GORD), THE CHARACTERISTICS OF MOTILITY DISORDERS IN PATIENTS DIABETES MELLITUS AND IN PRIMARY SJÖGREN'S SYNDROME.

Ph.D. Thesis

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LIST OF FULL PAPERS OF THE THESIS BASED UPON:

I. **Rosztóczy A,** Kovács L, Wittmann T, Lonovics J, Pokorny G. Manometric assessment of impaired esophageal motor function in primary Sjogren's syndrome. **Clin Exp Rheumatol. 2001; 19: 147-152.**
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II. **Rosztóczy A,** Róka R, Várkonyi TT, Lengyel C, Izbéki F, Lonovics J, Wittmann T. Regional differences in the manifestation of gastrointestinal motor disorders in diabetic patients with autonomic neuropathy.
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III. Róka R, **Rosztóczy A,** Izbéki F, Taybani Z., Kiss I., Lonovics J, Wittmann T. Prevalence of respiratory symptoms and diseases associated with gastroesophageal reflux disease.
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To my father, who devoted his life to science and research.

1. INTRODUCTION

The oesophagus is the most proximal part of the gastrointestinal tract, which is responsible for transporting food from the mouth to the stomach and for preventing retrograde movement of gastric contents. It is a hollow tube closed at both ends by the upper oesophageal sphincter (UOS) and lower oesophageal sphincter (LOS). Its lumen is lined with squamous mucosa, containing longitudinally oriented muscle fibers, and connected to the muscularis propria by a loose network of connective tissue fibers of the submucosa. The muscularis propria consists of an inner circular muscle layer, with fibers oriented along the circumference of the tube, and an outer longitudinal layer, with fibers oriented along its axis. The muscular structure of the pharynx and the proximal oesophagus contain striated muscle, while the lower two thirds of the oesophagus contain smooth muscle. The innervation of these parts is also different. While the striated muscles are controlled by the swallowing center, located in the brain stem, through the vagus nerves, the lower two thirds are controlled primarily by intrinsic neural networks located between the longitudinal and circular muscle layers and in the submucosa and only modulated by central mechanisms in the swallowing center.

The normal oesophageal peristalsis is resulted by the coordinated contractory function of the circular and longitudinal muscle layers. The circular layer is responsible for maintaining the pressure necessary for bolus transport, which is easy to measure by manometry. Normally peristaltic waves have the lowest amplitude at the level of the aortic arch, which is anatomically representing the transition zone between striated and smooth muscle containing parts of the oesophagus. The peristaltic wave amplitudes are increasing from this point to the LOS. The function of the longitudinal muscles which are responsible for the shortening of the oesophagus are more difficult to study. The function of the upper and lower sphincters are closely related to the function of the tubular part of the oesophagus. The sphincters are closed at rest, and so the LOS prevents the regurgitation of gastric contents to the oesophagus, while the UOS is avoiding regurgitation to the pharynx and consequently to the respiratory tract. During swallowing the relaxation of these circumferential muscular structures makes possible the transit of the bolus. Furthermore the LOS is able to relax transiently without previous swallowing when the fundus of the stomach is distended.

Oesophageal motility disorders are characterized by the derangement of this well coordinated function, and classified into primary and secondary motility disorders. **(Table 1.)**

Primary motility disorders:	Secondary motility disorders:
Gastro-oesophageal reflux disease	Diabetes mellitus
Achalasia cardiae	Autoimmune connective tissue disorders
Diffuse oesophageal spasm	- primary Sjögren's syndrome,
Nutcracker oesophagus	- progressive systemic sclerosis
Hypertensive lower oesophageal sphincter	- CREST-syndrome
Cricopharyngeal hypertonia and achalasia	- polymyositis-dematomyositis
Non-specific oesophageal motility disorders	- mixed connectivetissue disease
	Neuro-muscular disorders
	Chagas' disease
	Amyloidosis

Table 1. Classification of oesophageal motility disorders

Among the primary motility disorders gastro-oesophageal reflux disease (GORD) is the most prevalent and though the most extensively studied. By definition, GORD is a complex motility disorder of the upper gastrointestinal tract, which is characterized by the regurgitation of gastric/duodenal contents into the oesophagus, the mouth or to the respiratory tract and causing oesophageal or extraoesophageal symptoms or complications. Epidemiological studies made in Europe and in the United States confirmed that approximately 20 percent of the general population complains of its typical symptoms (heartburn, regurgitation) at least weekly, which has a significant impact on the quality of life of the patients. **[54, 59, 64, 84, 85]** Patients with GORD may have atypical symptoms as well. Atypical symptoms may arise from the oesophagus such as dysphagia and odynophagia, others have extraoesophageal origin. This latter group includes non-cardiac chest pain and respiratory symptoms. Furthermore, it is possible that the disease is presented by only atypical symptoms, and typical symptoms may be completely absent. This "silent" form of the disease is invisible for the epidemiologic studies and so the prevalence of GORD must be underestimated. **[47, XI, XIII]**

Data support that as high as 40 percent of patients with chest pain and normal coronary angiography may have acid reflux as a cause of their symptom, while in case of respiratory disorders the prevalence of silent GORD may exceed 50 percent. **[38, 61]**

Although the major pathophysiological factors of GORD, such as LOS incompetence, transient LOS relaxations, oesophageal body dysmotility, delayed gastric emptying, hiatal hernia are widely studied and well described, the pathogenesis of the extraoesophageal manifestations are still poorly understood. Among the extraoesophageal symptoms chest pain has a special importance, since it could be caused by myocardial ischaemia as well. Although it was believed earlier that chest pain episodes of oesophageal and cardiac origin could be clearly distinguished **[5, 6]**, the time that has subsequently passed has revealed that the differential diagnosis of chest pain is often difficult. In consequence of this, the evaluation of the

symptoms themselves is not sufficient to predict the underlying disease. It has been shown that up to 40 per cent of the patients, admitted to a coronary care unit with typical angina-like chest pain have a normal coronary anatomy. **[12]** The majority of their chest pain events might, however, be caused by gastro-oesophageal reflux disease or other oesophageal motility disorders as it has been established by functional oesophageal testing. **[8, 41, 53, 60, 61, 87, 111]** Furthermore, oesophageal causes of non-cardiac chest pain can also be observed in patients with coronary artery disease with a similar incidence. **[42, 67, 93]** This large overlap between patients with cardiac and non-cardiac chest pain events has raised the question whether this is only a coincidence or a causative link exists between them. **[62]** Since the oesophagus and the heart share their innervation, mechanical or chemical stimulation of the oesophagus may evoke coronary spasm and subsequent blood flow reduction, which may lead to myocardial ischemia and subsequent chest pain. This phenomenon has been designated as linked angina. **[4, 17, 65, 68, 101, 104, 110, IX]**

Data on the prevalence of the oesophageal acid exposure induced oesophago-cardiac reflex are still missing in humans; and only a few studies have evaluated the incidence of linked-angina i.e. the oesophageal acid exposure-induced myocardial ischaemia mediated by the oesophago-cardiac reflex. **[23, 62, 72]**

Respiratory diseases have a two way relationship with GORD. On the one hand, GORD is an important pathogenetic factor of chronic respiratory diseases. Many symptoms and diseases of the upper and lower respiratory tract, such as chronic cough, recurrent pneumonia, bronchial asthma, pulmonary fibrosis, chronic sinusitis, inflammation, ulceration or granuloma of the vocal cords, can be caused and maintained by GOR **[22, 43, 100].** Possible mechanisms are proximal reflux with subsequent microaspiration and a reflex mechanism – similar to the oesophago-cardiac reflex – when the regurgitating acid triggers the chemoreceptors of the distal oesophagus and induce reflectory bronchoconstriction. **[96, XII]** On the other hand respiratory disorders may also provoke GOR, due to the anatomical change of chest form, and the increased transdiaphragmatic pressure gradient.

Although the prevalences of chronic respiratory symptoms and diseases associated with GORD differ considerably, examinations have mainly been carried out on patients with pulmonary diseases. These investigations have shown that GORD is responsible for the generation or maintenance of chronic cough in 10–40% of the patients, while the prevalence of GORD among asthma patients has been reported to be 30–89%. **[32, 37, 77, 90, 92, XI, XII, XIII]** There have been only a few studies on the prevalence of respiratory complications in a non-selected population of GORD patients. The prevalence of pulmonary disorders among

patients with oesophagitis has been estimated to be between 0.5 and 17%, and bronchial asthma is reported by 9% of subjects with GORD symptoms **[25, 33, 39]**

Secondary motility disorders of the oesophagus are characterized by oesophageal hypomotility, and though they commonly show features of GORD. Diabetes mellitus is known to be the most common cause of secondary gastrointestinal motility disorders **[VII]**. Epidemiological studies have revealed that nearly 75% of patients with diabetes mellitus have gastrointestinal symptoms, independently of the type of the disease. **[86]** These may include swallowing difficulties, reflux symptoms, dyspeptic symptoms, and also problems with bowel transit and defecation. **[9]** Various types of gastrointestinal motility disturbances have been described at different levels of the gut, including an impaired oesophageal transit, delayed gastric emptying, impaired gallbladder function, bowel transit or defecation. **[1, 2, 9, 19, 44, 63, 69, 88, 97]** In the pathogenesis of diabetic gastrointestinal motility disturbances, autonomic neuropathy, hyperglycemia and altered visceral perception are believed to play significant roles. **[7, 49, 50, 51, 57, 70, 73, 82, 87]** Although autonomic neuropathy is thought to be important in the development of the gastrointestinal motor disorders, in most studies groups are not selected according to the presence of autonomic neuropathy.

Autoimmune connective tissue diseases are rare causes of oesophageal motility disorders. On the contrary oesophageal involvement is frequently present in most of these diseases and symptoms related to the oesophageal involvement may disturb the patient significantly. Though, the pathogenesis of these symptoms and their relation to other manifestations of the disease is an important question. Although patients with primary Sjögren's syndrome are often (up to 80%) complain of difficulties in swallowing, only a few studies have evaluated the oesophageal motor function in the disease. **[3, 34, 45, 56, 76, 103]** For the diagnosis the involvement of the salivary and lacrimal glands results in xerostomia and keratoconjunctivitis sicca are obligatory. Besides these local glandular manifestations, other organs (e.g. gastrointestinal, respiratory tract, joints, etc.) are often affected. **[30, 74, 106]**

2. AIMS

With this in mind, the aims of the studies summarized in the thesis were to investigate the pathogenesis of the extraoesophageal manifestations of GORD, with a special respect to the cardiac and respiratory complications. Secondly, the evaluate the characteristics of the esophageal motility disorders in patients with type 1 diabetes mellitus and in patients with primary Sjögren's syndrome.

Detailed aims of the experiments were:

1. To evaluate the cardiac manifestations of GORD. The establishment of the prevalence the oesophago-cardiac reflex characterised by gastro-oesophageal reflux induced coronary flow decrease, and its possible consequence: the “linked-angina” in patients with angina-like chest pain.
2. To evaluate the relationship between the presence of oesophago-cardiac reflex to different coronary artery diseases and gastro-oesophageal reflux disease.
3. To develop a new diagnostic method – transoesophageal Doppler echocardiographic coronary flow velocity measurement combined with oesophageal acid perfusion test – for the establishment of the oesophago-cardiac reflex.
4. To establish the prevalence of chronic respiratory symptoms and diseases in patients with GORD in Hungary.
5. To compare the results of upper gastrointestinal endoscopy, 24-hour oesophageal pH monitoring in patients with gastro-oesophageal reflux with and without respiratory manifestations.
6. To evaluate the association between gastrointestinal symptoms and motility disorders at different sites of the gastrointestinal tract, with special respect to the oesophagus in patients with type 1 diabetes mellitus.
7. To evaluate the characteristics of the oesophageal motility disorders in patients with type 1 diabetes mellitus.
8. To establish the oesophageal motility disorders in patients with primary Sjögren’s syndrome, and to examine its relationship to the systemic and oesophageal symptoms of the disease.

3. PATIENTS AND METHODS

3.1. Patient selection

Study protocols were approved by the Medical Ethics Committee of the University of Szeged. All patients signed the consent form prior to enrolment.

