EVALUATION OF NEW DIAGNOSTIC AND TREATMENT STRATEGIES IN THE MANAGEMENT OF THE ZOLLINGER-ELLISON SYNDROME

By

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Thesis Presented for the Degree of DOCTOR OF PHILOSOPHY

In the Department of SURGERY
UNIVERSITY OF CAPE TOWN
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The following papers and abstracts are related to the data presented in this thesis:


Radebold K, Adams BK, Bornman PC, Marks IN, Modlin IM, Terblanche J. Preliminary results of the Octreoscan in patients with Zollinger-Ellison syndrome. SAMJ 997;87:707 [Abstract]

Bornman PC, Radebold K, Krige JEJ, Van Wyk MEC, Essel H, Marks IN, Terblanche J. Changes in the diagnosis and treatment of Zollinger-Ellison syndrome at Groote Schuur Hospital over the last 17 years. SAMJ 1997;87:708 [Abstract]


Abbreviations used in the thesis:

APUD amine precursor uptake and decarboxylation
AO acid output
BAO basal acid output
CgA chromogranin A
CT computerized tomography
ECL enterochromaffin-like cells
EUS endoscopic ultrasonography
F female
GRP gastrin releasing hormone
GSH Groote Schuur Hospital
H2RA Histamine receptor 2 antagonists
LM liver metastases
M male
MAO maximal acid output
MEN-I multiple endocrine neoplasia type I
MRI magnetic resonance imaging
NIH National Institutes of Health
PP pancreatic polypeptide
PVS portal venous sampling
SMA superior mesenteric artery
SRS somatostatin receptor scintigraphy
SSTR somatostatin receptor
US abdominal ultrasonography
ZES Zollinger-Ellison syndrome
yrs years

Reference of Normal Values used at Groote Schuur Hospital

Serum gastrin: less than 115 pg/ml
Secretin stimulation: gastrin increase of less than 200 pg/ml
Basal acid output: men <10.5 mEq/h,
                  women <5.6 mEq/h
Maximal acid output: men <48 mEq/h
                     women <30 mEq/h
ABSTRACT

Despite major advances over the last two decades with the management of patients with Zollinger-Ellison syndrome (ZES), unanswered questions remain concerning the natural history of the disease, the impact of new diagnostics modalities on cure rates, and the role of surgery in the era of modern medical therapies.

The aims of the thesis are to (i) analyse the database of 40 ZES patients treated at Groote Schuur Hospital (GSH) over two decades, with special reference to the impact of various surgical strategies on outcome, (ii) investigate a possible correlation between biological tissue marker expression and tumour behaviour, (iii) study the effect of somatostatin receptor scintigraphy (SRS) on surgical cure rates, and (iv) assess the efficacy and safety of a new proton pump inhibitor (pantoprazole) in controlling acid hypersecretion.

GSH experience

Methods. The database consisted of 40 ZES patients managed at the GIT Clinic during the 20-year period 1978 to 1998. The diagnosis was based on acid studies, serum gastrin and secretin provocative tests. Tumour location was attempted by endoscopy, ultrasound, computerized tomography, angiography (in selected cases), and more recently SRS. Medical treatment consisted of H2RA until 1990 and thereafter of proton pump inhibitors.

Results. Follow-up data was obtained in 35 of 40 patients with a mean of 8.5 years (range 1-18 yrs). 9 (43%) of the 21 patients who underwent formal debulking procedures were cured. Patients with solitary lymph node gastrinoma (n=6) constituted the largest group and cure was achieved in 4. All 6 pancreatic gastrinomas were associated with lymph node metastases, with additional liver metastases in 3; only one of these patients was cured. Three of 8 patients with duodenal/gastric wall tumours were disease-free after debulking.

Of the 10 deaths only one patient succumbed from metastatic disease to the liver, associated with a large pancreatic primary. 6 of the 7 patients with liver metastases are
alive (mean follow-up 7.7 yrs), three with bilateral liver lobe metastases 10 and 14 years after diagnosis.

**Conclusion.** The survival of ZES patients treated at GSH was favourable, including those patients with liver metastases. A policy of tumour debulking yielded a cure rate comparable to other studies and was most successful in patients with extrapancreatic extraintestinal disease.

**Biological tumour markers**

**Methods.** Tissue samples of 24 gastrinomas from 19 patients were investigated for tumour marker expression (p53, bcl2, Mib1, Waf1, PCNA, and CD44). These were correlated with tumour location, size, metastatic behaviour and cure rate.

**Results.** There was no difference in tumour marker expression with respect to the above parameters nor were there differences in marker expression between the cured and non-cured groups. The only exception was CD44, which showed stronger staining in patients who were not cured.

**Conclusion.** Tumour markers provided no additional information with respect to tumour behaviour and predicting outcome after surgery.

**Somatostatin receptor scintigraphy (SRS)**

**Methods.** SRS was investigated in 12 patients, 7 of whom had surgical debulking operations.

**Results.** SRS was positive in 11 patients compared to only one with conventional imaging. SRS failed to image gastrinomas smaller than 6 mm in size, and 3 additional tumours were detected (2 during surgery and one at autopsy). Three patients were cured after surgery but one developed recurrence after 2 years. The cure rate was no better when compared to the pre-SRS era when 9 of 21 patients were disease-free after debulking operations.
Conclusion. While SRS is far superior to conventional imaging in detecting gastrinoma, this advantage did not translate into an improved surgical cure rate mainly because of the inability to detect small tumours.

Long-term PPI therapy (pantoprazole)

Methods. 10 ZES patients were included over a 2-year period. After a 14 day wash-out phase, the dose requirement of pantoprazole was determined by repeated measurements of basal acid output aimed at keeping BAO below 10meq/L. Gastric biopsies were taken at frequent intervals to assess changes in ECL status.

Results. A standard dose of 40 mg was required to achieve a safe BAO output in 6 patients. One patient required 80 mg and three 160 mg daily. BAO output in the latter 3 patients was > 30 mEq/L. One patient developed endoscopic signs of peptic ulcer disease during the trial despite a BAO of less than 5 mEq/h, and another required an upward dose adjustment from 80 to 160 mg pantoprazole. Enterochromaffin-like cell (ECL) hyperplasia was not observed in this study.

Conclusion:
The safety and efficacy of pantoprazole was demonstrated for the first time in patients with ZES. While the majority of patients were controlled with a standard dosage, patients with a BAO of > 30 mEq/L required quadrupled dosage to achieve a safe reduction of BAO below 10 mEq/L.

Overall conclusions

The GSH data on which this thesis was based provided a unique opportunity to investigate various important aspects of the management of ZES patients in the setting of a third world country. The study confirmed the important role of surgery as the only means of achieving a cure and that this is best achieved by formal debulking operations on extrapancreatic extraintestinal tumours situated in the gastrinoma triangle. The
importance of biological markers as a predictor of aggressive tumour behaviour has been questioned, as was the role of SRS to improve the surgical cure rate.

The study confirmed the overall good prognosis of the ZES including those patients with proven liver metastases. The safety and efficacy of long-term medical therapy with a PPI has been confirmed and will remain the mainstay of treatment in patients not cured by surgery and in those with the MEN I syndrome.
Chapter 1
Introduction and literature review

1.1 Introduction

In 1955, Zollinger and Ellison first described a syndrome characterized by fulminant peptic ulcer disease and marked hypersecretion of acid that was associated with a non-β-cell tumour of the pancreas. Numerous publications have followed since this first report, describing the clinical spectrum, pathological location and treatment of Zollinger-Ellison syndrome (ZES).

One of the major limiting factors in improving the surgical cure rate, which has remained unchanged at about 30%, is the failure to accurately detect all gastrinoma tumours. Conventional imaging studies such as computerized tomography and ultrasound are successful in only 30 to 50% of cases, but recent reports on somatostatin receptor scintigraphy (SRS) have claimed substantially better results, purported to localize tumours in 50 to 90% of cases. Only a few investigators have assessed the impact of SRS on the actual surgical cure rate.

Determining the optimal surgical strategy remains one of the most difficult aspects of the management of the ZES. In the majority of cases, total gastrectomy can be avoided with the availability today of potent acid suppressing agents. The focus of surgery has moved to tumour debulking, but even with this approach, the majority of patients are not cured. The effective management of these patients is particularly problematic in the context of Third World countries where lack of compliance, often for geographic reasons, and limited resources may hamper optimum management.

With the current limitations in surgical cure rate, maintenance acid suppression with a proton-pump inhibitor remains the cornerstone of ZES management. Concern has been expressed that life-long suppression of acid secretion at a level required to prevent ulcer complications may lead to ECL hyperplasia and carcinoid tumours.
1.2 AIMS OF THESIS

The purpose of the thesis was to utilize the GSH experience with 40 ZES patients as a basis for evaluating the evolving treatment strategies of this rare endocrine tumour. The emphasis of this thesis will be on:

1. The outcome of patients in terms of various surgical strategies in the context of healthcare in a Third World country.
2. Assessing the value of various biological markers with respect to tumour behaviour.
3. The accuracy of somatostatin receptor scintigraphy in localizing tumours, and the effect on surgical cure rate.
4. Determining the efficacy and long-term safety of pantoprazole with the emphasis on optimal therapeutic benefits.
5. Identifying aspects of the disease, which requires further research.

1.3 Literature review

1.3.1 1955 - The emergence of a clinical entity

On April 29, 1955, Robert M. Zollinger and Edwin H. Ellison presented their observations to the American Surgical Association in Philadelphia on two young women with fulminating peptic ulcer disease associated with jejunal ulceration and with non-insulin-producing tumours of the pancreas. They postulated that glucagon was the ulcerogenic humoral factor originating in pancreatic islet cells. This was the first time that a link between hormonal overproduction and gastric hypersecretion associated with severe ulcer diathesis had been proposed.

While gastric hypersecretion and fulminating peptic ulceration associated with an islet cell tumour suggested a humoral cause, the nature of this stimulus eluded investigators of that time. Insulin and glucagon were considered as candidates, but were soon discarded (Marks 1961). The initial failure to establish the nature of the hormone prompted some workers to question whether the syndrome existed as a clinical entity
An alternative hypothesis was that endocrine lesions and the hypertrophy of the gastric mucosa resulted from a genetic disorder, and that the marked gastric hypersecretion was simply due to an increase in the number of parietal cells, similar to what is seen in uncomplicated duodenal ulceration (Mackenzie 1959).

Eventually, Gregory (1960) extracted the polypeptide hormone gastrin first from pig stomachs in 1959, and a year later from human gastrinoma tissue. From these studies, he correctly identified gastrin as the humoral factor that mediates signalling between the endocrine tumour and parietal cells producing acid hypersecretion. The first case of a ZES patient at Groote Schuur Hospital was described by Marks (1961) in a 51-year-old Bantu woman. In this patient he managed to demonstrate a gastrin-like substance in the primary gastrinoma (a 4cm pancreatic tumour) and in the liver metastases.

The name Zollinger-Ellison syndrome was proposed by Dr. Ben Eiseman (1956) as an appropriate eponym one year after its first description by Zollinger and Ellison (Zollinger, Ellison 1955). The heightened awareness of ZES led to an immense accumulation of publications within a short time; six hundred cases had been reported in the world literature by 1968 (Zollinger, Tomkins 1968), and over a thousand by 1972 (Friesen 1972). Zollinger and Ellison are credited with first proposing the correlation between an islet cell tumour, its peptide product, and the resultant pathophysiology. Their hypothesis, which was later proven to be correct, provided a scientific basis for research in the field of gastrointestinal endocrinology.

Zollinger and Ellison, however, were not the first to describe patients with peptic ulcer disease, acid hypersecretion, and an islet cell tumour of the pancreas (Stabile 1997). Cases reported as early as in 1908 (Morse 1908) and 1927 (Wilder 1927) precluded definitive diagnosis of gastrinoma due to incomplete documentation. The study by Rabinovitch and Achs (1945) from the Jewish Hospital in Brooklyn, New York, provided evidence for the first clear description of a gastrinoma. They reported that a 37-year old female with a two-year history of epigastric pain and associated emesis had a gastric analysis showing “marked hyperchlorhydria” and a clinical diagnosis of “perforating duodenal ulcer”. Two weeks after a gastrojejunostomy, she had a radiological examination, went into shock and died. Post-mortem examination found a 2.3 cm tumour in the body of the pancreas, which, on histological evaluation, was shown to be a non-
beta islet cell tumour of the pancreas. At the time, the islet-cell tumour was not implicated as a cause of the fulminating ulcer disease in this patient.

1.3.2 Tumour biology

The primary distinctive feature of ZES is an elevation in serum gastrin levels, due to a gastrin-producing neuroendocrine tumour in either the pancreas, the duodenal or gastric wall, or in ectopic sites.

Gastrin. Two major forms of gastrin are secreted (G-34 and G-17), although smaller sizes exist. The predominant biologically active molecular form found in gastrinomas is G17. The common feature of all gastrins is an amitated tetrapeptide at the carboxyl terminus, which imparts full biological activity. The circulating half-life of gastrin is affected by the size of the various molecular forms. The full physiologic response is determined by the presence of the biologically active moiety and the time for receptor interaction.

Gastrin is present in the G cells of the gastric antrum and duodenum, but small amounts have also been found in the pituitary and certain fibers of the vagal nerve (Jensen 1991). The release of gastrin is triggered in response to aromatic amino acids or amines in the gastric lumen and also in response to cholinergic stimulation or gastrin releasing hormone (GRP) secreted from post-ganglionic fibers of the vagus nerve. Gastrin is also the major stimulatory peptide for the release of histamine from enterochromaffin-like (ECL) cells. The secretion of gastrin by tumours, however, is not governed by the normal acid-dependent feedback (Jensen 1991).

G cells. During embryonic development, G cells are derived from the neural crest and migrate via the neuroectoderm into the foregut before reaching their final destination in the intestinal tract. Histochemically, G cells are members of the APUD (Amine Precursor Uptake and Decarboxylation) system (Pearse 1975). These cells share the expression of various genes encoding certain markers and hormonal products. They have an early omnipotence with regard to hormone secretion. During migration, they lose this property in favour of a highly specific production of polypeptide hormones. Although not all aspects of the APUD cell theory are widely accepted, this classification of cell types
forms the basis of a useful unifying hypothesis for understanding syndromes including MEN (Pearse 1968; 1969).

**Origin of gastrinomas.** The exact cell of origin of gastrinomas remains unclear (Jensen, Gardner, 1993). Recent studies have suggested that duodenal and pancreatic gastrinomas may originate from distinct cell types, due to the apparent differences in their biology (Howard 1995). Howard proposed that, based on the different quantities of pancreatic polypeptide (PP) expressed by these tumours, duodenal gastrinomas might arise from the ventral pancreatic bud tissue and pancreatic body/tail gastrinomas from the dorsal bud tissue. PP was found in 80% of gastrinomas located to the right but not to the left of the superior mesenteric artery (Howard 1995).

**MEN I syndrome.** The observation that a number of ZES patients also suffer from other endocrine disturbances led to the description of multiple endocrine neoplasia type I (MEN I), first documented by Wermer (1954). MEN I is characterized by the occurrence of primary hyperparathyroidism in combination with pancreatic/duodenal endocrine and pituitary tumours. The association between MEN I and ZES is found in up to 26% of gastrinoma patients (Ruszniewski 1993). Tumours in MEN I often occur in multiple sites, while solitary gastrinomas are predominantly seen in the sporadic form of the disease (Donow 1991). It remains controversial whether gastrinoma patients with MEN I have a better prognosis than those with the sporadic form of ZES (Jensen 1999).

### 1.3.3 Pathological anatomy

Over the last four decades pathological observations have drawn attention to the location of gastrinomas outside the pancreas, namely in the duodenum, extrapancreatic/extra-intestinal, and, controversially as primaries in lymph nodes.

Earlier reports gave a ratio of intra- to extrapancreatic gastrinoma localization as 4:1 (Ellison, Wilson, 1964). Intrapancreatic tumours are found predominantly in the head or tail of the pancreas. The head:body:tail ratio has been reported by Gerstein (1975) to be 4:1:4.

Extrapancreatic gastrinomas are mainly found in the duodenum, often in the form of microgastrinomas (Becker 1984; Delcore 1992). These tumours are distributed in a decreasing gradient proceeding distally, with 71% in the first portion of the duodenum,
21% in the second, 8% in the third, and 0% in the fourth (Thom 1991). More recently, duodenal gastrinomas have been reported to occur in up to 77% of patients with the sporadic form of the disease (Frucht 1988).

In 70 to 90% of cases, gastrinomas are found in the so called "gastrinoma triangle", which is bordered laterally by the duodenum, medially by the head and body of the pancreas, cranially by the extrahepatic bile ducts, and inferiorly by the duodeno-jejunal flexure (Stabile 1984, Passaro 1998). Of particular interest in one series, duodenal gastrinomas occurred in all patients with ZES as a manifestation of MEN I syndrome (Pipeleers-Marichal 1990). Gastrinomas have also been identified in the stomach and a number of other sites, including extrapancreatic, extraintestinal, in peripancreatic lymph nodes (without a demonstrable primary), in the liver, kidney, ovary, and the retroperitoneal space (Bollen 1981; Sawicki 1990).

It remains controversial whether gastrinomas can arise in lymph nodes or whether these represent metastases from occult primary tumours (Arnold 1994). The possibility of lymph node primaries is supported by studies reporting cure after resection of a solitary lymph node. In one study (Arnold 1994), cure was observed in 43% of patients with so-called lymph node primaries with a mean follow-up period of 5.3 years.

Gastrinomas metastasise primarily to lymph nodes and to the liver. In previous reports 50 to 60% had metastasised to the liver (Ellison 1969; Friesen 1972; Creutzfeldt 1975; (McCarthy 1980; Zollinger 1984). In more recent studies the incidence is reported to be about 30% probably due to the earlier detection of the disease (Jensen, Gardner 1993). The term "malignancy" in the context of gastrinoma has not yet been clearly defined, since metastatic spread in ZES is not necessarily associated with poor prognosis. Indeed, the prognosis does not appear to be affected by the presence of lymph nodes metastases (Jensen 1999). It has been suggested by some (Hirschowitz 1995) that tumour biology and growth patterns are the most important factors, which determine the aggressiveness of the diseases.

1.3.4 Biological tissue markers

There is still a lack of accurate markers of malignant potential and prognosis of gastrinomas. Morphological criteria alone (Lee 1996; Jensen 1997) are unreliable but a
number of serum factors, including chromogranin A, pancreastatin, human chorionic
gonadotropin and forms of gastrin are currently being evaluated as potential prognostic
indicators. So far, the results have been inconclusive (Jensen, Gardner 1993), but
immunohistochemical indicators used for human cancers showed some promise (Bartek
1991; Hall 1995). Molecular tissue markers indicative of cell proliferation, cell adhesion
or cell death (apoptosis) have been investigated in a limited number of neuroendocrine
tumours. There is as yet no clear evidence whether these markers can be applied
specifically to gastrinomas (Solcia 1999).

1.3.5 Epidemiology

By far the most case reports on ZES come from the United States, where ZES is
estimated to occur with a yearly incidence of 0.1 to 3 cases per year per million people
(Hirschowitz 1997). Approximately 0.1% of patients diagnosed with duodenal ulcers
may suffer from ZES (Townsend 1985). It seems likely that many cases of ZES go
undiagnosed due to lack of screening. A variable incidence of ZES has been reported
from other countries: 0.5 patients per year per million people in Ireland, 1 to 3 patients
per year per million in Sweden, and 1.2 patient per year per million in Denmark
(Jacobson 1986). Official data reflecting the incidence in South Africa are not available.
In most series, ZES is slightly more prevalent in males with a male: female ratio of 3:2
(Ellison 1964; Friesen 1972). The tumour typically manifests itself between the third and
fifth decade of life, but there is no clear age prevalence (Zollinger 1987). The youngest
patients described so far have been two girls aged five and six years (Abe 1979; Stadil

Among neuroendocrine tumours, gastrinomas were originally thought to be less
common than insulinomas; however, some studies have shown that gastrinomas occur
with equal or even greater frequency (Tjon 1989). Gastrinomas are at least 0.5 to 1.5
times more common than non-functioning pancreatic neuroendocrine tumours and
pancreatic polypeptide-producing tumours; two to four times more frequent than
VIPomas (a neuroendocrine tumour of the pancreas secreting vasoactive intestinal
polypeptide); and 8 to 15 times more common than either glucagenomas or
somatostatinomas (Jensen 1997).
1.3.6 Clinical presentation

The devastating clinical course of ZES that was common in the 1960’s and 1970’s has been greatly reduced by earlier diagnosis with serum gastrin and secretin stimulation tests (Jensen, Doppman 1993), and importantly, effective medical treatments. Since the mid seventies 95% percent of ZES patients experience ulcerations at some stage of their disease (Ellison 1956; Zollinger 1986), of whom 10% develop multiple ulcers (Hallenback 1968). These are found predominantly in the post-pyloric or post-bulbar duodenum, with erosive duodenitis extending down into the second part of the duodenum. In 25% of cases, ulcers develop in atypical locations, e.g. the oesophagus, the gastric fundus, or the papilla of Vater (Miller 1990, 1992; Quatrini 1989) and proximal jejunum.

In patients with gastrinomas and MEN-I syndrome, hypocalcaemia from associated primary hyperparathyroidism is the most common clinical abnormality, occurring in 95 to 98% of patients (Metz, Jensen 1994). Pancreatic endocrine tumours develop in 80-100% of MEN I cases, with functional variants in 50 to 80% of cases. In MEN-I patients, gastrinomas are the most common functional tumours (54%), followed by insulinomas at 21%, glucagenomas at 3% and VIPomas at 1% (Metz, Jensen, 1994).

During the same 20-year period that we observed 40 patients with ZES, only one patient was diagnosed with a VIPoma at GSH, described by Marks (1976).

Epidemiological data for ZES, associated with MEN I are not available, because of the relatively low frequency of this condition. (Jensen, Doppman 1986). The clinical presentation of the disease is dominated by the aggressive peptic ulcer diathesis and, for most of the course of the disease, by the slow growing nature of the tumour. However, even in the presence of a large tumour bulk including liver metastases, patients may remain remarkably asymptomatic (Jensen, Gardner, 1983).

Early on, peptic ulcer-induced pain is the most common symptom, frequently accompanied by gastro-oesophageal reflux symptoms, or complications such as bleeding (10%) or intestinal perforation (7%) (Waxman 1991). Diarrhoea, caused by acid hypersecretion, is the second most common clinical feature, occurring as the main presenting symptom in 9-20% of patients (Jensen, Gardner 1991).
1.3.7 Diagnostic tests

1.3.7.1 Gastric acid studies

The availability of a reliable gastrin radioimmunoassay as developed by McGuigan in 1968, has, to a large extent, superseded gastric acid studies in the diagnosis of ZES. Previously, the diagnosis of ZES depended on the clinical history and acid studies. The determination of basal acid output (BAO) was followed by maximal acid output (MAO) measurements under conditions of maximal histamine stimulation (Waddell 1959). A BAO/MAO ratio in excess of 60% was regarded as strongly suggestive for the diagnosis of ZES (Marks 1961). According to a study by Aoyagi and Summerskill (1966), a BAO equal or greater than 15 mEq/h is highly suggestive of the diagnosis. Basic acid output, however, may vary widely in ZES patients (Jensen, Gardner 1993) and overlaps considerably with that of idiopathic duodenal ulcer patients. Currently, a BAO greater than 31 mEq/h is considered to have a 99% specificity and a 50% sensitivity for ZES diagnosis (Mignon 1995).

After surgery however, diagnostic criteria are even more difficult to determine due to biliary and/or pancreatic reflux (Aoyagi 1966). In these cases, a BAO equal or greater than 5 mEq/h is considered to be suggestive of the diagnosis. The results of acid studies alone may be inconclusive when not considered in relation to serum gastrin levels, or vice versa (Modlin 1984). At present, BAO measurements are mainly used in patients where serum gastrin levels and provocative secretin testing are equivocal (Jensen 1997), or where other causes of hypergastrinemia (e.g. achlorhydria) need to be excluded. BAO is also used to monitor adequate acid reduction in patients while on maintenance PPI therapy.

1.3.7.2 Biochemical studies

The first reliable radioimmunoassay for gastrin was developed in 1968 (Hansky 1968; Yalow 1970). In 1972, Isenberg (1972) reported that an intravenous infusion of secretin paradoxically increased both gastric acid secretion and serum gastrin concentrations in patients with gastrinomas. This procedure has been developed as a
diagnostic test, although the exact mechanism by which secretin mediates these effects is unknown (McGuigan 1980).

For some time, the use of crude preparations of secretin, contaminated with either bombesin or gastrin, produced false-positive results (Lamers 1981). The more economical calcium infusion test has been shown to be equally effective as the secretin test, but is rarely used because of its clinical side effects due to hypocalcaemia (Deveney 1977).

While secretin provocative testing is currently the most discriminating test to distinguish ZES patients from those with duodenal ulcer with either basal acid hypersecretion or raised basal serum gastrin, it should be noted that false-negative rates have been reported in up to 15% of cases (Jensen 1997).

1.3.7.3 Imaging techniques

Reliable imaging of gastrinomas remains an unresolved issue even at a time where sophisticated diagnostic technologies are available. Overall, successful tumour location by imaging studies varies greatly from 10 to 65% of cases (Gibril, Reynolds 1996). Forty years ago, the pre-operative work-up for ZES patients consisted mainly of barium contrast studies to document the sequelae of ZES: findings were coarse rugae of mucosal folds and multiple duodenal ulcers in ectopic locations (Marks 1961).

