ORIGINAL ARTICLES

Article received on March 17, 2015 and accepted for publishing on April 19 2015.

Diagnostic and treatment difficulties in insulinomas

Mariana Jinga [1,2], Irina I. Sima[4], Emilia Rusu[2,4], Aurelian E. Ranetti[1,2], Florentina Ioniţă Radu[1,3], Săndica Bucurică[1,2], Raluca S. Costache[1,2], Mihăiţă Pătrăşescu[1], Bogdan Macadon[1], Petruţ Nuţă[1], Vasile D. Balaban[1,2]

Abstract: *Background: Neuroendocrine tumors of the pancreas (NTP) comprise a unique and relatively rare group of tumors, of which gastrinoma and insulinoma are the most common types. Insulinomas tend to be small, solitary and benign, with surgical resection curable in most cases.*
Introduction: Insulinomas are localized preoperatively using conventional imaging studies as transabdominal ultrasonography (US), computed tomography (CT), and/or magnetic resonance imaging (MRI).
Purpose: Endoscopic ultrasound (EUS) is a valuable tool in the diagnosis of insulinomas.
Goals & methods: We performed a retrospective study on 21 patients with insulinoma (6 male and 15 female, 25 to 73 years of age), who were hospitalized and operated on between 2003 and 2012 at "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.
Results: US view was positive in 10% of patients (2 of 20), that presented proximal location. The sensitivity of CT was unsatisfactory, 21.05% (4 positive results of 19). CT failed to detect liver metastases, but identified nodal metastasis in one patient. MRI was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infra-centimetric tumor size. EUS has a high resolution which allows detection of lesions with very small diameter is safe and minimally invasive. EUS was performed in all patients, being able to identify formations in 17, was inconclusive in 3, showing a diagnosis sensitivity of 81%. Liver metastases were demonstrated in 3 patients, one by US and all 3 by MRI.
Conclusions:
- CT with intravenous iodinated contrast agent had a poor sensitivity in detecting the primary tumors, was insensitive in detecting liver metastases, but showed metastases in lymph nodes.
- MRI has higher sensitivity than CT in detecting primary tumors, including insulinomas with infracentimetric size, and is the imaging test of choice for possible liver metastases.
- EUS is the preoperative imaging test of choice.

Keywords: *insulinoma, neuroendocrine tumors of the pancreas, endoscopic ultrasound (EUS), preoperative localization*

INTRODUCTION

Neuroendocrine tumors of the pancreas (NTP) are rare, but increasingly recognized entities. Tumors may be functional or nonfunctional, which are frequently detected incidentally or with symptoms related to mass effect of the tumor or its metastases (1). NTP have been considered rare, with an estimated incidence of less than 1 per 100000 individuals (2). Insulinoma is the most common

[1] Carol Davila Central Emergency Military Hospital, Bucharest
[2] Carol Davila University of Medicine and Pharmacy, Bucharest
[2] Titu Maiorescu University, Faculty of Medicine, Bucharest
[4] National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania,

neuroendocrine tumor of the pancreas and cause of hypoglycaemia related to endogenous hyperinsulinism with an annual incidence of four in every 1 million persons (3, 4, 5). As many as 90% of insulinomas have been reported to be benign, 90% are solitary, >90% occur in intrapancreatic sites, and 90% are < 2 cm in diameter. Regarding the location of pancreatic tumors, 50% are in the head, 25% in the tail, and 25% caudally or permeates the entire pancreas (5, 6).

Most of these tumors occur sporadically, but they can also be associated with multiple endocrine neoplasia (MEN)-1 syndrome. Approximately 4% of patients with insulinoma have MEN-1 (3, 4, 6). In sporadic forms, the highest incidence is considered in the age group 40-60 years (mean age 50 years), with a slight predominance of females (sex ratio 3:2 for women). (3). The diagnosis of insulinoma can be established by determining plasma proinsulin, insulin, C-peptide, and glucose levels, which are usually performed during a 72-hour fast (4). Insulinomas classically present with "Whipple's triad:" a combination of symptoms of hypoglycaemia, inappropriately high insulin levels with associated documented blood glucose levels of <50 mg/dL, and symptom relief with administration of glucose (2).

Non-invasive imaging procedures, such as CT and MRI, are used when a diagnosis of insulinoma has been made to localize the source of pathological insulin secretion. Invasive modalities, such as endoscopic ultrasound (EUS) and arterial stimulation venous sampling, are highly accurate in the preoperative localization of insulinomas and have frequently been shown to be superior to noninvasive localization techniques. Topographic preoperative diagnosis is difficult due to small tumor size; EUS is the most sensitive diagnostic method for this purpose. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive (5, 7, 8). In the table below, we are presenting data from studies showing the accuracy of imaging methods in diagnosing insulinomas (Table 1).