3.1.1. The establishment of oesophago-cardiac reflex in patients with angina-like chest pain.

Fifty-one patients (24 males, 27 females, mean age 55±13 years, range: 36-74 years) with class II chest pain according to Canadian Cardiovascular Society were enrolled after a

detailed cardiologic evaluation including coronary angiography. Nineteen patients had significant epicardial coronary artery disease. Eleven of them had the involvement of the left anterior descending artery, and 8 had stenosis of other large vessels. Thirteen patients had microvascular disease and normal epicardial coronary arteries. Ten patients had coronary spasm, 7/10 had spontaneous spasm resolved by intracoronarial nitrate administration, and 3/10 had provokable spasm by ergonovine testing. Nine patients had negative cardiologic evaluation. These were considered to have non-cardiac chest pain. Patients with autonomic neuropathy (diabetics, chronic alcohol consumers, etc.), or with an impaired left ventricular function (ejection fraction ≤ 50%), or with left main coronary artery stenosis were not enrolled. The studied patients were screened for GORD by means of symptom analysis, and were asked to fill a standardized questionnaire regarding their symptoms related to gastro-oesophageal reflux disease, such as heartburn, chest pain and gastro-oesophageal acid regurgitation. Upper gastrointestinal endoscopy, 24-hour pH monitoring were performed. Spastic oesophageal motility disorders (such as diffuse oesophageal spasm, and nutcracker oesophagus) were excluded by oesophageal manometry.

3.1.2. The evaluation of chronic respiratory symptoms and diseases in patients with GORD.

Two hundred and ninety-nine consecutive patients with symptomatic GORD (154 women, 145 men) were enrolled in the study between 1992 and 2000. The diagnosis of GORD was based on the results of upper gastrointestinal endoscopy and 24-hour pH monitoring, extended by oesophageal manometry and video barium oesophagography if the 24-hour pH monitoring study failed to reveal a significant gastro-oesophageal reflux. For the evaluation of reflux and respiratory symptoms, the patients filled in a detailed questionnaire that contains the patient's history, medications, life habits and all symptoms of GORD including the oesophageal and the extraoesophageal ones. The patients filled in this questionnaire at two separate occasions, with the help of a doctor and then of a nurse. The questionnaire was an adopted version that has extensively been used in the literature. **[105]** The diagnosis of upper or lower airway diseases was established from the results of further pulmonological and oto-rhino-laryngological investigations.

3.1.3. The evaluation of oesophageal and other gastrointestinal motility disorders in patients with type 1 diabetes mellitus.

Sixteen patients with long-standing type 1 diabetes mellitus and 29 age and sex matched healthy controls were studied. **(Table 2.)** The presence of organic gastrointestinal

diseases and non-diabetic causes of neuropathy were excluded in all patients. Controls were free of any gastrointestinal symptoms. As concerns the presence of gastrointestinal symptoms, patients were asked to complete a questionnaire. Swallowing disorders, reflux symptoms (heartburn, and regurgitation), abdominal pain, dyspeptic complaints (fullness, early satiety, bloating and nausea), and problems with bowel movements and stool evacuation were scored according to Talley's questionnaire. Frequency and severity scores were obtained. The absence of symptoms was scored as zero. The severity score was 4 if the symptom was intolerably severe. The frequency was scored as 4 if the symptom presented regularly after each meal. **[98]** Patients were evaluated in an inpatient manner. Blood glucose levels were normalized initially, and manometric studies had already been performed under normoglycemic conditions. According to this, blood glucose levels were checked at the beginning of the studies and were between 4.5 and 7.0 mmol/L in all patients. During the short procedures (procedure time less than 30 minutes), such as oesophageal and ano-rectal manometry blood glucose levels were measured only at the beginning of the study. On the contrary, during the gastric manometry blood glucose levels were checked at 2 hours and at the end (4 hours) as well. Patients were fasted overnight before the procedures. The appropriate blood glucose level was achieved by the administration short acting sc. insulin injections and/or intravenous glucose (5%) administration prior to the procedures as needed.

	Diabetics	**Controls**	**p**
n (M/F)	16 (8/8)	29 (13/16)	= 0.76
age (years)	53±15	47±14	= 0.19
BMI	25.8±5.5	27.3±5.0	= 0.36
DM duration (years)	22±15	-	-
HbA1c (%)	9.6±1.6	-	-
Blood glucose (mmol/l)	5.8±0.8	5.5±0.6	= 0.16
Ewing's autonomic neuropaty score	5.73±2.34	0.33±0.82	< 0.01

Table 2. Demographic parameters of the studied patients. Data expressed as mean±SD. The blood glucose levels were measured just before the manometric procedures. During gastric manometry blood glucose levels were controlled 2 and 4 hours after the beginning of the procedure, and were 5.9±0.8 and 6.1±0.7 mmol/l respectively.

3.1.4. The evaluation of oesophageal motor function in primary Sjögren's syndrome.

Twenty-five patients (22 women, 3 men) with systemic symptoms, who met the criteria of the European Community Study Group were studied in 1997 and 1998. **[107]** Patients had a detailed clinical evaluation including routine and immunological laboratory tests and lower lip histology at the time of the diagnosis of primary Sjögren's syndrome several months before the enrolment to this study. At the time of enrolment, their mean age was 55 (range 31-75) years,

and the mean duration of the disease was 14 (range 2-30) years. All patients were submitted to detailed symptom analysis regarding the swallowing function, oesophageal manometry and salivary function test. The swallowing symptom analysis was performed by means of a symptom scoring questionnaire. Swallowing difficulty, i.e. dysphagia, comprises symptoms ranging from a moderate sensation of slowing of the oesophageal passage to the typical feeling of the sticking of solid foods or even liquids. The patients were asked about the typical site (from the mouth to the cardia) and the frequency of dysphagia (0: no dysphagia; 1: less than one episode of dysphagia/week; 2: one or more episodes of dysphagia/week,3: one or more episodes of dysphagia/day; 4: one or more episodes of dysphagia/meal; and 5: problems at each swallow), the quality of food sticking (solids , liquids or both), the typical food causing a symptom, the requirement of liquid for swallowing (0: never, 1: sometimes, and 2: always), the presence of odynophagia (pain associated with swallowing) (0: never, 1: occasionally, and 2: regularly), and the frequency of aspiration during a meal (0: never, 1: less than one episode/ week, 2: one or more episodes/week, 3: one or more episodes/day, and 4: one or more episodes/meal). For the manometric examinations a control group of 42 subjects – consisted of healthy volunteers and of patients admitted to the hospital because of minor abdominal complaints, who did not exhibit any upper gastrointestinal symptoms, all of whom likewise gave their signed informed consent – were enrolled. These latter patients did not suffer from metabolic disorders or autoimmune connective tissue diseases.

3.2. Methods

3.2.1. Upper gastrointestinal endoscopy

Upper gastrointestinal endoscopy was performed to establish the presence and the severity of oesophagitis (GIF Q130, Olympus, Japan), and to rule out organic upper gastrointestinal abnormalities (e.g. tumors). For the grading of oesophagitis the Savary-Miller classification system was used when respiratory complications of GORD had been evaluated. **[89]** This scoring system (Grade I – diffuse hyperaemia, isolated macular/linear erosions at the gastro-oesophageal junction, Grade II – confluent erosions involving only a part of the oesophageal circumference, Grade III – confluent erosions involving the whole oesophageal circumference, Grade IV – presence of chronic lesions, such as ulcer, stricture, Barrett's metaplasia or adenocarcinoma) was supplemented with the stage "0" representing the macroscopic integrity of the mucosa. As the new Los Angeles classification system became widely accepted it has replaced the Savary-Miller system in our laboratory also. Though this new endoscopic classification system was used when the cardiac manifestations of GORD

were evaluated. **[66]** This allowed us a more appropriate semi-quantitative grading of the severity of oesophagitis: no erosions = 0, grade LA-A = 1 (isolated, less than 5mm long macular or linear erosions at the top of the mucosal folds), grade LA-B = 2 (isolated, more than 5mm long erosions at the top of the mucosal folds), grade LA-C = 3 (erosions extending between the tops of the mucosal folds, involving less than 75% of the luminal circumference), grade LA-D = 4 (erosions extending between the tops of the mucosal folds, involving more than 75% of the luminal circumference).

3.2.2. 24-hour intraoesophageal pH monitoring

The 24-hour intraoesophageal pH monitoring studies were carried out in an inpatient basis using the classical DeMeester criteria. **[20, 21]** A single channel, naso-oesophageal, antimony pH-probe (Synectics Medical, Sweden) was positioned 5 cm above the lower oesophageal sphincter, and was connected to a portable data logger (Digitrapper Mark II Gold or Digitrapper MD, Synectics Medical, Sweden). The computer-assisted data analysis was carried out with the EsopHogram 5.60 or Polygram for Windows 3.1 software (both supplied by Gastrosoft Inc., Sweden). The parameters measured were: the percentage of the time below pH 4, the total time below pH 4, the number of pH<4 episodes, the number of pH<4 episodes longer than 5 minutes, the longest pH<4 episode, and the DeMeester score. Postprandial periods were evaluated according to the Mason criteria. **[71]** The administration of drugs, which have an effect on acid secretion, or on gastrointestinal motility were appropriately stopped 48-168 hours before the study.

3.2.3. Manometric studies

Oesophageal motility was studied on the basis of Castell's criteria. **[10, 11, 18, 35]** by standard, water perfusion, stationary manometry (Polygraph HR, Synectics Medical, Sweden) with computer-assisted analysis of the tracings (Polygram 5.06C2 UGI Edition, Gastrosoft Inc., Sweden). For the procedure, a multi-lumen, low-compliance, naso-oesophageal catheter was applied. The competence of the lower oesophageal sphincter (LOS) was estimated by station pull-through technique. The oesophageal body function was studied at 4 points: 3, 8, 13 and 18 cm-s above the LOS by measuring the amplitude, the duration, the propagation velocity of the contractions and the frequency of simultaneous waves after wet ($5cm^3$ of room-temperature tap water) and dry swallows. 10 swallows of each type were performed at least 30 seconds apart. In the upper oesophageal sphincter (UOS), the pressure profile and relaxation were studied. In the pharynx (PHX) the amplitude of contraction was measured, with the analysis of the UOS-PHX coordination. Prior to the manometric examinations the presence of

organic oesophageal diseases (webs, strictures and malignancies) were excluded by upper gastrointestinal endoscopy in all cases, and the administration of the drugs affecting oesophageal motility was suspended appropriately 48 hours before the study.

For gastric manometry, a similar, water-perfused multi-lumen, low-compliance catheter was positioned into the gastric antrum under fluoroscopic control. 4-hour periods of fasting were recorded, and the presence of the migrating motor complex (MMC) I-II-III phases was analyzed. The gastric motility was considered pathologic if no phase III activity was found. Total gastroparesis was diagnosed when neither phase III nor phase II activity was detected.

For the recto-anal studies, the Polygram 5.06C2 LGI Edition (Gastrosoft Inc., Sweden) software was used. A Marquat probe was placed into the anus. The balloon on the tip of the probe was used for air inflation and positioned into the ampoule of the rectum. The two other balloons were placed into the internal (IAS) and external (EAS) anal sphincters to measure the sphincteric functions. The resting pressures of both sphincters, and the presence of recto-anal inhibitory (RAI) and contractory (RAC) reflexes was established during 5 increasing volumes (10, 20, 30, 40 and 50 ml) of air inflation. The voluntary contraction (VC) capacity and the efficacy of expulsion (VE) were also studied. VE was considered effective if the EAS pressure simultaneously dropped with elevation of the IAS pressure.

Our normal ranges, which were set upon the results obtained in healthy subjects or in patients referred to our hospital because of minor abdominal complaints, and who had no evidence of gastroenterological disease, corresponded to the internationally accepted values. **[80] [24]**

3.2.4. Coronary angiography

The coronary angiography was carried out in all the patients prior to the enrolment to the study, according to the standard Seldinger technique. Lesions were considered to be significant in case of at least 70% narrowing of the coronary artery lumen, causing typical chest pain and myocardial ischaemia confirmed by one of the following non-invasive tests: tredmill exercise electrocardiography, myocardial isotope perfusion test, stress echocardiography. When signs of suspected coronary spasm were detected (n=7), intracoronarial glyceril-trinitrate (200μg) was administered to prove the presence of coronary spasm. Patients with a previous history of suspected coronary spasm, but having no signs of this at the time of coronarography (n=3), were submitted to a provocative test, involving the intracoronarial administration of increasing doses of ergonovine (5, 10, 25 and 50 μg) to evaluate the presence of provokable coronary artery spasm.