1.3.7.3.1 Noninvasive techniques

Transabdominal ultrasonography (US) is generally unreliable in detecting the primary tumour. In a recent study of 80 ZES patients, US detected extrahepatic disease in only 9% of cases (Weber, Venzon 1995). US completely failed to detect primary tumors less than 1 cm in size and detected only 15% of lesions 1 to 3 cm in size. However, US proved to be equally effective to computerized tomography in detecting metastases to the liver (46% versus 42%, respectively). Gibril (1996) showed that CT has a slightly higher sensitivity for the detection of metastatic disease to the liver (38%) than extrahepatic disease (31%). The size of the lesion remains a limiting factor even when high resolution dynamic scanning is performed. Currently, tumours less than 1 cm cannot be detected, and only 30% of those ranging between 1-3 cm in size are successfully imaged.
Magnetic resonance imaging (MRI). In one series MRI and STIR (short-time inversion-recovery) detected extrahepatic disease in 30% of cases, and in 45% when hepatic disease was included (Gibril, Reynolds 1996). However, when comparing MRI to conventional studies, the specificity of MRI has been slightly lower because a number of small liver haemangiomas were interpreted as gastrinomas (Pisegna, Doppman 1993).

Somatostatin receptor scintigraphy (SRS). In 1987, Reubi (1987) demonstrated that somatostatin receptors are expressed on gastrinomas using in vitro techniques. Subsequently, $^{[123]}$I-labeled octreotide followed by $^{[111]}$In-labeled pentetreotide radionucleotide scanning has been successfully used to identify gastrinomas. The true sensitivity of SRS remains unclear. Whereas initial reports documented 100% yield (Krenning, Kwekkeboom 1993), a more recent publication showed a more modest detection rate of 53% (Kisker 1998).

1.3.7.3.2 Invasive techniques

Portal venous sampling, with gastrin estimations introduced by Ingemansson in 1977, was previously considered to be the localization procedure of choice with a varying positive yield of 46% to 90% (Ingemansson 1977; Galiber 1988; Meko 1994, 1995). This method, however, is difficult to perform and as such may be unreliable. In addition, the procedure carries an appreciable morbidity of 5% to 20% (Thom 1992; Norton 1994).

Angiography has been used with limited success because of difficulties in discriminating between the relative vascularity of the lesion and the surrounding tissue. Small tumours (less than 1 cm in size) or tumours that do not produce a distinct vascular blush are frequently missed by angiography. A recent investigation by the National Institutes of Health in 80 patients showed angiographic detection of gastrinoma in only 40% of cases (Weber, Venzon 1995).

Intra-arterial secretin stimulation with hepatic venous gastrin measurements has also been described (Imamura 1987). In a study of 36 consecutive patients, this method yielded a sensitivity rate of 89% (Thom 1992). However, because of its invasive nature, this modality is mainly used in cases where other imaging techniques remain inconclusive, or after previous failed surgery (Cohen 1997).

Endoscopic ultrasonography (EUS) has attracted considerable attention, because repeated investigations have confirmed its high sensitivity, particularly in the localization
of pancreatic neuroendocrine tumours (Roesch 1991). With high-frequency resolution, EUS has successfully identified structures as small as 2 to 3 mm in size and Roesch and co-workers (1992) have reported successful localization in 82% of cases. Unfortunately, contrary to what was expected, EUS is of limited value in the detection of small duodenal wall tumours. In one study (Ruszniewski 1995), EUS failed to detect 50% of these tumours. In addition, the high expense of the equipment and long learning curve with inevitable observer variation have limited its widespread application.

1.3.8 Past and current management strategies

1.3.8.1 Medical treatment

The evolution of the medical treatment in ZES with regard to acid suppression is characterized by two landmark developments: the introduction of histamine receptor 2 antagonists (H2RA) in 1974 and the advent of proton pump inhibitors (PPI) in 1980. Prior to the availability of potent medical acid suppression, ulcer therapy consisted of a diet of milk and cream, antacids, anticholinergics, supplemented by sedation or even narcotics for pain (Zollinger, Ellison 1955). This regimen only partially controlled symptoms, and did not prevent life threatening complications.

The introduction of H2RA revolutionized the treatment of gastrinomas (McCarthy 1978). Long-term use of these drugs, however, had disadvantages. A high number of patients required very large doses (up to 9 grams per day) to control acid secretion effectively. Others had to take the drug every 4 to 6 hours, or needed yearly dose increases (McCarthy 1977). The complication rate of maintenance treatment remained high; according to studies from the National Institutes of Health, up to 25% of patients developed complications (bleeding, perforation) due to poor control with H2RAs (McCarthy 1978).

The development of more potent, long-acting PPIs has greatly simplified the medical management of ZES (McArthur 1985). These agents are given once or twice daily and are effective in almost all ZES patients (Metz 1995). Omeprazole and lansoprazole are currently the agents of choice for the long-term management of acid hypersecretion (Maton, Gardner 1989). However, there are reports where dosages of 120mg and 360mg omeprazole did not provide adequate acid control (Lloyd-Davis 1988;
Frucht 1991). Individuals with ZES complicated by severe gastrooesophageal reflux or previous partial gastrectomies seem to fall into this category (Metz 1993).

Pantoprazole, a relatively new PPI is unique in that it targets the metabolic pathway of the liver cytochrome P450 system to metabolise drugs (Hanauer, Graf 1991; Kromer 1990; Steinijans 1994). Thus, pantoprazole has the theoretical advantage over omeprazole in that it exhibits fewer interactions with other drugs (e.g. phenytoin, warfarin, diazepam, and digoxin). While the efficacy of pantoprazole in peptic ulcer disease has already been proven (Rendsburg 1992; Rehner 1993), there is currently no data on its usefulness and safety in ZES patients.

1.3.8.2 Surgical procedures

Surgery for ZES has undergone major changes since Zöllinger initially described a series of operations performed on the first reported cases (Zollinger, Ellison 1955). Medical therapies and standard ulcer operations available at that time were ineffective in controlling acid hypersecretion, and soon total gastrectomy was adopted as the only effective surgical treatment to prevent life-threatening complications of the aggressive ulcer diathesis. Attempts to remove gastrointestinal tumours, which often included some form of pancreatectomy, were also abandoned in favour of total gastrectomy. In rare instances, total gastrectomy has resulted in a biochemical cure, presumably due to the inadvertent removal of a gastrinoma in the stomach or first part of the duodenum.

Total gastrectomy remained the treatment of choice until H2RA was introduced in 1974. As these drugs dramatically reduced acid levels and provided excellent relief of symptoms with prevention of ulcer recurrence and complications, a new era emerged of conservative treatment of ZES. At that time some leaders in the field suggested that surgery is no longer required other than for complications. This approach was supported by the poor surgical cure rates. The trend would probably have continued if it were not for the important work by Stabile and co-workers (1984) on the anatomical pathology of gastrinoma. These researchers first described the "gastrinoma triangle" where up to 80% of tumours are localized. In addition, they found that most tumours were located outside the pancreas, thereby permitting excision and debulking procedures without resorting to major resection procedures.
For a short period before the PPI era, vagotomy, either in the form of truncal vagotomy and pyloroplasty or highly selective vagotomy, was revisited. The aim of vagotomy was to augment acid reduction in those cases where insufficient acid reduction was achieved with H2RA, particularly in cases where no tumour or residual tumour was found at laparotomy. As pointed out by Richardson (1985), vagotomies reduced the mean BAO by 41% and thereby also reduced the dose of antisecretory drugs required by 38%. A similar observation was made by our group (Bornman 1987).

Vagotomy was soon abandoned with the introduction of PPIs, which in combination with tumour debulking opened a new era in the management of ZES. The concept of tumour debulking has been further supported by recent publications (Fraker 1994; Norton 1999) showing a possible reduction in the development of liver metastases.

1.3.8.3 Management of advanced disease

There is no clear evidence that chemotherapy, embolization, irradiation or biotherapy increase survival in patients with widespread metastatic disease. Most treatments have usually been reported in studies involving different types of neuroendocrine tumours. Furthermore, criteria for assessing anti-tumour responses often vary among different studies.

External radiation in neuroendocrine tumours is only effective to treat brain and bone metastases. Embolization of the feeding hepatic artery by using Spongostan or Gel foam resulted in a temporary response rate of 45%-70%, with a moderate impact on tumour reduction, amelioration of symptoms and reduced circulating hormone levels (Marlink 1990). Chemoembolization with cisplatin, mitomycin C, and 5-fluorouracil showed no long-term effect on survival (Hajarizadeh 1991).

Chemotherapy in the form of streptozotocin combined with 5-flourouracil generates a response rate of about 60% for the duration of 2 years and therefore remains the most important treatment for progressive unresectable disease.

Biotherapy comprises treatment with somatostatin analogues and interferon-alpha (Nold 1994; Arnold 1996). Somatostatin can exert antiproliferative effects on endocrine tumours by various mechanisms. At the usual dose of 100-150 microgram given two or three times a day somatostatin shows symptom relief in 35-50% of patients. Tumour
shrinkage was observed in 12% at experimentally higher doses of 3000-18,000 microgram per day (Arnold 1993, 1996; Saltz 1993).

Interferon-alpha in combination with somatostatin analogues shows a 50% biochemical response in various neuroendocrine tumours (Oeberg 1999), in 60% of patients symptomatic improvement, and a 12%-15% tumour reduction (Pisegna, Slimak 1993; Eriksson 1986, 1995). The effects of these therapies are currently subject to further investigations.

Gene therapy is conducted in phase I studies for various malignancies, but no trials have been initiated for neuroendocrine tumours. A lack of knowledge of the tumour biology prevents its use at this stage.

1.3.9 Postoperative outcome
1.3.9.1 Cure rates

The overall cure rate for resection of gastrinoma over the last 40 years with a variable follow-up between 1 - 40 years ranges from 5% to 82% (Ellison EC 1995). However, cure rates can only be determined by long-term follow-up since Norton and co-workers (1992) found that 50% of initial cures recurred within five years.

1.3.9.2 Survival rates

A study from the National Institutes of Health (NIH) (1995) demonstrated that the presence of liver metastases (LM) may be dependent on both the size and location of the gastrinoma (Weber 1995). In their study of 185 ZES patients, 54% of pancreatic gastrinomas had spread to the liver, versus 5% of duodenal tumours. Furthermore, tumours larger than 3 cm in size were associated with increased hepatic metastases. However, there were not enough large duodenal tumours and small pancreatic gastrinomas in their series to establish statistically whether size and the primary tumour location were independent determinants of metastatic potential to the liver (Weber 1995).

The presence of LM was found to be the most important determinant of survival. In patients without LM, the 10-year survival rate was 90% compared to 30% in those with LM (p<0.0001). The significance of liver metastases on clinical outcome remains controversial, as survival of 20 years and longer has been reported (Hirschowitz 1995).
1.4 Conclusions

Three issues remain unresolved in our understanding of gastrinomas. These include the natural history of the disease, the best diagnostic approach, and the most effective management.

It is not yet clear to what extent the natural history of the disease is determined by management or biological factors. In this thesis, the influence of management, which has changed over the two decades, is studied by analysing data of 40 ZES patients from a 20-year period collected at a single centre.

Biological markers are applied to gastrinoma specimens obtained during surgery at GSH and compared to outcome. If such markers prove to be of prognostic value, they could lead to an individual management approach according to the natural behaviour of each tumour.

In the field of diagnostics, somatostatin receptor scintigraphy (SRS) has been recently introduced and seems to be of advantage in localizing gastrinomas, since it detects small tumours generally not imaged by conventional imaging modalities. However, very little information is available with regard to its impact on surgery and post-surgical outcome, since few investigators have focused on whether the better detection of gastrinomas translates into an improved cure rate. Depending on the yield obtained by SRS, the importance of conventional imaging techniques within the diagnostic algorithm needs to be re-established.

Previously, medical treatment of ZES consisted of treatment with the relatively less potent H2RA for acid suppression. The current medical management is based on acid control with the PPIs omeprazole and lansoprazole. However, these medications are associated with potential disadvantages such as metabolic interference with other drugs and the need of repeated dose adjustments. A third PPI, pantoprazole, has recently been introduced. So far, pantoprazole was found to be highly effective in peptic ulcer disease, but has not yet been investigated in ZES. Thus, our ZES patients at the Gastrointestinal Clinic offer a unique setting to study prospectively the efficacy and safety of pantoprazole in ZES.
In summary, the goal of this thesis is to elaborate new aspects of the natural history of ZES, its diagnosis, and its management. With the new information derived from the GSH data enriched by results from prospective clinical studies, a new algorithm for the diagnosis and management of ZES will be proposed.
Chapter 2
Clinical data from Groote Schuur Hospital 1978-1998

The combined medico-surgical clinic at Groote Schuur Hospital serves as a region and national referral centre for patients with ZES. The management of these patients by a dedicated team of surgeons and gastroenterologists has provided a large clinical database from which treatment outcome and prognosis could be evaluated.

2.1 Methods
2.1.1 Patients

Between 1978 and 1998, 40 patients with ZES were managed at the Gastro-Intestinal Clinic at Groote Schuur Hospital (GSH), and their data were prospectively collected (see “Mastersheet of 40 ZES Patients”). Five of these patients (12.5%) had MEN I syndrome. There were 26 men and 14 women with a mean age of 33.8 years at the time of diagnosis, with six patients under the age of 16 when diagnosed with ZES (Table Clin1). Patients were classified as children under the age of 12, and adolescents when 13-16 years of age.

2.1.2 Diagnosis
2.1.2.1 Serum gastrin

Serum gastrin levels (normal range <115 pg/ml) were determined by radioimmunoessay (Sorin, Inc, France), and all patients underwent secretin provocative testing. Two units of secretin (Ferring, Malmo, Sweden) per kilogram body weight were injected intravenously, and serum gastrin levels were measured at -10, 0, 2, 5, 10, 15 and 30 minutes after injection. A serum gastrin increase of more than 200 pg/ml was considered to be diagnostic for ZES (Jensen 1983; Jensen 1991).
2.1.2.2 Measurement of gastric acid output

Basal acid output (BAO) was analysed in all patients (Mee 1983; Bornman 1987). Prior to the test, all acid-inhibitory agents were discontinued. When PPIs where taken, they were stopped 7 days before the test and replaced by high dose H2RA. H2RA therapy was discontinued 24 hours before BAO measurements, while hyperacidity was controlled by sucralfate (1 gram twice a day). After this wash-out phase, a nasogastric tube placed in the antrum and the stomach was emptied. Four consecutive samples of gastric fluid were collected from each patient. Quadruplicate aliquots of each sample were titrated to pH 7.0 with 0.2 N sodium hydroxide and the BAO determined with the Radiometer Copenhagen TTT 85 Titrator (ABU 80 Autoburette).

The diagnostic criteria for ZES included a BAO >15 mEq/h or >5 mEq/h in cases where there was a history of previous acid-reducing gastric surgery.

2.1.2.3 Endoscopy

All patients had upper gastrointestinal endoscopy to determine the sequelaee of increased acid production. A standard technique was used under sedation with 2.5 mg midazolam (Roche) using a forward viewing endoscope (GIF Q, Olympus Corporation). All mucosal changes were documented (See ‘Pantoprazole study’ chapter 5.1.2 for details of biopsies and histological evaluation).

2.1.3 Imaging studies for the localisation of gastrinomas

Abdominal ultrasonography was performed with a 5 mHz linear transducer and computerized tomography (CT) using either a Siemens Somatom DR or an Elscint Ecel 2400. Continuous scans of 10-mm thickness were obtained through the abdominal cavity from the dome of the liver to the pelvis. An oral contrast agent (gastrografin) was administered two hours prior to the CT. A bolus injection of 150 ml of intravenous contrast agent (omnipaque 300[lomheoxol] or ultravist 300 [lopramide]) was infused at a rate of 1 ml/sec for liver scans.

Selective angiography (Philips Diagnost ARC) was performed with Omnipaque 300 or Ultravist 300 contrasting agents to the splenic, superior mesenteric, gastroduodenal, and hepatic arteries. This test was selectively performed in cases where other imaging studies were negative or unequivocal. Endoscopic ultrasonography (EUS)
and magnetic resonance imaging (MRI) were not available at GSH at the time of the study. Portal venous sampling (PVS) was not performed, due to its invasive nature. SRS was performed since the beginning of 1996 (see Chapter 4).

2.1.4 Treatment

2.1.4.1 Medical treatment

Medical treatment of ZES evolved considerably during the course of the observation period of this study. Between 1984 and 1989 patients received HR2A, and PPIs since 1990. The dose of the acid-reducing medication was adjusted to a safety level of less than 10 mEq/h in patients without previous acid reducing surgery, and less than 5 mEq/h in those with.

2.1.4.2 Surgical treatment

A policy of formal tumour debulking was adopted at GSH since the mid eighties. The term “tumour debulking”, which is used throughout the thesis, means debulking of all suspicious macroscopic tumours. Patients underwent an explorative laparotomy once metastatic liver disease was excluded. A careful laparotomy was carried out with the emphasis on the liver, pancreas, stomach, duodenum, and ‘gastrinoma triangle’. The duodenum was mobilized (Kocher’s manoeuvre) and the pancreatic body dissected from the bed to allow a bimanual palpation. All suspicious lymph node groups along the upper and lower pancreatic border and the duodenum were resected for histological investigation.

The type of tumour resection was determined by the specific location of each tumour. For lesions within the duodenum, a full-thickness wall excision was performed. Lesions located in the pancreas were treated by enucleation or distal pancreatectomy. Tumours in the liver were removed by a wedge resection. Major resections, including a Whipple resection, were not performed.

During the H2RA era (1984-1989), some patients underwent an additional vagotomy (truncal or proximal gastric) in order to enhance the pharmacological suppression of acid production. A total gastrectomy was performed on cases where medical treatment was ineffective due to poor compliance or geographical factors.
2.1.5 Surgical outcome after tumour debulking

The term “cure” has been defined as normal serum gastrin levels (<115 pg/ml), and a negative secretin test (increase <200 pg/ml) at a given time. “Permanent cure” was only claimed when serum gastrin levels and the secretin test remained normal after 5 years. However, to better characterize outcome of GSH gastrinoma patients, several categories have been created. Surgical outcome was divided into 4 different categories: (1) no cure, with persistent abnormal serum gastrin levels and a positive secretin test; (2) temporary cure post-surgery with recurrence; (3) cured at time when lost for follow-up; (4) cured at last follow-up with [a] less than 5 years and [b] more than 5 years.

2.1.6 Pathological tumour classification

In this study gastrinomas were classified as (a) pancreatic primaries; (b) ‘ectopic’ or extrapancreatic/extraintestinal/extralymphatic; (c) lymph node gastrinomas without primaries, either solitary or multiple; (d) liver gastrinomas without primary, and (e) duodenal and gastric wall tumours.

Gastrinomas in lymph nodes with surrounding follicular tissue were considered to be ‘primary lymph node gastrinomas’ when no other primary tumour was identified.

Pancreatic gastrinomas were defined as those tumours, which were either located within the pancreatic tissue, or showed a rim of pancreatic tissue around the tumour. Tumours without evidence of pancreatic or lymphatic origin but located on the pancreatic surface were classified as ‘ectopic’ (extrapancreatic, extraintestinal, extralymphatic) gastrinomas.

2.2 Results

2.2.1 Patient characteristics

Table Clin1 shows the overall data of the 40 ZES patients treated at Groote Schuur Hospital between 1978 and 1998. The “Mastersheet of 40 patients treated at GSH 1978-1998” (see Appendix) provides information on each ZES patient.

The diagnosis of Zollinger Ellison Syndrome was mostly made at Groote Schuur Hospital. Concerning demographics, fifteen patients came from outside Cape Town and
the Western Cape region, which is the usual referral basis for patients treated at GSH. Fourteen of the 15 patients referred from outside the Western Cape area came from rural parts of the country. Nine of these 15 patients came from the Eastern Cape, which is a referral region for GSH via two satellite hospital.

The most important clinical symptoms at the time of diagnosis were pain in 33 (82%), diarrhoea in 4 (10%), and oesophagitis in 3 (7.5%). Half of patients had endoscopic signs of peptic ulcer disease at the time of diagnosis. Others presented with complications of PUD, such as perforated duodenal ulcer (20%) and upper gastrointestinal bleeding (18%). The remaining 12% had pain and/or diarrhoea without endoscopic evidence for PUD.

2.2.2 Validation of diagnostic tests

Serum gastrin levels (normal <115 pg/ml) at the time of diagnosis were elevated in all 40 patients (mean, 671.07 ± 636 pg/ml; range 122-3582 pg/ml). A moderately elevated level (115-500 pg/ml) was found in the majority of patients (21/40, 53%). In 10 cases (25%) it was 500-1000 pg/ml and in 9 (23%) gastrin levels were >1000 pg/ml.

Provocative testing with secretin was positive in 38/40 (95%). One patient with a histological proven tumour had an increase of only 50 pg/ml. The other case with MEN I had elevated basal/maximum acid outputs of 30/46 and 33/62.5 mEq/h, on two separate occasions.

Basal acid output was markedly elevated (>15 mEq/h) in all but 6 patients; 4 of the 6 had a partial gastrectomy prior to the ZES diagnosis, and a truncal vagotomy in the remaining 2.

2.2.3 Imaging

A total of 105 conventional imaging procedures including abdominal ultrasonography, computerized tomography, and angiography were performed in 31 patients who underwent gastrinoma resection (Table Clin2). The sensitivity of ultrasonography was 17%, of computed tomography 15%, and of angiography 27%. The sensitivity of all conventional studies combined was 18%.

The results of the recently introduced somatostatin receptor scintigraphy (SRS) are outlined in chapter 5.2.
2.2.4 Surgical therapy

2.2.4.1 Emergency procedures

14 emergency procedures were performed in 10 patients for complications of peptic ulcer disease, all before the diagnosis of ZES was established (Table Clin3). 10 of them were performed in the eighties, and the last one in 1995 (patient 38 of the "Mastersheet").

2.2.4.2 Tumour debulking and acid reducing procedures

Table Clin3 gives an overview of the surgical procedures performed in 36 patients, including emergency procedures and gastrinoma resections. 4 of the 40 patients had no surgery during the entire course of their disease: 2 (P23,32) had widespread metastatic disease derived from a pancreatic primary gastrinoma, one (P30) refused surgery, and the other (P21) had severe concomitant medical disease, which precluded surgery.

36 ZES patients underwent a total of 77 surgical procedures; 36 procedures (47%) were aimed at reducing gastric acid output, (18 before and 18 after the diagnosis of ZES). Gastrinoma were removed during 31 operations; however, 21 procedures were considered to be formal gastrinoma debulking operations (Table Clin6). In the remaining ones, gastrinoma were removed incidentally with the gastric specimens during total gastrectomies on four occasions before the policy of formal debulking was introduced at GSH.

18 of 36 surgical patients (50%) had a vagotomy during the course of the disease; in 11 before, and in 7 after the diagnosis of ZES (Clin4). The vagotomy was combined with an antrectomy in 7 patients; all had this procedure done before the diagnosis of ZES was established.

One patient (P14) who had a highly selective vagotomy after the diagnosis of ZES continued to have peptic ulcer disease despite high doses of H2RA. A truncal vagotomy was carried-out after a year. During a third operation, the removal of a retrogastric
gastrinoma lymph node resulted in a cure. However, the patient was lost for follow-up after two years.

Although post-vagotomy BAO was significantly lower (5.06 versus 28.6 mEq/h; p<0.003), 16 of the 18 patients (89%) developed further acid related complications after the vagotomy, which included recurrent duodenal ulcers, stomal ulcers and oesophagitis with stricture formation. In 7 patients total gastrectomy was ultimately performed for recurrent peptic ulceration due to poor acid control.

Partial gastrectomies were performed in 9 patients (P6,7,12,22,24, 28,35,36,37) exclusively before the ZES diagnosis was established. Continued uncontrolled acid related complications necessitated total gastrectomy in 3 (P6,24,28).

11 out of 40 patients (28%) had a total gastrectomy during the course of their disease (Table Clin3), one (P25) of them before the diagnosis was established. All gastrectomies except in P28 were performed before PPIs became available in 1990. The total gastrectomy performed in 1994 was necessary in a patient (P28) from a rural area who was cured after tumour debulking surgery but continued to have intractable oesophagitis despite intensive PPI therapy. His initial operation was a vagotomy and antrectomy.

Functional outcome after gastrectomy was based on conventional clinical assessment at various stages during follow-up. All patients reported some weight loss and minimal restrictions concerning food intake. However, serum albumin levels obtained during follow-up remained within normal limits.

A total of 21 tumour debulking operations (Table Clin 5) were performed. The majority of procedures were excisions of lymph nodes, either solitary (6) or multiple (2), resections of duodenal and gastric wall gastrinomas (7), 6 of whom were associated with regional lymph node metastases. Only one debulking procedure was performed on a pancreatic gastrinoma situated on the surface in the body region. Two debulking procedures were performed on extrapancratic/extraintestinal gastrinomas.

2.2.5 Results

Follow-up data are available in 35 (88%) of 40 patients, with a mean of 8.5 years;
range 0-18 years ("Mastersheet"). One patient was lost to follow-up immediately after surgery, and 4 after 4 years. In 14 patients follow-up is longer than 10 years, and in 9 more than 15 years.

Of the 9 patients who died during the study period only one death (P23) could be attributed to advanced metastatic disease. One death was alcohol related, one died postoperatively after septic complications, and another patient of homicide. In the remaining 6, the cause of death remains uncertain; 4 of these patients had a total gastrectomy. At the time of their gastrectomy there were no signs of widespread metastatic disease. Autopsies were not performed in these 6 patients.

2.2.5.1 Surgical results

When we exclude those 5 patients who had no formal debulking operation, the cure rate was 9 of 21 (43%) patients (Table Clin5). 8 patients were cured for more than 5 years, (5 with biochemical confirmation) whereas the remaining patient (p22) was lost to follow-up after 27 months (Table Clin6). Two additional patients (P4,8) had normal postoperative gastrin after surgery, but their disease recurred within 3 years.