Table 1. Accuracy of imaging methods in diagnosing insulinomas (5, 9, 10, 11, 12, 13, 14, 15, 16)

	US	CT scan	MRI	EUS	Intraoperative US	Intraoperative palpation
Pitre J, 1996				90%		
Angeli E, 1997	79.3%	44.8%	65.5%			
Ardengh JC, 2000		16.7%		83.3%		
Machado MCC, 2001	30%	25%	17%	27%		
Mirallié E, 2002				85%		
Jyotsna VP, 2006		57-83%	31%		92%	76%
Sotoudehmanesh R, 2007				89.5%		
Joseph AJ, 2013		69%		89%		
Takehiro O, 2013		33-64%	40-90%		80-100%	75-95%

There are still great difficulties in correct positive and topographic diagnosis of insulinomas. Advances in the pre-and intraoperative diagnosis allow precise localization and excision of the tumors, avoiding blind pancreatic resections (5, 17). Objectives of treatment are: symptomatic treatment of the tumor hypersecretion syndrome and surgical treatment which consists in enucleation of benign tumor or extensive resections. The surgery may use laparoscopic or open techniques. It is essential to perform intraoperative ultrasound, which will have an important role in choosing the optimal resection technique. Enucleation is the excision method of choice, whenever it is technically possible. Currently, resection of insulinomas can be successful with the techniques of minimally invasive surgery - laparoscopy or robotics, combined with intraoperative ultrasound performance. Medical treatment addresses to control symptoms or complications, for surgery preparation or as palliative treatment in cases where it can be done. When technically feasible, tumor enucleation is the

procedure of choice; however, a more formal resection may be necessary for certain tumors (3, 5, 7, 14, 18, 19).

GOALS

Given the low incidence of pancreatic neuroendocrine tumors and the relatively limited medical experience regarding this group of tumors, there is concern in the medical world to find methods of diagnosis and appropriate treatment.

This study aims to identify characteristic features of pancreatic neuroendocrine tumors, especially of insulinoma, the most frequently diagnosed of them. We analyzed in terms of demographics, clinical, laboratory, imaging, pathology and treatment 21 cases of pancreatic insulinoma, all diagnosed and treated at "Dr. Carol Davila" Central Military Emergency University Hospital", Bucharest, highlighting the best diagnostic and therapeutic attitude and the comparison with literature data.

We will try to determine which are most sensitive and specific methods applied at this time to establish early diagnosis and what treatment is recommended considering the particular features of the disease, the low incidence, appropriate index of suspicion, non-specificity of symptoms with often delayed diagnosis, involving a complex team (endocrinology, internal medicine, gastroenterology, oncology, intensive care, oncology, surgery) and the difficulties of this pathology.

MATERIAL AND METHODS

This article aims to develop a retrospective study to track the best diagnosis and treatment for insulinomas. In the period 25.01.2003 – 26.11.2012, 21 patients diagnosed with insulinoma were hospitalized and/or operated at the "Dr. Carol Davila" Central Military Emergency University Hospital in Bucharest. Medical records of patients with insulinoma seen in the period 2003-2012 were retrieved from the hospital's medical records department. From these, data on clinical and diagnostic features, localization and surgical outcome were extracted. All mathematical analysis was performed using Microsoft Excel 2007.

The study analyzes the following data: epidemiology (distribution by sex and age), clinical (symptoms), laboratory (laboratory diagnosis, topography, location, locoregional extension and/or distant metastases), histological (macroscopic, microscopic and immunohistochemical) and treatment (surgery, medical), postoperative evolution, complications and prognosis. Features of patients were highlighted and analyzed by comparison with the data currently available in the literature. Biological, imaging and histological methods of diagnosis and techniques and results of surgical treatment and care were analyzed in particular.

RESULTS

Over a period of nine years, between January 2003 and November 2012, 21 patients diagnosed with pancreatic insulinoma were admitted to "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.

Gender distribution was 15 women (71.4%) and 6 men (28.6%) and sex ratio women: men of 2.5:1. A particular case was the association with Wermer syndrome (MEN I) found in a patient of 36 years, who was hospitalized for symptoms of insulin hypersecretion. The mean age at diagnosis of insulinoma was 46.66 years (25-73 years), being lower for women than men (41.06 vs. 53.66). Distribution by age showed a peak between 40 and 49 years.

Positive diagnosis is suggested by clinical manifestations. In 1938, Whipple described a triad with a high specificity: 1) neurological crises that occur on an empty stomach or after exercise 2) decrease in blood glucose below 50 mg% during the crisis, 3) disappearance of neuropsychiatric disorders following administration of glucose orally or intravenously.

The diagnosis is based on simultaneous measurement of blood glucose and insulin levels (5, 6, 7). In our cases, positive diagnosis was suggested by Whipple triad. All patients had presented with fasting hypoglycemia and fulfilled the triad to suspect a diagnosis of insulinoma.