3.2.5. Coronary flow reserve (CFR) measurement

The coronary flow reserve was established by transoesophageal Doppler echocardiography (Toshiba Power Vision). A 5 MHz multiplane transducer was introduced into the oesophagus. Flow velocities were measured in the LAD according to our previously published protocol **[75]** originally described by Iliceto et al. **[46]** Briefly, 0.56 mg/kg dipyridamole was administered intravenously in 4 minutes. The CFR was calculated as the ratio of the peak and the baseline diastolic flow velocities. In line with to our previous results, the CFR was accepted as normal if it exceeded 2.5. **[75]** In patients with intact large coronary arteries at coronary angiography the decrease of CFR represents microvascular disease by definition.

3.2.6. The establishment of the oesophago-cardiac reflex

Patients were fasted overnight before the study. Their regular anti-ischaemic treatment (such as Ca-channel blockers, beta-receptor blockers or nitrates) was suspended 24-48 hours before the study. Only anti-platelet aggregation and statin therapy was preserved. During this period, the patients were allowed to use sublingually administered short-acting glyceryl-trinitrate for their chest pain as required up until 12 hours before testing. The test was postponed if the patient needed this treatment during the last 12 hours. Drugs affecting gastrointestinal motility, acid secretion or visceral sensitivity were also stopped appropriately before the study. First a naso-oesophageal perfusion catheter was positioned into the middle oesophagus, 15 cm above the lower oesophageal sphincter, 30 minutes before the insertion of the transoesophageal echocardiographic transducer. For the transoesophageal echocardiographic measurement, a multiplane 5 MHz transducer was placed into the oesophagus in a left lateral decubitus position. Flow velocities were measured in diastole in the left anterior descending artery (LAD), and continuously recorded on videotape during the study. To detect any critical myocardial perfusion changes, the standard 12-lead electrocardiogram (ECG) was monitored continuously throughout the study. In case of any significant ST segment changes and/or dysrhythmias the study was terminated immediately. Baseline coronary flow measurements were made after a 5 minutes adaptation period. During the acid stimulation test 0.1 N HCl and 0.9% NaCl solutions were perfused in a double-blinded manner into the middle oesophagus, at a rate of 12 millilitres per minute for 10 minutes. The ratio between the LAD flow velocities during HCl and NaCl perfusion was calculated. In preliminary studies, receiver operating characteristic (ROC) plots were done on the acid perfusion induced flow changes with respect to the accompanying chest pain. We set the cut-

off at a reduction of 15% in the coronary flow velocity for establishing the presence of the oesophago-cardiac reflex with a sensitivity of 92.3% and a specificity of 53.3%. The area under the ROC curve was 0.874 indicating a good ability of the test to distinguish between the presence and absence of the oesophago-cardiac reflex. On this basis, the patients, who exhibited a higher than 15% decrease in coronary flow velocity during oesophageal acid stimulation were considered to have oesophago-cardiac reflex, while the others were not.

3.2.7. Cardiovascular autonomic neuropathy testing

The autonomic neuropathy was evaluated according to the standard cardiovascular reflex tests of Ewing. **[27]** Of the five parameters measured, 3 represented the parasympathetic function (beat-to-beat variation of the heart rate on deep breathing, the heart rate variation on standing up (30/15 ratio), and the Valsalva-manoeuvre) and 2 revealed the sympathetic function (change in blood pressure on standing up (lying-to-standing ratio) and in response to a sustained handgrip). The normal values are summarized in **Table 4.** Each parameter was scored between 0 to 2, for normal (=0), borderline (=1) or abnormal (=2) absolute values. An overall score between 1 and 3 corresponded to mild, between 4 and 6 corresponded to moderate, and the interval 7-10 corresponded to severe autonomic neuropathy.

3.2.8. Evaluation of salivary function

Both the unstimulated (basal) and the stimulated whole saliva production were measured in all patients. The salivation test was carried out under standardized conditions between 9 and 11 a.m. Patients we re instructed to fast before the procedure. Neither eating nor drinking nor smoking was allowed for at least 1 1/2 hours before the saliva collection. For the study, a sterile absorbent gauze was placed between the lower lip and the teeth of the patient, who was sitting in an upright position leaning slightly forward. Patients were not allowed to swallow, masticate or speak during the examination. Both unstimulated and stimulated whole saliva collection lasted for 10 minutes. During the stimulated saliva production, 1 drop of 2% citric acid solution was placed on the surface of the tongue at the beginning and in the fifth minute of the examination. The difference in the weights of the gauze before and after the procedures gave the amount of saliva produced. When the methods were established earlier, we also determined the saliva production in 96 normal subjects. With our method, the unstimulated saliva production was considered to be reduced, if it had been ≤1.0 ml in 10 minutes. This corresponded well to the internationally accepted value **[107]** The stimulated production was determined abnormally low, if it had been ≤4.0 ml in 10 minutes.

3.2.9. Laboratory investigations and lower lip histology in patients with primary Sjögren's syndrome.

Routine laboratory and immune serological examinations we re performed in all patients: anti-nuclear antibody (ANA; indirect immune fluorescence on rat liver substrate), IgM rheumatoid factor (latex test, positive if titer ≥1:40) , anti-native DNA (radio immune assay) , anti-SSA , anti-SSB, anti-RNP and anti-Sm antibodies (enzyme linked immune sorbent assay; Immuno DOT), and concentrations of complement C3 (rocket immune-electrophoresis). A lower lip biopsy was performed in 23 of the 25 patients for histological examination of the minor salivary glands. The result was considered positive, if at least one focus of ≥50 mononuclear inflammatory cells per 4 mm^2 was found. **[107]** The lip biopsies were carried out several months or even years prior to this oesophageal manometric study.

3.3. Statistical analysis

Analyses were done by running GraphPad Prism 3.02 (GraphPad, San Diego, CA). Frequency distribution analysis was carried out by the Chi-square test or the Fischer's exact test; group means were compared by the Student's unpaired T-test, with the Welch's correction if needed. If means of more than two groups were compared, one-way analysis of variance (ANOVA) was carried out first, followed by Newman-Keuls post hoc test. Correlation analysis was done by the Pearson's test. The level of significance was set at $p<0.05$. Data are presented as means. Standard deviations (SD) or errors (SEM) are marked appropriately.

4. RESULTS

4.1. The establishment of oesophago-cardiac reflex in patients with angina-like chest pain.

Table 3. summarizes the results of the demographic data of the patients. The gastroenterologic evaluation of the studied patients showed, that typical reflux symptoms, such as heartburn and acid regurgitation were present regularly (>1/week) in 37 and 35 percent of the patients respectively. These data were similar in all of the studied patient subgroups.

On endoscopy, erosive oesophagitis was present in 23/51 (45%) cases (16 patients with Los Angeles grade A, 6 with grade B, and 1 with grade C oesophagitis). Patients with positive oesophago-cardiac reflex test had higher oesophagitis score values (LA-A 8/25, LA-B 5/25, LA-C 1/25), than those with negative test (LA-A 8/28, LA-B 1/28, LA-C 0/28) **(Figure 1A.)**.

The 24-hour pH monitoring revealed an incidence of 23/51 (45%) for pathologic gastro-oesophageal acid reflux. Although abnormal 24-hour pH monitoring results were found more often in patients with NCCP or coronary spasm, than in the other patient groups this was not statistically significant.

		All	CAD	MD	Spasm	NCCP	p=
	n (males)	51 (24)	19 (9)	13 (6)	10 (4)	9 (5)	NS
	Age	54.6±1.3	57.6±2.1	57.1±1.6	49.9±2.3	51.4±3.6	NS
	BMI	28.5±0.8	28.1±1.6	30.2±1.0	28.5±1.8	26.7±2.3	NS
	Chest pain	51/51	19/19	13/13	10/10	9/9	NS
	Heartburn	19/51	5/19	5/13	4/10	5/9	NS
	Regurgitation	18/51	6/19	4/13	4/10	4/9	NS
Oesophagitis	**No erosions**	28/51	12/19	5/13	5/10	6/9	NS
	LA-A	16/51	5/19	7/13	1/10	3/9	NS
	LA-B	6/51	2/19	1/13	3/10	0/9	NS
	LA-C	1/51	0/19	0/13	1/10	0/9	NS
	LA-D	0/51	0/19	0/13	0/10	0/9	NS
	Abnormal 24h pH	23/51	7/19	4/13	7/10	5/9	NS
	OCR	25/51	7/19	4/13	10/10	4/9	0.01

Table 3. Characteristics of the studied patient groups. CAD: any significant epicardial coronary artery disease, LAD: significant LAD disease, MD: microvascular disease, NCCP: non-cardiac chest pain, OCR: oesophago-cardiac reflex. Data are expressed as mean±SEM.

Of the different pH parameters studied the "DeMeester score" **(Figure 1B.)**, the "number of prolonged (>5 minutes) pH<4 episodes" **(Figure 1C.)**, the "number of pH<4 episodes" (data not shown) and the fraction time below pH 4 (data not shown) showed correlation with the presence of oesophago-cardiac reflex. In patients with non-cardiac chest pain, i.e. macroscopically normal coronary arteries and normal CFR (n=9), the prevalence of gastro-oesophageal reflux disease was 5/9 (55%). In the remaining 4 cases the oesophageal origin of the chest pain could not be proved, since not only the 24-hour pH monitoring, but the upper gastrointestinal endoscopic and oesophageal manometric evaluation yielded normal results as well.

Figure 2. depicts the prevalence of the oesophago-cardiac reflex based on the results of the acid stimulation test. Twenty-five patients (49%) had positive test, while the remaining 26 (51%) cases had no change in the coronary flow velocity. Two-third of the patients with oesophago-cardiac reflex (16/25; 64%) reported chest pain during the oesophageal acid stimulation, but none in the group of not having oesophago-cardiac reflex. The coronary flow reduction and/or chest pain have been accompanied by ST segment alterations in 10/25 cases.

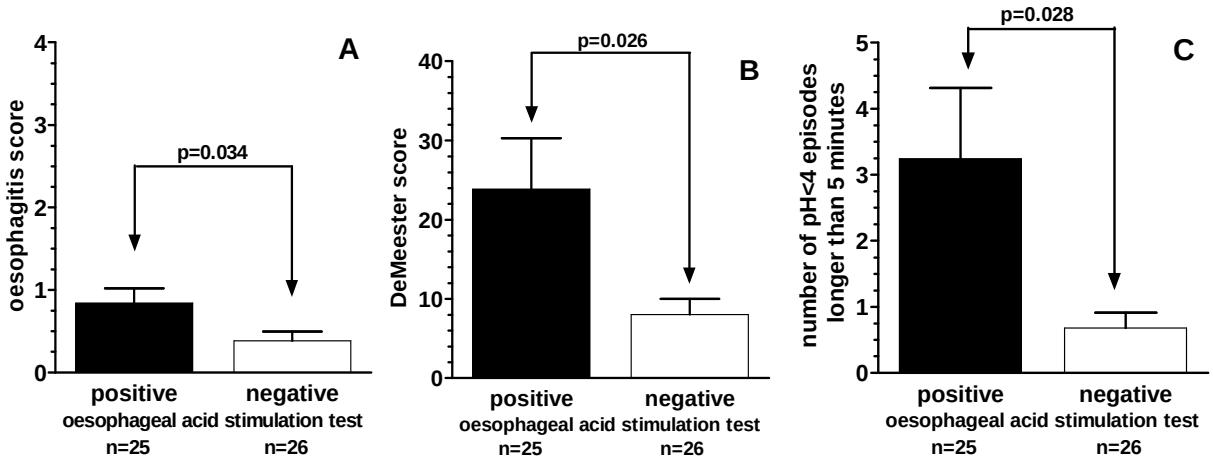

Figure 1. The association between oesophageal endoscopic and pH monitoring results and the presence of oesophago-cardiac reflex in patients with chest pain. A: DeMeester score (intraoesophageal pH monitoring), B: number of pH<4 episodes longer than 5 minutes (intraoesophageal pH monitoring). C: Oesophagitis scores. Closed bars – positive oesophageal acid stimulation test, open bars – negative oesophageal acid stimulation test. Data are expressed as mean±SEM