4 of the 9 patients who were cured had solitary lymph node involvement by gastrinoma. The remaining 5 were extra-intestinal/extra pancreatic (1), duodenal/gastric wall with lymph nodes (3) and pancreas with lymph node, (1). According to anatomical sites the best results were obtained in patients with solitary lymph nodes where 4 out of 6 patients were cured (Table Clin5).

7 patients presented with liver metastases (Table Clin8), of whom 4 had gastrinoma resections from the liver (P10,12,15,16). None of these patients were cured. Only one patient (P23) died as a consequence of extensive metastatic liver disease. 3 with bilateral liver disease survive now for more than 10 years, one of them 14 years.

2.2.6 Anatomical distribution of gastrinoma at surgery

There were 6 patients (P1,10,22,23,32,33) with a pancreatic tumour of whom 2 (23,32) did not have surgery (Clin7). One patient (p33) who had previously removal of 2 synchronous duodenal wall tumours had a pancreatic tumour detected at autopsy. 3 of the
4 patients had metastatic disease to regional lymph nodes (P1,10,22) and to the liver (10). Only one patient (22) was cured.

Of the conservatively treated patients, one (P23) died three years after the diagnosis from extensive tumour bulk; the other (P32) is well after an 8-year follow-up. None of these two patients had chemotherapy or embolization of the hepatic artery.

'Ectopic' gastrinomas were observed twice (P26,29), both were located on the pancreas body outside the 'gastrinoma triangle'. One of these patients (P26) was cured after simple excision.

"Primary lymph node gastrinomas" were found in 11 patients, eight of them were solitary (Table Clin7). Two of these patients did not have formal debulking procedures and it is possible that a primary was missed. Two patients (P5,6) presented with tumours on the pancreatic surface and the pancreatic upper border that could easily be "shelled out". They contained a 'halo' of lymphoid tissue, and were therefore classified as "gastrinoma lymph nodes" since there was no evidence of a primary tumour.

The patient with resection of a gastric wall gastrinoma tumour (P18) with metastasis to a regional lymph node was cured at 1 year but lost to follow-up thereafter. 7 patients had duodenal wall gastrinomas (Figure 6C). As mentioned earlier, one MEN I patient (P33) had two synchronous duodenal wall lesions but a pancreatic tumour was discovered later at post-mortem. 6 of the duodenal wall gastrinomas were associated with regional lymph node metastases; and 2 were cured with debulking procedures.

Of the 57 gastrinomas resected ("Mastersheet of 40 ZES Patients"), 30 (52.6%) were located in the so-called 'gastrinoma triangle', and the remaining 27 on the left side of the pancreas (left to the superior mesenteric vein; n= 15), the lesser curve of the stomach (5), duodenojejunal flexure (1), and liver (6).

2.2.7 ZES associated with the MEN I syndrome

In 5 patients (12.5%) ZES was part of the MEN I syndrome (P1,6,20,25,33) (Table Clin11). All had documented hyperparathyroidism at some stage of their disease. A parathyroidectomy was carried out in 4 of them (except P20). All but one (P1)
presented with elevated prolactin levels at the time of MEN I diagnosis. However, only one (P25) showed a left-sided pituitary tumour with erosion of the sella turcica on CT scan. All patients with elevated prolactin levels were temporarily treated with bromocryptine.

Two MEN I patients (P20,25) refused a laparotomy with potential gastrinoma resection. Three (P1,6,33) had gastrinomas removed: 2 (P1,33) had pancreatic tumours, and one (P33) two duodenal wall tumours removed during the first operation (see below). In patient P6 a lymph node containing gastrinoma was incidentally removed with the gastrectomy specimen. No patient was cured after gastrinoma resection. In 3 (P1,6,25), a total gastrectomy was necessary to avoid recurrent complications of gastric acid hypersecretion.

One patient (P33) also had other neuroendocrine lesions. During the first operation, he had two duodenal wall gastrinomas and one insulinoma resected. Five years later, he underwent recurrent exploration for a suspected insulinoma but instead a somatostatinoma was removed next to the second part of the duodenum. Postoperatively, the patient developed a jejunal perforation, complicated by peritonitis, which culminated in multi-organ failure and death. A pancreatic tail gastrinoma was discovered at autopsy.

The mean follow-up in the 5 patients is 8.4 years (range 1-15 yrs). 2 patients died; P1 in 1992 from unknown cause, and P33 post-operatively after septic complications. One patient (P20) developed lung cancer and was lost to FU. The remaining 2 (P6,25) are well, although one (P25) is known to have liver metastases since 1986, while the other (P6) demonstrated two hot spots on SRS indicating possible liver metastases.

2.2.8 ZES in childhood and adolescence

Six patients (P6,8,15,16,18,31) were under the age of 16 years when the diagnosis was made (range 11-16 years). None of their parents had a history with ZES or MEN I. All patients in this group had at least one gastrinoma resection at some stage of their disease. 2 (P6,16) were subjected to a total gastrectomy in the eighties before the introduction of PPIs. Both had gastric outlet obstruction. Liver metastases were
histologically confirmed in two (P15,16) adolescents. One (P15) had one liver metastasis removed 6 years before he died from an unnatural cause (homicide). The second (P16) had two liver metastases removed in 1986; a CT scan in 1996 revealed additional metastases but he remained clinically well. A third patient (P6) with MEN I had a positive lymph node removed during the total gastrectomy in 1982. At follow-up in 1996, 2 hot spots on SRS were shown in the right liver lobe indicating possible liver metastases. His serum gastrin level was 296 pg/ml, and the secretin test was positive.

The mean follow-up in the 6 patients was 11.2 years (range 8-15 years). 3 (P6,18,31) had normal serum gastrin levels after gastrinoma resections; P6 had recurrence after 35 months, P18 was lost to follow-up one year after surgery, and patient (P31) was a long-term cure after resection of a solitary lymph node from the gastrohepatic ligament.
2.3 Discussion

2.3.1 Introduction

Only few reports, mostly from multicenter study groups such as the National Institutes of Health (NIH) in the United States and the Zollinger-Ellison Study Group in France, have been able to incorporate large numbers of patients. While these large studies have significantly improved our understanding of this rare and unique functional endocrine tumour, the heterogeneous nature of the patient data accrued from many participating centres has limited their value to some extent.

The 40 patients with ZES that form the basis of this thesis were managed and prospectively studied over a 20-year period by a dedicated team of medical and surgical gastroenterologists in the Gastrointestinal Clinic at Groote Schuur Hospital. The data reflects the evolving changes that occurred in the diagnostic and therapeutic approach to the disease during the study period. Importantly, the long-term follow-up permits a detailed analysis of the natural history of gastrinomas, the efficacy of various treatment strategies and the prognosis.

2.3.2 Epidemiology

This series constitutes one of the largest single centre experiences with the ZES. While the large numbers accrued in this series may be construed as representing a higher incidence when compared to other countries we believe that a more likely explanation is the tertiary referral pattern to our centre with a particular interest in the disease. While most ZES patients were diagnosed at GSH, 15 came from outside Cape Town and the Western Cape region. Fourteen of the 15 were referred from rural parts of the country, 9 of whom were from the Eastern Cape. Reliable epidemiological data are also not available to determine the incidence of ZES in South Africa.

Compared to other series (Table Clin9) our patients were on the average 10-15 years younger, had proportionately more males and there were fewer patients with the MEN 1 syndrome. Again, these differences may be explained on demographic variations as most reports are based on multicenter studies.
2.3.3 Surgical management

2.3.3.1 Intra-operative methods to localize gastrinoma

We investigated the role of intra-operative endoscopic trans-duodenal illumination prospectively in the 5 patients who had surgery after somatostatin receptor scintigraphy. No additional tumours were found in the duodenal wall in our series. Frucht and co-workers (1990) reported an 83% sensitivity of this method for duodenal gastrinomas although it remains unclear whether the duodenal gastrinomas were detected either before or after duodenotomy.

2.3.3.2 The role of vagotomy

Richardson (1979, 1985; McArthur, Richardson 1996) and co-workers were the first to introduce vagotomy to augment the acid suppression by H2RA. They showed in 1985 that the addition of a parietal cell vagotomy at the time of an exploratory laparotomy could result in a 75% decrease in the basal acid output. It should be noted, however, that tumour debulking was also carried out in 43% in their series, which makes it difficult to distinguish the effect of tumour debulking from that of vagotomy.

There are other investigators who continue to use vagotomy in selected cases. Jensen (1997) argues in favour of vagotomy when no tumour is found and when acid hypersecretion is poorly controlled by PPIs. Indeed, 35% of his patients on long-term PPIs became hypochlorhydric associated with vitamin B12 and iron deficiency (Dutta 1994). However, Kopp could not confirm a similar finding in his series (1992).

In our vagotomy series (Tables Clin3 and Clin4) 14 patients did not have gastrinomas resected but a significant drop in BAO (from a mean of 30.8 mEq/h before to 6.35 mEq/h after vagotomy; p<0.004) was achieved. A substantial decrease of BAO was also noted after tumour debulking without vagotomy (Tables Clin4 and SRS3).

The rationale for vagotomies must be seen in the context of an era when potent acid suppression was not available, where long-term H2RA showed substantial side effects, and when surgical cure was achieved in few patients. With the advent of PPIs vagotomy with the purpose to augment acid suppression is now no longer indicated and in our practice total gastrectomy is a better option in the rare cases where acid suppression is erratic with PPI therapy.
2.3.3.3 Partial gastrectomy

All 9 partial gastrectomies with or without vagotomy were carried out before the diagnosis of ZES was made. Our experience confirms the observation made by RM Zollinger in 1985 that this operation increases the risk of complications despite much improved medical therapy today. 4 of the 9 patients ultimately required a total gastrectomy for complications, 2 of whom for intractable oesophagitis.

2.3.3.4 Total gastrectomy

Total gastrectomy is now rarely performed in first world countries (Brennan 1985, Jensen, Gardner 1991). Most of our 11 patients had their total gastrectomies before PPIs became available. Nevertheless, life-long maintenance on PPIs poses major fiscal and compliance problems particularly in a population group such as ours who cannot afford expensive drug therapy and where patients often live in remote places. In these patients a total gastrectomy remains a viable, if not preferred, option.

Total gastrectomy unfortunately still carries a stigma of crippling sequelae, but this has been associated mostly in patients with gastric cancer (Cuschieri 1996). Malagelada (1983) reported side effects in 50% of cases that included oesophageal reflux, early satiety, cramping and anastomotic strictures in 17% of patients. However, our results mirror that of Thompson & Lewis (1983) and the experience by Wilson (1991) in children showing few side effects with excellent functional results in 7 of our 11 patients. The 4 remaining patients died of unrelated disease.

The patients who came from rural area often did not have reliable supplies of PPI's from clinics, hence the decision to do a total gastrectomy in some of them.

We conclude that a gastrectomy may be considered as a last resort in cases that do not respond adequately to acid inhibitory medication or in those who remain exposed to life threatening complications because of unreliable supply of proton pump inhibitors.

2.3.3.5 Is more aggressive surgery indicated in ZES?

Explorative laparotomy with local gastrinoma resection is currently recommended as the procedure of choice in patients without metastatic disease. Biochemical cure can be expected in 20-30% of patients (Mignon 1995). However, this approach offers two
benefits as outlined previously. First, reduction of the tumour burden decreases the risk of metastatic spread to the liver with the potential of improving survival (Fraker 1994; Ellison 1995), and secondly, it has the beneficial effect of significantly decreasing basal acid output, which facilitates medical acid control (see SRS chapter 4.2.4).

Whether more aggressive surgical approaches will increase the cure rate remains a point of debate. Oberhelman (1960) proposed the Whipple operation as early as 1960, only 5 years after the first description of ZES. At that time, it failed to gain widespread acceptance due to the procedure’s high morbidity and mortality. With the significant reduction of operative mortality (Trede 1990) pancreatoduodenectomy has been strongly advocated by some institutions, which report cure rates of 82% (Howard 1990) to 100% (Delcore, Friesen 1992) in a small number of patients. Nevertheless, with the benign nature of gastrinomas, the adoption of such a major procedure remains controversial unless a subgroup of patients can be identified who will clearly benefit from this approach.

2.3.4 Surgery in ZES associated with MEN I

The indication for gastrinoma resections in patients with ZES/MEN I remains controversial. Those who argue against surgery point out that cure is rarely achieved in MEN I patients due to the multicentricity of tumours (Pipeleers-Marichal 1993; Norton 1999), and that the prognosis in gastrinomas associated with MEN I may even be better than in sporadic gastrinomas (Mignon, Cadiot 1996). Cadiot observed that surgery had no significant impact on survival when he compared 48 patients with surgery to 29 without (Cadiot 1999). This is true particularly for patients without large pancreatic tumours. However, his group concedes that resection is indicated for large gastrinomas because there is a greater risk of developing liver metastases (Fraker 1994; Cadiot 1999).

Recent support for explorative laparotomy in MEN-I patients comes from the NIH (Norton 1999) and a German group (Kisker 1998). They recommend tumour debulking in all cases, provided that the tumour is located by pre-operative imaging (Kisker, Fries 1997; Jensen 1997). However, in a recent report by the NIH group (Norton 1999) this strategy yielded no cure in 28 MEN I patients.
As with other reports the majority of our MEN I patients had associated hyperparathyroidism. A parathyroidectomy was the initial surgical procedure in 4 of 5 patients. One patient had temporarily normalization of calcium and gastrin levels, which is similar to the experience by Norton (1987). There was only one patient (p33) in this series who presented with additional neuroendocrine tumours namely an insulinoma and a somatostatinoma.

2.3.5 **Outcome in accordance to tumour site**

Pancreatic gastrinoma. 6 of our 40 patients (15%) had gastrinoma in the pancreas; 2 of them were not operated upon because of extensive disease (Table Clin7). This is a similar figure compared to the 17% reported by the NIH (Norton 1999). In previous studies, 30-50% of gastrinomas were found to be present in the pancreas (Deveney 1978; Bonfils 1981; Zollinger 1984). The decrease in proportionate number of pancreatic gastrinoma in the last two decades could be attributed to the increasing awareness of extrapancreatic tumours in the gastrinoma triangle (Stabile 1984) and, what was previously thought to be pancreatic tumours, were in fact gastrinomas sitting on the surface of the pancreas. In this respect we reclassified 2 gastrinomas located on the surface of the pancreas (P5,6) as lymph node primaries when reviewing the histology; both tumours were surrounded by a “halo” of lymphoid tissue.

All 6 pancreatic tumours were associated with lymph node metastases, 3 had additional liver metastases. There was only one surgical cure (P22) in a patient where a superficial tumour and a lymph node were removed; he was lost to follow-up after 27 months. In a series published by Kisker (1998), 12 of 24 pancreatic gastrinomas had metastases of which 6 had liver metastases. However, in a more recent communication from the NIH (Norton 1999) only 3 of 21 patients with pancreatic tumours were associated with metastases. This raises the question whether differences in the interpretation of anatomical location and histopathological classification could account for the discrepancies between different centres.

Tumours located in the pancreas seem to have a more aggressive behaviour in terms of liver metastases, and present a lesser chance of achieving surgical cure.
Nevertheless, the prognosis in these patients in our series was still good. There was only one tumour death in our series in a patient (P23) with a large primary in the pancreatic head associated with liver metastases. The other deaths (P1,33) were not tumour related. 2 patients (P10,32) with associated liver metastases are alive at 6 and 13 years, respectively.

‘Ectopic’ gastrinoma. 2 patients were identified with ‘ectopic’ gastrinomas defined as extrapancreatic, extraduodenal. These tumours were located on the surface of the pancreas. Wu (1997) in the NIH experience found ectopic gastrinomas in 5.6% of patients but unlike ours located in the liver, common bile duct, omentum and jejunum. These patients seem to have a favourable outcome with surgical excision. Norton and Doppman (1992) reported a cure rate of 63% while in our small series of two one patient was cured.

As in the case of ‘primary’ lymph node gastrinomas, the origin of these ‘ectopic’ tumours remains an enigma. While the duodenum and the stomach contain a large number of gastrin-producing neuroendocrine G-cells, it remains difficult to explain the origin of tumours in the pancreas apart from that these gastrinomas may derive from a different cell line than those gastrinomas found at other locations.

Stabile and Passaro (1984) noted that gastrinomas lie predominantly extrapancreatic in the anatomical area referred to as the “gastrinoma triangle”. In a more recent publication (Passaro 1998) they hypothesize that gastrinomas within the triangle arise from stem cells from the ventral pancreatic bud which become dispersed and incorporated into lymphoid tissue and the duodenal wall during the ventral bud’s embryogenic dorsal rotation within the described area.

Their hypothesis is based on the observation that gastrinomas within the triangle express pancreatic polypeptide (PP), whereas tumours outside the area are PP-negative. This observation, however, is based on a very small number of tumours (n=14); furthermore, the theory does not explain the origin of sporadic gastrinomas in the pancreatic body/tail area or in lymph nodes found outside the triangle (e.g. lesser curve of stomach).

Chaudry (1994) favours another theory for the origin of gastrinomas. It is based on the expression of the marker CD44. He found CD44 in gastrinomas and pancreatic
duct cells, but not elsewhere in the pancreatic tissue. He concludes that the pancreatic ductal cell positivity for CD44 strengthens the ductal origin concept of gastrinomas (Chaudry 1994).

The question of where gastrinomas derive from remains highly speculative and will require future studies, particularly on the genetical level, to shed more light on this poorly understood process. This will hopefully improve treatment strategies, which may ultimately be non-surgical.

**Gastrinoma in lymph nodes without primary** were found in 11 patients; 4 of the 6 who had formal debulking operation were cured (Clin7). Interestingly, patients with solitary lymph node gastrinoma constituted the largest group in our series, which is different to the experience by the NIH series where only 14% of their gastrinomas fell into this category. However, of their 15 patients, 12 remained disease-free at 5 years. Other reports are anecdotal, with most of them reporting only one to 3 patients with short follow-up (Wolfe 1982; Thompson 1985).

The use of the term ‘primary lymph node gastrinomas’ is debatable. Lymph node gastrinomas should only be designated as ‘primary’ when follicular tissue is present without a detectable primary tumour, and cure is achieved after resection. From a pathological perspective it remains difficult to explain the phenomena of primary lymph node gastrinoma and why many of these patients are cured with simple excision. These tumours may arise from neuroendocrine cells in the lymph nodes but in a study from the Mayo Clinic (Perrier 1995) this was a rare occurrence (less than 1% of 1026 investigated lymph nodes). Other explanations are regression of an occult primary, resection of a primary at the time of previous gastric surgery or inadequate follow-up (Perrier 1995). In patients who were not cured and those who developed recurrence with long-term follow-up, the primary was most likely occult in nature (analogous to the lateral aberrant thyroid nodule) at the time of the initial operation. Most of these tumours are located in the duodenal wall.

**Liver metastases.** The prognostic implications of liver metastases in ZES remain a subject of debate. Sutliff and co-workers (1997) observed that a large proportion of patients with LM (42%) experienced rapid growth with a high mortality rate (62%).
Kisker (1998) observed an actuarial 5-year survival of 28% in patients with LM, compared to 100% in those without. However, the majority (Ellison 1987; Hirschowitz 1997) including our own series suggest a much more benign course. Ellison (1987) reported a 15-20 year survival of patients with LM, and a similar experience was reported by Hirschowitz (1997).

With the probability of cure in patients with liver gastrinomas, Moriura and others recommend removal of secondaries (Moriura 1993; Goletti 1992). While local excision of solitary or limited number of LM would seem reasonable, the role of surgical removal in the presence of multiple liver metastases with or without other tumour bulk should be questioned because of the good prognosis with conservative treatment. By the same token chemotherapy would seem to be an unnecessary radical approach.

The present role of different adjuvant treatments for metastatic disease to the liver remains unclear. There is no clear evidence that chemotherapy, embolization, irradiation or biotherapy increase survival. Most treatments have usually been reported in studies involving different types of neuroendocrine tumours. Furthermore, criteria for assessing tumor response to chemotherapy vary considerable amongst different studies.

**Duodenal Gastrinomas.** The reported incidence of duodenal gastrinoma has increased substantially in recent years. 6 of our 7 patients with duodenal wall gastrinomas had metastatic spread to regional lymph nodes. This observation is similar to other reports (Howard 1990; Arnold 1994), but not to the recent NIH series (Norton 1999), where only 29% of duodenal tumours presented with regional LM metastases. 2 of our 6 patients with metastatic LN spread were long-term cures stressing again the importance of regional LN resections.

The frequent spread to regional lymph nodes and the difficulties in detecting small (and microscopic) duodenal wall tumours prompted some investigators to recommend a Whipple procedure with the aim to increase the chance of a surgical cure (Delcore 1992, 1994; Udelsman 1993; Stadil 1995). However, the indication for aggressive resections remains controversial since clinical experience is limited, and the specific subgroup of patients who benefit from such an approach has not yet been identified (Delcore 1992; Fraker 1994). The strongest argument against aggressive procedures is the good prognosis of ZES patients with local resections alone and the
increased morbidity and mortality associated with extended resections including pancreaticoduodenectomy. However, an argument can be made to perform a pancreaticoduodenectomy in the sporadic form for multifocal duodenal wall tumours with or without lymph node metastases in the gastrinoma triangle.

2.3.6 Follow-up in ZES

10 (25%) patients died during the follow-up. There was only one death directly related to tumour metastases. Other causes included cancer, alcohol intoxication, and homicide. The ZES related mortality might well be slightly higher since 5 patients were lost for follow-up.

Nevertheless, our results on survival are similar to the recently large series published by the NIH (Norton 1999) on 151 ZES patients. The 10-year survival in sporadic gastrinomas was 95%, and for MEN I patients, 88%. There is only one study where 25% of ZES patients had a more progressive course leading ultimately to death (Weber 1995). The disease was characterized by the rapid development of liver metastases, large pancreatic tumours, higher serum gastrin levels, and occurring predominantly in females.

The favourable outcome observed at Groote Schuur Hospital may be explained by a policy of tumour debulking which was introduced since the early 80’s. Several studies provided evidence that tumour debulking in ZES patients significantly decreases the chance of developing hepatic metastases (p<0.003) and, improved survival (p<0.008) (Fraker 1994; Ellison 1995). Thus the rationale for tumour debulking is not only based on facilitating acid control but also improving the prognosis.

2.3.7 ZES in childhood and adolescence

The concern in young patients with ZES is the compliance and safety of life-long medical treatment with proton-pump inhibitors on the one hand and the reluctance to perform radical surgery on the other.

Reporting on a 25-year follow-up on 8 young ZES patients, Wilson (1982,1992) preferred total gastrectomy to life-long medical management when surgical cure is not achieved. Concerns have been expressed on the long-term safety of PPIs including the
potential development of gastric carcinoids from enterochromaffin-like cell hyperplasia, or bacterial overgrowth as a result of achlorhydria (see “Pantoprazole Trial” 5.3). Colorectal adenocarcinoma has also been linked to hypergastrinemia. However, current data suggest that PPIs are safe without side effects (Jensen 1996; Freston 1997). The development of carcinoid tumours which has been observed in rat models after prolonged hypergastrinemia has only been associated with ZES in the context of familial MEN I (Freston 1992) but not in sporadic gastrinomas. The hypothesis of development of gastrointestinal adenocarcinoma generated by hypergastrinemia is tenuous at best (Freston 1991; Lechago 1993; Feston 1997). Although the number of patients is small, none of these effects have been observed in this series during a mean follow-up period of more than 11 years.

Wilson’s support for total gastrectomy in young ZES patient should thus be questioned except for those who are not compliant, or where as in our situation, geographical reasons may preclude a regular supply of medication. Young ZES patients should otherwise be diagnosed and managed in every respect like adult gastrinoma patients.

2.4 Summary and conclusions

While the Groote Schuur experience with the ZES mirrors to a large extent the reports from multicenter studies (Norton 1999; Cadiot 1999), the study is unique in that it is the largest reported series from a single centre in a Third World setting. One of the strengths of this experience is the long-term follow-up (mean of 8.5 yrs) with relatively few patients defaulted from study.

While vagotomy was introduced to augment acid suppression during the H2RA era with good effect, this approach was soon abandoned with the introduction of PPIs. From an early stage (1980’s) the emphasis on surgical treatment has shifted to formal tumour debulking procedures, supported by PPI therapy. The outcome of this approach has yielded good results not only in terms of preventing life-threatening peptic ulcer related
complications but also achieved a cure rate comparable to the large experience by the NIH (Norton 1999) and the Paris ZES Study Group (Cadiot 1999). The prognostic implications of tumour site have been highlighted in this series. This study has identified a subgroup of patients who are most likely to be cured by debulking operations namely, those with extrapancreatic extraintestinal gastrinoma in particular, and somewhat surprisingly those with solitary lymph node gastrinomas. In this regard careful re-examination of all pathological specimens has changed the spectrum of tumour distribution in our series and highlights the pitfalls of classifying tumours in the immediate region of the pancreas as pancreatic of origin, which seem less likely to be cured by surgery.

This study also confirmed that MEN I patients are not cured by surgical debulking nor those with multiple liver metastases. However, the prognosis was still excellent in these patients and we can therefore not support the treatment of these patients with potential harmful chemotherapeutic regimes.

Children and adolescents constituted a relatively large proportion of this series. Although some of these young patients who were not cured by debulking operations required total gastrectomy this was done mostly for reasons other than poor acid control. While total gastrectomy is now less often performed in our practice this operation still has a place for patients living in remote parts of the country where medical supplies are unreliable.