CLINICAL PRESENTATION

Patients had various associations of symptoms, shown after prolonged overnight fasting and physical activity or stress. The fact that the symptoms occur early in the morning, on waking, and disappear after ingestion of carbohydrates or glucose administration oriented the diagnosis (7). Delays in the diagnosis of insulinoma are common because the symptoms usually precede detection of a tumor and there may be misattribution of the symptoms to psychiatric, cardiac, or neurological disorders and, on average, it takes up to 2 years before the correct diagnosis is made (5, 7, 8). Neuroglicopenic symptoms delayed diagnosis in 8 patients (38%), as they were hospitalized in the neurology and psychiatry wards and mistreated. Of them, four were diagnosed with epilepsy and received anticonvulsant treatment, and the others were labeled with neurological disorders, anxiety disorders or thyroid dysfunction. Finally, the diagnosis was correct, the time period between onset of symptoms and diagnosis ranging from several days to 10 years. The symptomatic evolution of the disease before diagnosis had a mean of 25.14 months. More than half of patients in the study (61.9%) were diagnosed as having insulinoma after less than a year of evolution.

Table 2. Frequency of symptoms and signs that patients presented with in the study

Symptoms	Number of patients (%)
Preprandial hypoglycaemia	21 (100)
Loss of consciousness	13 (62)
Dizziness	12 (57)
Diaphoresis	12 (57)
Tremor	10 (48)
Obesity	8 (38)
Fatigue / asthenia	8 (38)
Increased appetite / hunger	7 (33)
Palpitations	7 (33)
Headache	6 (28)
Loss of balance	6 (28)
Diplopia, blurred vision	6 (28)
Seizures	5 (24)
Extremity paresthesia	5 (24)
Impaired memory or concentration	5 (24)
Dysarthria	5 (24)
Confusion	4 (19)
Abnormal behavior	4 (19)
Anxiety, irritability, psychomotor agitation	4 (19)
Difficulty in waking up in the morning	4 (19)
Amnesia	3 (14)
Hypoglycemic coma	3 (14)
Temporo-spatial disorientation disorder	2 (9)
Affective disorder	2 (9)
Distal neuropathy associated with hypoglycaemia	1 (5)

Positive biological diagnosis

Insulinomas are the most common cause of hypoglycemia resulting from endogenous hyperinsulinism. B cell insulin secretion does not decrease in the presence of hypoglycemia, and so plasma insulin greater than or equal to 3µU/ml along with a plasma

glucose less than or equal to 50 mg/dL suggest an insulinoma. The diagnosis is made by simultaneous measurements of blood sugar and insulin levels in the blood (5).

The 72-h fasting test is a demonstration of Whipple's triad of symptoms, which is considered as the gold standard for the diagnosis. This test is conducted under supervised conditions, which requires hospitalisation of the patient. During the fasting period, the patient is allowed to drink calorie-free fluids and physical activity is encouraged. Blood glucose should be measured every 6 hours until it reduces to 60 mg/dL or less, when the interval is reduced to every 1–2 h. The fast is terminated when the plasma glucose level is 45mg/dl or less and the patient has symptoms and signs of hypoglycemia.

When symptoms of hypoglycemia appear, blood should be sampled for measurement of glucose, insulin, C-peptide, and proinsulin. Insulinoma is diagnosed when the following criteria are fulfilled: plasma concentrations of glucose less than 55 mg/dL (3.0 mmol/L), insulin of at least 3.0 µU/mL (18 pmol/L), C-peptide of at least 0.6 ng/mL (0.2 nmol/L), proinsulin of at least 5.0 pmol/L. Some authors described modified fasting test where duration of the fasting period is reduced to 48 h (3, 5, 7, 8, 21).

Glycemia during the crisis was on average 30.37 mg/dl (range 20-45 mg/dl). Plasma insulin measured was increased, the average being 25.9 µU/ml (6 to 79.2 µU/ml). A supervised fasting test was performed in 11 patients and was positive in 10 of them. Median time to a positive test was 15.5 hours (4-28 hours). In 3 patients (30%) the test was positive in the first 10 hours (after 4 hours fastest in 1 patient), in 6 patients (60%) was positive within 24 hours and in all patients (100%) was positive within 48 hours. No patient passed the boundary of 48 hours.

Differential diagnosis

Hypoglycemia due to excessive endogenous insulin secretion can be caused by a primary pancreatic beta cell disorder including an insulinoma, beta cell hyperplasia or nesidioblastosis (presence of diffuse microadenomatosis, in which multiple small non-encapsulated tumors or nodules are distributed throughout the pancreas), a beta cell secretagogue including a sulphonylurea or a beta cell stimulating antibody, or an antibody to insulin. The differential diagnosis of hypoglycaemia includes hormonal deficiencies, hepatic insufficiency, exogenous hyperinsulinism, medication, drugs and enzyme defects. Occasionally differentiating insulinoma from these other causes of hypoglycaemia can be quite difficult (7).