The prevalence of oesophago-cardiac reflex in the different patient subgroups was evaluated on the basis of the presence or absence of coronary artery disease. Results were similar in patients with large epicardial coronary artery stenosis, microvascular disease and negative cardiologic evaluation. **(Table 3.)**

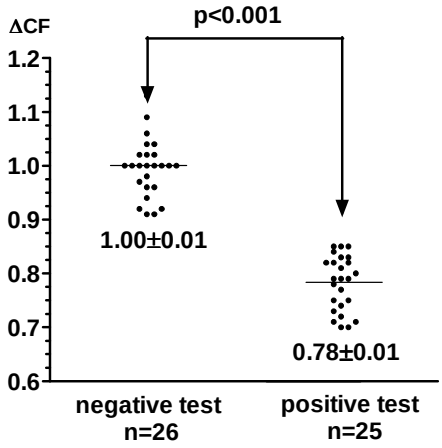

Figure 2. Coronary flow velocity changes during oesophageal acid stimulation test in patients with chest pain. ΔCF: coronary flow velocity change during oesophageal acid stimulation

Furthermore, the prevalence of oesophago-cardiac reflex appeared to be independent of the location – LAD, circumflex branch, right coronary artery – of the coronary artery disease

as well (data not shown). On the other hand all of the studied patients with coronary spasm had decreased coronary flow velocity during oesophageal acid perfusion. **(Figure 3.)**

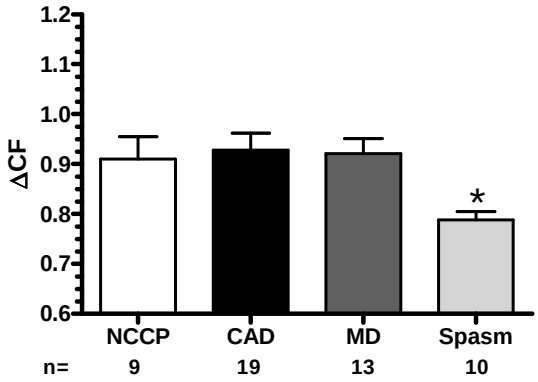

Figure 3. The association between coronary artery disease and the presence of oesophago-cardiac reflex in patients with chest pain. NCCP: patients with non-cardiac chest pain (negative cardiologic evaluation), CAD: significant coronary artery disease, MD: microvascular disease, Spasm: coronary spasm, NS: not significant, ΔCF: coronary flow velocity change during oesophageal acid stimulation. Data expressed as means±SEM. *: p=0.02

4.2. The evaluation of chronic respiratory symptoms and diseases in patients with GORD.

Eighteen percent of the GORD patients (56/299; 29 women, 27 men; age: 51.1±14.9 years; 7 smokers, 49 non-smokers) had chronic upper and lower respiratory complaints or respiratory disease. Of the symptoms evaluated, chronic cough was present in 75% (42/56) and hoarseness in 11% (6/56). Of the reflux-associated chronic respiratory diseases, chronic bronchitis was found in 21% (12/56), asthma in 18% (10/56), recurrent pneumonia in 18% (10/56), chronic sinusitis in 12% (7/56), and chronic laryngitis in 2% (1/56).

The reflux symptoms associated with respiratory complications were heartburn (54%, 30/56), acid regurgitation (43%, 24/56), nausea (20%, 11/56), chest pain (12%, 7/56), acidic belching (12%, 7/56) and vomiting (9%, 5/56), while 12% of the patients (7/56) did not have reflux symptoms.

24-hour oesophageal pH monitoring was carried out in 51 of the 56 patients. For the other 5 patients, technical reasons or the lack of patient consent made it impossible to carry out pH monitoring, but in these patients the presence of GORD was proved by the endoscopic findings, manometry and the video barium oesophagography results. The DeMeester score was pathological in 57% (29/51; DeMeester score: 66.33±48.38, mean±SD), while the fraction time below pH 4 appeared abnormal in 55% (28/51; fraction time below pH 4: 18.70±12.77%) of the patients. In patients with an abnormal DeMeester score, nocturnal reflux was predominantly observed (52%, 15/29). Diurnal reflux or postprandial acid reflux occurred in

28 (8/29) and 20% (6/29), respectively. Seventeen patients (31%) had a normal DeMeester score, but abnormal values of one or more of the studied pH parameters. In this group, postprandial reflux was detected in 82% (14/17), nocturnal reflux in 12% (2/17), and diurnal reflux in 6% (1/17) of the patients. **(Table 4.)**

	Patients with respiratory symptoms % (n = 51)	***Patients without respiratory symptoms % (n = 225)***
Abnormal DeMeester score	***57 (29/51)***	***63 (142/225)***
Postprandial reflux	20 (6/29)	34 (48/142)
Nocturnal reflux	52 (15/29)	18 (25/142)
Diurnal reflux	28 (8/29)	48 (69/142)
Normal DeMeester score with abnormal reflux events	***31 (17/51)***	***21 (48/225)***
Postprandial reflux	82 (14/17)	85 (41/48)
Nocturnal reflux	12 (2/17)	8 (4/48)
Diurnal reflux	6 (1/17)	7 (3/48)
Normal 24-hour pH curve	***9 (5/51)***	***16 (35/225)***

Table 4. Results of 24-hour oesophageal pH-monitoring in patients with or without respiratory symptoms

In 9% (5/56) of the subjects, the 24-hour intra-oesophageal pH profile was completely normal, while manometry showed a decreased pressure of the lower oesophageal sphincter, and the video barium oesophagography confirmed a significant gastro-oesophageal regurgitation of the contrast medium. Of the symptoms and reflux-associated respiratory diseases evaluated in patients with an abnormal DeMeester score, chronic cough was present in 86% (25/29), hoarseness in 14% (4/29), chronic bronchitis in 10% (3/29), asthma in 18% (5/29), recurrent pneumonia in 14% (4/29), chronic sinusitis in 7% (2/29), and chronic laryngitis in 3% (1/29).

Among patients with a normal DeMeester score, chronic cough was found in 59% (13/22), chronic bronchitis in 32% (7/22), asthma in 18% (4/22), recurrent pneumonia in 14% (3/29), and chronic sinusitis in 18% (4/22). In this group of patients chronic laryngitis and hoarseness were not observed. **(Table 5.)** Twenty-four hour pH monitoring was performed in 225 of 243 patients without respiratory symptoms. The DeMeester score was pathological in 63% (142/225), while the fraction time below pH 4 appeared abnormal in 60% (136/225) of the patients. In patients with an abnormal DeMeester score, postprandial, nocturnal or diurnal acid reflux occurred in 34% (48/142), 18% (25/142) and 48% (69/142), respectively. Forty-eight patients (21%) had a normal DeMeester score, but the values for one or more of the studied pH parameters were abnormal.

Respiratory symptoms	*Patients with abnormal DeMeester score % (n = 29)*	*Patients with normal DeMeester score % (n = 22)*
Chronic cough	86 (25/29)	59 (13/22)
Hoarseness	14 (4/29)	0
Chronic bronchitis	10 (3/29)	32 (7/22)
Bronchial asthma	18 (5/29)	18 (4/22)
Recurrent pneumonia	14 (4/29)	14 (3/22)
Chronic sinusitis	7 (2/29)	18 (4/22)
Chronic laryngitis	3 (1/29)	0

Table 5. Prevalence of respiratory symptoms in patients with abnormal or with normal DeMeester scores

In this group, postprandial reflux was detected in 85% (41/48), nocturnal reflux in 8% (4/48) and diurnal reflux in 7% (3/48) of the patients. In 16% (35/225) of the subjects, the 24-hour intra-oesophageal pH profile was completely normal, however the manometry demonstrated impaired lower oesophageal sphincter function and endoscopy or video barium oesophagography revealed reflux. **(Table 4.)**

Of the 56 patients with respiratory complications, upper gastrointestinal endoscopy did not detect any abnormality in 11% (6/56) and revealed erosive oesophagitis Savary-Miller stage I in 40% (23/56), stage II in 29% (16/56), stage III in 9% (5/56) and stage IV in 11% (6/56). On endoscopy 5% (12/243) of the patients without respiratory complications proved negative and 32% (78/243) had stage I, 39% (95/243) had stage II, 12% (29/243) had stage III and 12% (29/243) had stage IV erosive oesophagitis. **(Table 6.)**

Endoscopy oesophagitis stage (Savary-Miller)	*Patients with respiratory symptoms % (n = 56)*	*Patients without respiratory symptoms % (n = 243)*
0	11 (6/56)	5 (12/243)
I	40 (23/56)	32 (78/243)
II	29 (16/56)	39 (95/243)
III	9 (5/56)	12 (29/243)
IV	11 (6/56)	12 (29/243)

Table 6. Results of upper gastrointestinal endoscopy in patients with or without respiratory symptoms

4.3. The evaluation of oesophageal and other gastrointestinal motility disorders in patients with type 1 diabetes mellitus.

The results of the cardiovascular autonomic neuropathy tests are presented in **Table 7**. Of the parasympathetic parameters, more abnormalities were found in the heart rate variation on deep breathing (10/16) and during standing-up (10/16). In contrast, the results of the

Valsalva-test (7/16 abnormal) were mainly in the borderline range. Of the sympathetic tests, the orthostatic blood pressure response (lying-to-standing) was markedly impaired in the majority of patients (9/16), while the sustained handgrip test proved fewer abnormalities (5/16). The mean(±SD) of the overall scores, 5.73±2.34, indicated a moderate autonomic neuropathy on average.

Test	***Diabetics (n=16)***	***Controls (n=29)***	***p***	***Ewing's normals***
Parasympathetic function				
Beat-to-beat variation of the heart rate on deep breathing (beats/min)	8.7±6.9	23.1±7.1	< 0.01	≥ 15.0
Heart rate variation on standing up (beats/min)	1.01±0.04	1.17±0.13	< 0.01	≥ 1,04
Heart rate variation in response to Valsalva-maneuver (beats/min)	1.24±0.26	1.60±0.32	< 0.01	≥ 1,21
Sympathetic function				
Change in blood pressure in response to sustained handgrip (mm Hg)	15.2±6.0	26.0±8.1	< 0.01	≥ 16.0
Change in blood pressure on standing up (mm Hg)	22.5±14.2	7.0±7.9	< 0.01	≤ 10.0
Ewing's total autonomic score	***5.73±2.34***	***0.33±0.82***	***< 0.01***	***≤ 2***

Table 7. Results of cardiovascular autonomic neuropathy tests (mean±SD).

The symptom analysis revealed the chronic presence of a number of gastrointestinal symptoms. Twelve of the 16 patients had such symptoms for several (>5) years. In contrast, a significant weight loss was reported only in 4 cases. If a swallowing difficulty was present, it was linked to solid meals. 7/16 patients reported such a symptom; 3 of them were severe (score=4). Only one patient associated his dysphagia with the pharyngeal region; the others did so with the oesophagus. The mean severity score was 2.50±1.27 (mean±SD). Reflux symptoms were reported in 10 cases. The heartburn frequency score was 1.56±1.55. Only 2 patients reported heartburn and regurgitation after each meal. The duration of heartburn episodes exceeded 30 minutes in 7 patients, with a mean symptom severity score of 1.80±0.42. Abdominal pain episodes were present in 12 patients (mean frequency score 2.12±1.67). The pain episodes were associated with the epigastric area in a majority of the cases (8/12). The severity score was 2.00±0.43. Within the dyspeptic symptoms reflecting gastrointestinal dysmotility, a loss of appetite was reported by only 2 patients. Others occurred more frequently: early satiety in 5, and fullness, bloating and nausea 9/16 cases each. These were

represented by frequency scores of 0.69±0.87, 1.81±1.47, 1.88±1.45 and 1.88±1.63, respectively. Problems with the bowel discharge were also reported. Diarrhoea (>3 stools/day) was observed in 4/16 patients, with a mean daily discharge of 7.5 (5-14). Three of them had fecal incontinence. Constipation (<3 stools/week) was not observed in this patient group, but difficult evacuation/hard stools were reported by 3 patients.