The overall prognosis in the GSH series is excellent and compares favourably with other reported series. We believe that the results achieved by a policy of formal debulking operations in the sporadic form of the disease without overt liver metastases is vindicated.
Chapter 3

Biological tissue markers

There remains a lack of accurate markers of malignant potential and prognosis of gastrinomas. Morphological criteria alone are unreliable, and results derived from serum markers have been inconclusive. The availability of a large number of pathological specimens has allowed a detailed assessment of the possible correlation between various biological markers and tumour behaviour.

The tumour markers that have been used in this study had either been studied by other investigators, providing inconclusive results (PCNA, CD44), or they were already established as prognostic indicators in other tumours, but had not been tested in gastrinomas (bcl-2, Mib1/K67, p21).

3.1 Methods

3.1.1 Tissue samples

This study was based on 24 specimens obtained from 19 patients who had gastrinoma resections between 1981 and 1994. Table Bio1 summarizes the basic clinical data of the patients. 8 of the 19 patients were cured, five are currently on long-term medical treatment with proton-pump inhibitors, one was lost to follow-up, and 5 died. Their deaths were not related to ZES (see ‘Mastersheet’).

The specimens consisted of 15 gastrinoma lymph nodes, 6 duodenal wall tumours, and one wedge resection of a gastrinoma liver metastasis. The remaining 2 samples were pancreatic tumours “shelled out” from the pancreatic surface.

2 specimens were available in 3 patients, and 3 in one patient. All samples were fixed in formalin and embedded in paraffin shortly after the resection.

3.1.2 Staining Techniques

All specimens had been fixed in paraformaldehyde immediately after surgery, and embedded in paraffin. Only paraffin sections with the best-preserved tumour were selected for the study. The sections were cut and mounted on APES-coated slides, then
deparaffinized in xylenes and rehydrated with graded alcohols. Endogenous peroxidase was blocked by incubating sections in 1% hydrogen peroxide in methanol for 15 minutes. After washing in tap water, sections were subjected to antigen retrieval with 0.1% trypsin in Tris buffer (1M, pH 7.8) at 37° C for 5 minutes. This was followed by incubation in 1M citric acid buffer (pH 6) that had been microwaved for 10 to 20 minutes. Slides were then rinsed in phosphate buffered saline (PBS) at pH 7.6 and then sensitized with 10% normal rabbit or swine serum for 10 minutes at room temperature.

The sources of primary antibodies and their dilutions are designated below. Sections were incubated with primary antibodies (2-8 μl/ml depending on the antibody) for 60 minutes at room temperature, except for p53 and Waf 1, which were incubated at 4° C overnight. Sections were then rinsed in PBS and incubated with biotinylated secondary antibodies. Rabbit anti-mouse and swine anti-rabbit antibodies were applied (concentrations 1/50 to 1/200 depending on the antibody) for 30 minutes at room temperature, followed by a PBS wash and a 30-minute incubation with peroxidase conjugated avidin at room temperature. The formation of antigen/antibody (Ag/Ab) complexes was visualized with diaminobenzadine (DAB). Sections were washed in tap water, and counterstained in Mayer’s hematoxylin, then dehydrated, cleared, and finally mounted. Ag/Ab complexes stained dark brown with DAB.

Staining with p53, bcl-2, p21 (Waf1/CIP1) was interpreted either positive or negative. For PCNA, we interpreted the staining either being weak, moderate, or strong, and for CD44 either weak or strong (according to Chaudry 1994). The percentage of Mib1/K67 positive cells was determined twice in each sample with a microscope scoring a minimum of 2000 tumour cells in areas of highest immunostaining (according to Pelosi 1992). All slides were interpreted by one experienced pathologist (P.K.) and the author.

Immunocytochemical detection was obtained with monoclonal anti-p53 (D07) antibody (Dako A/S, Copenhagen), anti-Mib-1 antibody (Calibiochem, CA, USA), anti-bcl-2 antibody (Dako A/S, Copenhagen, Denmark), anti-p21 (Waf1/CIP1) antibody (Novocastra, Newcastle-on-Tyne, England), and anti-CD 44 antibody (clone A3D8, Whitehead Sc.

The software used to evaluate the data was MINITAB (Version 12 from Minitab Inc., State College, PA, USA). The Pearson product moment correlation coefficient was used to measure the degree of linear relationship between two variables. A value of
p<0.05 was considered to be statistically significant.

3.2 Results

Table Bio1 summarizes the staining results obtained in each tissue sample, together with the corresponding clinical data.

All gastrinomas stained positive for gastrin, but only half of them for somatostatin. Of the total of all 24 specimens, p53 was positive in 29%, bcl-2 in 13%, and p21 (Waf1/CIP1) in 21%. CD44, Mib-1, and PCNA were positive in all tumours, although the staining intensities differed (Table Bio2).

p53 was positive in one third of the lymph nodes, and in 1/6 duodenal wall gastrinomas. The marker was positive in about 30% in cured and non-cured patients (Table Bio3). p53 was positive in 3/5 p21-positive samples (correlation coefficient \( r=0.348, p=0.096 \)), and in all three bcl-2-positive specimens (\( r=0.589, p=0.002 \)).

No bcl-2 staining was found in duodenal tumours, with minimal staining in 2/15 lymph nodes. For bcl-2 there was also no difference among cured vs. non-cured patients. However, all positive bcl-2 staining was strongly associated to PCNA staining (\( r=0.589, p=0.002 \)).

The weakest staining for Mib1/K67 was found in duodenal wall gastrinomas, whereas staining intensity was almost equally distributed when all 24 tumours were taken together.

An equal distribution of staining intensity was found for PCNA, particularly in lymph nodes, and CD44. The 2 pancreatic tumours showed strong PCNA, but weak CD44 staining intensity. There was a strong association between p21 and PCNA staining (\( r=0.574, p=0.003 \)), and p21 and bcl-2 (\( r=0.427, p=0.038 \)). When CD44 staining is compared in cured versus non-cured patients, there is a trend of more tumours showing weak staining in the cured, compared to stronger staining in the non-cured group (Table Bio3). We also found an inverse correlation between positive bcl-2 and weak CD44 staining (\( r=-0.411, p=0.046 \)).

Any other correlations between the different biological markers not mentioned were statistically not significant.

Unfortunately, it was not possible to compare patients with single tumours and
those who had multiple gastrinomas, because in 8/19 patients, not all tumours resected at surgery were available for immunostaining.

3 patients (P5,14,31) had removal of solitary lymph nodes with subsequent cure. We compared their staining patterns (a) to lymph nodes in patients who were not cured (P4,19,17) and (b) to those that were associated with a primary (cured: P22,13,18; no cure: P6,8,10,35,17,33). Staining in all groups was heterogeneous and did not allow identifying a trend in either group.

Table Bio4 provides information about those patients who had a primary gastrinoma and a lymph node removed either at the same time (in 3 pts) or on two different occasions (1 pt). The gastrinomas in patient P8 resected in 1990 are the only ones in our small series of 4 patients where the primary and a metastasis showed identical staining pattern with all antibodies. This pattern, however, was different when compared to the lymph node that was resected eight years earlier.

The mean size of pancreatic gastrinomas was 19 mm (range 18-20mm), lymph nodes 13.1 (5-30 mm), and duodenal wall tumours 10.1 mm. Due to the small number of pancreatic tumours a difference between pancreatic and duodenal tumour size could not be determined statistically.

3.3 Discussion

There is a paucity of pathological criteria and tumour markers in determining the malignant potential and aggressive behaviour of gastrinoma tumours (Jensen 1997). Serum tumour products such as pancreastatin, alpha-human chorionic gonadotropin (HCG) and β-HCG as well as various forms of gastrin (progastrin, amidated gastrin, glycine extended forms) were correlated to the presence of malignancy, but their usefulness remains unclear (Jensen, Gardner 1993).

The rationale for this study was the experience from other oncological areas where biological tissue markers are increasingly used in clinical practice to guide therapeutical decisions; particularly the cell cycle marker p53 has gained importance in breast and colonic cancer (Zolota 1999; Turner 2000; Sinicrope 1999; Bukholm 2000). Similar investigations in neuroendocrine tumours have been performed occasionally, with inconclusive results (Pelosi 1992; Chaudhry 1994; Ruschhoff 1993; Imam 2000).
p53

Abnormalities of the gene p53 represent the most common alteration identified in human malignancies (Chang 1993). The wild-type p53 is a regulator of cell cycle. It prevents cells with abnormal DNA from entering mitosis (Jacks 1996). Should the damage be irreparable, p53 targets the cell for self-inflicting death or apoptosis. While normal p53 is not immunohistochemically detectable at a significant level, the mutated protein is stabilized and thus presents at much higher levels that makes it easily identifiable (Levine 1991).

Our study demonstrates that about one third of gastrinomas stained positive for p53. A similar distribution of positive staining was found in the two groups, cured versus non-cured patients. Tomita (1993) detected a slightly higher rate of 49% p53-positive gastrinomas, but no correlation to survival. In contrast, the Paris ZES Study Group (Jais 1998) detected circulating anti-p53 antibodies in sera of 16% of 44 ZES patients, and was able to associate this finding to liver metastases ($p=0.0017$) and shorter survival ($p=0.012$). In contrast, only 1 out of 7 p53 positive patients in our study had a single liver metastases removed. The Paris study is the only investigation today where p53 expression, although in serum, has been positively linked to outcome.

It should be mentioned that p53 staining has been observed not only in pancreatic gastrinoma tumour cells but in normal adjacent islets as well. The significance of this finding is unknown at present (Tomita 1997).

Immunocytochemical tissue staining for p53 has to be interpreted carefully, because it may not directly reflect genetic p53 base changes (Louis 1993; Bartz 1996). At this stage, overexpression of p53 in gastrinomas should still be considered with caution when used as a marker for malignancy.

bcl-2

The bcl-2 family consists of both inducers and repressors of apoptosis (Boise 1993). One potential function of the proteins is to mediate/guard against alteration in mitochondrial transmembrane potentials (Yang 1997).

There are currently no reports on gastrinoma staining with bcl-2. We found positive staining in 13% of tumours. There was no difference between the two groups, cured versus non-cured. If we take the benign nature of gastrinomas in our series into
consideration, our findings do not correlate to bcl-2 staining results obtained in other oncological areas. Zolota (1999) observed a near lack of staining particularly in aggressive tumours of the breast; however, he found a correlation between p53 and bcl-2, as in our series \((p= 0.002)\). In another study on colon carcinomas (Sinicrope 2000), bcl-2 overexpression was associated with more favourable outcome \((p=0.04)\). With the limited data available, we cannot explain the lack of bcl-2 staining, which is linked to a relatively good prognosis in our gastrinomas.

**Mib1/Ki-67**

Proliferative activity can be estimated by immunohistochemical demonstration of proteins expressed in cycling but not in resting cells. In the past, Ki-67 antigen has been shown to be the most reliable marker of cell proliferation (Brown 1990). The Mib-1 antibody has replaced Ki-67 because it can be used in paraffin sections, which allows investigations of archival material.

Mib1 has not been investigated in gastrinomas yet. Whereas expression was equally distributed among lymph nodes, minimal staining \(<1\%)\) was found in the majority of duodenal gastrinomas. However, when we compared the two clinical groups cured versus non-cured (Table Bio3), there was no pattern evident that would indicate a staining preference of one group versus the other.

When staining for Mib1/K67 and PCNA was compared in neuroendocrine tumours (Pelosi 1992), no significant differences were found. We made the same observation except in duodenal gastrinomas, which showed minimal staining with Mib1/K67 but stronger expression of PCNA.

**p21 (Waf1/CIP1)**

A more recently identified gene, Waf 1/Cip1, is activated by wild-type p53 (Jacks 1996). Its product is an inhibitor of cyclin-dependent kinases, which regulates entry into the DNA synthetic phase of the cell cycle. Although p53 independent induction of the gene Waf 1 has also been shown, it has been suggested that p21 (Waf1/CIP1) protein expression could reflect the functional status of p53 (Bukholm 1997). This marker has been mainly investigated in different types of carcinomas but not in gastrinomas.

p21 (Waf1/CIP1) has not been studied in gastrinomas before. The marker was
positive only in a small fraction of lymph nodes and duodenal gastrinomas, but in both pancreatic tumours. It was more often positive in patients who were not cured, which is contrary to findings in colon carcinomas, where the absence of p21 was strongly associated with development of metastases and death (Bukholm 2000). The same author observed a strong association between p53 and p21, which was not the case in our study (p=0.096). A larger series could find evidence for the role of p21 in gastrinomas.

PCNA

Proliferating cell nuclear antigen (PCNA), is a nuclear polypeptide that is associated with cell proliferation (Landberg 1990). It is an auxiliary protein to DNA polymerase delta, and its presence appears to be necessary for DNA replication (Jaskuselski 1988). PCNA is present in various degrees in all stages of the cell cycle (Bravo 1987). The PCNA staining was found to reflect on the tumour growth fraction, which may allow to distinguish local from advanced tumours and predict malignancy (Imam 2000; Tomita 1997).

PCNA was positive in all our gastrinomas. Weak, moderate, and strong expression was equally distributed in all locations, except for the 2 pancreatic tumours, which both stained strongly positive. Our findings are in accordance to the investigation by Tomita (1997) who also stained all gastrinomas with PCNA.

CD44

Intensive research on CD44 has been done in malignant melanomas and neuroendocrine tumours of the lung. Dietrich (1997) reported that in melanoma patients high levels of CD44 are associated with increased metastatic risk and reduced survival (p<0.05). Coppola and co-workers (1996) investigated various splice variants of CD44 in neuroendocrine tumours of the lung. Interestingly, they found that expression of the standard form of CD44 and the v6 isoform of CD44 are inversely correlated with more aggressive types of neuroendocrine tumours. In pulmonary carcinoids for example, the loss of CD44v6 was associated with nodal metastases, but no difference was noted in survival in CD44v6-positive and negative patients (Coppola 1996).

Chaudry (1994, 1997) belongs to the few investigators who reported on the expression of CD44 in gastrinomas. Similar to our series, Chaudry detected CD44
expression in all gastrinomas, but not in other gastroenteropancreatic tumours except for some non-functioning endocrine pancreatic tumours. Although CD44-positive tumours seem to metastasise to regional lymph nodes, it had no significant impact on survival (Chaudhry 1994). Our own investigation found CD44 expression in patients to be stronger in those who were not cured compared to those who had a favourable outcome after surgery (Table Bio3). In a recent publication by Imam (2000), CD44 expression could be linked to a lack of metastases \( p=0.001 \) in 26 endocrine pancreatic tumours, including 5 gastrinomas.

Weak CD44 staining was observed in the 2 pancreatic tumours (one patient cured), with all other tumours showing an almost equivalent distribution of weak and strong staining. It is difficult to draw any conclusions from this limited observation. CD44 may be useful to indicate higher malignancy in neuroendocrine tumours, as indicated by others (Chaudhry 1994; Komminoth 1996), although metastatic spread to lymph nodes may not necessarily translate into a poor prognosis.

CD44 expression has also been linked to the development of gastrinomas. Since expression of CD44 is observed mainly in the pancreatic duct of some non-functioning islet-cell tumours and in gastrinomas, a Swedish group from Uppsala hypothesized that multipotent stem cells in the pancreatic duct might be the origin of gastrinomas (Chaudhry 1994). This hypothesis awaits confirmation.

### 3.4 Conclusions

Our study demonstrated that gastrinomas from different locations did not show a specific pattern of antigen expression (Table Bio2), and that the expression of each marker did not differ in the cured versus the non-cured patients, except for CD44, which showed slightly stronger staining in the non-cured group (Table Bio3). Identical staining patterns of the primary and its metastases was found in only one out of 4 patients where multiple specimens were obtained at the same time. However, this pattern was different from a tumour resected 8 years earlier, suggesting that the biological tumour behaviour may change over time.

We also could not find any staining differences when we compared solitary lymph nodes of patients who were cured to lymph nodes that were associated with a primary in
either cured or non-cured patients. However, there might be some interesting associations between certain markers, although the significance of this finding cannot be fully evaluated due to the small number of specimens studied.

It should be mentioned that the results of immunohistochemistry depend on various fixation regimens, sensitivities and specificities of different antibodies, and the detection systems employed. Efforts to standardize the immunohistochemical techniques and interpretation, possibly by automation, quantitation and image analysis may help to reduce the variation in data interpretation.

Besides the small number of samples, the fact that no patient died during the observation period due to ZES limited the statistical evaluation of the study. The relatively benign course of ZES found in our series was recently confirmed by a larger study from the NIH, where only 3 out of 151 ZE patients died as a consequence of ZES (Norton 1999). For these reasons, it was only possible to evaluate gastrinoma aggressiveness, expressed by the tendency to metastasise. However, none of the markers as defined by immunocytochemical analysis were useful predictors of aggressive tumour behaviour, except for CD44, which may indicate an increased tendency of gastrinomas to spread.

At this stage it seems that immunohistochemical markers have little or no role to play in dictating treatment strategies.
Chapter 4
Somatostatin receptor scintigraphy (SRS)

The introduction of SRS at GSH in the 1990’s has allowed a critical appraisal of whether this expensive imaging modality has improved the surgical cure rate when compared to a period when only conventional imaging was available.

4.1 Methods
4.1.1 Patients
12 patients were studied prospectively (10 males, 2 females; mean age 46.3 years; range 24 to 76 years; two with MEN-I syndrome). 7 of the 12 patients previously had surgery; 5 had a gastrinoma resection without cure, and in 3, no tumours were detected.

4.1.2 Scintigraphic techniques
$[^{111}\text{In}]$ pentetreotide (DTPA-Phe-1-octreotide; Octreoscan), a radiolabeled analog of somatostatin, was obtained from Mallinckrodt Medical, Petten, Holland. SRS was performed after intravenous injection of a mean dose of 5.8 mCi $[^{111}\text{In}]$ pentetreotide (155-270 Mbq or 4.2-7.3 mCi). Patients were administered a laxative prior to the injection to reduce accumulation of radioactivity in the intestines. Planar images were taken of the abdomen and chest after 4 hr, 24 hr, and 48 hr, and a single photon emission computer tomogram (SPECT) was obtained at 24 hours. Images were obtained using a General Electric 400 ac large field of view gamma camera equipped with a medium energy parallel-hole collimator with the pulse height windows centred over both $^{111}$In photon peaks (172 keV and 245 keV). Anterior and posterior planar images of the chest, upper abdomen, and lower abdomen were taken to 500,000 counts. The SPECT study of the abdomen was taken from 64 projections at 60 seconds acquisition time per projection on a 64 x 64 matrix. Positive SRS studies were defined by the occurrence of tracer in areas not normally associated with its accumulation.
4.1.3 Surgery

Seven patients without diffuse liver metastases were submitted to laparotomy with the intent of removing all gastrinoma tumours. All 7 patients had intra-operative upper GI endoscopy down to the ligament of Treitz with simultaneous transillumination after Kocherization of the duodenum. Debulking procedures were performed as described above (Chapter 2.1.4.2).

Postoperatively, serum gastrin and secretin tests were evaluated at regular intervals. Biochemical cure after excision of the gastrinoma was defined as normalization of the fasting serum gastrin concentrations (<115 pg/ml), and a negative secretin provocative test result (i.e., < 200 pg/ml increase of serum gastrin). All patients were followed as outpatients at the Gastrointestinal Clinic at Groote Schuur Hospital.

4.2 Results

4.2.1 Conventional imaging studies

12 patients participated in the study (Table SR1). Upper gastrointestinal endoscopy was negative in all of them while ultrasound and computerized tomography revealed 2 tumours in one patient. These 2 lesions were >3 cm in size on imaging and confirmed by surgery.

Figure 7a demonstrates location of a periduodenal tumour in patient P29 on a CT scan; the pancreatic gastrinoma, however, was only imaged by SRS (Figure 7b) and confirmed by surgery.

4.2.2 SRS

A total of 17 scintigraphic studies were performed in the 12 patients. SRS was repeated in 2 patients (P33,38) with a higher nucleotide dose because no lesion was imaged on the initial scan (Table SR1). In one patient (P38) the scan turned positive, in the other (P33), it remained negative despite an increase of the radiopharmaceutical from 160 Mbq to 240 Mbq. 3 SRS studies were done post-operatively (see below).

SRS identified 13 hot spots (including one false-positive spot in P8) in 11 out of 12 (92%) patients (Table SR1). SRS was the only positive pre-operative imaging
technique in 10 out of 11 patients (91%), excluding the false-positive result in P8. 9 of 13 (69%) hot spots were located in the ‘gastrinoma triangle’, 3 in the liver, and one inferior to the right kidney. It was not possible either by planar view or SPECT images to accurately localize the anatomical site of the tumour when situated in the pancreatic head or the second/third part of the duodenum.

SPECT studies were performed in all patients. They did not increase the number of positive findings when compared to planar view images. The superimposition of CT scan and SRS, as done in a single patient (P29), delineated the additional tumour in the area of the pancreas (Figure 7).

Liver metastases. SRS identified hot spots in the liver in 2 patients (P6,37) who were not operated upon. CT and US were negative in both of them. In one patient with MEN-I (P6) who had a gastrectomy in 1982, the scinitigram showed two small liver foci 16 years thereafter. In a second patient (P37) with previous vagotomy and antrectomy, SRS revealed a small scintigraphic spot in the left liver lobe, twelve years after surgery. Both patients are currently well.

4.2.3 Correlation between operative findings and SRS

7 of the 12 study patients had a laparotomy shortly after SRS (Table SR2 and SR3). The reasons for nonoperative treatment in the remaining 5 patients were concomitant medical disease (P21,38), age-related reasons (P24,37), and one patient (P6) with liver metastases.

In the 7 surgical patients, ultrasound and CT scans identified 2, SRS 7, and surgical exploration 9 gastrinomas (Table SR2).

One surgeon (PCB) experienced in gastrinoma surgery performed the resections in six of the seven patients. Two additional tumours in one patient were removed in the gastrinoma triangle. These tumours were found in a lymph node located periduodenal and in a second node on the surface of the pancreas (P27).

Number and area of hot spots matched with surgical findings in 3 patients (P34,39,40). Post-operatively, serum gastrin levels and secretin provocative testing were normal in these 3 patients.

Discrepancies between SRS and surgery were observed in one patient with MEN I syndrome (P33). He had surgery for recurrent life-threatening hypoglycaemic episodes,
which were thought to be caused by a recurrent insulinoma. Planar and SPECT images remained negative despite a dose increase of the radionucleotide from 160 to 240 Mbq. A pre-operative secretin-stimulation angiogram, however, was positive for a gastrinoma in the left pancreatic area. At surgery, no insulinoma was found, but a 15x10 mm (SRS negative) somatostatinoma lateral to the 2nd part of the duodenum was removed. The patient died post-operatively after developing a jejunal perforation and severe septic shock. At autopsy, a 12 mm gastrinoma was found in the tail of the pancreas embedded in the hilum of the spleen.

In another patient (P8) extensive surgical exploration could not detect a tumour in the region where a hot spot was shown up adjacent to the right kidney by SRS.

Intraoperative transduodenal illumination did not detect any further duodenal wall lesions.

Postoperative SRS studies. 3 patients (P8, 29, 39) had a postoperative scan. One lesion not identified at surgery (P8) was again visualized at the same location near the right kidney. SRS was negative in a second patient (P39), who was cured with surgery. SRS turned negative in the third patient (P29) after removal of two lesions previously diagnosed by SRS (Figure 5). Serum gastrin levels, however, remained elevated (319 ng/ml).

4.2.4 Outcome after surgery

Acid studies, secretin provocative testing and serum gastrin levels taken 3 weeks and 3 months after surgery were normal in 3 (P30, 40, 34) patients (43%). However, one patient (P34) living abroad at present developed a recurrent duodenal ulcer complicated by bleeding one year after the resection of a lesser curve lymph node gastrinoma. Although more detailed information with regard to serum gastrin could not be obtained it must be assumed that this patient was not cured by surgery.

In the remaining 4 patients without biochemical cure, the removal of gastrinomas resulted in a substantial drop of serum gastrin levels from a mean of 1424 pg/ml to 229 pg/ml (Table SR3), and the post-operative basal acid output dropped from a mean of 31.2 to 13.9 mEq/h. (Means were provided to illustrate the difference in gastrin levels before and after surgery, although this may not be statistically appropriate because of the small
number of cases). The dose of pantoprazole was also substantially reduced.

4.3. Discussion

Conventional imaging studies were positive in only a minority of our patients studied compared to a positive SRS test in 92%. SRS was false negative in one patient (demonstrated at autopsy), false positive in another, and detected less tumour than was found at surgery. The cure rate (2/7) did not improve when compared to the results obtained at GSH before the introduction of SRS, when 7/21 (33%) with formal debulking procedures were cured (Table Clin6).

4.3.1 The yield of conventional imaging studies

Conventional imaging (computed tomography, abdominal ultrasound and angiography) was positive in only one of the patients studied in this series, which is comparable to the results achieved before SRS was introduced at GSH (13%) and in other studies (Table SR4). Gibril (1996) found conventional techniques to be positive in 15-35% of patients, detecting mainly in those lesions larger than 2 cm in diameter. If more invasive techniques are employed the detection rate may increase to 62% of patients (Termanini 1997; Kisker 1998). Endoscopic ultrasonography or portal venous sampling provide a similar yield, but have the disadvantage of being expensive and associated with morbidity, while endoscopic ultrasonography is investigator dependent (Wiedenmann 1998).

4.3.2 Results of SRS in surgical and non-surgical trials

Several studies have demonstrated that SRS is the single most sensitive imaging modality for detecting the primary gastrinoma or hepatic metastases (Krenning 1993; Gibril 1996; Lebtahi 1997; de Kerviler 1994). SRS was reported to visualize gastrinomas in up to 100% of patients, although recent publications showed less impressive results (Table SR4).

The true sensitivity of SRS remains controversial since some investigators calculated the sensitivity per patient, and others per lesion. Alexander (1998) showed in his series of 35 surgical ZES patients a 78% sensitivity per patient, and a slightly lower
sensitivity of 67% when assessed per tumour.