Differentiation for nondiabetic-associated hypoglycemia is broad and includes insulinoma, drugs, hormone deficiencies, and critical illness. For stratification, the causes of hypoglycemia can be divided into those associated with diabetes mellitus and those who do not suffer from this condition, as the pathophysiology of each is distinct. Drugs are the most common cause of hypoglycemia (antidiabetic agents – insulin, sulfonylurea and meglitinides, salicylates, quinine derivatives, disopyramide, pentamidine, ethanol). Hypoglycemia is not an infrequent finding in the critically ill patient and may be related to sepsis, hepatic or renal failure, or to general malnourishment. Hepatic or renal failure, deficiency of cortisol and/or growth hormone can lead to hypoglycemia, leads to an inability to maintain adequate fasting glucose levels despite adaptive renal gluconeogenesis. Hypoglycemia associated with endogenous hyperinsulinaemia is very rare. Differential diagnosis of fasting hypoglycemia in adults should also include nonislet tumor (mesenchymal tumors, hepatocellular carcinoma or hematological malignancies) and autoimmune hypoglycemia (insulin receptor autoantibodies, insulin autoantibodies) (22, 23, 24, 25).

Topographic diagnosis

Transabdominal ultrasonography (US) was performed in 20 of the 21 patients and was positive in two of them (10%), which shows no accuracy of topographic diagnosis. A positive US result was obtained in a patient with proximal insulinoma (the pancreatic region most accessible for abdominal ultrasound exploration) – at the pancreatic head-body junction, and the other in a patient with caudally located tumor, but large (63/43 mm).

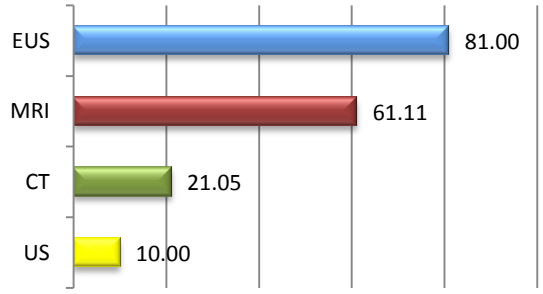
In one patient with a history of metastatic insulinoma, previously operated, followed by a second surgical intervention for hepatic metastases, US still showed liver metastases. US showed a tumor with regular contour, hyperechoic, homogeneous, with a diameter of 12 mm, in contact with the left suprahepatic vein.

Computed tomography (CT) could not be performed in one patient because she exceeded allowable weight, so of the 20 patients undergoing this investigation, one result was inconclusive, and from the remaining 19, 4 results were positive, with tumor size over 1 cm.

The sensitivity of CT was unsatisfactory: 4 positive results out of 19 (21.05%). CT failed to detect liver metastasis, but identified lymph node metastasis in one patient.

Magnetic resonance imaging (MRI) was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infracentimetric tumor size.

Figure 1. Sensitivity of imaging methods (%)



Endoscopic ultrasound (EUS) was performed in all patients, being able to identify formations in 17 out of 21, showing a diagnostic sensitivity of 81%.

In two patients, the results were inconclusive, as inhomogenous, poorly delineated areas were seen in the head and tail of the pancreas, but without clear identification of a tumor.

Excluding the other two patients in whom EUS failure was caused by lack of cooperation or poor conditions related to patient preparation, reporting the results of the remaining 19 patients who underwent EUS, we can say that its sensitivity was 89.47%.

Regarding EUS location of insulinomas, in our study, 44.44% were proximal (head and uncinate process), 27.77% in the body and the same percentage in the tail. The mean size of insulinomas was 1.958 cm (range 0.91 cm - 5.37cm). Most of them were between 1 and 3 cm (13 out of 18, meaning 72.2%).

Preoperative diagnosis and treatment

The preoperative topographic diagnosis was possible in 18 (86%) of the 21 patients with insulinoma. It was not possible in 3 patients, in whom all localization imaging investigations were negative, but in the presence of biological and symptomatic confirmed insulinoma.

In these patients surgical intervention was not performed, the solution consisting of reevaluation, follow-up and palliative treatment.

17 of 21 patients with insulinoma were referred to surgery. In addition to the 3 patients where the tumor could not be located, another patient with rare symptoms, drug controlled, was not operated because he did not accept surgery; he is being monitored clinically and by imaging methods.

At surgical exploration, the abdomen is initially investigated for evidence of metastatic disease. The pancreas is then completely exposed, allowing palpation of the entire pancreas.

Intraoperative ultrasonography and palpation can be performed at this time in order to effectively localize and guide in the dissection of the tumor. Since most insulinomas are benign and solitary, tumor enucleation is the procedure of choice, when is technically feasible (3).