The manometric evaluation of the oesophagus **(Table 8.)** did not reveal significant differences in the UOS-PHX region between the diabetics and controls, with the exception of a slightly premature UOS relaxation in one patient in the diabetic group. In the esophageal body, the peristaltic waves had lower amplitudes in the diabetics than in the controls, but a statistically significant difference was found at only 2 measurement points (8 and 18 cm above the LOS). Abnormally low amplitudes were detected in all segments in 8/16 patients. The peristaltic wave duration appeared significantly longer in the diabetics at all measurement points. Abnormally prolonged duration was observed in 7/16 patients. The diabetic group had a significantly decreased peristaltic wave propagation velocity, which appeared to lie outside the normal range in 11/16 cases. The rate of simultaneous contractions was increased, with abnormal values in 9 patients. In the LOS, the mean pressure was significantly decreased, and the relaxation time was prolonged in the diabetic group. Abnormal pressures and relaxation times were each found in 10/16 patients.

The correlation analysis showed that longer LOS relaxation times associated with higher reflux symptom scores (r^2=0.32, p=0.05). In contrast, correlation was not found between LOS pressures and reflux symptoms (r^2=0.15, p=0.22). Correlation analysis of dysphagia scores and manometric parameters of the oesophageal body revealed that higher scores associated with increased rate of simultaneous waves (r^2=0.49, p=0.01), and the duration of peristaltic waves was more prolonged at 3, 13 and 18cm-s above the LOS. The r^2 values were 0.49, 0.41 and 0,39; while p values were 0.01, 0.03 and 0.04 respectively. Furthermore the mean the propagation velocity of the contractions were decreased in patients with more severe dysphagia scores (r^2=0.35, p=0.05). The amplitude of the contractions showed no correlation with the dysphagia scores.

The manometry of the gastric antrum demonstrated impaired fasting state motility patterns in 11 patients. 5 of them involved only the absence of the MMC phase III activity, with a preserved phase II activity, while 6 had a markedly diminished antral motility, with MMC phases II and III absent, clearly representing complete gastroparesis. Significant differences were not found between the mean dyspepsia score values of the patients with or without fasting gastric motility disorders (p=0.39).

	Diabetics (n=16)	*Controls (n=29)*	*p*
LOS			
Pressure (mm Hg)*	17±6	26±8	< 0.01
Relaxation time (s)	11.7±4.4	8.7±1.5	= 0.02
OB peristaltic wave			
Amplitude at			
3 cm-s:	84±58	115±37	= 0.07
8 cm-s:	75±38	101±30	= 0.01
13 cm-s:	54±38	66±28	= 0.23
18 cm-s:	32±20	58±31	< 0.01
above the LOS (mm Hg)			
Duration at			
3 cm-s:	4.9±2.2	3.8±1.1	= 0.03
8 cm-s:	4.5±1.4	3.6±0.8	< 0.01
13 cm-s:	4.2±1.4	3.1±0.6	< 0.01
18 cm-s:	3.4±1.6	2.6±0.6	= 0.02
above the LOS (s)			
Propagation velocity (cm/s)	2.6±0.7	3.3±0.6	< 0.01
Simultaneous contractions (%)	11±13	1±4	< 0.01
UOS			
Pressure (mm Hg)*	63±19	69±30	= 0.47
Relaxation time (s)	1.68±0.40	1.55±0.34	= 0.25
PHX			
Contraction amplitude (mm Hg)	47±11	42±16	= 0.29
UOS-PHX			
Coordination (s)	-0.20±0.10	-0.15±0.07	= 0.05

Table 8. Manometric differences in esophageal motility between patients with long-standing type 1 diabetes mellitus and controls (mean±SD). LOS: lower esophageal sphincter, OB: oesophageal body, UOS: upper oesophageal sphincter, PHX: pharynx. * Mean pressure in high-pressure zone of sphincter.

The recto-anal manometry **(Table 9.)** did not reveal any significant differences between the diabetics and the controls as concerns the EAS and IAS mean resting pressures. Of the 16 diabetic patients, 4 had abnormally low values for each parameter. An impaired RAI reflex was observed in 3, and a RAC reflex failure in 4/16 cases, while the group means for the diabetics and the controls were statistically similar. During squeezing, the mean pressures were found to be mildly ($p=0.08$) decreased in the diabetics in both sphincters of the anus. Abnormally low values were obtained in 7/16 patients. The squeezing-resting pressure difference (Δp) of the IAS was significantly ($p=0.02$) reduced in the diabetics. This parameter was abnormal in 7/16 cases. An impaired fecal expulsive function was found in 4/16 diabetics,

the distribution of which reached the level of statistical significance (p=0.04) as compared to controls.

When the manometric data of the studied regions of the gastrointestinal tract were considered overall, an impaired motility was found simultaneously in all studied regions (oesophagus, gastric antrum and recto-anal sphincters) in 4/16 patients. At least two 2 regions were affected in 9 cases and 1 region in 3 cases. None of the patients gave normal results in all tests.

	Diabetics (n=16)	***Controls (n=29)***	***P***
Mean resting pressure			
EAS (mm Hg)	39±20	47±19	= 0.18
IAS (mm Hg)	66±33	67±23	= 0.84
Mean squeezing pressure			
EAS (mm Hg)	61±32	78±30	= 0.08
IAS (mm Hg)	94±47	118±44	= 0.08
Mean pressure difference (Δp)			
EAS (mm Hg)	23±20	31±23	= 0.19
IAS (mm Hg)	28±25	51±34	= 0.02
RAI reflex (%) *	68±22	63±21	= 0.46
RAC reflex †	12 / 16	27 / 29	= 0.16
VE	12 / 16	28 / 29	= 0.04

Table 9. Manometric differences in recto-anal motility between patients with long-standing type 1 diabetes mellitus and controls (mean±SD).EAS: external anal sphincter, IAS: internal anal sphincter, RAI: recto-anal inhibitory, RAC: recto-anal contractory, VE: voluntary expulsion, Δp: $p_{squeezing} - p_{resting.}$ * IAS relaxation (% relative to baseline) for 40 ml air inflation. † Appearance of EAS contraction for 30 ml air inflation.

4.4. The evaluation of oesophageal motor function in primary Sjögren's syndrome.

Of the main clinical manifestations, which appeared during the course of the disease, parotid enlargement occurred in 15 of the 25 patients (60%), articular involvement in 19 (76%), purpura in 7 (28%) and Raynaud's phenomenon in 13 (52%). Renal tubular acidosis was diagnosed in 7 patients (28%), including 2 patients with histologically proved tubulointerstitial nephritis. Lower airway involvement was found in 10 patients (40%), with lung fibrosis in 7 (mild in 5, advanced in 2) of the 10, verified by high-resolution computed tomography. Of the laboratory changes, leukopenia (with or without anaemia) was detected in 13 cases (52%), hypergammaglobulinemia in 15 (60%), IgM rheumatoid factor positivity in 23 (92%) and ANA positivity in 15 (60%). Anti-SSA antibody positivity was found in 20 (80%) patients [alone in 8 (32%), and coupled with anti-SSB antibody positivity in 12 (48%)] and anti-SSB antibody positivity in 13 (52%) cases [alone in 1 (4%) and together with anti-SSA

antibody positivity in 12 (48%)]. The histology of the minor salivary glands was indicative of Sjögren's syndrome in all biopsied patients.

The symptom evaluation revealed some grade of dysphagia [score: 2.7±1.37 (mean±SD)] in all but 2 patients (23/25, 92%). **(Table 10)** Of these 23 patients, 22 had swallowing difficulties for only solids, and 1 for both solids and liquids. The patients predominantly stated that their dysphagia was situated in the upper oesophageal (9/23) or pharyngeal region (5/23), whereas symptoms relating to the mid-oesophagus were reported in 4/23 cases. The most typical foods causing swallowing problems were bread and other bakery products (17/23, 74%). Meat or other solid foods (fruits, potato, cheese, etc.) were reported less frequently (6/23, 26%). All but one patient with dysphagia needed the aid of liquids to help them swallow solids, 17 had a constant, and 5 an occasional need for this (score: 1.68±0.56 mean±SD). Episodes of odynophagia occurred in 11 of the 25 patients (in 5 regularly and in 6 occasionally), giving an overall score of 0.67±0.82 (mean ± SD). Aspiration was rare. At least daily episodes were reported by only 1 patient, and 3 patients had weekly episodes (score: 0.58 ± 0.88, mean±SD).

Dysphagia	***Number of patients***
Presence:	23/25 (92%)
Location:	
- mouth	3/23 (13%)
- pharynx	5/23 (22%)
- upper third of oesophagus	9/23 (39%)
- middle third of oesophagus	4/23 (17%)
- lower third of oesophagus	1/23 (4.5%)
- cardia/diaphragm	1/23 (4.5%)

Table 10. Distribution of different locations of dysphagia in patients with primary Sjögren's syndrome.

Both basal and stimulated saliva production were reduced in 23/25 (92%) patients. One patient displayed an impairment of stimulated secretion only. Normal saliva secretion was detected in 1 patient. Of the studied manometric parameters, the mean LOS pressures were significantly lower ($p<0.01$), and the relaxation times were significantly prolonged ($p<0.02$) as compared with the controls**.** However abnormally low LOS pressures were found in 9/25, and prolonged relaxation in 8/25 cases. **(Table 11.)**

In the OB, an increased duration of peristaltic contractions, a decreased propagation velocity and a higher rate of simultaneous contractions were observed for dry and wet swallows ($p<0.01$, **Table 11.)**. The duration of peristaltic contractions was abnormally long in 10/25 cases for wet swallows, and less frequently (7/25) for dry swallows. The rate of

simultaneous contractions exceeded the normal rate in 7 patients for wet swallows, and in 4 patients for dry swallows. The rate of simultaneous contractions for dry swallows correlated with the frequency of dysphagia.

	Primary SS (n=25)		***Control (n=42)***		***p***	
LOS						
- pressure (mmHg)*	20±8		26±7		< 0.01	
- relaxation time (sec)	9.7±2.1		8.6±1.4		< 0.02	
	DS	WS	DS	WS	DS	WS
OB peristaltic wave						
- amplitude at						
3 cm-s:	71±38	105±40	86±35	113±34	ns	ns
8 cm-s:	69±35	92±35	66±35	99±29	ns	ns
13 cm-s:	53±31	67±30	50±22	65±26	ns	ns
18 cm-s:	41±30	46±30	51±35	59±32	ns	ns
above the LOS (mmHg)						
- duration at						
3 cm-s:	4.7±1.1	4.8±0.8	3.6±1.0	3.7±0.9	< 0.01	< 0.01
8 cm-s:	4.5±1.1	4.7±0.9	3.2±0.8	3.4±0.7	< 0.01	< 0.01
13 cm-s:	4.0±0.9	3.9±0.7	3.2±0.7	3.1±0.6	< 0.01	< 0.01
18 cm-s:	3.3±0.7	3.5±1.0	2.5±0.7	2.6±0.6	< 0.01	< 0.01
above the LOS (sec)						
- propagation velocity (cm/s)	2.8±0.8	2.6±0.6	3.7±0.9	3.3±0.6	< 0.01	< 0.01
- simultaneous contractions (%)	14±22	10±12	5±12	1±3	0.05	< 0.01
UOS						
- pressure (mmHg)*	66±27		73±29		ns	
- relaxation time (sec)	1.43±0.39		1.54±0.31		ns	
PHX						
- contraction amplitude (mmHg)	45±15		42±16		ns	
UOS-PHX						
- coordination (sec)	-0.15±0.09		-0.15±0.05		ns	

Table 11. Manometric differences in the oesophageal motility between primary Sjögren's syndrome patients and controls (mean±SD).LOS: lower oesophageal sphincter, OB: oesophageal body, DS: dry swallow, WS: wet swallow, UOS: upper oesophageal sphincter, PHX: pharynx, ns: not significant. * mean pressure at the high pressure zone of the sphincter.