To date there are few published series (Table SR5) where the values of SRS was evaluated in terms of improving surgical cure rates. As with other studies (Cadiot, Lebthai 1996; Alexander 1998) we detected additional tumours to what was shown on pre-operative SRS (Table SR5). In a recent investigation by Kisker (1998), SRS was positive in 53% of patients, but resections of gastrinomas were performed in 96%. The difference of SRS sensitivity on the one hand, and surgical yield on the other emphasizes the importance of not compromising on standard exploratory procedures for apparent solitary gastrinomas. The chances to detect small lesions, which are currently not detected on SRS, vindicate the performance of an explorative laparotomy in those patients where pre-operative imaging studies including SRS are negative (Alexander 1998).

SRS has the potential advantage of identifying gastrinomas in areas that are not part of the screening by conventional techniques, such as chest (Cadiot 1997), bones (Termanini 1997), and ovaries (Maton 1989). However, SRS may also provide misleading information. We could not identify the hot spot in one patient inferior to the right kidney. Gibril (1999) found 12% of SRS examinations to be false positive in 146 patients.

The impact of SRS findings on treatment strategy has been documented in several studies. In a protocol that included 122 ZES patients, Termanini and co-workers (1997) observed changes in clinical management in 47% of patients. SRS clarified questionable results of conventional imaging studies in more than one half of all the cases in which SRS changed management. This was especially important where reoperations were considered. Without positive imaging, these patients would not normally undergo repeat surgical exploration at their institution. SRS also facilitated to distinguish liver metastases from haemangiomas.

The ZES Study Group in Paris examined the impact of SRS on gastrointestinal endocrine tumours including 78 ZES patients, 38 with carcinoids, and 44 with other types of neuroendocrine tumours (Lebthai 1997). With the additional information obtained from SRS surgical strategies were changed in 25% of 160 cases.
4.3.3 Cure rate before and after the advent of SRS

Despite the improvement in the preoperative gastrinoma localization, the overall cure rate in ZES patients at GSH was not improved since the introduction of SRS. There were only 2 (28.5%) definite cures in our series at 2 \(\frac{1}{2}\) years, which was less than before the introduction of SRS at GSH when the cure rate was 8 out of 21 patients (38%, with a mean follow up of 10.2 years). Other investigators report a disease-free rate with SRS of 43% (Alexander 1998; mean FU 2.1 years), 44% (Kisker 1998; mean FU 5.2 years) and 63% (Cadiot 1996). However, most reports did not provide long-term cure rate data. Follow-up periods were well short of the acceptable 5 years follow-up to assess cure rate (Fishbeyn 1993). Data from the NIH (Alexander 1998) showed the disease-free status at 2 years of 38% before and 43% after introduction of SRS. Unfortunately, other recent studies did not provide information concerning cure rates before and after the introduction of SRS. The unsatisfactory cure rates may be primarily due to small (<5 mm) "micro-gastrinomas" that escape scintigraphic and surgical detection.

Our study also highlights the limited role of SRS in patients who undergo re-exploration after previously failed surgery. Among our seven surgical patients, three had previously gastrinoma removed, but none was cured with the additional information provided by SRS. Similarly, only 30% of patients studied at the NIH study were cured after a re-exploration (Jaskowiac 1996). There are currently no established criteria to identify a subgroup of patients most likely to benefit from further surgery when a positive scan is obtained after previous failed surgery. However, even without cure, surgery is beneficial in terms of lowering acid output and PPI dose requirements.

4.3.4 Limitations of SRS

The failure of SRS to identify gastrinomas may depend on a number of factors (Table SR6). First, the tumour may express no or a paucity of somatostatin receptors, or, the particular somatostatin receptor subtype expressed may not be identified by SRS. John (1996) and co-workers found that all gastrinoma tissues they investigated expressed high affinity binding sites for octreotide and high levels of somatostatin receptor subtype 1 and 2 messengerRNA. He concluded that false negative scintigraphic results seem to be
influenced by factors independent of the expression of specific somatostatin receptors, such as tumour blood supply or down regulation of somatostatin binding sites by various factors (for example, corticosteroids).

Perhaps more importantly, false-positive or negative SRS results may be explained by neighbouring high-uptake areas, such as the spleen, the gallbladder and the kidneys as for example in our patient with MEN I.

Tumour size, however, would seem to be the most important reason for failure to detect gastrinoma. All additional tumours discovered at surgery in this series were smaller than 6 mm in size except for the 12 mm gastrinoma located in the pancreatic tail. Alexander (1998) found a significant correlation between median gastrinoma size and tumour detection \( (r = 0.97, p = 0.01) \). The detection rate of tumours < 1.1 cm was 30%, in those between 1.1 to 1.9 cm 64%, and when > 2 cm 96%. He speculated that the tumour size may be an important variable with regard to the amount of radioactivity that needs to be taken up to provide adequate resolution.

It has been suggested that the accuracy of SRS may be improved by three-dimensional SPECT images (Krenning 1994). In recent series, SPECT detected up to 20% more tumours than planar images alone (Schirmer 1995; Zimmer 1996; Corletto 1996). However, in our series as well as in Cadiot’s experience (1996) in 21 ZES patients, no additional tumours were detected by this method.

The use of CT images superimposed with those of transaxial SPECT studies using computer-scanning techniques requires further evaluation before introducing this expensive technology in the diagnostic workup of ZES patients.

4.4 Conclusions

While SRS simplifies the diagnostic approach to gastrinoma patients, this expensive study has not clearly influenced our management of ZES patients in terms of the decision to operate. It had no impact on improving our cure rate, and was also not useful when re-explorations were done for recurrent disease. The main limiting factor with the current technique is failure to detect small tumours. To this end new technology such as hand-held or laparoscopic devices for the intraoperative detection of radiolabelled somatostatin analogues may improve the detection of small gastrinomas.
CHAPTER 5

Phase III drug trial: Safety and efficacy of pantoprazole

The Gastrointestinal Clinic participated in a prospective study on the efficacy and safety of pantoprazole in ZES for the purpose of registering the drug for long-term use.

5.1 Methods

5.1.1 Patient selection

Ten patients with ZES were included in the study (8 males, 2 females; 1 patient had MEN-I; mean age 48.9 years). 4 patients had previously gastric surgery, including a vagotomy and antrectomy or a vagotomy and pyloroplasty. All patients had been treated with other PPIs sometime between 6 months and 7 years prior to the pantoprazole study, and none had active peptic ulcers on entry to the study. The diagnosis of ZES was established by the criteria outlined above (see Chapter 2.1).

Patients with ZES were considered for the study if they were over 18 years of age. Pregnant patients and those with a history of alcohol abuse were excluded from the study. All patients gave written consent to a protocol approved by the Research Ethics Committee of the University of Cape Town (May 5, 1995).

The initial evaluation included a comprehensive history, physical examination, upper gastrointestinal endoscopy, serum gastrin levels, and haematologic, liver and renal functions. A urine analysis was performed prior to the treatment with pantoprazole. Females were also subjected to a pregnancy test.

6 patients had previous histologic confirmation of the gastrinoma by laparotomy prior to the study. Another 4 (3 had negative laparotomies, 1 had no surgery) had positive secretin stimulating tests.

5.1.2 Study design

This prospective, open label, dose-titration study was designed for the period of 3 years. All patients underwent a 14-day washout phase, which consisted of 11 days on
high dose H2-receptor blockers (ranitidine) and 3 days antacid therapy, prior to the assessment of the BAO. The individuals were instructed to discontinue the wash-out period immediately if they experienced severe symptoms (e.g., heartburn, diarrhoea or severe epigastric pain). On day 15, gastric acid secretion was determined after radiological positioning of a nasogastric tube and collection of 4 consecutive 15-minutes samples after emptying the stomach. The volume was documented and an aliquot of each sample was titrated to pH 7.0 with 0.2 N sodium hydroxide, and the BAO determined as described previously (Radiometer Copenhagen TTT 85 Titrator, ABU 80 Autoburette).

After the wash-out phase, patients received a single standard 40 mg enteric-coated tablet of pantoprazole (Byk Gulden, Konstanz, Germany) per day as a minimum. The dose titration study was carried out at days 5, 9, or 13 depending on the level of acid suppression. Four-day intervals were selected because pantoprazole reaches a steady-state acid secretory level for a specific dose after 4 daily doses. Adequate control was defined as <10 mEq/h prior to the next dose of antisecretory drug in patients without and <5 mEq/h in patients with previous acid reducing surgery (Jensen 1997). In patients in whom a daily dose was not sufficient to obtain a BAO of less than 10 mEq/h, a daily dosage of 160 mg pantoprazole was administered (dosage instruction: 2 x 40 mg tablets every morning before breakfast and 2 x 40 mg tablets every evening before dinner). Because there was no experience with a dose as high as 160 mg, the protocol initially restricted the maximum dose to a period of 3 months. In the meantime, further measurements of BAO were taken. A temporary protocol extension was performed after consultation with Byk Gulden Pharmaceuticals. Blood samples were taken once every 3 months during the trial period to evaluate hepatic and renal function. The fasting serum gastrin values were determined by radioimmunoassay (Sorin, Inc., France).

Upper gastrointestinal endoscopy was performed every 3 months during the study after sedation with midazolam (Dormicum, Roche). A careful visual assessment of the oesophagus, stomach, and duodenum was made. Biopsies were taken from the antrum (x2) to evaluate the presence of Helicobacter pylori and from the body of the stomach and the greater curve (x4) to evaluate the oxyntic mucosa histologically. Tissue samples were placed in formalin, embedded in paraffin, and stained with haematoxylin and eosin. Giemsa stains for H.pylori and chromogranin staining (Dako-1/100 with microwave antigen retrieval) for neuroendocrine cells were also performed.
The interpretation of ECL-cell status was evaluated according to the recommendation of Solcia (1995). The Sydney system was used for evaluation of the degree of gastritis (Genta 1995). Parietal cell hypertrophy was assessed morphologically without quantification, as described by Driman (1996). Briefly, fundic glands showing serrated edges in cross section with bulging of the parietal cell cytoplasm into the lumen of the glands (lingulate projections) were considered to represent parietal cell hypertrophy. All histopathological material was reviewed and interpreted by a single investigator without the prior knowledge of the status of acid-output studies or gastrin levels.

5.1.3 Maintenance dose requirements

Following an initial dose of 40 mg, the pantoprazole dose regimen was established by measuring BAO on day 5. This measurement was done at the last hour prior to the next daily dose of pantoprazole. Safe control of the acid hypersecretory state was considered to be at a BAO of less than 5 mEq/h in patients who had previous gastric surgery and less than 10 mEq/h in those who did not. Individuals who failed to achieve these criteria at 40 mg pantoprazole were titrated upwards in 40 mg increments, and BAO measurements were repeated on days 9 and 13.

Following the initial evaluation and the establishment of the individually required dose, each patient was re-evaluated on a monthly basis. Re-evaluation included a complete clinical investigation to identify any possible adverse effects. Blood was collected once every 3 months for determination of haematological, hepatic, and renal parameters. In addition, all patients underwent a BAO measurement and upper endoscopies at three-month intervals to assess the control of gastric acid secretion and the mucosal status, respectively.

Inadequate control of gastric acid secretion was defined as: (1) previously documented (BAO >10 mEq/h without or > 5 mEq/h with prior gastric surgery in the hour prior to the next dose of pantoprazole); (2) endoscopic evidence of oesophagitis, duodenitis, or peptic ulceration; or (3) the presence of peptic symptoms. If any of these criteria were met, the pantoprazole dose was titrated upwards as during the initial evaluation, until resolution of symptoms or decrease of BAO values to the required study level definitions.
5.1.4 Statistics

The software used to evaluate the data was MINITAB (Version 12 from Minitab Incorporation, State College, PA). The Student's t-test was used for comparison of benign and malignant gastrinomas and for comparison of duodenal and pancreatic gastrinomas. A value of p<0.05 was considered to be statistically significant. The chi-square test was used to determine the association between the various markers, the clinicopathological variables, and survival. The Fisher exact test was used to compare independent percentages.

5.2 Results

5.2.1 Patient characteristics

The characteristics of the 10 ZES patients who participated in the oral pantoprazole trial are summarised in Table Pant1. The following data reflect preliminary study results that were obtained during the first 2 years of the 3-year trial.

5.2.2 Initial dose requirements

The standard daily dose of 40 mg pantoprazole reduced BAO to <10 mEq/h in 6 of the 10 patients (Table Pant2 and Pant5). One patient (P39) initially required 80 mg pantoprazole per day, and the remaining 3 (P27,33,40) 160 mg (80 mg twice daily).

The standard dose of pantoprazole (40 mg daily) resulted in the required level of acid suppression for patients with an initial BAO of <20 mEq/h, but patients who had an initial BAO >35 mEq/h required at least twice the standard dose.

There was a strong relationship between BAO and pantoprazole dose (r=0.806, p<0.005) indicated by the Pearson correlation coefficient. However, the pantoprazole dose did not correlate to fasting gastrin concentrations.

5.2.3 Long-term dose requirements

One of the 10 patients (P29) was removed from the study after 12 weeks because of alcohol related problems that prevented him from adhering to the protocol. His BAO was well controlled on 40 mg pantoprazole before he was excluded from the study (Table
Pant2). At the time of writing, 2 patients (P21,40) had been on the study for 48 and 76 weeks, and 7 between 60-74 weeks. 2 patients (P37,38) required a temporary increase of pantoprazole: one (P37) developed reflux symptoms not associated with overt oesophagitis at endoscopy, and the other (P38) had duodenal erosions, which were discovered at routine endoscopy.

Both events occurred despite a BAO <5 mEq/h. Resolution occurred immediately after the temporary increase of pantoprazole to 80 mg. One patient (P39) who was controlled on 80 mg pantoprazole for 8 months subsequently required a dose increase to 160 mg (80 mg twice daily) to achieve the desired acid reduction (see below).

5.2.4 Endoscopy findings

At the end of the initial washout phase, all but one patient (P40) had endoscopic signs of mucosal disease (Table Pant2) (duodenitis: n=8, gastritis: n=1, oesophagitis: n=4, stomal erosions: n=1). After initiation of the trial, all mucosal abnormalities resolved within 3 months. One patient (P38) on 40 mg pantoprazole remained asymptomatic despite identification of multiple duodenal erosions at week 24. Endoscopy returned to normal after increasing the dose to 80 mg pantoprazole daily for one month. The patient continued the trial on 40 mg pantoprazole without a similar event.

5.2.5 Histology

Three patients (P8,21,29) were initially Helicobacter pylori positive and had concomitant moderate to severe gastritis. They received eradication therapy during the trial. Changes of gastrin levels after eradication of H.pylori were not observed. Four other patients (P7,27,37,39) had mild chronic gastritis on at least 2 biopsies during the study period but no evidence for H. pylori. Parietal cell hyperplasia was a consistent finding in 6 patients (P21,27,29,33,39,40), but this did not change significantly during the trial. ECL-cell hyperplasia was present in 8 patients at the initiation of the study (Table Pant3). In 2 patients (P7,21), this was severe enough to diagnose endocrine cell dysplasia, but without evidence for micro-infiltrative lesions. No morphological changes could be detected in chief cells or mucous cells within the gastric pits. Antral biopsies were also unremarkable except for those patients with a H.pylori gastritis.

Random biopsies from the greater curve obtained regularly for up to 76 weeks...
showed no microscopic evidence of carcinoid development, nor was there any trend indicating increasing ECL-cell hyperplasia (Table Pant3).

5.2.6 Basal acid output and gastrin levels before and during the study
Basal acid output varied from 0.5 – 97.8 mEq/hr (mean 27 mEq/h, ± 30 mEq/h) (Table Pant2). 4 patients who had had a previous vagotomy had a much lower BAO (0.5 – 12.4, mean 6.7 mEq/hr) than those without previous gastric surgery (3.7-97.8, mean 40.7 mEq/hr; p<0.03). The patient (P21) without previous surgery but with a low BAO of 3.7 mEq/hr showed severe H. pylori gastritis with atrophy of fundic glands.

Gastrin levels were all elevated and ranged from 152-4738 pg/ml. The mean serum gastrin level before treatment was 1545 pg/ml (± 1856), and during pantoprazole treatment 1645 pg/ml (± 1893). This was not a significant difference according to the Student’s paired t-test (p= 0.697). Gastrin levels and BAO showed no correlation (r=-0.418; p = 0.229).

5.2.7 Haematological and biochemical studies
There were no effects on red blood cell count, red cell indices, total white cell count, differential white cell count or platelet count that could be attributed to pantoprazole. Hepatic biochemistry was unchanged during the trial. Two patients (P37,38) had elevated liver enzymes at the start of the study and this did not change during the trial. There were also no alterations of any haematological or biochemical studies in 2 elderly patients (P7,37) both aged 74, or in a patient with alcohol-induced impaired liver function (P40).

8 patients showed no significant increase in serum gastrin levels for the duration of the study (Table Pant3). One patient (P21) showed an increase in serum gastrin levels from 398 to 586 pg/ml (+47%). He subsequently underwent resection of a 2 cm pancreatic body gastrinoma. His serum gastrin levels and BAO became normal postoperatively and he was taken off the study. Another patient (P21) became achlorhydric during the study (Table Pant2). His serum gastrin levels rose from 2776 to 4821 pg/ml (+73%).
5.2.8 Adverse events

We did not observe severe adverse events attributable to pantoprazole. Adverse reactions observed included dizziness (3 episodes), rash (2), diarrhoea (3), and headaches (x4), which were transient and mild in nature, and not necessarily associated with the drug. Furthermore, these adverse reactions were not dose related.

5.3 Discussion

The purpose of this study was to evaluate the potential usefulness of oral pantoprazole in ZES patients by assessing its efficacy and safety during prolonged administration. Pantoprazole was found to be safe in all patients, even when the maximum dose of 160 mg/day was taken. However, in ZES patients with a high BAO of 30 mEq/h or more, a 25% higher dose than the officially recommended dose of 120 mg per day was required to effectively reduce acid secretion.

5.3.1 Inclusions

The inclusion of ZES patients with previous gastric surgery in studies with proton pump inhibitors remains controversial. It is challenged because of the difficulties in obtaining reliable BAO measurements due to factors such as bile reflux, early gastric emptying, and disturbances in motility, or delayed emptying due to anastomotic strictures (Maton, Frucht 1988; Metz, Pisegna 1993). The reason for including 4 cases with previous gastric surgery in this study was not so much to assess the efficacy of acid suppression, particularly in the 2 cases with low acid output, but to analyse the long-term safety record of pantoprazole. These patients were not cured, as assessed by gastrin levels and secretin tests, and continued medical therapy was warranted to avoid potential complications. There was concern that the acid output could have been falsely low as a result of duodeno-gastric reflux.

Interestingly, all 4 patients with previous gastric surgery and those with a low BAO (< 5 mEq/h) became symptomatic during the washout phase. Furthermore, they all developed mucosal abnormalities at endoscopy. Both the symptoms and the mucosal changes disappeared immediately after administration of pantoprazole. A similar
observation was reported recently where complications such as bleeding and perforation occurred in ZES patients with previous partial gastrectomies who had almost no detectable basal acid output (Hirschowitz 1997). The mechanisms that cause mucosal damage in the absence of acid secretion remain unclear.

5.3.2 Efficacy

The main finding of our study was that standard dosage of pantoprazole does not suppress acid secretion sufficiently in ZES patients with high BAO. The officially allowed maximum dose of 120 mg/day had to be temporarily increased to a dose of 160 mg after consultation with Byk Gulden Pharmaceuticals. The higher dose reduced BAO to about 10-11 mEq/h. A similar relationship between BAO and drug dose was observed in a study with omeprazole (Maton, Vinayek 1989), whereas no such relationship was found in 20 ZES patients investigated with lansoprazole (Metz, Pisegna 1993). A larger study of ZES patients is needed to assess the efficacy of pantoprazole in ZES patients with a high BAO.

In this study our aim was to reduce acid secretion to <10 mEq/h. We based this on a safety level, which was a consensus based on empirical data derived from several studies (Jensen, Gardner 1993; Metz 1995). Some investigators, however, see the necessity to reduce BAO to even lower levels. Hirschowitz and others (Hirschowitz 1992, 1997; Metz, Pisegna 1993; Sprenger 1997) recommend a BAO of <5mEq/h in uncomplicated and <1-2 mEq/h in complicated ZES or postgastrectomy cases. However, this does not rule out the possibility that a higher BAO may be safe in some cases, such as patient (P39) who required an upward dose adjustment during the course of the study. After acid control (BAO 5.1 mEq/h) with 80 mg pantoprazole for 8 months, his BAO suddenly increased to 27.8 mEq/h within 4 weeks. BAO was reduced to 14.8 mEq/h on 120 mg pantoprazole and 11mEq/h on 160 mg pantoprazole. He remained clinically asymptomatic with no mucosal disease on endoscopy. He was cured after resection of a 35 mm lymph node gastrinoma located on the pancreatic surface. The sudden rise of acid secretion in this patient accompanied by a moderate increase of gastrin is difficult to explain. Tumour growth may have played a role since tachyphylaxia has not been described in connection with PPIs.

The present study suggests that once an effective dose of pantoprazole has been
established in ZES patients, upward dose adjustments are not likely to be required during prolonged therapy. Only 10% of the patients in this study required a dose increase, which compares favourably to other studies on PPIs. Individuals treated on a long-term basis with omeprazole required upward dose adjustments in 23% of cases, with another 20% requiring varying dose adjustments (Maton, Vinayek 1989). Similar results were obtained in studies with lansoprazole, where 25% of patients required dose adjustments (Metz, Pisegna 1993).

Split dose pantoprazole regimens were required in 40% of patients, similar to the 20-60% of patients treated with omeprazole or lansoprazole in other studies (Maton 1989; Metz, Pisegna 1993).

5.3.3 Safety profile

Our study suggests that the use of pantoprazole in patients with ZES is free of severe adverse effects and causes neither short- nor long-term haematological or biochemical changes. Mild adverse events (headaches, dizziness, rash or diarrhoea) were infrequent and comparable to those seen when using other proton pump inhibitors (Frucht 1991). In particular, patients who presented with abnormally high serum transaminases at the beginning of the study showed no further increase while taking pantoprazole.

A relapse of mucosal disease, despite successful control of acid secretion, was observed in one of our patients. This phenomenon has also been observed in patients treated with other PPIs such as omeprazole and lansoprazole (Maton 1989; Hirschowitz 1996). In those cases the BAO was less than 5 mEq/h and the patients were H. pylori negative. Hirschowitz (1996) observed mucosal relapses in 6 of his 26 (23%) patients treated with lansoprazole. The mechanism that causes mucosal damage in low acid conditions remains unknown. Hirschowitz (1995) therefore recommends frequent periodic evaluations with endoscopy, gastric analysis and clinical assessments 2 to 4 times a year during the first year of therapy, and twice a year thereafter.

5.3.4 Effects of elevated serum gastrin levels during treatment

It has been suggested that long-term administration of proton-pump inhibitors, may be associated with an increase in serum gastrin levels and that the trophic effect of this hormone on gastrointestinal organs could lead to ECL hyperplasia and carcinoid
tumour.

We observed additional increase in elevated serum gastrin twice in 2 of the 10 patients in our study but without a trophic effect on ECL cells. In one patient the rise of gastrin was probably due to tumour progression, whereas in the other achlorhydria may have been responsible. Studies with omeprazole showed conflicting results. Maton (1989) could not demonstrate any increase of gastrin levels in his long-term study of 40 ZES patients, whereas Cadiot (1994) observed a serum gastrin increase of up to 374% in some of his patients. The reason for this discrepancy is not clear but it is possible that Cadiot's patients had advanced disease. Patients with ZES are unlikely to develop increases in serum gastrin concentrations on the basis of acid reducing affect of PPI.

The trophic effect of gastrin on the development of carcinoid tumour remains controversial (Berkowitz 1991). In experimental settings, gastrin induces hyperplasia and hypertrophy of surface epithelial cells, stem cells, and the endocrine, enterochromaffin-like (ECL) cells (Modlin 1998). In rats, hypergastrinemia can lead to progressive argyrophil cell hyperplasia with the development of carcinoid tumours particularly when the antisecretory agents cause achlorhydria (Frucht 1991; Cadiot 1993). The rat ECL cell response to secretory inhibition, however, does not reflect that of humans (Sachs 1994).

The administration of PPI had no effects on gastric morphology in our series or in other investigations (Maton 1990). In the sporadic form of ZES, the development of gastric carcinoids has not been observed despite the fact that gastrin levels in gastrinoma patients tend to be much higher than those observed during PPI treatment (Cadiot 1993). ZES patients develop gastric carcinoids exclusively where the syndrome is associated with MEN I, indicating an additional genetic defect as a prerequisite for this type of tumour formation (Lehy 1989; Frucht 1991; Hirschowitz 1992; Solcia 1995; Bordi 1995).

Some investigators have associated elevated gastrin levels with an increased incidence of gastrointestinal adenocarcinoma (Sobhani 1993; Modlin 1998). However, the induction of specific types, such as colonic cancer, by the trophic effect of gastrin remains a topic of speculation. Rectal cell proliferation defects have been found in association with high serum gastrin levels similar to those observed in patients at high risk for colon cancer (Renga 1997). Conversely, an increase in colon cancer could not be documented by Orbuch and co-workers (1996) in an 11-year study of 97 ZES patients.
with extremely high gastrin levels. In conclusion, the risks of epithelial and endocrine cell neoplasia in hypergastrinemic patients receiving powerful antisecretory agents are not yet completely known, but should not be dismissed until more long-term follow-up studies are available.