In patients undergoing surgery, intraoperative diagnosis was made by intraoperative palpation of the tumor and/or intraoperative ultrasound. In 8 patients the intraoperative diagnosis was established by manual palpation of the pancreas and in 8 patients the two techniques were combined (pancreas palpation and intraoperative ultrasound).

In our cases, intraoperative ultrasound combined with a manual exploration of the pancreas showed a

diagnostic sensitivity of 94.11%. The tumor could not be identified at the first surgery on a patient, but intraoperative ultrasound managed to locate it at the second intervention.

Table 3. Imaging methods and EUS location of insulinomas

	Patient	Gender	Age	US	CT	MRI	EUS tumor localization within the pancreas
1	PA	F	43	negative	negative	negative	between the body and the tail, near the posterior part of the gland
2	CC	F	37	negative	negative	negative	neck
3	ATI	F	26	negative	patient's weight was above the upper limits accepted for CT	patient's weight was above the upper limits accepted for MRI	located in the tail, posterior, near the left kidney
4	ME	F	46	positive, after EUS	negative	positive, after EUS	near the confluence, posterior
5	TC	M	53	negative	negative	negative	head
6	BM	M	41	negative	negative	positive, after EUS	head (uncinate)
7	AA	F	27	negative	negative	negative	fare tail, posterior, between spleen, left kidney and adrenal gland
8	SG	M	75	liver metastatis	negative	positive	head area
9	AZA	F	52	negative	negative	positive	border between body and tail, posterior
10	RL	F	50	negative	positive	positive	inhomogeneous head; cystic tumor in the tail
11	MNC	F	30	negative	positive	positive, after EUS	neck, posterior, near the confluence
12	SM	F	61	negative	negative	positive	neck
13	MI	M	72	not performed	negative	positive	body
14	LM	F	63	negative	negative	negative	tumor not located
15	GC	M	45	negative	negative	not performed	inhomogeneous head
16	VM	F	52	negative	negative	negative	head (uncinate), posterior
17	AD	F	38	negative	inconclusive	not performed	inhomogeneous tail
18	UV	M	43	negative	positive	negative	head (uncinate)
19	ELE	F	36	positive	negative	positive	tail
20	CG	F	38	negative	positive	positive	tumor not located
21	GC	F	43	negative	negative	positive, after EUS	between body and tail

Grade and differentiation

According to European Neuroendocrine Tumors Society (ENETS), detailed description of macroscopic, microscopic and immunohistochemical features is absolutely necessary to confirm the diagnosis of NTP and allow correct classification. The grade of a tumor refers to its biologic aggressiveness. For NTP, the grading system is based on the rate of proliferation, which is defined by the number of mitoses per 10 high-power microscopic fields or per 2 mm^2 (mitotic rate), or as the percentage of tumor cells that immunolabel positively for the Ki-67 antigen (Ki-67 index). An important role in insulinoma histological diagnosis is played by immunohistochemical analysis. NTP can also be classified based on differentiation, which refers to the extent to which cancerous, or neoplastic, cells resemble normal cells. Well differentiated NET cells produce large amounts of secretory granules with diffuse immunoexpression of

neuroendocrine markers. In contrast, poorly differentiated NTP have atypical, sheet-like, diffuse and irregular nuclei, less cytoplasmic secretory granules, and limited biomarker immunoexpression. General markers of well-differentiated NET include cytosolic markers (neuron-specific enolase and PGP-9.5), markers associated with small vesicles (synaptophysin and synaptotagmin), markers associated with secretory granules (chromogranin A and HISL-19). Specific cell markers include insulin and proinsulin hormones. Poorly differentiated tumors do not express cell markers but can be positive for some of general markers. Endocrine differentiation may be confirmed at immunohistochemical analysis with neuroendocrine markers such as chromogranin A or synaptophysin. In well-differentiated NTP, determination of malignancy is straightforward only if the tumor invades adjacent organs or a distant metastasis is present. Histologic confirmation of complete excision and the benign nature of the insulinoma are essential (7).

Histological findings were available for our study in 7 of the 17 operated patients (tests were not available for the patients that were included in the first years of the study and we did not manage to recover the paraffin blocks to make them new tests). In 6 of these, the neuroendocrine tumor was identified; in one patient the tumor was not found and the absence of exact location of reccurent insulinoma led to a palliative treatment.