Patients with at least daily episodes of dysphagia (score ≥3) had significantly more simultaneous contractions for dry swallows, than those with fewer episodes (score ≤2). The rates were 19.7±25.5% vs. 3.3±7.1%, (mean±SD), $p<0.05$, respectively. This difference was not seen for wet swallows. Concerning the amplitude of the peristaltic waves, the values for the

patients appeared to be similar to those for the controls. In the upper region of the oesophagus (at the level of the UOS and the PHX) the manometric standards indicated no differences in the studied parameters between the primary Sjögren's syndrome patients and the controls. **(Table 11.)**

As decreased OB peristaltic velocity was the predominant motor abnormality, two groups of patients were formed for further study: those with normal and those with abnormal values. **(Table 12.)**

	GROUP I Impaired (≤2.7cm/s) OB peristaltic velocity (n=11)	***GROUP II Normal (> 2.7cm/s) OB peristaltic velocity (n=14)***	***p***
LOS pressure (mmHg, mean±SD)	15±6	24±8	<0.01
LOS relaxation time (sec, mean±SD)	10.7±2.5	9.0±1.4	<0.05
Simultaneous contractions in OB (%, mean±SD)	DS: 25±29 WS: 10±14	DS: 6±6 WS: 10±11	0.05 ns

Table 12. Manometric abnormalities associated with an impaired oesophageal body peristaltic velocity in patients with primary Sjögren's syndrome. OB: oesophageal body, DS: dry swallow, WS: wet swallow, ns: not significant

In group I (11 patients), each patient had a decreased (≤2.7 cm/s) oesophageal peristaltic velocity, while the patients in group II (14 patients) had normal values. The LOS pressure was found to be significantly lower ($p<0.01$) and the LOS relaxation significantly longer ($p<0.05$) in group I than in group II. We also observed a higher rate of simultaneous contractions in the OB in group I for dry swallows ($p=0.05$), whereas this difference was not seen for wet swallows. There was no statistical difference between the two groups as concerns the values of the amplitude and duration of the OB contraction, and the studied parameters of the UOS-PHX region.

Both the unstimulated and the stimulated whole saliva production were significantly lower in group I ($p<0.05$ for both) than in group II. **(Table 13.)** The characteristics of the dysphagia were identical in the two groups, with the exception of one parameter: the group I patients had a significantly higher liquid requirement for swallowing than the patients in group II, with scores of 1.91±0.30 vs. 1.50±0.65; (mean±SD), $p=0.05$, respectively. **(Table 13.)** The distributions of the systemic manifestations of primary Sjögren's syndrome, the results of routine and immunological laboratory tests and the studied demographic parameters (age and duration of the disease) did not demonstrate any statistically significant difference between the two groups.

Saliva production test	*GROUP I Impaired OB peristaltic velocity (n=11)*	*GROUP II Normal OB peristaltic velocity (n=14)*	*p*
Basal whole saliva volume (ml/10min, mean±SD)	0.23±0.17	0.69±0.75	<0.05
Stimulated whole saliva volume (ml/10min, mean±SD)	0.44±0.29	1.45±1.63	<0.05
Symptom:	***GROUP I Impaired OB peristaltic velocity (n=11)***	***GROUP II Normal OB peristaltic velocity (n=14)***	***p***
Frequency of dysphagia episodes (symptom score, mean±SD)	2.60±1.51	2.71±1.33	NS
Frequency of odynophagia episodes (symptom score, mean±SD)	0.70±0.82	0.64±0.84	NS
Liquid requirement for swallowing (symptom score, mean±SD)	1.91±0.30	1.50±0.65	0.05
Frequency of aspiration (symptom score, mean±SD)	0.90±0.88	0.36±0.84	NS

Table 13. Results of saliva production tests and oesophageal symptom analysis in primary Sjögrens's syndrome patients with or without an impaired oesophageal body (OB) peristaltic velocity.

5. DISCUSSION

Gastro-oesophageal reflux disease is known to be a common cause of chest pain. Since the differential diagnosis is often difficult, a number of studies carried out to evaluate this problem. It has been shown that GORD and coronary artery disease may coexist in up to 40 percent of the patients **[41, 42, 62, 67, 93, VI, IX, X]** and similar incidence of GORD can be observed in patients with non-cardiac chest pain **[26, 64, 109]**, but until now neither aimed to evaluate prospectively the possible causal link – the oesophago-cardiac reflex – in patients with gastro-oesophageal reflux disease and different types of coronary artery diseases or in non-cardiac chest pain.

In our series, gastro-oesophageal reflux disease was proved in 37 percent of patients with epicardial coronary artery disease, and in 55 percent in those with non-cardiac chest pain. These results are in concordance with the previous observations. There were no difference in the prevalence of typical reflux symptoms, such as heartburn and acid regurgitation in the different patient groups. The majority (56%, 28/55) of the patients had no such symptom at all, only chest pain. Forty-three percent (12/28) of them had significant gastro-oesophageal acid reflux by 24-hour intraoesophageal pH monitoring. This may suggest that the detailed gastro-

enterological diagnostic workup is useful in patients with unexplained chest pain even in the absence of typical symptoms of gastro-oesophageal reflux disease.

Intraoesophageal acid perfusion evoked the oesophago-cardiac reflex and caused subsequent coronary flow velocity reduction in 49% (25/51) of the studied patients. We found similar prevalence of this reflex in patients with different types of epicardial coronary artery stenosis, microvascular disease (CFR ≤ 2.5), and in cases with non-cardiac chest pain. This suggests, that the presence of this reflex is independent of the underlying organic coronary artery disease. These results correspond to our previous observation that oesophageal acid perfusion has failed to change the baroreflex function non-selected patients with GORD. **[VIII]** By contrast, patients with proven coronary spasm were significantly more liable to acid perfusion induced coronary flow reduction and chest pain. This may reflect a sympathetic autonomic hyperreactivity in this subset of patients.

Since the oesophago-cardiac reflex is suspected of being a primary pathogenetic factor in the development of linked-angina, anatomical and physiological studies have been carried out to characterise its main features. Morphological studies in rats reported that the heart receives collateral projections from the nucleus ambiguus neurons. These innervate the oesophagus **[17]** supporting thereby the previous physiological observation that a mechanical stimulation triggered oesophago-cardiac reflex circuit involves vagal afferents and efferent sympathetic preganglionic pathways. **[65]**

Evidence has been provided also in humans that both electrical and mechanical stimulation of the oesophagus appears to amplify respiratory-driven cardiac vagoafferent modulation, while decreasing sympathetic modulation. **[4, 101]** This shift of sympathovagal balance towards parasympathetic component might lead to diminished myocardial perfusion.
Studies on chemically induced nociceptive oesophago-cardiac signals by direct coronary flow measurements during coronary angiography showed that the coronary flow could be decreased in patients with preserved innervation of the heart, but not in patients with a denervated heart. **[14, 15, 16, 17, 102]** Furthermore, Mellow et al were able to induce myocardial ischaemia by prolonged oesophageal acid stimulation. **[72]**

In the present work, we combined transoesophageal Doppler echocardiographic coronary flow measurement, with the oesophageal acid perfusion test in order to obtain data on the prevalence of oesophago-cardiac reflex in our patients. The rationale behind the application of this method was the fact that, during the past decade, transoesophageal Doppler echocardiography has become an important and widely accepted tool for the establishment of proximal coronary flow disturbances. This technique permits the prediction of significant proximal coronary artery stenoses. Moreover, its combination with the dipyridamole stress test

has made it possible to detect functional coronary abnormalities such as an impaired CFR in patients with microvascular angina. **[75]**

As compared with the results of Chauchan et al. **[13, 14, 15, 16]**, our method is less invasive, but similarly effective as the direct intracoronarial flow measurement technique for the evaluation of oesophago-cardiac reflex. Since previous studies and observations had demonstrated that exposure of the oesophagus to acid may eventually lead to cardiac dysrhythmias **[78]** or significant ST segment alterations **[72, IX, X]** the ECG was monitored continuously throughout the study. Regarding the safety of the procedure, we did not observe any significant complication, including critical myocardial ischaemia, infarction or dysrhythmia.

The first studies of combined 24-hour ECG and intraoesophageal pH recordings failed to prove direct correlation between gastro-oesophageal acid reflux episodes and events of myocardial ischaemia, but both of them found that gastro-oesophageal reflux disease commonly coexisting in patients with coronary artery disease. **[42, 93]** Recently, Dobrzycki et al. **[23]** detected pathologic acid reflux episodes at the time of myocardial ischaemia in approximately 20 percent of the cases. Moreover, they showed that proton pump inhibitor therapy is able to decrease significantly the number of ischaemic events. Our observation, that oesophago-cardiac reflex is present in approximately one third of the patients with epicardial coronary artery disease, supports these results.

The oesophago-cardiac reflex test was positive more often in patients with moderate forms of erosive oesophagitis (6/7 LA-B/C cases), than in patients with mild erosive forms (8/16 LA-A cases) or without erosions (11/28 cases). This appears to be in a good concordance with the observation of Fass et al., who demonstrated that mild to moderate chronic tissue injury in gastro-oesophageal reflux disease increases the sensitivity of chemosensitive neural pathways. **[28]** Additionally, we found that several pH monitoring parameters were significantly higher in patients with oesophago-cardiac reflex, than in those who had no change in coronary flow velocity during oesophageal acid perfusion test. This may mean that the endogenous trigger of this reflex – the regurgitating gastric acid – is frequently present in these patients, predisposing them for the induction of linked-angina.

Since the oesophago-cardiac reflex may be present either in patients with or without epicardial coronary artery disease the appropriate acid suppression therapy seems to be important to prevent reflux induced episodes of myocardial ischaemia in these patients. Although this hypothesis is supported by the observation of Dobrzycki – as proton pump inhibitor treatment reduced the number of ischaemic cardiac events in their patients **[23]** –

further prospective studies carried out in large patient groups are needed to prove the beneficial effect of acid suppressive therapy in patients with coronary artery disease.

Regarding the role of GORD in the pathogenesis of upper and lower respiratory tract diseases and symptoms a number of investigations to date have been carried out. Although the majority of these were done on selected populations of patients suffering from airway diseases, the results have remained heterogeneous. This may be related in part to the diversity of the methods applied in the diagnosis of GORD. In studies based on 24-hour oesophageal pH monitoring, Palombini et al. **[77]** detected a pathological GOR in 41%, while Gastal et al. **[32]** did so in 50% of patients with chronic cough. In asthmatic patients, Schnatz et al. **[90]** and Sontag et al. **[94]** reported a higher prevalence of GORD (78–80%), while Gastal et al. **[32]** observed a lower prevalence (44%). In a study based on endoscopy, Sontag et al. **[95]** detected reflux oesophagitis or Barrett's oesophagus in 39%, and hiatal hernia in 58% of asthmatic patients. By exploration of the clinical symptoms, Field et al. **[29]** found that 77 and 55% of asthmatic patients experience heartburn and acid regurgitation, respectively. Because of the diversity of the results, the diagnostic value of the different methods, including 24-hour pH-metry and endoscopy, requires further investigations as proposed in a recent review. **[36]** Over and above the methodological interest, the prevalence of airway complications of GORD should have an important medical impact, though this question has only rarely been examined. Our study has provided data on the prevalence of airway disease in association with GORD in the Hungarian population and assesses the diagnostic value of different clinical methods applied in the diagnosis of GORD. Examinations were performed on 299 patients, in whom the diagnosis of GORD was established by endoscopy and 24-hour pH-metry. Fifty-six patients were selected from among the 299 patients on the basis of clinical criteria, as having respiratory symptoms and airway diseases, all of which had been treated earlier by specific medication.