Other side effects of long-term acid suppressive therapy, such as a decrease of vitamin B12 levels, have been described elsewhere but were not investigated in our trial. Termanini (1998) observed a significant decrease of serum vitamin B12 levels, but not of folate levels, in 6% of 111 ZES patients observed over a mean of 4.5 years on omeprazole. The B12 deficiency occurred mainly in patients with omeprazole-induced hyposcretion or complete achlorhydria. Therefore, he suggested that serum vitamin B12 levels should be monitored in ZES patients on long-term treatment with H+/K+-ATPase inhibitors.

5.4 Conclusions

We have shown that pantoprazole is a safe and effective proton pump inhibitor in the medium to long-term treatment of patients with the Zollinger-Ellison syndrome. Most patients required standard dosage of pantoprazole and prolonged increase in dosage requirement over the observation period was only necessarily in one. There appears to be a correlation between the level of BAO and dosage requirement to achieve safe control of the acid hypersecretory state. While regular clinical and endoscopic surveillance is necessary to avoid peptic ulcer related complications, the need for repeated BAO measurements to assess treatment efficacy remains uncertain.
6.1 Contemporary management algorithm (Figures 8 and 9)

6.1.1 Diagnosis

The initial diagnosis is biochemical. Serum gastrin level is the first step in patients with suspected ZES. Unless gastrin is in the diagnostic range of >1000 pg/ml, a secretin provocative test is mandatory to exclude other conditions mimicking the disease. Acid output studies are not readily available anymore as a diagnostic test but remains desirable to optimise medical therapy. All patients should be screened for the MEN I syndrome by measuring serum calcium and prolactin levels for hyperparathyroidism and a pituitary tumour.

6.1.2 Imaging

Abdominal US and spiral CT are routinely performed but the use of more sophisticated imaging will be determined by their availability at a given centre. Currently SRS is the most widely recommended test but endoscopic US in expert hands is a good substitute except for detecting tumours in ectopic sites. Arteriography with secretin stimulation and hepatic vein sampling is reserved mainly when the above tests are negative particularly when re-exploration is contemplated.

6.1.3 Treatment

Control of acid hypersecretion by a PPI is priority and a safe dose requirement needs to be determined. Since gastric acid secretion tests are no longer readily available, optimising acid suppression is now assessed by symptomatic control and endoscopic surveillance.

Patients with extensive disease (e.g. multiple liver metastases) and those with MEN I (after parathyroidectomy) should be treated conservatively. All other patients should undergo surgical exploration, including those where imaging procedures remain negative.
The emphasis of surgical therapy is on tumour debulking without resorting to major pancreatic and or liver resections. Patients who are cured by surgery should be monitored at 6 to 12 month intervals with serum gastrin estimations. Those who are not cured should continue with PP therapy with regular follow-up to ensure adequate acid control (preferably with BAO monitoring). Patients who are poorly controlled on high dose PPI therapy should be considered for re-exploration. Repeat exploration should be considered prior to surgery to identify ectopic tumours.

Further surgical strategy will depend on the tumour status at surgery; if no tumour is localized total gastrectomy should be considered when there is poor compliance such as experienced in third world countries. Some investigators may argue in favour of a vagotomy in this situation. In cases of residual disease, a further debulking procedure is indicated but there may be a place for a pancreatoco-duodenectomy with regional lymphadenectomy when the tumour bulk is located in the gastrinoma triangle.
6.2. Final conclusions

The GSH data on which this thesis was based has provided an unique opportunity to critically review the evolving treatment strategies in 40 patients with the ZES over a 20 years period. This study has vindicated the policy of debulking procedures in patients with the sporadic form of the disease and showed that the best results were achieved in those patients with solitary extrapancreatic extraintestinal gastrinoma while few patients with pancreatic primaries and those who were re-explored were cured. However, those patients who were not cured by debulking operations still benefited in terms of easier control of acid hypersecretion with PPI therapy. While the data has not allowed assessment of the effect of debulking operations on prognosis, the excellent long-term survival in the series without the development of overt liver metastases would seem to support this benefit claimed by other studies. This study also showed that total gastrectomy still has an important role to play in third world countries where close monitoring and reliable medical supplies may not be available.

The failure of pre-operative SRS to improve surgical cure rate despite a significantly improved detection rate of gastrinoma was disappointing. Indeed, some of the limitations of this test were exposed, in particular the failure to detect small and ectopic lying tumours. In the light of these findings, the role of this expensive test requires further evaluation before incorporating this as part of the routine pre-operative diagnostic work-up.

The medium to long-term study with pantoprazole confirmed the efficacy and safety of PPI therapy in the ZES. The study highlighted the correlation between the level of acid output and PPI dosage requirement which was appreciably higher in those patients with a BAO of >30 mEq/L.
6.3. **Recommendations for further research**

While PPIs have simplified the management of patients who are not cured by surgery, life-long maintenance treatment in a disease with an excellent prognosis remains problematic, particularly in third world countries. Future research should focus on improving the pre- and intra-operative methods of detecting small and multiple tumours by more sophisticated targeted imaging techniques. The ultimate goal should be to use this technology for target therapy particularly in cases with multiple and metastatic disease including those patients with the MEN I syndrome. In the meantime the role of a more aggressive surgical approach in the form of a pancreatoco-duodenectomy with regional lymphadectomy should be evaluated in selected patients with multifocal disease with or without regional lymph node metastases localized in the gastrinoma triangle.
“Mastersheet” of the 40 patients with Zollinger-Ellison syndrome treated at Groote Schuur Hospital 1980 - 1998
<table>
<thead>
<tr>
<th>No</th>
<th>Patient</th>
<th>Year</th>
<th>Age</th>
<th>Surgery before ZES Diagnosis</th>
<th>Surgery after ZES Diagnosis</th>
<th>Status</th>
<th>Follow-up years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEN I</td>
<td></td>
<td></td>
<td></td>
<td>1978 1 pancreatic gastrinoma; distal pancreatectomy, 2 LN next to pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1983 TG, 1 pos LN in specimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>FT</td>
<td>1978</td>
<td>39</td>
<td></td>
<td>1978 TG, 1 pos LN removed with specimen</td>
<td>1996 well</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>PB</td>
<td>1980</td>
<td>61</td>
<td></td>
<td>1980 truncal vagotomy, pyloroplasty</td>
<td>1998 died, carcinoma of the lung</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1981 1 periduodenal LN primary removed</td>
<td>cured for 27 mths, had then recurrence was on Losec 40 mg per day until 1995</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MEN I</td>
<td></td>
<td></td>
<td>1980 re-gastr</td>
<td>1981 parathyroidectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>truncal vagotomy</td>
<td>1982 TG (for peptic gastric outlet stenosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pos LN upper border of the pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>HJ</td>
<td>1982</td>
<td>61</td>
<td>recur PUD</td>
<td>no surgery (no gastrinoma localization)</td>
<td>1998 well on 30 mg lansoprazole</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1974 vagotomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1982 rev gastr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>SA</td>
<td>1982</td>
<td>14</td>
<td></td>
<td>1983 1 pos LN at duodenojejunal flexure</td>
<td>was cured for 35 mo, then recurrence</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pos LN peri-duodenal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1990 1 duodenal wall tumor and</td>
<td>1998 well on 30 mg lansoprazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pos LN on pancreas removed</td>
<td>SRS with hot spot next to right kidney (false-positive?)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1996 neg explorative laparotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>FR</td>
<td>1989</td>
<td>61</td>
<td></td>
<td>no surgery since diagnosis made</td>
<td>1998 caecal resection for carcinoma</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>CK</td>
<td>1983</td>
<td>45</td>
<td></td>
<td>1983 1 pancreas tumor, distal pancreatectomy</td>
<td>1998 well, multiple liver metastases</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1984 TG, 1 pos LN in specimen, liver metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Patient</td>
<td>Year ZES diag.</td>
<td>Age</td>
<td>Surgery before ZES Diag.</td>
<td>SURGERY after ZES Diagnosis</td>
<td>Status</td>
<td>Follow-up years</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>---------------</td>
<td>-----</td>
<td>--------------------------</td>
<td>----------------------------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>12</td>
<td>CB</td>
<td>1985</td>
<td>21</td>
<td>1982 polya gastr</td>
<td>1985 2 liver metastases removed</td>
<td>1985 Lost for FU (moved out of town)</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>KD</td>
<td>1985</td>
<td>38</td>
<td></td>
<td>1985 1 duodenal wall gastrinoma removed with 2 peripancreatic lymph nodes</td>
<td>1995 cured, normal gastrin</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>PS</td>
<td>1986</td>
<td>13</td>
<td></td>
<td>1989 vagotomy and pyloroplasty: 1 pos LN lesser curve 1 pos LN along left gastric artery 1 liver metastasis</td>
<td>1995 died (homicide) was on Losec 20 mg until 1995</td>
<td>9</td>
</tr>
<tr>
<td>16</td>
<td>JS</td>
<td>1986</td>
<td>11</td>
<td>1986 perfor DU 1986 trunc vag</td>
<td>1986 2 liver metastases removed TG (peptic duodenal stenosis)</td>
<td>1996 well, developed additional liver metastases as shown on CT scan</td>
<td>10</td>
</tr>
<tr>
<td>18</td>
<td>ZZ</td>
<td>1987</td>
<td>15</td>
<td></td>
<td>1987 1 gastric wall gastrinoma and 2 pos LN in the omentum removed</td>
<td>1988 cured 1995 clinically well</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>RL</td>
<td>1988</td>
<td>48</td>
<td></td>
<td>1989 TG, 1 pos LN in specimen</td>
<td>1993 died, obviously not ZES related</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>MP</td>
<td>1988</td>
<td>46</td>
<td></td>
<td>no surgery due to severe concomitant medical disease (liver cirrhosis)</td>
<td>1999 well on 30 mg lansoprazole SRS: one hot spot in duodenal area</td>
<td>11</td>
</tr>
<tr>
<td>No</td>
<td>Patient</td>
<td>Year ZES diag.</td>
<td>Age</td>
<td>Surgery before ZES Diag.</td>
<td>SURGERY after ZES Diagnosis</td>
<td>Status</td>
<td>Follow-up years</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
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<td>-----</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>--------</td>
<td>----------------</td>
</tr>
<tr>
<td>22</td>
<td>BS</td>
<td>1989</td>
<td>21</td>
<td>bleeding DU</td>
<td>1989 truncal vagotomy; 1 pancreatic tumor shelled out (containing pancreatic tissue) 1 pos LN from hepato-duodenal ligament</td>
<td>1992 lost to follow-up, normal gastrin for 27 mo until lost for FU</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>FD</td>
<td>1989</td>
<td>46</td>
<td>no surgery due to metastatic disease to the liver derived from a pancreatic primary</td>
<td></td>
<td>1992 died from ZES</td>
<td>3</td>
</tr>
<tr>
<td>24</td>
<td>GR</td>
<td>1989</td>
<td>53</td>
<td>1984 polygastr 1986 truncal vag 1987 revisio gastr</td>
<td>1989 TG (for 'geographical' reasons)</td>
<td>1997 well, on SRS one hot spot in the duodenal area US and CT negative</td>
<td>8</td>
</tr>
<tr>
<td>26</td>
<td>DD</td>
<td>1990</td>
<td>51</td>
<td>1990 1 pancreatic surface gastrinoma resected (extrapancreatic/extraintestinal/extralymphatic)</td>
<td>1995 cured, normal gastrin lost for follow-up</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>SD</td>
<td>1990</td>
<td>39</td>
<td>1993 1 duodenal wall gastrinoma resected 1996 3 pos LN (2 periduodenal, 1 on pancreas)</td>
<td>1998 well on 120 mg lanzoprazole</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>PL</td>
<td>1990</td>
<td>38</td>
<td>1992 negative explorative laparotomy 1996 1extraintestinal/extrapancreatic tumour on pancreatic body removed + 1 pos LN periduodenal</td>
<td>1998 well on 40 mg pantoprazole</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>KP</td>
<td>1991</td>
<td>43</td>
<td>refused surgery (no tumour localized)</td>
<td>1998 well, on Losec 40 mg per day</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>PV</td>
<td>1991</td>
<td>16</td>
<td>1991 1 pos LN from gastrohepatic lig removed</td>
<td>1997 cured for 4 yrs until lost for FU</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>TA</td>
<td>1991</td>
<td>45</td>
<td>no surgery, larger gastrinoma head of pancreas with liver metastases</td>
<td>1997 well, dose of Mx not known (living abroad)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Patient</td>
<td>Year ZES diag.</td>
<td>Age</td>
<td>Surgery before ZES Diag.</td>
<td>SURGERY after ZES Diagnosis</td>
<td>Status</td>
<td>Follow-up years</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>----------------</td>
<td>-----</td>
<td>--------------------------</td>
<td>----------------------------</td>
<td>--------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1990 perfor DU</td>
<td>2 duodenal wall gastrinomas removed with one insulinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1996 one somatostatinoma next to 2nd part of duodenum removed. Pancreatic tail gastrinoma not detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>PW</td>
<td>1992</td>
<td>32</td>
<td></td>
<td>1997 1 pos LN along lesser curve</td>
<td>1999 well on Mx, dosis not known patient is living abroad</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1994 TG for esophagitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>MV</td>
<td>1995</td>
<td>40</td>
<td>1995 V&amp;A</td>
<td>no surgery (no tumour localized)</td>
<td>1995 died, unlikely ZES related</td>
<td>0</td>
</tr>
<tr>
<td>37</td>
<td>JO</td>
<td>1995</td>
<td>74</td>
<td>1985 V&amp;A</td>
<td>no surgery</td>
<td>1999 well on 30 mg lansoprazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1995 hot spots on SRS in liver area CT and ultrasound negative</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>PW</td>
<td>1995</td>
<td>48</td>
<td>1992 perfor DU</td>
<td>no surgery</td>
<td>1999 well on 30 mg lansoprazole</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1995 perfor DU</td>
<td></td>
<td>1995 hot midline spot on SRS CT and ultrasound negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>RM</td>
<td>1995</td>
<td>58</td>
<td></td>
<td>1997 1 pos LN on surface of pancreatic body</td>
<td>1999 cured</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>RW</td>
<td>1996</td>
<td>56</td>
<td>1994 perfor DU</td>
<td>1997 1 duodenal wall tumour removed</td>
<td>1999 elevated gastrin, on PPIs</td>
<td>3</td>
</tr>
</tbody>
</table>
Legend to "Mastersheet of 40 ZES patients treated at Groote Schuur Hospital 1980-1998"

The "Mastersheet of 40 ZES patients" contains the relevant clinical information of all the ZES patients that were treated at the GIT Clinic.

The year numbers in the two columns "Surgery before ZES diagnosis" and "Surgery after ZES diagnosis" indicate one surgical procedure each.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastr</td>
<td>gastrectomy</td>
</tr>
<tr>
<td>PUD</td>
<td>peptic ulcer disease</td>
</tr>
<tr>
<td>TG</td>
<td>total gastrectomy</td>
</tr>
<tr>
<td>LN</td>
<td>lymph node</td>
</tr>
<tr>
<td>Rev gastr</td>
<td>revision gastrectomy</td>
</tr>
<tr>
<td>SRS</td>
<td>somatostatin receptor scintigraphy</td>
</tr>
<tr>
<td>PGV</td>
<td>proximal gastric vagotomy</td>
</tr>
<tr>
<td>BAO</td>
<td>basal acid output</td>
</tr>
<tr>
<td>MEN I</td>
<td>Multiple Endocrine Neoplasia type I</td>
</tr>
<tr>
<td>V&amp;A</td>
<td>vagotomy and antrectomy</td>
</tr>
<tr>
<td>DU</td>
<td>duodenal ulcer</td>
</tr>
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TABLES
Table Clin1. Characteristics of the 40 patients with Zollinger-Ellison syndrome treated at Groote Schuur Hospital

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no of ZES patients</td>
<td>40</td>
</tr>
<tr>
<td>Study period</td>
<td>1980 - 1998</td>
</tr>
<tr>
<td>Patients with MEN I syndrome</td>
<td>5/40 12.5%</td>
</tr>
<tr>
<td>Male sex</td>
<td>30/40 75%</td>
</tr>
<tr>
<td>Age at onset of symptoms (mean, yrs)</td>
<td>34.8 range 11-62</td>
</tr>
<tr>
<td>Age at diagnosis of ZES (mean, yrs)</td>
<td>38.9 range 13-62</td>
</tr>
<tr>
<td>ZES patients under age of 16</td>
<td>6/40 15%</td>
</tr>
<tr>
<td>Duration of symptoms before diagnosis (yrs)</td>
<td>3.8 range 0.2-22</td>
</tr>
<tr>
<td>Total number operated*</td>
<td>36/40 90%</td>
</tr>
<tr>
<td>Resection of gastrinomas</td>
<td>27 68%</td>
</tr>
<tr>
<td>Tumour debulking</td>
<td>9/21 43%</td>
</tr>
<tr>
<td>Cured &lt; 5 years**</td>
<td>5</td>
</tr>
<tr>
<td>Cured &gt; 5 years***</td>
<td>4</td>
</tr>
<tr>
<td>Cure rate in total series</td>
<td>9/40 23%</td>
</tr>
</tbody>
</table>

* Includes emergency surgery for complications of peptic ulcer disease and gastrinoma resections
** Refers to patients P14,18,39,22 and 31 of the “Mastersheet”
*** Refers to P5,13,26 and 28 of the “Mastersheet of 40 ZE Patients”
† Cure includes normal serum gastrin levels (<115 pg/ml) and a negative secretin test (increase of serum gastrin <200 pg/ml)
Table Clin2. Results of conventional imaging studies for the localisation of gastrinomas

<table>
<thead>
<tr>
<th>Test</th>
<th>No of tests performed*</th>
<th>No of pat.</th>
<th>false-negative</th>
<th>true-positive</th>
<th>false-positive</th>
<th>Sensitivity of each test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>47</td>
<td>40</td>
<td>39</td>
<td>8</td>
<td>-</td>
<td>17%</td>
</tr>
<tr>
<td>CT-scan</td>
<td>46</td>
<td>36</td>
<td>38</td>
<td>7</td>
<td>1</td>
<td>15%</td>
</tr>
<tr>
<td>Angiography</td>
<td>11</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>27%</td>
</tr>
<tr>
<td>Angiography with secretin injection</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>Overall</td>
<td><strong>105</strong></td>
<td><strong>82</strong></td>
<td><strong>19</strong></td>
<td><strong>3</strong></td>
<td></td>
<td><strong>18%</strong></td>
</tr>
</tbody>
</table>

* The table shows results per test, not per patient.

The somatostatin receptor scintigraphy was performed in a subgroup of 12 patients. See results on tables SR1-SR6.
Table Clin3. Types of surgical procedures performed before and after ZES diagnosis

<table>
<thead>
<tr>
<th>BEFORE ZES DIAGNOSIS</th>
<th>Number of procedures</th>
<th>AFTER ZES DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 77 (in 36 pts)</td>
<td></td>
</tr>
<tr>
<td>32 (20 pts)</td>
<td></td>
<td>45 (30 pts)</td>
</tr>
<tr>
<td>14 (10 pts)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>emergency procedures</td>
<td>18 (14 pts) Acid reducing procedures</td>
<td>31** (27 pts) local excision of gastrinoma</td>
</tr>
<tr>
<td></td>
<td>Total of 36 procedures in 30 patients</td>
<td></td>
</tr>
<tr>
<td>vagotomy</td>
<td>7 (7 pts)</td>
<td>8 (7 pts) vagotomy &amp; pyloroplasty</td>
</tr>
<tr>
<td>revision gastrectomy</td>
<td>3 (3 pts)</td>
<td></td>
</tr>
<tr>
<td>polya gastrectomy</td>
<td>4 (4 pts)</td>
<td></td>
</tr>
<tr>
<td>vagotomy/antrectomy</td>
<td>4 (4 pts)</td>
<td></td>
</tr>
<tr>
<td>total gastrectomy</td>
<td>1 (1pt)</td>
<td>10 (10 pts) total gastrectomy</td>
</tr>
</tbody>
</table>

Since most patients had several procedures during the course of their disease, they are listed repeatedly under different categories.

* One patient had a revision gastrectomy and a vagotomy at the same time, which increases the total number of operations to 19.

** Number includes also 4 total gastrectomies where a gastrinoma was resected incidentally with the gastrectomy specimen.

*** One patient had a vagotomy twice, another a vagotomy, followed by a total gastrectomy at a later stage. Some patients had both, a local gastrinoma resection combined with a vagotomy.
Table Clin4. Acid output and follow-up in 18 ZE patients with vagotomy

<table>
<thead>
<tr>
<th>No in Mastersheet</th>
<th>Procedure</th>
<th>Vagotomy† before/after diag.</th>
<th>Gastrinoma Resection</th>
<th>BAO* pre-vagotomy</th>
<th>BAO* post-vagotomy</th>
<th>Outcome post-vagotomy</th>
<th>Medical/Surgical treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.) 1</td>
<td>Vagotomy/pyloroplasty** before</td>
<td>-</td>
<td>N/A</td>
<td>32</td>
<td>DU</td>
<td>DU</td>
<td>TG (H2RA)</td>
</tr>
<tr>
<td>2.) 6</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>23</td>
<td>Stomal ulcerations</td>
<td>TG (H2RA)</td>
</tr>
<tr>
<td>3.) 7</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>15</td>
<td>2.8</td>
<td>Stomal erosions</td>
<td>Rx</td>
</tr>
<tr>
<td>4.) 8</td>
<td>HSV</td>
<td>before</td>
<td>-</td>
<td>39</td>
<td>6.8</td>
<td>Recurrent DU</td>
<td>Rx</td>
</tr>
<tr>
<td>5.) 16</td>
<td>Truncal vagotomy</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>2.3</td>
<td>Duodenal erosions</td>
<td>Rx</td>
</tr>
<tr>
<td>6.) 17</td>
<td>Truncal vagotomy</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>20</td>
<td>DU, oesophagitis</td>
<td>TG (H2RA)</td>
</tr>
<tr>
<td>7.) 24</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>43.5</td>
<td>N/A</td>
<td>Stomal ulcerations</td>
<td>TG (PPI)</td>
</tr>
<tr>
<td>8.) 28</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>33</td>
<td>5.0</td>
<td>3° Oesophagitis</td>
<td>TG (H2RA)</td>
</tr>
<tr>
<td>9.) 35</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>N/A</td>
<td>Oesophagitis, stricture</td>
<td>TG (H2RA)</td>
</tr>
<tr>
<td>10.) 36</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>N/A</td>
<td>DU</td>
<td>Rx</td>
</tr>
<tr>
<td>11.) 37</td>
<td>V/A</td>
<td>before</td>
<td>-</td>
<td>N/A</td>
<td>10.4</td>
<td>-</td>
<td>Rx</td>
</tr>
<tr>
<td>12.) 3</td>
<td>Vagotomy/pyloroplasty after</td>
<td>-</td>
<td>47</td>
<td>14</td>
<td>Duodenal erosions</td>
<td>Rx</td>
<td></td>
</tr>
<tr>
<td>13.) 4</td>
<td>Truncal vagotomy</td>
<td>after</td>
<td>-</td>
<td>37</td>
<td>2.9</td>
<td>DU</td>
<td>Rx</td>
</tr>
<tr>
<td>14.) 9</td>
<td>Truncal vagotomy</td>
<td>after</td>
<td>-</td>
<td>14</td>
<td>6.6</td>
<td>Recurrent DU</td>
<td>Rx</td>
</tr>
<tr>
<td>15.) 11</td>
<td>Vagotomy/pyloroplasty after</td>
<td>+</td>
<td>57</td>
<td>2.4</td>
<td>Duodenal fistula</td>
<td>TG (H2RA)</td>
<td></td>
</tr>
<tr>
<td>16.) 14</td>
<td>HSV</td>
<td>after</td>
<td>+</td>
<td>13.4</td>
<td>1.0</td>
<td>Recurrent DU</td>
<td>Truncal vagot.</td>
</tr>
<tr>
<td>17.) 15</td>
<td>Vagotomy/pyloroplasty after</td>
<td>+</td>
<td>22</td>
<td>8.3</td>
<td>Diarrhoea</td>
<td>Rx</td>
<td></td>
</tr>
<tr>
<td>18.) 22</td>
<td>Truncal vagotomy</td>
<td>after</td>
<td>+</td>
<td>8.6</td>
<td>0.8</td>
<td>-</td>
<td>Lost for FU</td>
</tr>
</tbody>
</table>

Mean 28.6 (SD16.4)  5.06 (SD 4.0; p=0.001)

The patient numbers refer to the "Mastersheet of 40 ZES Patients". N/A, data not available; * BAO in mEq/h; H2RA, histamine receptor 2 antagonist; PPI, proton pump inhibitor. H2RA and PPI indicate the medication the patient was on when he had the total gastrectomy. ** not performed at Groote Schuur Hospital; HSV, highly selective vagotomy; DU, duodenal ulcer; †, vagotomy before or after the ZES diagnosis was made. Mean, standard deviation and significance (paired t-test) includes only cases with pre-and postoperative basal acid output. * This patient had additional surgery the same year after the two vagotomies with resection of a gastrinoma lymph node (see "Mastersheet"). Rx, medication, either H2RA or PPIs.
### Table Clin5. Results of debulking operations according to anatomical location

<table>
<thead>
<tr>
<th>Patient no in “Mastersheet”</th>
<th>Number of operations</th>
<th>Cure status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.) Pancreas (superficial) with lymph node</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>2.) Extrapancreatic/extraintestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- no metastases</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>- lymph node metastases</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>3.) Lymph nodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- solitary</td>
<td>4,5,14,31,34,39</td>
<td>6</td>
</tr>
<tr>
<td>- multiple</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>- plus liver metastases</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>4.) Liver metastases, no primaries</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>5.) Duodenal and gastric wall*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- without LN metastases</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>- with LN metastases</td>
<td>8,11,13,18,27,28,35</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