Table 4. Surgical interventions performed in patients from the study

	Patient	Previous surgery for insulinoma	Surgical intervention	Type of intervention
1	PA	no	yes	Surgical enucleating
2	CC	yes	yes	Surgical enucleating
3	ATI	no	yes	Surgical enucleating
4	ME	no	yes	Surgical enucleating
5	TC	yes	Patient refused surgical intervention	Patient refused surgical intervention
6	BM	no	yes	Tumor resection
7	AA	no	yes	Tumor resection
8	SG	yes	yes	Follow up (liver metastasis)
9	AZA	no	yes	Tumor resection
10	RL	no	yes	Tumor resection
11	MNC	no	yes	Duodenopancreatectomy
12	SM	no	yes	Laparoscopic enucleation
13	MI	no	yes	Tumor resection
14	LM	no	no	Follow up
15	GC	no	no	Follow up
16	VM	no	yes	Surgical enucleating
17	AD	no	no	Follow up
18	UV	no	yes	Tumor resection
19	ELE	no	yes	Tumor resection
20	CG	yes	yes	Tumor resection
21	GC	yes	yes	Surgical enucleating

Dietary treatment and medication were recommended especially for preoperative preparation of patients, aiming to reduce the frequency and severity of hypoglycemic symptoms and consisted of administration of frequent meals rich in carbohydrates and possibly an intravenous glucose solution and Verapamil.

In patients in whom the tumor could not be located with any imaging technique and for the patient who refused the surgery because of light and rare symptoms, and last but not least, in patients whose surgery was not curative, treatment was palliative. Drugs used for this purpose were: Verapamil tablets 80 mg or 40 mg 3 times daily, Octreotide (Sandostatin 0.1 mg every 8 hours subcutaneously) and 1 mg Glucagon kit (glucagon) intramuscular injection (the thigh or deltoid) in case of impaired consciousness (the latter was used in one case). These were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom surgery was not possible as a solution.

Surgical treatment was applied to 17 of 21 patients with insulinoma. In all 17 patients the tumor was localized by EUS. Some patients had two or even three surgical interventions. In 4 of them, EUS was performed before the second intervention, in another EUS was diagnostic and performed several times, but it did not provide an exact location of reccurent insulinoma. One patient underwent two surgeries, the first following suspicion of insulinoma, so partial pancreatic resection was performed, but no evidence of neuroendocrine tumor was found in the excised pancreatic tissue (blind partial pancreatic resection did not include tumor). Operative failures were due to lack of precise localization of the tumor and presence of metastases at EUS (inhomogeneous head/tail), but there were only 3 such cases. Regarding the 4 patients with negative EUS results, 3 of them were not operated because of failure to locate the tumor, therefore they are followed up. The fourth patient underwent surgery (tumor resection) with a good subsequent recovery. Among patients with positive EUS diagnosis surgery was successful, intraoperative diagnosis being made by intraoperative palpation of the tumor and/or intraoperative ultrasound showing the same location of the tumor as in EUS. There was one exception in a patient with metastasis that was followed up.

Recurrence of symptoms has led, ultimately, to a secondary surgical intervention (resection of pancreatic head and duodenum); this time intraoperative ultrasound confirmed location of the tumor and its excision was performed successfully. It is important to note that EUS was not performed in this patient. Another case is of a patient who underwent corporeo-caudal pancreatectomy without remission of hypoglycemic syndrome even if the tumor was localized preoperatively by EUS. Three years later EUS revealed tumor recurrence in the remaining parenchyma, which was enucleated with good recovery. Of the 7 enucleations, 3 (43%) were for tumors located in the pancreatic head.

Complications

The immediate postoperative complications occurred in 8 of the 17 patients who had surgical intervention, so we can say that postoperative morbidity was 47.05%. The complications are presented in table 5.

Table 5. Postoperative complications

Complication	Number
Transient hyperglycemia	4
Late postoperative pancreatic pseudocyst	2
Postoperative anemic syndrome	1
Transient pancreatitis	1
Postoperative eventration	1
Permanent diabetes mellitus	1
Exocrine pancreatic insufficiency	1
Malabsorption	1

The most common complication was transient hyperglycemia that occurred in 4 patients (23.52%), ranging between 3 and 15 days, with values between 130-170 mg/dl.

Diabetes mellitus occurred in a patient who underwent cephalic duodeno-pancreatectomy and exocrine pancreatic insufficiency in a patient who

underwent a corporeo-caudal pancreatectomy followed by enucleation after three years.

Postoperative eventration and pancreatic pseudocysts were resolved surgically.

Metastases and recurrence

Malignancy is suggested by large tumor size, presence of metastases or vascular invasion. Tumors larger than 3 cm raise the suspicion of a malignant insulinoma. Other biological explorations advocating for malignancy are: high insulinemia, proinsulin large proportion (90%), elevated C-peptide. Ki-67 index is also a predictive factor for malignancy. Necrosis and high mitotic rate also correlate with malignancy. (5, 6, 7)

Concerning metastasis, ultrasound identified a hyperechoic area, with regular contour, on hepatic segment IV. This patient was previously operated for insulinoma with liver metastases: corporeo-caudal pancreatectomy and splenectomy were performed and liver metastases were resected in segments V, VI, VII of the liver. The therapeutic approach involves close patient monitoring and guidance for oncology service for cancer therapy. Liver metastases were demonstrated in 2 more patients by MRI. MRI confirmed the one found at US too. 5 patients had postoperative recurrences at varying intervals of time. In one patient in whom symptoms reappeared after seven years, imaging methods could not identify the tumor so he received palliative treatment (Verapamil 240 mg per day orally, Sandostatin 0.1 mg every 8 hours subcutaneously, diet, avoiding intense exercise). In another case, 3 years after surgery, at the EUS reevaluation a tumor was observed suggesting a possible recurrence; the patient refused surgical intervention. Excluding those 2 patients with liver metastases and the 5 with recurrence, we can say that the remaining 10 received curative surgery. And out of the 21 patients with insulinoma in the study, the remaining 4 underwent a palliative treatment, with periodic evaluation to locate the tumor. Where recurrent tumor could be identified, but the patient refused surgical intervention, anatomical location was the same as the primary tumor.