The patients were referred for consultation to our clinic by family doctors or specialists at other medical units. The prevalence of airway diseases among the GORD patients was found to be near to 20%, a relatively high level. El-Serag et al. **[25]** carried out similar investigations on the prevalence of airway diseases among a large population of United States military veterans: GORD was established by endoscopy and the prevalence of pulmonary diseases was found to be about 17%, while the recent ProGERD study reported a 13% prevalence of chronic cough and a 4.8% prevalence of asthma in GORD patients. **[48]**

Our observations provide complementary information concerning the diagnostic value of the different methods applied to reveal GOR in patients with GORD associated respiratory diseases. Earlier studies indicated that pathological reflux parameters (the DeMeester score and

the fraction time below pH 4 during 24 h) could be expected in 41–78% of patients with GORD-related pulmonary diseases. **[77, 90]** Our results confirm this: the De-Meester score was abnormal in 57% of our patients, with a predominance of nocturnal acid reflux (52%). However, in patients with a normal DeMeester score, a more detailed analysis of the pH curve provided important additional information: pathological reflux events were detected in 33% of these patients. In this group of patients with less severe GORD, the reflux events occurred predominantly in the postprandial period (82%). Moreover, in case of a pathological DeMeester score, we observed a significant difference in reflux activity between patients with and without respiratory complications. In case of respiratory complications nocturnal reflux was predominantly observed, while in patients without respiratory complaints nocturnal reflux occurred less frequently than diurnal and postprandial reflux. In case of a normal DeMeester score, postprandial reflux was predominantly detected in both groups of patients. This indicates that other than nocturnal acidic reflux may be responsible for the respiratory symptoms of these patients. Recently, studies provided data, that in patients with asthma oesophago-bronchial reflex may have a pathophysiological role. **[XIII]** On the other hand non-acidic or weakly acidic reflux may be important factors in patients with chronic cough. **[92]** Since standard 24-hour intraoesophageal pH monitoring can not detect these latter type of reflux events combined intraoesophageal pH and multi channel impedance monitoring or if this is not applicable video barium oesophagography has to be applied. **[V, XII]**

In patients with airway diseases, the diagnostic value of typical reflux complaints and of oesophageal endoscopy has been widely discussed. Field et al. **[29]** reported the prevalence and severity of GORD symptoms in asthmatics: 77% had heartburn and 55% had regurgitation. In his review Richter **[81]** concluded that classical reflux symptoms might be present in 40–60% of asthmatics and 43–75% of patients with ear, nose and/or throat complaints. Irwin et al. **[47]** found that 43–75% of patients with GORD-related cough were reflux symptom free. In our study heartburn and/or acid regurgitation were detected in 54 and 43% of patients with respiratory complications of GORD. This was validated by a parallel method: the symptoms were investigated at two different times, once by the physician and later by a nurse by carrying out a questionnaire survey before pH-metry. Our results underline the low value of a questionnaire based on the existence of the classical reflux symptoms of GORD-related airway diseases. Our endoscopic study revealed Savary-Miller stage I reflux oesophagitis in 40%, stage II in 29%, stage III in 9%, and stage IV in 11%, i.e. in 89% of the patients overall. Erosive or advanced forms of oesophagitis were detected in 49% of the patients. These results closely resemble those obtained by Sontag et al. **[95]**, who described oesophageal erosions and ulcerations in 39%, and Barrett's oesophagus in 13% of asthmatic patients. In our study, the

distribution of the endoscopic stages of reflux oesophagitis did not show any difference between patients with or without respiratory complications. In addition our data overall confirm the relatively low diagnostic value of endoscopy in the diagnosis of GORD-associated airway diseases.

Among disorders, responsible for secondary oesophageal motor abnormalities diabetes mellitus is the most common. Recent studies indicated that gastrointestinal manifestation of diabetes mellitus is more frequent, than previously proposed. **[9]** The pathogenesis of these motility disorders is partly understood. Autonomic neuropathy, impaired enteric nervous system, and hyperglycemia are suspected factors. **[40, 55, 79, 88, 97]** Although autonomic neuropathy is thought to play an important role in the development of diabetic gastrointestinal motility disturbances, few studies have been performed on selected patients with diabetic autonomic neuropathy**. [55, 88, 97]** Moreover none of them evaluated different regions of the gastrointestinal tract in parallel. In our patients, the cardiovascular autonomic neuropathy tests proved the presence of significant parasympathetic and sympathetic autonomic dysfunctions.

The presence of gastrointestinal symptoms may have a significant impact on the quality of life of patients with diabetes mellitus. **[99]** On the other hand, many patients do not report such symptoms, and only objective measurements are able to reveal the silent gastrointestinal motor abnormalities in these cases. As gastrointestinal motility is known to play a role in the regulation of the carbohydrate homeostasis, its preservation is an important factor in the management of the disease.

The recent development of gastrointestinal motility measurements allows a detailed description of diabetic gastrointestinal motility disturbances. Although clinical and experimental data support that motility disorders may arise at different regions of the gastrointestinal tract in patients with diabetes mellitus, their coexistence in the same patient is still poorly studied.

Of the symptoms reflecting pharyngo-oesophageal motility disorders, dysphagia is not rare in patients with diabetes mellitus. Its reported prevalence in the literature is around 25%. **[44]** This is slightly less than ours, since 44% subjects reported at least the occasional (≥1/month) presence of dysphagia. As motor disorders of the UOS and the PHX were rare and not significant in this study the mechanism is unlikely to be explained by defective UOS-PHX motility. On the other hand, these results suggest that the development of autonomic neuropathy has only a minor, if any effect on the motility in this region.

In contrast with the preserved UOS-PHX function, oesophageal body motor abnormalities were found frequently in our patients. Alterations in the peristaltic wave

amplitudes 50% and duration 44% were common. These abnormalities were associated with a decreased peristaltic velocity 69%, and increased numbers of simultaneous contractions 56%, which are known to facilitate the development of an impaired bolus transit. Since the scintigraphic data revealed that the prolonged oesophageal transit is associated with swallowing complaints in patients with diabetes mellitus and autonomic neuropathy **[50]**, this supports our finding, as the impaired oesophageal contractile activity has a major role in the development of the swallowing complaints of these patients. Since the vagal nerve is known to exert an appreciable excitatory effect in the oesophageal body motility, the observed motility changes may reflect its impairment.

The motor abnormalities of the LOS were predominantly characterized as a decreased mean pressure and prolonged relaxation. This may explain the frequent episodes of gastro-oesophageal reflux in patients with diabetes mellitus, **[63]** but other mechanisms such as transient relaxations of the lower oesophageal sphincter due to the impaired gastric emptying and increased gastric fundus distension, also play a pathophysiological role. **[31]** In our series, 62% of the patients complained of reflux symptoms with a moderate severity score. However, the overall results of the LOS manometry demonstrated a borderline range mean pressure, and a mildly prolonged relaxation on average; a decreased LOS pressure and prolonged relaxation were observed in 62% of the patients. This impaired LOS function may reflect the diminished cholinergic excitatory mechanisms, and also the abnormalities of the NO-mediated pathways.

Since not only oesophageal, but other – gastric, enteric, gallbladder, ano-rectal – motility disorders may also develop in patients with diabetes mellitus we performed the manometric evaluation of gastric and ano-rectal motility in our patients. Gastric motility abnormalities and gastroparesis are severe gastrointestinal complications of diabetic autonomic neuropathy, and a number of studies (e.g. radioisotope and ultrasound emptying measurements, different techniques with manometry or a barostat or electrogastrography) have been performed to understand its representative features. **[57]**

The fasting state gastric motility has been reported to be frequently pathologic. In contrast with the rather mild dyspepsia scores, pathologic MMC patterns were observed frequently (70%) in our patients, including 6 cases with complete gastroparesis. This difference confirms the hypothesis that the prevalence and severity of the symptoms reflecting gastric dysmotility, such as fullness, early satiety, nausea or bloating, are less pronounced than gastric motor disturbances demonstrated by manometry. The lack of correlation between fasting motility abnormalities and dyspeptic symptoms can be explained by two ways. Since dyspeptic symptoms are rather postprandial in nature, it needs further evaluation to see whether they have a better correlation with postprandial gastric motility patterns. On the other

hand, the impaired visceral sensory function or central perception resulted by the diabetic neuropathy can also be responsible for these findings. **[105]**

Since vagotomy inhibits the MMC activity of the gastric smooth muscle **[69]**, the frequent occurrence of diminished MMC phase II and phase III activity may reflect a vagal nerve dysfunction as a serious consequence of parasympathetic autonomic neuropathy.

In the recto-anal region, abnormalities of the sphincteric resting pressures were infrequent. The occurrence of decreased squeezing capacity was more common, but the differences between the diabetic and the control group means were not quite significant. The studied reflex mechanisms were rarely affected. On the other hand, the decreased EAS and IAS resting pressures were well associated with symptoms of diarrhoea and fecal incontinence. These data correspond to the previous observation that recto-anal dysmotility is more prevalent in cases complicated by diarrhoea **[97]**, but a recto-anal dysfunction does not seem to be a primary marker for diabetic autonomic neuropathy.

In 81% of the patients, abnormal motility patterns were observed in more than one studied regions of the gastrointestinal tract. This underlines the role of autonomic neuropathy as a common mechanism in the pathogenesis of various motility disorders.

Among other systemic disorders causing oesophageal dysmotility we evaluated primary Sjögren's syndrome. Although swallowing difficulties are common in patients with primary Sjögren's syndrome, the first report on oesophageal abnormalities was published only in 1967 **[56]**. Since then, a number of studies have been carried out, but the results are still controversial. The high prevalence of dysphagia is explained as a consequence either of the lack of saliva or of oesophageal dysmotility **[3, 34, 56, 91, 103].** Less often, in approximately 10% of the cases, upper oesophageal webs have also been reported **[45, 56].** In contrast, oesophageal motility measurements failed to reveal any specific dysmotility patterns in Sjögren's syndrome **[3, 34, 56, 76].** Grande *et al*. found no manometric differences between patients with Sjögren's syndrome and controls, except for a decrease in the distal oesophageal peristaltic velocity for dry swallows in Sjögren's syndrome patients with dysphagia. Their other finding, that patients with Sjögren's syndrome have a significantly higher LOS pressure than controls, may be questionable, since their value (15.6 mmHg) was at the lower end of the internationally accepted normal range **[34, 80].** Anselmino *et al*. studied 27 patients and detected only an increased number of simultaneous contractions when severe dysphagia was present **[3].** In contrast, Palma *et al*. observed no significant differences in oesophageal body motility **[76].**

Our results are in accordance with the literature as regards the prevalence of dysphagia (70-90%) in primary Sjögren's syndrome. On the other hand, we observed an impaired oesophageal body peristaltic velocity in nearly half of the patients (44%), which appeared to be the predominant oesophageal motor abnormality. This alteration was coupled with a mild decrease in the LOS pressure and a prolongation of the LOS relaxation time, which has not been described previously. We must note that the absolute values of LOS pressure and relaxation time were on the borderline of the internationally accepted normal ranges **[80]** and the normal ranges in our laboratory. Additionally, other oesophageal motor abnormalities, such as a prolonged duration of peristaltic contractions and an increased rate of simultaneous contractions in the oesophageal body, were also found in our patients. Most of our patients had a markedly diminished salivary function, which was even more prominent in the group with an impaired oesophageal peristaltic velocity. Anselmino *et al*. **[3]** did not find a correlation between saliva production and oesophageal motor disorders. The difference from our results could be explained by the less markedly diminished saliva production in their patients, and/or by the fact that the various parameters in the oesophageal motility measurements were not compared individually with the saliva production. Similarly as in the series of other investigators, the manometric abnormalities of the oesophageal function did not correlate with the presence of the different systemic manifestations, the laboratory findings or the demographic parameters of our patients with primary Sjögren's syndrome. The questionnaire analysis revealed a higher liquid requirement for swallowing in patients with an impaired peristaltic velocity and saliva production. This suggests that both oesophageal motor abnormalities and a diminished salivary function are factors in the development of swallowing difficulties in primary Sjögren's syndrome. As there are data indicating a parasympathetic dysfunction in Sjögren's syndrome **[58],** its role in the development of an oesophageal motor disturbance and/or decreased saliva production can not be ruled out.

6. NEW RESULTS ESTABLISHED IN THE THESIS

1. The oesophago-cardiac reflex may be present in patients with patients with normal coronary arteries and in patients with different coronary artery diseases.
2. The oesophago-cardiac reflex was more frequently observed in patients with coronary spasm than in those with epicardial coronary artery stenosis or microvascular disease.
3. The combination of oesophageal acid perfusion test and transoesophageal Doppler echocardiographic coronary flow measurement seems to be a useful method for the detection of this reflex.
4. Patients with oesophago-cardiac reflex positivity had more, and longer gastrooesophageal acid reflux episodes and had higher oesophagitis scores (more severe erosive forms of oesophagitis).
5. In Hungarian patients with GORD we obtained epidemiological data on the prevalence of respiratory symptoms.
6. Patients with GORD and respiratory symptoms had significantly more often abnormal nocturnal reflux than those without respiratory symptoms. This difference was not observed in patients with normal DeMeester scores.
7. Motility disorders are frequently present simultaneously at different regions of the gastrointestinal tract in patients with type 1 diabetes mellitus.
8. In patients with type 1 diabetes mellitus dysphagia scores were associated with the decrease of peristaltic wave propagation velocity, the increase of peristaltic wave duration and the increase of the number of simultaneous contractions.
9. In patients with primary Sjögren’s syndrome a decreased oesophageal body peristaltic velocity was the most common manometric abnormality.
10. This showed an association with the impaired saliva production and with the higher liquid requirement for swallowing, but not with the laboratory parameters or the systemic manifestations of the disease.