Note: solitary lymph nodes and extrapancreatic/extraintestinal gastrinomas combined

8 | 5 (62.5%)

*Cure status: 1 = > 5 years; 2 = < 5 years; 3 = cure but lost for follow-up; 4 = cure with recurrence.
Excluded were patients (a) who had surgery before a policy of debulking operation was adopted (2,6,10,19); (b) those with MEN I; (c) when only incomplete information was available (12,34,40).
Numbers in bold indicate patients who were cured after surgery.
### Table Clin6. OUTCOME OF SURGICAL DEBULKING (I)

#### A. Cure > 5 years - Clinical & normal gastrin and/or secretin test

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age Yrs</th>
<th>Tumour site</th>
<th>Total FU Years</th>
<th>Normal Gastrin Years</th>
<th>Clinically</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>VM</td>
<td>27</td>
<td>SLN</td>
<td>17</td>
<td>17 yrs</td>
<td>well</td>
<td>No Rx</td>
</tr>
<tr>
<td>13</td>
<td>DK</td>
<td>38</td>
<td>DW + 2LN</td>
<td>10</td>
<td>6 yrs</td>
<td>well</td>
<td>No Rx</td>
</tr>
<tr>
<td>26</td>
<td>DD</td>
<td>51</td>
<td>extraintest/expancr.</td>
<td>5</td>
<td>5 yrs</td>
<td>well</td>
<td>No Rx</td>
</tr>
<tr>
<td>28</td>
<td>DM</td>
<td>49</td>
<td>2 DW + 1 LN</td>
<td>8</td>
<td>5 yrs</td>
<td>well</td>
<td>No Rx, TG</td>
</tr>
<tr>
<td>31</td>
<td>PV</td>
<td>16</td>
<td>SLN</td>
<td>4</td>
<td>4 yrs</td>
<td>well</td>
<td>No Rx</td>
</tr>
</tbody>
</table>

#### B. Cure Gastrin < 5 yrs, Clinical > 5 yrs

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age Yrs</th>
<th>Tumour site</th>
<th>Total FU Years</th>
<th>Normal Gastrin Years</th>
<th>Clinically</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>JS</td>
<td>28</td>
<td>SLN</td>
<td>9</td>
<td>1 yr 5 mo</td>
<td>well</td>
<td>No Rx</td>
</tr>
<tr>
<td>18</td>
<td>ZZ</td>
<td>15</td>
<td>1 gastric + 2 LN</td>
<td>8</td>
<td>1 yr</td>
<td>well</td>
<td>No Rx</td>
</tr>
<tr>
<td>39</td>
<td>RM</td>
<td>58</td>
<td>SLN</td>
<td>5</td>
<td>4 yrs</td>
<td>well</td>
<td>No Rx</td>
</tr>
</tbody>
</table>

#### C. Cure < 5 years, Lost to follow-up < 5 years

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age Yrs</th>
<th>Tumour site</th>
<th>Total FU Years</th>
<th>Normal Gastrin Years</th>
<th>Clinically</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>SB</td>
<td>21</td>
<td>Pancreas + 1LN</td>
<td>2 yrs 3 mo</td>
<td>2 yrs 3 mo</td>
<td>2 yrs 3 mo</td>
<td>No Rx, lost</td>
</tr>
</tbody>
</table>
## OUTCOME OF SURGICAL DEBULKING (II)

### D. Initial cure with recurrence

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age Yrs</th>
<th>Tumour site</th>
<th>Interval to recurrence Yrs</th>
<th>Total Follow up Years</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>SA</td>
<td>14</td>
<td>1° 2x LN/ 2° 2LN</td>
<td>3 yrs (35 mo)</td>
<td>16</td>
<td>Lanzor 30 mg/day</td>
</tr>
<tr>
<td>4</td>
<td>EP</td>
<td>36</td>
<td>SLN</td>
<td>2 yrs (27 mo)</td>
<td>15</td>
<td>Died alcohol related</td>
</tr>
</tbody>
</table>

### E. Uncertain outcome/ incomplete data

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age Yrs</th>
<th>Tumour site</th>
<th>Total FU Years</th>
<th>Gastrin</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>BC</td>
<td>21</td>
<td>2 liver metastases</td>
<td>lost</td>
<td>Lost</td>
<td>Lost</td>
</tr>
<tr>
<td>34</td>
<td>PW</td>
<td>32</td>
<td>SLN</td>
<td>3</td>
<td>Normal BAO post-op</td>
<td>Recurrent DUs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No gastrin value</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>RW</td>
<td>56</td>
<td>1 DW</td>
<td>3</td>
<td>Normal postop gastrin 186 ng/ml at 3 yrs</td>
<td>PPI for heartburn</td>
</tr>
</tbody>
</table>
### OUTCOME OF SURGICAL DEBULKING (III)

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Age (yrs)</th>
<th>Tumour site</th>
<th>Total FU (yrs)</th>
<th>Additional treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.</td>
<td>No cure after debulking procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>EM</td>
<td>35</td>
<td>DW + 2 LN</td>
<td>5</td>
<td>V+P</td>
<td>died, not ZES related</td>
</tr>
<tr>
<td>15</td>
<td>PS</td>
<td>15</td>
<td>2 LN, 1 LM</td>
<td>9</td>
<td>V + P</td>
<td>died - homicide</td>
</tr>
<tr>
<td>16</td>
<td>SJ</td>
<td>11</td>
<td>2 LM</td>
<td>10</td>
<td>TG</td>
<td>well</td>
</tr>
<tr>
<td>17</td>
<td>RA</td>
<td>49</td>
<td>2 LN</td>
<td>5</td>
<td>TG</td>
<td>death not ZES related</td>
</tr>
<tr>
<td>27</td>
<td>SD</td>
<td>39</td>
<td>1 DW, 2 LN</td>
<td>8</td>
<td>-</td>
<td>well 120 lanzoprazole</td>
</tr>
<tr>
<td>29</td>
<td>PL</td>
<td>38</td>
<td>1 extraintest, 1 LN</td>
<td>8</td>
<td>-</td>
<td>well 40 mg pantoloc</td>
</tr>
<tr>
<td>35</td>
<td>KR</td>
<td>49</td>
<td>1 DW, 2 LN</td>
<td>4</td>
<td>TG</td>
<td>well</td>
</tr>
<tr>
<td>G.</td>
<td>No cure, no formal debulking procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>RP (MEN)</td>
<td>29</td>
<td>Pancreas, LN, TG</td>
<td>1</td>
<td>TG</td>
<td>died, cause unknown</td>
</tr>
<tr>
<td>33</td>
<td>CP</td>
<td>52</td>
<td>Pancreas, 2 DW</td>
<td>5</td>
<td>-</td>
<td>died post-operatively</td>
</tr>
<tr>
<td>6</td>
<td>JP (MEN)</td>
<td>13</td>
<td>1 LN, TG</td>
<td>15</td>
<td>TG</td>
<td>well</td>
</tr>
<tr>
<td>10</td>
<td>CK</td>
<td>45</td>
<td>Pancreas, LM, LN, TG</td>
<td>15</td>
<td>TG</td>
<td>well</td>
</tr>
<tr>
<td>2</td>
<td>FT</td>
<td>39</td>
<td>1 LN</td>
<td>18</td>
<td>TG</td>
<td>well</td>
</tr>
<tr>
<td>19</td>
<td>LR</td>
<td>48</td>
<td>1 LN</td>
<td>5</td>
<td>TG</td>
<td>died (unrelated)</td>
</tr>
</tbody>
</table>

TG, total gastrectomy; DW, duodenal wall tumor; LN, lymph node; SLN, solitary lymph node; yrs, years; mo, month(s); no Rx, no treatment; PPI, proton pump inhibitor.
<table>
<thead>
<tr>
<th>Tumour site</th>
<th>Pat No on “Mastersheet”</th>
<th>Number of ZE patients cured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surgical exploration:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic primary gastrinomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with lymph node metastases</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>with LN and liver metastases</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>MEN I</td>
<td>1,33</td>
<td></td>
</tr>
<tr>
<td>Extrapancreatic/extraintestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without metastases</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>with LN metastases</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph nodes (without primary)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>solitary</td>
<td>5,14,31,39, 4,34, (2),(19)</td>
<td></td>
</tr>
<tr>
<td>multiple</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>plus liver metastases</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>MEN I</td>
<td>(6)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Liver gastrinoma/metastases, no primary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12,16,</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MEN I</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td><em><em>Duodenal (7) and gastric wall (1</em>)</em>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without LN metastases</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>with LN metastases</td>
<td>18*, 8,11,13, 27,28,35</td>
<td></td>
</tr>
<tr>
<td><strong>No location at surgery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before diagnosis of ZES</td>
<td>9,37,38</td>
<td>3</td>
</tr>
<tr>
<td>no attempt at location</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>attempted at location</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Location by imaging (no surgery)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No tumour found</td>
<td>7,20,21,30,36</td>
<td>5</td>
</tr>
<tr>
<td>Pancreas and liver metastases</td>
<td>23,32</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td><strong>23%</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The patient numbers refer to the “Mastersheet of 40 ZES Patients”
* Patient numbers in bold indicate patients that were cured after surgical gastrinoma removal
( ) Patients had no formal debulking operations
** or 4/6 (67%) if patients 2 and 19 are not considered
### Table Clin8. Clinical data including outcome in ZES patients with liver metastases

<table>
<thead>
<tr>
<th>Liver tumours/metastases associated with</th>
<th>Total n</th>
<th>Patient No in &quot;Mastersheet&quot;</th>
<th>Surgery</th>
<th>Outcome (years after the diagnosis of LM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.) Pancreatic primary</td>
<td>3</td>
<td>10</td>
<td>pancreatic tail tumour resection</td>
<td>well 14 yrs, multiple LM during FU died from ZES, multiple LM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>none</td>
<td>well 6 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>2.) No primary</td>
<td>3</td>
<td>12</td>
<td>2 LM removed</td>
<td>lost for FU same year</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>2 LM removed, TG</td>
<td>well 10 yrs, more LM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25*</td>
<td>TG</td>
<td>well 10 years with LM</td>
</tr>
<tr>
<td>3.) No primary, but positive lymph nodes</td>
<td>3</td>
<td>6*</td>
<td>LN upper border pancreas</td>
<td>two hot spots on SRS left liver lobe died of homicide after 6 yrs FU</td>
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<td></td>
<td></td>
<td>15</td>
<td>two gastric LN removed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>37</td>
<td>V&amp;A</td>
<td>two liver spots on SRS</td>
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</table>

All metastases were confirmed histologically except the two cases that had hot spots on SRS. The follow-up started at the time of the diagnosis of the liver metastases, not at the time of the ZES diagnosis. The patient numbers refer to the "Mastersheet of 40 ZES patients". LM, liver metastasis; yrs, years; TG, total gastrectomy; V&A, vagotomy and antrectomy; SRS, somatostatin receptor scintigraphy; LN, lymph node; FU, follow-up. * Patient with MEN I syndrome. More follow-up data are presented in the "Mastersheet of 40 ZE patients".
Table Clin9. Clinical features of ZES patients
– A literature review –

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<tr>
<td>Total patients</td>
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<td>26</td>
<td>40</td>
<td>144</td>
<td>165</td>
<td>40</td>
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<td>MEN type I, %</td>
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<td>18</td>
<td>23</td>
<td>24</td>
<td>18</td>
<td>13</td>
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<tr>
<td>Duration of symptoms before diagnosis, years</td>
<td>1-4</td>
<td>5.8</td>
<td>6.5</td>
<td>ND</td>
<td>6.4</td>
<td>3.8</td>
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<td>Initial symptoms, %</td>
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<tr>
<td>Abdominal pain</td>
<td>93</td>
<td>73</td>
<td>98</td>
<td>26</td>
<td>24</td>
<td>82</td>
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<td>Pain &amp; diarrhoea</td>
<td>30</td>
<td>19</td>
<td>28</td>
<td>49</td>
<td>55</td>
<td>22</td>
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<td>Diarrhoea only</td>
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<td>27</td>
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<td>15</td>
<td>18</td>
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<td>Mean age at onset, years</td>
<td>ND</td>
<td>50</td>
<td>50</td>
<td>47</td>
<td>45</td>
<td>34</td>
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<td>Sex, % male</td>
<td>60</td>
<td>50</td>
<td>60</td>
<td>68</td>
<td>8</td>
<td>75</td>
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</table>
- A literature review -

<table>
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<tr>
<th>References*</th>
<th>Year of publication</th>
<th>No. of Patients</th>
<th>Length of Follow-up (years)</th>
<th>No. of Patients Cured</th>
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<td>3</td>
<td>0.5-5</td>
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<td>Oberhelman</td>
<td>1972</td>
<td>4</td>
<td>0.1-11</td>
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<td>Deveney</td>
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<td>1980</td>
<td>40</td>
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<td>Bonfils</td>
<td>1981</td>
<td>92</td>
<td>1-6</td>
<td>5</td>
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<td>Wilson SD</td>
<td>1982</td>
<td>28</td>
<td>3-21</td>
<td>5</td>
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<tr>
<td>Friessen</td>
<td>1982</td>
<td>23</td>
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<td>Wolfe</td>
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<td>18</td>
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<td>5</td>
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<td>Malagelada</td>
<td>1983</td>
<td>53</td>
<td>3-10</td>
<td>7</td>
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<td>Stabile</td>
<td>1985</td>
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<td>1.5-10</td>
<td>6</td>
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<tr>
<td>Norton</td>
<td>1986</td>
<td>52</td>
<td>0.5-4</td>
<td>7</td>
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<tr>
<td>Vogel</td>
<td>1987</td>
<td>22</td>
<td>0.6-15</td>
<td>8</td>
</tr>
<tr>
<td>Kaplan</td>
<td>1990</td>
<td>27</td>
<td>0.7-16</td>
<td>6</td>
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<tr>
<td>Farley</td>
<td>1992</td>
<td>90</td>
<td>N/A</td>
<td>10</td>
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<tr>
<td>Norton</td>
<td>1992</td>
<td>56</td>
<td>1-12</td>
<td>41</td>
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<td>Ellison EC</td>
<td>1995</td>
<td>34</td>
<td>5-14</td>
<td>6</td>
</tr>
<tr>
<td>Norton</td>
<td>1999</td>
<td>151</td>
<td>8-12</td>
<td>52</td>
</tr>
<tr>
<td><strong>Own series</strong></td>
<td><strong>1998</strong></td>
<td><strong>40</strong></td>
<td><strong>1-20</strong></td>
<td><strong>9</strong></td>
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</table>

Modified from Anderson (1989) and Ellison (1995). Most authors did not distinguish in their publications between cure <5 or >5 years. * Refers to ‘References’ section.
* 43% in patients who had tumour debulking.
Table Clin11. Overview on ZES/MEN I patients treated at GSH

<table>
<thead>
<tr>
<th>No</th>
<th>Patient No in “Mastersheet”</th>
<th>Parathyroid gland involvement</th>
<th>Pituitary tumours</th>
<th>Pancreatic endocrine tumours</th>
<th>Status</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1978 parathyroidectomy</td>
<td>1983 CCT normal</td>
<td>1978 pancreatic gastrinoma</td>
<td>1992 died, cause unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 2 LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1983 TG, 1 LN in specimen</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1981 parathyroidectomy</td>
<td>1981 prolactin up</td>
<td>1982 TG, removal gastrinoma</td>
<td>1997 well, on SRS 2 hot liver spots</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCT abnormal sella</td>
<td></td>
<td>LN upper border pancreas</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1983 hyperparathyroidism</td>
<td>1983 prolactin up</td>
<td>refused surgery despite PUD</td>
<td>1989 died, lung cancer (?)</td>
</tr>
<tr>
<td></td>
<td>no surgery</td>
<td></td>
<td></td>
<td>1983 BAO 30 mEq/h,</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MAO 46 mEq/h</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>pituitary tumour prolactin up</td>
<td></td>
<td>1988 gastrin 1000 pg/ml</td>
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<td></td>
<td></td>
<td>no Sx</td>
<td></td>
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<td>5</td>
<td>33</td>
<td>1991 parathyroidectomy</td>
<td>1984 prolactin up</td>
<td>1991 2 duodenal wall tumours+</td>
<td>1996 died postop. after septic complications</td>
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<tr>
<td></td>
<td></td>
<td>destruction of sella</td>
<td></td>
<td>insulinoma removed</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1996 1 somatostatinoma</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pancreatic gastrinoma</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>found at autopsy</td>
<td></td>
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</table>

TG, total gastrectomy; PUD, peptic ulcer disease; BAO, basal acid output; MAO, maximal acid output; SRS, somatostatin receptor scintigraphy; LN, lymph node; CCT, cranial computerized tomography.
<table>
<thead>
<tr>
<th>No</th>
<th>No in &quot;Mastersheet&quot;</th>
<th>Year of surgery</th>
<th>Gastrinoma location</th>
<th>Size (mm)</th>
<th>Outcome</th>
<th>p53</th>
<th>bcl2</th>
<th>Mib1/K67 (Waf1/CIP1)</th>
<th>p21</th>
<th>PCNA</th>
<th>CD44</th>
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<tr>
<td>1</td>
<td>22</td>
<td>1989</td>
<td>pancreas</td>
<td>18</td>
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<td>-</td>
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<td>1.1</td>
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<td>weak</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>LN</td>
<td>10</td>
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<td>-</td>
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<td>1.0</td>
<td>-</td>
<td>mod</td>
<td>strong</td>
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<td>2</td>
<td>8</td>
<td>1983</td>
<td>LN</td>
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<td></td>
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<td>duodenum</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>mod</td>
<td>strong</td>
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<td>LN</td>
<td>25</td>
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<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>mod</td>
<td>strong</td>
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<td>3</td>
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<td>20</td>
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<td>+</td>
<td>+</td>
<td>2.4</td>
<td>+</td>
<td>strong</td>
<td>weak</td>
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<td>5</td>
<td>lost for FU</td>
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<td>1</td>
<td>-</td>
<td>strong</td>
<td>strong</td>
</tr>
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<td>5</td>
<td>27</td>
<td>1993</td>
<td>duodenum</td>
<td>15</td>
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<td>-</td>
<td>strong</td>
<td>strong</td>
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<td>-</td>
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<td>strong</td>
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<td>weak</td>
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<td>1987</td>
<td>LN</td>
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<td>-</td>
<td>-</td>
<td>2.9</td>
<td>-</td>
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<td>weak</td>
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<td>8</td>
<td>31</td>
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<td>LN</td>
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<td>-</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
<td>weak</td>
<td>strong</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>1987</td>
<td>LN</td>
<td>10</td>
<td>cured</td>
<td>+</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
<td>weak</td>
<td>strong</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>1989</td>
<td>LN</td>
<td>14</td>
<td>died, not rel.</td>
<td>+</td>
<td>+</td>
<td>1.6</td>
<td>+</td>
<td>strong</td>
<td>weak</td>
</tr>
<tr>
<td>No</td>
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<td>Year of gastrinoma</td>
<td>Gastrinoma location</td>
<td>Size (mm)</td>
<td>Outcome</td>
<td>p53</td>
<td>bcl2</td>
<td>Mib1/ K67</td>
<td>p21</td>
<td>PCNA</td>
<td>CD44</td>
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<td>0.5</td>
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<td>weak</td>
<td>weak</td>
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<td>17</td>
<td>1986</td>
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<td>5</td>
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<td>+</td>
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<td>-</td>
<td>strong</td>
<td>weak</td>
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<td>LN</td>
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<td>died, nor rel.</td>
<td>+</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>mod</td>
<td>strong</td>
</tr>
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<td>5</td>
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<td>LN</td>
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<td>-</td>
<td>strong</td>
<td>weak</td>
</tr>
<tr>
<td>15</td>
<td>28</td>
<td>1991</td>
<td>duodenum</td>
<td>7</td>
<td>cured</td>
<td>+</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
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<td>16</td>
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<td>17</td>
<td>35</td>
<td>1994</td>
<td>LN</td>
<td>12</td>
<td>died, not rel.</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>strong</td>
<td>strong</td>
</tr>
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<td>18</td>
<td>10</td>
<td>1984</td>
<td>LN</td>
<td>15</td>
<td>not cured</td>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>mod</td>
<td>strong</td>
</tr>
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<td>19</td>
<td>33 (MEN I)</td>
<td>1991</td>
<td>duodenum</td>
<td>10</td>
<td>died, not rel.</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>+</td>
<td>weak</td>
<td>weak</td>
</tr>
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</table>

The staining results are summarized in Table Bio2

+, positive staining; -, negative staining; LN, lymph node; temp, temporarily; not rel., not related to ZES; FU, follow-up; Mx, medication.
Table Bio2. Gastrinoma location and corresponding marker expression

<table>
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<tr>
<th>Location</th>
<th>n</th>
<th>p53 positive</th>
<th>bcl-2 positive</th>
<th>Mib1/K67 positive cells</th>
<th>p21 (Waf1/CIP1) staining intensity</th>
<th>PCNA staining intensity</th>
<th>CD44 staining Intensity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>15</td>
<td>5 (33)</td>
<td>2 (13)</td>
<td>&lt;1% 4 27</td>
<td>weak 7 47</td>
<td>weak 7 47</td>
<td>weak 7 47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-2% 9 60</td>
<td>mod. 4 27</td>
<td>mod. 4 27</td>
<td>strong 8 53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;2% 2 13</td>
<td>strong 4 27</td>
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<tr>
<td>Pancreas</td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1-2% 1 50</td>
<td>strong 2 100</td>
<td>weak 2 100</td>
<td>weak 2 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;2% 1 50</td>
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</tr>
<tr>
<td>Duodenum</td>
<td>6</td>
<td>1 (17)</td>
<td>(0)</td>
<td>&lt;1% 5 83</td>
<td>weak 1 17</td>
<td>weak 2 33</td>
<td>weak 2 33</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>1-2% 1 50</td>
<td>mod. 3 50</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>&gt;2% 1 17</td>
<td>strong 2 33</td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>(0)</td>
<td>(0)</td>
<td>&lt;1% 1 100</td>
<td>strong 1 100</td>
<td>strong 1 100</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>24</td>
<td>7 (29)</td>
<td>3 (13)</td>
<td>&lt;1% 10 42</td>
<td>weak 8 33</td>
<td>weak 11 46</td>
<td>weak 11 46</td>
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<td></td>
<td></td>
<td>1-2% 10 42</td>
<td>mod. 7 29</td>
<td>mod. 13 54</td>
<td>mod. 13 54</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;2% 4 16</td>
<td>strong 9 37</td>
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</table>
Table Bio3. Expression of biological markers in cured versus non-cured ZES patients

<table>
<thead>
<tr>
<th></th>
<th>p53 positive n %*</th>
<th>bcl-2 positive n %</th>
<th>Mib1/Ki67 positive cells n %</th>
<th>p21 positive n %</th>
<th>PCNA n %</th>
<th>CD44 n %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cured</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 patients</td>
<td>3 30</td>
<td>1 10</td>
<td>&lt;1% 3 30</td>
<td>1 10</td>
<td>weak 4 40</td>
<td>weak 6 60</td>
</tr>
<tr>
<td>10 specimens</td>
<td>1-2% 5 50</td>
<td></td>
<td></td>
<td></td>
<td>mod 2 20</td>
<td>strong 4 40</td>
</tr>
<tr>
<td></td>
<td>&gt;2% 2 20</td>
<td></td>
<td></td>
<td></td>
<td>strong 4 40</td>
<td></td>
</tr>
<tr>
<td><strong>Not cured</strong></td>
<td>4 29</td>
<td>2 14</td>
<td>&lt;1% 7 50</td>
<td>4 28</td>
<td>weak 4 28</td>
<td>weak 5 36</td>
</tr>
<tr>
<td>11 patients</td>
<td>1-2% 5 36</td>
<td></td>
<td></td>
<td></td>
<td>mod 5 36</td>
<td>strong 9 64</td>
</tr>
<tr>
<td>14 specimens</td>
<td>&gt;2% 2 14</td>
<td></td>
<td></td>
<td></td>
<td>strong 5 36</td>
<td></td>
</tr>
</tbody>
</table>

* n and percentages refer to specimens, not to the number of patients.
Table Bio4. Status of biological markers in ZES patients with multiple gastrinomas

<table>
<thead>
<tr>
<th>Gastrinoma location</th>
<th>p53</th>
<th>bcl-2</th>
<th>Mib1/Ki67 % positive cells</th>
<th>Waf1</th>
<th>PCNA Staining Intensity</th>
<th>CD44 % of positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pat I (22)</td>
<td></td>
<td></td>
<td>1.1</td>
<td>+</td>
<td>strong</td>
<td>weak</td>
</tr>
<tr>
<td>LN</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>moderate</td>
<td>strong</td>
</tr>
<tr>
<td>Pat II (13)</td>
<td></td>
<td></td>
<td>0.5</td>
<td>-</td>
<td>moderate</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Duodenal wall</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>weak</td>
<td>5-10</td>
</tr>
<tr>
<td>LN</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
<td>weak</td>
<td></td>
</tr>
<tr>
<td>Pat III (35)</td>
<td></td>
<td></td>
<td>0.2</td>
<td>-</td>
<td>moderate</td>
<td>strong</td>
</tr>
<tr>
<td>Duodenal wall</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>+</td>
<td>strong</td>
<td>weak</td>
</tr>
<tr>
<td>LN</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pat IV (8)</td>
<td></td>
<td></td>
<td>0.5</td>
<td>-</td>
<td>weak</td>
<td>strong</td>
</tr>
<tr>
<td>LN (1982)</td>
<td>-</td>
<td>-</td>
<td>2.3</td>
<td>-</td>
<td>moderate</td>
<td>strong</td>
</tr>
<tr>
<td>Duodenal wall</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN (both 1990)</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>moderate</td>
<td>strong</td>
</tr>
</tbody>
</table>

Patterns of marker expression in 4 ZE patients who had a primary gastrinoma and a lymph node removed at the same time, or as in patient IV, on two different occasions. LN, lymph node
(0), patient number refers to the “Mastersheet of 40 ZES Patients”
Table SR1. Results of conventional imaging studies and somatostatin receptor scintigraphy in 12 ZES patients

<table>
<thead>
<tr>
<th>Patient no in “Mastersheet”</th>
<th>Conventional Imaging studies (Number of lesions)</th>
<th>Number of SRS hot spots</th>
<th>Location of hot spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.) 39</td>
<td>neg</td>
<td>1</td>
<td>Pancreas body region</td>
</tr>
<tr>
<td>2.) 33</td>
<td>Angiography**</td>
<td>false-negative</td>
<td>Inferior to right kidney</td>
</tr>
<tr>
<td>3.) 8</td>
<td>neg</td>
<td>false positive*</td>
<td>Duodenal area</td>
</tr>
<tr>
<td>4.) 27</td>
<td>neg</td>
<td>1</td>
<td>Duodenal area</td>
</tr>
<tr>
<td>5.) 40</td>
<td>neg</td>
<td>1</td>
<td>Duodenal area</td>
</tr>
<tr>
<td>6.) 29</td>
<td>CT (1) US (1)</td>
<td>2</td>
<td>Pancreatic body &amp; duodenal area</td>
</tr>
<tr>
<td>7.) 34</td>
<td>neg</td>
<td>1</td>
<td>Upper pancreatic border</td>
</tr>
<tr>
<td>8.) 21</td>
<td>neg</td>
<td>1</td>
<td>4th part of duodenum</td>
</tr>
<tr>
<td>9.) 37</td>
<td>neg</td>
<td>1</td>
<td>Focus left lobe of the liver</td>
</tr>
<tr>
<td>10.) 38</td>
<td>neg</td>
<td>1*</td>
<td>Hot spot mid abdominal region</td>
</tr>
<tr>
<td>11.) 24</td>
<td>neg</td>
<td>1</td>
<td>2nd part of duodenum/ pancreatic head</td>
</tr>
<tr>
<td>12.) 6</td>
<td>neg</td>
<td>2</td>
<td>Hot spots over left liver lobe</td>
</tr>
</tbody>
</table>

Comparison of conventional imaging studies (CT scan, transabdominal ultrasound, and occasionally angiography) and somatostatin receptor scintigraphy (SRS). The first seven patients underwent surgery (further details next table SR2), the remaining five were managed with proton pump inhibitors.