Evolution and mortality

Postoperative evolution was generally favorable, with 0% mortality. Curative surgery was followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss.

DISCUSSION

Patients with insulinoma have symptoms of excessive insulin release with consecutive hypoglycemia, namely neuroglicopenic and adrenergic symptoms. The delay in establishing the diagnosis is explained by misleading and nonspecific clinical presentation (5, 7, 8). A high index of clinical suspicion is therefore essential. Eight of our patients were hospitalized in the neurology and psychiatry clinics and mistreated. Of them, four patients were diagnosed with epilepsy and received anticonvulsant treatment, and the other four patients were labeled with neurological disorders, anxiety disorders or thyroid dysfunction.

Between the onset of symptoms and diagnosis days, months, years or even decades can pass. This situation is found in our patients, the correct diagnosis being established over a period of symptomatic evolution averaged 25.14 months, but there are delays that have reached 10 years.

Diagnosis is based on Whipple's triad. The biochemical diagnosis of insulinoma is supported by the presence of hypoglycemia and inappropriately elevated insulin levels during prolonged fasting. In most patients (90%), insulinomas appear as single pancreatic tumors. In our study all patients were classified in this category – single pancreatic tumor – and the most common location was the proximal one (head and uncinate process) - 44.44%.

Most insulin-secreting tumors are small, so they are a diagnostic challenge for physicians, endocrinologists, gastroenterologists, surgeons and radiologists. In our case, most of them were between 1 and 3 cm (average size 1.9 cm). The role of imaging is to detect and provide precise information on anatomical location and staging before surgery.

Ultrasound, CT and MRI showed poor sensitivity. In our case it was 10% for US (literature 9-79.3% (6,

10)), positive results being obtained in patients with tumor located proximally (pancreatic region most accessible for abdominal US exploration), at the junction head – pancreatic body, and in only one tumor located caudally, but large (63/43 mm).

The diagnostic utility of somatostatin receptor scintigraphy is limited by the fact that only about 40-50% of them have somatostatin receptors, so that a negative scan cannot exclude the presence of an insulinoma. Octreoscan was performed in two patients; in one was not conclusive, in the other one revealed metastatis.

Studies show that PET (Positron Emission Tomography) is a sensitive imaging method to locate insulinoma with higher sensitivity than CT or MRI (26). PET was performed in three patients in our study, identifying metastatic insulinoma in only one of them. It should be noted that both Octreoscan and PET-CT became available later; however none of them have helped us greatly in clarifying the diagnosis.

EUS is particularly useful. EUS detection rates vary in different studies from 40% to 93% (6). EUS located the tumor in a patient where US and CT were negative. The typical lesion at EUS is round, smooth, hypoechoic, located mostly in the pancreas. Neuroendocrine tumors of the pancreas are usually slow growing, indolent, and growth is still a major determinant of survival in these patients. Because they usually grow slowly, their metastatic potential is relatively low and there are specific criteria to predict their behavior (the distinction between benign and malignant tumors is based on the presence of metastasis), so long-term monitoring is necessary. Currently, the most important prognostic factor is the presence or absence of metastases. Even metastatic tumors grow slowly, the prognosis in these patients being relatively good compared with non-endocrine pancreatic neoplasms (27, 28).

10% (5.8-15%) of insulinomas are malignant and already present liver and locoregional lymph node metastases at diagnosis. In our study three patients with insulinoma had liver metastases (14.28%).

Treatment options for pancreatic neuroendocrine tumors require a multidisciplinary approach and depend on the presence or absence of metastases. Surgical resection is the only potentially curative approach. (29)

The main technical options available are: enucleation and pancreatic resections, the choice of either method being determined by location, size and number of tumors. Enucleation is generally preferred because it strictly removes the lesion without unnecessary sacrifice of healthy pancreatic tissue; in our study was performed in 7 of the 19 surgeries (30).

Intraoperative diagnosis was made by intraoperative palpation of tumor and/or intraoperative ultrasound. Intraoperative ultrasonography is valuable for locating small lesions and has an overall sensitivity of 80-100% (5), being more sensitive than all other preoperative imaging techniques and better than physical examination performed intraoperatively by the surgeon. In our case, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%.