7. SUMMARY

BACKGROUND:

Gastro-oesophageal reflux disease (GORD) is one of the most prevalent and though the most extensively studied disorder of the modern gastroenterology. By definition, GORD is a complex motility disorder of the upper gastrointestinal tract, which is characterized by the regurgitation of gastric/duodenal contents into the oesophagus, the mouth or to the respiratory tract and causing oesophageal or extraoesophageal symptoms or complications. Epidemiological studies made in Europe and in the United States confirmed that approximately 20 percent of the general population complains of its typical symptoms (heartburn, regurgitation) at least weekly, which has a significant impact on the quality of life of the patients. Recently, more attention is paid to the extraoesophageal manifestations of the disease which may result in upper or lower respiratory tract, cardiac, oral complications or in different sleep disorders. Since typical symptoms of GORD are often missing in such cases, the recognition of the extraoesophageal GORD is often difficult. The prevalence of these complications is still not known exactly, and data are incomplete on their pathogenesis. Furthermore, the value of the standard diagnostic tests of GORD is still unclear in such conditions.

Among the extraoesophageal symptoms chest pain has a special importance, since it could be caused by myocardial ischaemia as well. Although it was believed earlier that chest pain episodes of oesophageal and cardiac origin could be clearly distinguished, the time that has subsequently passed has revealed that the differential diagnosis of chest pain is often difficult. Since the oesophagus and the heart share their innervation, mechanical or chemical stimulation of the oesophagus may evoke coronary spasm and subsequent blood flow reduction, which may lead to myocardial ischemia and subsequent chest pain. This phenomenon has been designated as linked angina and involves the oesophago-cardiac reflex. Neither the prevalence, nor the pathophysiology of this complication is known. It is unclear whether it can be elicited on anatomically normal or abnormal (atherosclerotic) coronary arteries. Furthermore, this reflex is difficult to study since the only method for its evaluation is the invasive direct flow measurement during coronary angiography.

Regarding the respiratory symptoms associated to GORD there have been only a few studies carried out to establish the prevalence of respiratory complications in a non-selected population of GORD patients. The prevalence of pulmonary diagnoses among patients with oesophagitis has been estimated to be between 0.5 and 17% and bronchial asthma is reported by 9% of subjects with GORD symptoms. In the pathophysiology of respiratory complications

there are at least two major pathways involved. The first is microaspiration, which occurs when the regurgitated gastric acid reaches the pharynx and drops into the airways of patients. The second one involves a reflex mechanism, when acid triggers the sensory nerve endings of the distal oesophagus. Their role is still not fully established, and the diagnostic value of the standard tests of GORD should also be evaluated in such conditions.

Secondary motility disorders of the oesophagus are characterized by oesophageal hypomotility, and though they commonly show features of GORD. Diabetes mellitus is known to be the most common cause of secondary gastrointestinal motility disorders.

The increasing prevalence of the disease in the developed countries results rapidly growing health care expenses. Epidemiological studies have revealed that nearly 75% of patients with diabetes mellitus have gastrointestinal symptoms, independently of the type of the disease. These may include swallowing difficulties, reflux symptoms, dyspeptic symptoms, problems with bowel transit and defecation. Since these symptoms are often mild, the motility disorder can be hidden for a long time, which makes the treatment of the disease more difficult and may worsen the prognosis. Subsequently the establishment of the characteristics and the pathogenetical features of the motility disorders as well as their early recognition are important for the better management of the disease.

Autoimmune connective tissue disorders are rare causes of oesophageal motility disorders. On the contrary oesophageal involvement is frequently present in most of these diseases, and symptoms related to the oesophageal involvement may disturb the patient significantly. Though, the pathogenesis of these symptoms and their relation to other manifestations of the disease is an important question. Patients with primary Sjögren's syndrome are often (up to 80%) complain of difficulties in swallowing, but only a few studies have evaluated the oesophageal motor function in the disease. The relationship between oesophageal motility disorders and other systemic manifestations of the disease or the involvement of the salivary glands is still unclear.

AIMS:

(1) To evaluate the cardiac manifestations of GORD. The establishment of the prevalence the oesophago-cardiac reflex characterised by gastro-oesophageal reflux induced coronary flow decrease, and its possible consequence: the "linked-angina" in patients with angina-like chest pain. (2) To evaluate the relationship between the presence of oesophago-cardiac reflex to different coronary artery diseases and gastro-oesophageal reflux disease. (3) To develop a new diagnostic method – transoesophageal Doppler echocardiographic coronary flow velocity measurement combined with oesophageal acid perfusion test – for the

establishment of the oesophago-cardiac reflex. (4) To establish the prevalence of chronic respiratory symptoms and diseases in patients with GORD in Hungary. (5) To compare the results of upper gastrointestinal endoscopy, 24-hour oesophageal pH monitoring in patients with gastro-oesophageal reflux with and without respiratory manifestations. (6) To evaluate the association between gastrointestinal symptoms and motility disorders at different sites of the gastrointestinal tract, with special respect to the oesophagus in patients with type 1 diabetes mellitus. (7) To evaluate the characteristics of the oesophageal motility disorders in patients with type 1 diabetes mellitus. (8) To establish the oesophageal motility disorders in patients with primary Sjögren's syndrome, and to examine its relationship to the systemic and oesophageal symptoms of the disease.

PATIENTS AND METHODS:

For the establishment of oesophago-cardiac reflex in patients with angina-like chest pain 51 patients with chest pain were enrolled after detailed cardiologic evaluation including coronary angiography. The prevalence of gastro-oesophageal reflux disease was established by symptom analysis, upper gastrointestinal endoscopy, 24-hour oesophageal pH monitoring, and oesophageal manometry. The oesophago-cardiac reflex was established by oesophageal acid perfusion test (0.1 N HCl and 0.9% NaCl, 120–120 ml/10 min in a blinded manner) combined with transoesophageal Doppler echocardiographic coronary flow measurement in the left anterior descending artery.

For the evaluation of the chronic respiratory symptoms and diseases in patients with GORD 299 consecutive patients with GORD were referred for oesophageal function testing. The diagnosis of GORD was based on the results of upper gastrointestinal endoscopy and 24-hour pH monitoring, extended by oesophageal manometry and video barium oesophagography. For the evaluation of respiratory and reflux symptoms, the patients filled in a detailed questionnaire on patient history, medications, life habits and all symptoms of GORD including the oesophageal and the extra-oesophageal ones.

For the establishment of secondary gastrointestinal motility disorders 16 with long-standing type 1 diabetes mellitus and 29 age and sex matched healthy control subjects were studied to assess their oesophageal, gastric and ano-rectal motility disorders. Patients had moderate autonomic neuropathy. The gastrointestinal symptom scores were established by using the Talley dyspepsia questionnaire. The motor function of the digestive tract was tested in the oesophagus, in the stomach and in the ano-rectum by manometry.

For evaluation of oesophageal motor function in primary Sjögren's syndrome 25 consecutive patients who met the criteria of the European Community Study Group for

Sjögren's Syndrome and 42 control subjects were enrolled and submitted to oesophageal symptom analysis, sialometry and oesophageal manometry.

RESULTS:

Gastro-oesophageal reflux disease was established in 45% (23/51) of the patients with chest pain. Oesophageal acid perfusion decreased the coronary flow velocity in 49% (25/51) of the patients indicating the presence of oesophago-cardiac reflex. Oesophago-cardiac reflex was present more frequently in patients with coronary spasm, than in patients with either epicardial coronary artery disease or microvascular coronary disease . Patients with oesophago-cardiac reflex had higher DeMeester scores, increased number of reflux episodes, fraction time below pH 4, and prolonged acid reflux episodes.

In the second study, chronic respiratory symptoms or diseases were present in 18% (56/299) of the studied patients with GORD. Chronic cough was observed in 42/56 patients, while typical reflux symptoms such as heartburn and acid regurgitation were observed in 30/56 and 24/56 cases, respectively. The prevalence of airway diseases was chronic bronchitis 12/56, asthma 10/56, recurrent pneumonia 10/56, chronic sinusitis 7/56 and chronic laryngitis 1/56. In patients with respiratory complications pathologic acid reflux was established in 29/51 cases on the basis of the DeMeester score, while 17/51 had pathologic postprandial, nocturnal or diurnal reflux events. Upper gastrointestinal endoscopy revealed a normal oesophageal mucosa in 6/56, Savary-Miller stage I oesophagitis in 23/56, stage II in 15/56, stage III in 5/56 and stage IV in 6/56 patients.

In patients with long standing type 1 diabetes mellitus and autonomic neuropathy, manometric evaluation of the oesophagus did not reveal abnormalities in the region of the pharynx and the upper sphincter except slightly premature UOS relaxation. In contrast, they had decreased peristaltic wave amplitude, prolonged duration, decreased wave propagation velocity, and increased number of simultaneous contractions in the oesophageal body, and decreased lower oesophageal sphincter pressures with prolonged relaxation compared to the age and sex matched controls. Symptom analysis showed correlation between reflux symptoms and LOS relaxation times, and between dyphagia scores and oesophageal body peristaltic wave duration, propagation velocity and the rate of simultaneous contractions. In the gastric antrum, frequent, and often severe fasting motility disorders were observed, which had no correlation with dyspeptic symptoms. In the ano-rectal region patients with diabetes mellitus had lower squeezing-resting pressure difference in the IAS, and impaired fecal expulsive function. Motility disorders were simultaneously present at multiple parts of the gastrointestinal tract in the vast majority of the cases (13/16).

Patients with primary Sjögren's syndrome had decreased pressure and prolonged relaxation of the lower oesophageal sphincter. In the oesophageal body decreased peristaltic velocity (≤2.7cm/s) was the dominant motility disorder. Apart of that increased duration of contractions and higher occurrence of simultaneous waves were detected. Patients with primary Sjögren's syndrome and decreased propagation velocity of the peristaltic waves in the oesophageal body had significantly lower LOS pressures and prolonged LOS relaxation time than those with normal oesophageal body peristaltic velocity. Furthermore they had higher rate of simultaneous contractions in the oesophageal body. Of the clinical parameters, the decreased oesophageal body peristaltic velocity was associated with a smaller whole saliva production either at basal state or after stimulation, and such patients needed significantly higher liquid requirement for swallowing than those who had normal peristaltic velocities.

CONCLUSIONS:

Gastro-oesophageal reflux disease is frequently established in patients with either epicardial or microvascular coronary artery disease or with coronary spasm. The oesophago-cardiac reflex was more frequently observed in patients with coronary spasm. The combination of oesophageal acid perfusion test and transoesophageal Doppler echocardiographic coronary flow measurement seems to be a useful method for the detection of this reflex. Patients with prolonged gastro-oesophageal acid reflux episodes, erosive oesophagitis and coronary spasm may be at higher risk for the development of linked-angina.

The prevalence of respiratory symptoms and diseases was 19% in patients with GORD. More than half of the patients with GORD and respiratory symptoms had abnormal 24-hour pH monitoring scores, predominantly due to night time reflux. The large number of patients with normal 24-hour pH scores indicates, that short GOR episodes may also be able to induce respiratory complications. The prevalence of erosive oesophagitis was similar in patients with GORD regardless of the presence or absence of respiratory symptoms.

In patients with type 1 diabetes mellitus and autonomic neuropathy gastrointestinal motility disorders were observed frequently, and in most of the cases simultaneously. While oesophageal and ano-rectal symptoms correlated better with the manometric abnormalities, the lack of correlation between the impaired fasting gastric motility and dyspeptic symptoms shows, that on the basis of the clinical symptom analysis the prevalence of such motor disorders could be underestimated. The early recognition of gastointestinal motility disorders may be important for the better long-term management of patients with type 1 diabetes mellitus.

In advanced cases of primary Sjögren's syndrome several and often significant oesophageal motility abnormalities can be found, of which the decreased peristaltic wave velocity in the oesophageal body showed an association with the impaired whole saliva production. The other manifestations of SS were independent from the presence of oesophageal dysmotility.

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10. ANNEXES

ANNEX I.