CT, computerized tomography; US, transabdominal ultrasound.

* The first scan with 165 Mbq was negative, whereas the second, with an increased dose of 270 Mbq, became positive.

** SRS remained negative despite a dose increase of the radiopharmaceutical from 160 Mbq to 240 Mbq. A selective angiography with secretin stimulation and portal venous sampling revealed a tumour in the pancreatic body/tail region.
### Table SR2. Results of conventional imaging studies, somatostatin receptor scintigraphy, and surgery in seven ZES patients

<table>
<thead>
<tr>
<th>Patient “Master gastrinoma resection”</th>
<th>Previous Conventional imaging studies (Number of lesions)</th>
<th>Conventional imaging studies</th>
<th>Number of SRS hot spots</th>
<th>Number of resected tumours</th>
<th>Tumour Localization*</th>
<th>Size (mm)</th>
<th>Post-operative cure (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 39</td>
<td>neg</td>
<td></td>
<td>1</td>
<td>1</td>
<td>Surface pancreas</td>
<td>20</td>
<td>yes (30)</td>
</tr>
<tr>
<td>2) 33</td>
<td>DW Angiography**</td>
<td>false-negative</td>
<td>1</td>
<td>Not located</td>
<td>Tail of pancreas</td>
<td>12</td>
<td>no</td>
</tr>
<tr>
<td>3) 8</td>
<td>LNs; DW neg</td>
<td>false positive†</td>
<td>-</td>
<td></td>
<td>Not located</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
<td>4) 27</td>
<td>DW neg</td>
<td>1</td>
<td>3</td>
<td>LN 2nd part duodenum</td>
<td>LN 3rd part duodenum</td>
<td>12</td>
<td>no</td>
</tr>
<tr>
<td>5) 40</td>
<td>neg</td>
<td>1</td>
<td>1</td>
<td>Duodenum</td>
<td>LN Surface pancreas</td>
<td>6</td>
<td>yes (30)</td>
</tr>
<tr>
<td>6) 29</td>
<td>Neg. Explo. CT (1) US (1)</td>
<td>2</td>
<td>2</td>
<td>Pancreas; duodenum</td>
<td>15;35</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>7) 34</td>
<td>neg</td>
<td>1</td>
<td>1</td>
<td>Lesser curve</td>
<td>12</td>
<td>yes (12)* **</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of conventional imaging studies (CT scan, transabdominal ultrasound, and angiography), somatostatin receptor scintigraphy, and surgical findings in the 7 ZES patients. The overview also includes the data of previous surgery for gastrinoma resection. All hot spots corresponded to the tumours found at surgery.

SRS, somatostatin receptor scintigraphy; CT: computerized tomography; US: transabdominal ultrasound; DW, duodenal wall; LN, gastrinoma containing lymph node; Neg. Explo, negative exploration. † an atypical hot spot on SRS inferior to the right kidney could not be detected at surgery; MEN I: multiple endocrine neoplasia type I; LN: lymph node; L: left; R: right; * Italic, site and size of tumours detected at surgery but not imaged by SRS. **Angiography with secretin injection and consecutive gastrin measurements in the hepatic vein. *** presumably recurrent disease after 12 months.
Tab SR3. Comparison of pre- and postoperative serum gastrin levels, basal acid output, and dose regimen of proton-pump inhibitors in ZES patients diagnosed by SRS

<table>
<thead>
<tr>
<th>Patient No in “Mastersheet”</th>
<th>Gastrin levels (pg/ml) preop</th>
<th>postop</th>
<th>Basal Acid Output (mEq/h) preop</th>
<th>postop</th>
<th>Dose of PPI (mg) preop</th>
<th>postop</th>
</tr>
</thead>
<tbody>
<tr>
<td>With surgical cure (post-op)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>586</td>
<td>58</td>
<td>43.5</td>
<td>3.5</td>
<td>160 Panto</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>448</td>
<td>107</td>
<td>36.5</td>
<td>0.7</td>
<td>160 Panto</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>156</td>
<td>55</td>
<td>55.2</td>
<td>1.0</td>
<td>40 Losec</td>
<td>-</td>
</tr>
<tr>
<td>Without surgical cure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>233</td>
<td>187</td>
<td>97.8</td>
<td>ND</td>
<td>160 Panto</td>
<td>*</td>
</tr>
<tr>
<td>27</td>
<td>551</td>
<td>244</td>
<td>43.5</td>
<td>14.3</td>
<td>160 Panto</td>
<td>80 Panto</td>
</tr>
<tr>
<td>29</td>
<td>4726</td>
<td>319</td>
<td>19</td>
<td>13.4</td>
<td>80 Losec</td>
<td>40 Panto</td>
</tr>
</tbody>
</table>

PPI, proton-pump inhibitor; panto, pantoprazole; ND, not done; gastrin levels were measured in the fasting state. The preoperative basal acid output was measured after a wash-out phase of 10 days, the postoperative in patients with surgical cure after one week from cessation of medication and in those not cured after 24 hours off proton-pump inhibitors. 20 mg of losec were considered to be of an equivalent of 40 mg pantoprazole (personal information from Byk Gulden, Konstanz, Germany). * The patient died postoperatively due to septic complications. The one patient (P8) whose tumour was not found at surgery is not listed above.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>US</th>
<th>Computerized tomography</th>
<th>SRS positive of total number of patients investigated</th>
<th>Sensitivity of SRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kisker, Bastian</td>
<td>1998</td>
<td>44</td>
<td>56</td>
<td>9/17</td>
<td>53</td>
</tr>
<tr>
<td>Alexander</td>
<td>1998</td>
<td>16</td>
<td>51</td>
<td>29/37</td>
<td>78</td>
</tr>
<tr>
<td>Termanini, Gibril</td>
<td>1997</td>
<td>-</td>
<td>-</td>
<td>75/122</td>
<td>61</td>
</tr>
<tr>
<td>Cadiot, Bonnaud</td>
<td>1997</td>
<td>**</td>
<td>**</td>
<td>62/85</td>
<td>73</td>
</tr>
<tr>
<td>Meko, Doharty</td>
<td>1996</td>
<td>13</td>
<td>33</td>
<td>6/8</td>
<td>67</td>
</tr>
<tr>
<td>Gibril, Reynolds</td>
<td>1996</td>
<td>-</td>
<td>-</td>
<td>56/80</td>
<td>70</td>
</tr>
<tr>
<td>Krenning, Kwekkeboom</td>
<td>1994</td>
<td>23</td>
<td>50</td>
<td>61/79</td>
<td>77</td>
</tr>
<tr>
<td>de Kervellier, Cadiot</td>
<td>1994</td>
<td>21</td>
<td>47</td>
<td>39/48</td>
<td>81</td>
</tr>
</tbody>
</table>

SRS, somatostatin receptor scintigraphy. US, transabdominal ultrasonography. * the sensitivity of ultrasound and computerized tomography was calculated on a per patient basis. ** the tests had been performed, but the results were not mentioned in the publication. † the authors did not distinguish between the two tests in their results section. a including MRI, selective angiography, and bone scan.
Table SR5. Sensitivity of somatostatin receptor scintigraphy in ZES patients compared to surgical findings
- Review of the literature -

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Sensitivity of SRS in ZES patients</th>
<th>Resections in patients</th>
<th>Hot spots imaged by SRS</th>
<th>Gastrinomas resected</th>
<th>Post-operative cure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kisker</td>
<td>1998</td>
<td>9/17 53</td>
<td>24/25 96</td>
<td>9</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td>Alexander, Fraker</td>
<td>1998</td>
<td>35/35 100</td>
<td>35/35 100</td>
<td>47</td>
<td>74</td>
<td>59*</td>
</tr>
<tr>
<td>Cadiot, Lebtai</td>
<td>1996</td>
<td>12/21 57</td>
<td>19/21 90</td>
<td>17</td>
<td>27</td>
<td>63**</td>
</tr>
<tr>
<td>Zimmer, Stoelzl</td>
<td>1996</td>
<td>8/10 80</td>
<td>9/10 90</td>
<td>12</td>
<td>14</td>
<td>50**</td>
</tr>
<tr>
<td>Schirmer, Melvin</td>
<td>1995</td>
<td>8/9 89</td>
<td>9/9 100</td>
<td>21</td>
<td>23</td>
<td>N/A</td>
</tr>
<tr>
<td>Our series</td>
<td>1998</td>
<td>6/7 86</td>
<td>6/7 86</td>
<td>7</td>
<td>9</td>
<td>43***</td>
</tr>
</tbody>
</table>

N/A, not available. SRS, somatostatin receptor scintigraphy. ZES, Zollinger-Ellison syndrome

* Results are based on a per patient, not per lesion basis
*The cure rate at 2 years decreased to 41%.
** No follow-up data available
*** 29% at two years
### Table SR6. Possible causes for false negative results after somatostatin receptor scintigraphy

<table>
<thead>
<tr>
<th>Category</th>
<th>Possible Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.) Technical</td>
<td>1.) Insufficient dose of radioactivity</td>
</tr>
<tr>
<td></td>
<td>2.) No SPECT images obtained</td>
</tr>
<tr>
<td></td>
<td>3.) One-headed camera used instead of two-headed one</td>
</tr>
<tr>
<td>B.) Anatomical</td>
<td>1.) Hot spot obscured by physiological uptake of neighbouring structures (i.e. area tail of pancreas, gallbladder)</td>
</tr>
<tr>
<td></td>
<td>2.) Small tumour size (&lt;3-5 mm)</td>
</tr>
<tr>
<td>c.) Biological</td>
<td>1.) Low density of somatostatin receptors</td>
</tr>
<tr>
<td></td>
<td>2.) Low affinity of somatostatin receptors</td>
</tr>
<tr>
<td></td>
<td>3.) Locally high concentration of somatostatin</td>
</tr>
<tr>
<td></td>
<td>4.) Impaired tumour blood supply</td>
</tr>
<tr>
<td></td>
<td>5.) Down-regulation of binding sites by corticosteroids</td>
</tr>
<tr>
<td></td>
<td>6.) Receptor subtypes for octreotide not expressed</td>
</tr>
</tbody>
</table>
Table Pant1. Characteristics of ZES patients who participated in the pantoprazole trial

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yr)</td>
<td>48.9 (range 28-74)</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>8/2</td>
</tr>
<tr>
<td>ZES associated with MEN I (n)</td>
<td>1</td>
</tr>
<tr>
<td>Mean BAO (mEq/h)</td>
<td>27.1 (range 0.5-97.8)</td>
</tr>
<tr>
<td>Mean fasting serum gastrin (pg/ml)</td>
<td>1554 (range 136-4762)</td>
</tr>
<tr>
<td>Previous gastric surgery (n)</td>
<td>4</td>
</tr>
</tbody>
</table>

BAO, basal acid output; MEN I, multiple endocrine neoplasia, type 1;
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Previous Gastric Surgery</th>
<th>ZES History (yrs)</th>
<th>Wash-out phase</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gastrin (pg/ml)</td>
<td>BAO (mEq/h)</td>
</tr>
<tr>
<td>1.)</td>
<td>33</td>
<td>58</td>
<td>-</td>
<td>4</td>
<td>152</td>
</tr>
<tr>
<td>2.)</td>
<td>27</td>
<td>46</td>
<td>-</td>
<td>7</td>
<td>1135</td>
</tr>
<tr>
<td>3.)</td>
<td>29</td>
<td>45</td>
<td>-</td>
<td>3</td>
<td>4762</td>
</tr>
<tr>
<td>4.)</td>
<td>37</td>
<td>74</td>
<td>V/A</td>
<td>1</td>
<td>236</td>
</tr>
<tr>
<td>5.)</td>
<td>38</td>
<td>50</td>
<td>V/P</td>
<td>1</td>
<td>366</td>
</tr>
<tr>
<td>6.)</td>
<td>8</td>
<td>29</td>
<td>V/P</td>
<td>13</td>
<td>443</td>
</tr>
<tr>
<td>7.)</td>
<td>7</td>
<td>74</td>
<td>V/A</td>
<td>13</td>
<td>4738</td>
</tr>
<tr>
<td>8.)</td>
<td>39</td>
<td>61</td>
<td>-</td>
<td>1</td>
<td>398</td>
</tr>
<tr>
<td>9.)</td>
<td>21</td>
<td>65</td>
<td>-</td>
<td>8</td>
<td>2776</td>
</tr>
<tr>
<td>10.)</td>
<td>40</td>
<td>57</td>
<td>-</td>
<td>1</td>
<td>448</td>
</tr>
</tbody>
</table>

Patient data giving age, previous gastric surgery, initial serum gastrin, endoscopy findings and basal acid output before and during treatment with pantoprazole. All pathological findings on endoscopy were resolved after 3 months. V/A, vagotomy and antrectomy; V/P, vagotomy and pyloroplasty; BAO, basal acid output; D, duodenitis; SE, stomal erosions; E, esophagitis; G, gastritis. * The second row of patient numbers refers to the “Mastersheet of 40 ZES Patients”. **BAO increased in this patient (probably due to tumour growth) to 14.8 mEq/h under 120 mg pantoprazole/day and finally 11 mEq/h with 160 mg pantoprazole/day.
Table Pant3. ECL-cell status and serum levels before and during the treatment with pantoprazole

<table>
<thead>
<tr>
<th>Pre-Treatment (wash-out phase)</th>
<th>During Treatment (after at least 1 year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pat.*</td>
<td>ECL-Cell Status</td>
</tr>
<tr>
<td>1.) 33</td>
<td>normal</td>
</tr>
<tr>
<td>2.) 27</td>
<td>nodular</td>
</tr>
<tr>
<td>3.) 29</td>
<td>nodular</td>
</tr>
<tr>
<td>4.) 37</td>
<td>normal</td>
</tr>
<tr>
<td>5.) 38</td>
<td>linear</td>
</tr>
<tr>
<td>6.) 8</td>
<td>nodular</td>
</tr>
<tr>
<td>7.) 7</td>
<td>dysplasia</td>
</tr>
<tr>
<td>8.) 39</td>
<td>linear</td>
</tr>
<tr>
<td>9.) 21</td>
<td>dysplasia</td>
</tr>
<tr>
<td>10.) 40</td>
<td>linear</td>
</tr>
</tbody>
</table>

The mean treatment time with pantoprazole was 61.5 weeks (range 13-76 weeks; ± 26.15 weeks). *The second row of patient numbers refers to the “Mastersheet of 40 ZES patients”. The categorisation of the enterochromaffin-like (ECL) cell status was performed by one pathologist (PK) according to the classification provided by Solcia (1995).

The mean serum gastrin level before treatment was 1545 pg/ml (± 1856), and during the treatment 1645 pg/ml (± 1893), which was not significantly different, as assessed by the Student’s paired t-test (p= 0.697).
Table Pant4. Basal acid output before and during the pantoprazole trial at different doses of pantoprazole in 10 ZES patients

The graph reflects the initial basal acid output during the washout phase and the effect of different doses of oral pantoprazole (40-160mg) on acid secretion in 10 patients with ZES. The basal acid output (BAO) was determined after a washout phase of 10 days. The dotted line represents an acid output of 10 mEq/h, the accepted level of control in patients without previous gastric surgery.
FIGURES
Robert M. Zollinger

Robert M. Zollinger was born on 4 September 1903, in Millersport, Ohio. He graduated from Medical School in Ohio in 1927. After his surgical training he was appointed assistant professor at Harvard Medical School. During World War II, he served overseas in the Medical Corps of the Army. In 1947 he became professor and chairman of the Department of Surgery, Ohio State University, and occupied this post until he retired with emeritus status in 1974. He has published more than 340 articles, covering not only different surgical topics (gallstone disease, breast and colonic cancer) but wrote also articles of historical interest (Doctor Loyal Davies, William Beaumont). Since 1957 he has been editor-in-chief of the American Journal of Surgery.

Edwin H. Ellison

Edwin Ellison was born in Dayton, Ohio, on 4 September 1918 and graduated in medicine with distinction from Ohio State University. He performed postgraduate studies in biochemistry prior to his residency training in surgery. In 1948, he joined the faculty of the Ohio State University, where he pioneered the use of the flame photometer in determining electrolyte levels in surgical patients. By investigating surgical procedures in peptic ulcer disease, he noticed recurrent surgical failures in one patient, who later proved to be one of the two making up the original report later in 1955. In 1958, he was appointed as professor and chairman of surgery, Marquette School of Medicine. Amongst various scientific activities, Ellison developed together with a medical artist an atlas for surgical procedures of the stomach. He died 1970 in Milwaukee, Wisconsin, at the age of 52.

(References: Beighton 1986; Zollinger 1970)
Figure 2. Historical landmarks in the evolution of diagnosis and treatment of ZES

<table>
<thead>
<tr>
<th>Date, year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1955</td>
<td>Original description of ZES</td>
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<tr>
<td>1958</td>
<td>Oberhelman excises duodenal gastrinomas</td>
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<tr>
<td></td>
<td>Cure rates of 2-5 percent reported</td>
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<tr>
<td>1960</td>
<td>Identification of gastrin as the secretory product of the islet-cell tumor by Gregory</td>
</tr>
<tr>
<td>1968</td>
<td>A gastrin-like substance derived from a pancreatic primary and liver metastases at GSH</td>
</tr>
<tr>
<td>1976</td>
<td>Gastrin radioimmunoassay</td>
</tr>
<tr>
<td>1977</td>
<td>H2 receptor antagonists shift focus to the medical management of patients with ZES</td>
</tr>
<tr>
<td>1980</td>
<td>Portal venous gastrin sampling</td>
</tr>
<tr>
<td>1982</td>
<td>Duodenal gastrinomas identified intraoperatively</td>
</tr>
<tr>
<td>1984</td>
<td>Biologic differences identified in gastrinomas from different locations</td>
</tr>
<tr>
<td>1984</td>
<td>Proton pump inhibition (omeprazole) introduced</td>
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<tr>
<td>1984</td>
<td>Gastrinoma triangle defined</td>
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<tr>
<td>1988</td>
<td>Augmented localization of duodenal tumors</td>
</tr>
<tr>
<td>1988</td>
<td>Selective intra-arterial secretin injection test</td>
</tr>
<tr>
<td>1988</td>
<td>Gene responsible for MEN I mapped to chromosome 11q13</td>
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<tr>
<td>1990</td>
<td>Intraoperative endoscopic transillumination</td>
</tr>
<tr>
<td>1992</td>
<td>Cure rates of 30-82% percent are reported</td>
</tr>
<tr>
<td>1994</td>
<td>Somatostatin receptor scintigraphy</td>
</tr>
<tr>
<td>1997</td>
<td>Successful cloning of the MEN I gene</td>
</tr>
<tr>
<td>1997</td>
<td>Intravenous proton pump inhibitor (pantoprazole)</td>
</tr>
</tbody>
</table>

(Modified after Modlin, Lawton 1994; the references are mentioned in the text)
Figure 3. Surgical procedures and their effect on acid secretion in the first ZES patient reported by Zollinger and Ellison 1955

Schematic representation of Zollinger and Ellison’s first case from their original presentation, including the operative procedures until total gastrectomy (above), and the effects on gastric acid output (below).
Figure 4 A. Immunohistochemical staining for neuroendocrine tumours (including gastrinomas)

Gastrin (Magnification 20x)

Somatostatin (40x)

Chromogranin A (40x)
Figure 4 B. Gastrinoma tissue staining for biological markers

CD44 (Magnification 40x)

PCNA (20x)  PCNA (40x)

Mib1 (20x)  Mib1 (40x)
Figure 4 C. Gastrinoma tissue staining for biological markers

The figures show staining for neuroendocrine tumours including gastrinoma (4 A), and the expression of various biological markers in a gastrinoma lymph node (4 B and C). Figure 4 A shows examples of gastrin (upper) and somatostatin (middle) staining of gastrinoma tumour cells, whereas the lower picture reflects positive chromogranin A staining of gastrinoma cells (dark).

Figure 4 B, upper picture, gives an example of intense CD44 membrane staining of gastrinoma tumor cells. CD44 may play a role in the regulation of cell substrate interactions as well as cell migration. The picture in the middle reflects staining for PCNA, the lower for Mib-1. Waf1 is regulated by the cell cycle marker p53 (4 C). Staining for p53 (4C) was weak and occurred in less than a third of the tumours. The lower picture demonstrates staining for bcl-2.
Figure 5. Example of pre- and postoperative scintigrams in a ZES patient

A. This preoperative scan, taken at 4 hours after injection of the radionuclide, shows two hot spots in patient 29 from “Mastersheet of 40 ZES Patients”. The bigger one (arrow) is located in the area of the duodenum, the smaller one (arrowhead) in the pancreatic region. Additional physiologic uptake is seen in the spleen (S) and the kidneys (K).

B. A scan of the same region of the patient in A., two weeks after resection of a paraduodenal lymph node gastrinoma and a pancreatic tumour. The two tumour-related hot spots no longer appear. Only the physiologic uptake over both kidneys (K) is detected. Despite the negative scan, this patient remained hypergastrinemic (serum gastrin 239 pg/ml) with a positive secretin test, probably due to remaining microscopic gastrinoma tissue.
Figure 6. Intraoperative tumour locations

A. Location of the periduodenal gastrinoma in patient 29 (from “Mastersheet”). B, the same tumour excised and bivalved. Note the cystic pattern of the gastrinoma. C, the duodenal wall tumor in P40 (from “Mastersheet”) before removal.
Figure 7. Fusion of computed tomography and SRS for localization of gastrinomas

**Image A.** A CT scan showing a 3cm lesion (arrow) next to the duodenum. The body of the pancreas looks normal.

**Image B.** Somatostatin receptor scintigraphy using SPECT imaging (transaxial view), same layer as CT scan in Image A. The left arrow indicates the known lesion in the pancreatic head/duodenal area; the right arrow reveals an additional smaller tumour in the body region of the pancreas.

**Image C.** Computerized fusion of the same layer of the CT scan and the SPECT image to delineate the anatomical position of the two lesions (arrows).
Figure 8. Algorithm for the diagnosis of ZES

**Clinical Symptoms**
- Complicated duodenal ulcer
- Atypical ulcer site
- Diarrhoea

**Biochemical Diagnosis**
- Serum gastrin
- Secretin provocative test
- Basal acid output

**Exclude MEN I Syndrome**
- Hypocalcaemia
- Pituitary Abnormalities
- Other neuroendocrine tumours

ZES (sporadic)  ZES with MEN I

**Topographical Localization**

MEN I, multiple endocrine neoplasia type I; NET, neuroendocrine tumor
Figure 9. Contemporary management algorithm in the Zollinger-Ellison syndrome

- Diagnosis confirmed
  - Serum-gastrin/secretin test
  - MEN I screen

- Imaging
  - US, CT, SRS

- MEN I
  - Multiple liver metastases

- Conservative therapy
  - PPI

- Formal debulking

- Assess PPI dosage
  - Requirement BAO < 10 mEq/h

- Laparotomy

- Cured
  - 6-12 months serum gastrin

- Not cured

- Continue medication
  - Poorly controlled
  - SRS
  - Re-exploration
  - Vagotomy/ total Gastrectomy


Morse ME. Two adenomata of the islands of Langerhans. JAMA, 51: 1075-1076, 1908.


Pearse AGE. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. Proc R Soc 170:71-80, 1968.