Postoperative complications – transient hyperglycemia, anemia, transient pancreatitis, pancreatic pseudocyst, diabetes mellitus, exocrine pancreatic insufficiency, malabsorption, eventration – occurred in 8 of 17 patients (postoperative morbidity 47.05%).

In one case, the tumor was not found by intraoperative palpation, probably due to its deep location, but was easily detected by intraoperative ultrasound evaluation.

In metastatic disease the most important therapeutic goal is to reduce the life-threatening hypoglycemic syndrome. This can be achieved by surgical removal of metastases or a variety of other cytoreductive techniques, radiofrequency ablation, with good results. An active approach to patients with advanced disease may result in longer survival and prevention of hypoglycemia.

Medical treatment was used to prepare the surgical one aiming at reducing the frequency and severity of hypoglycemic symptoms in patients whose tumor could not be located with any imaging techniques and in the patient who refused surgery because of mild

and rare symptoms and, not least, in patients whose surgery was not curative (palliative treatment).

Drugs used for this purpose were: Verapamil, Octreotide (Sandostatin) and Glucagon (glucagon). They were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom curative surgery was not possible as a solution (31).

Evolution was generally good, with 0% mortality, and curative interventions were followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss. Unfortunately, there were some failures of surgery, especially when the location of the tumor has not exactly been made preoperatively or intraoperatively, more likely associated with metastatic disease.

CONCLUSIONS

The challenging aspect of treating insulinomas is localizing the tumors. Diagnosis has three steps: identifying the clinical syndrome, biological diagnosis with hormonal determination and topographic diagnosis. Due to nonspecific symptoms, a high index of suspicion is required. The supervised fast is the classic diagnostic test for insulinoma. Histopathological diagnosis is essential for diagnosis and therapeutic management. Native CT or CT with intravenous iodinated contrast material had low sensitivity in detecting insulinomas and liver metastases, but identified in our study, lymph node metastases. MRI is more sensitive than CT and can diagnose insulinomas with infracentimetric size; MRI is also a technique of choice for highlighting possible metastases in the liver. EUS can locate the tumors and describes the size, shape, contour, echostructure and relationship with adjacent structures. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive. EUS is the imaging investigation of choice in preoperative diagnosis of insulinomas. Surgical resection remains the mainstay of treatment for patients with localized disease. Future studies must lead to further improving therapeutic options for patients with this disease.

References:

1. Kim MK. Endoscopic Ultrasound in Gastroentero-pancreatic Neuroendocrine Tumors. Gut Liver 2012;6(4): 405-410.

2. Kulke MH, Bendell J, Kvols L, Picus J, Pommier R, Yao J. Evolving Diagnostic and Treatment Strategies for Pancreatic Neuroendocrine Tumors. J Hematol Oncol. 2011;4:29

3. Shin JJ, Gorden P, Libutti SK. Insulinoma: pathophysiology, localization and management. Future Oncology 2010;6(2):229–237.

4. Kulke MH, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, Jensen RT; North American Neuroendocrine Tumor Society (NANETS). NANETS Treatment Guidelines: Well-Differentiated Neuroendocrine Tumors of the Stomach and Pancreas. Pancreas 2010;39(6): 735–752.

5. Okabayashi T, Shima Y, Sumiyoshi T, Kozuki A, Ito S, Ogawa Y, Kobayashi M, Hanazaki K. Diagnosis and management of insulinoma. World J Gastroenterol. 2013;19(6):829-37.

6. Goswami J, Somkuwar P, Naik Y. Insulinoma and anaesthetic implications. Indian Journal of Anaesthesia 2012;56(2):117–122.

7. Abboud B, Boujaoude J. Occult sporadic insulinoma: Localization and surgical strategy. World Journal of Gastroenterology 2008;14(5):657-665.

8. Mathur A, Gorden P, Libutti SK. Insulinoma. Surg Clin North Am, 2009;89(5):1105–1121.

9. Pitre J, Soubrane O, Palazzo L, Chapuis Y. Endoscopic ultrasonography for the preoperative localization of insulinomas. Pancreas. 1996;13(1):55-60.

10. Angeli E, Vanzulli A, Castrucci M, Venturini M, Sironi S, Zerbi A, Di Carlo V, Pozza G, Del Maschio A. Value of abdominal sonography and MR imaging at 0.5 T in preoperative detection of pancreatic insulinoma: A comparison with dynamic CT and angiography. Abdom Imaging 1997;22(3):295-303.

11. Ardengh JC, Rosenbaum P, Ganc AJ, Goldenberg A, Lobo EJ, Malheiros CA, Rahal F, Ferrari AP. Role of EUS in the preoperative localization of insulinomas compared with spiral CT. Gastrointest Endosc. 2000;51(5):552-5.

12. Machado MC, da Cunha JE, Jukemura J, Bacchella T, Penteado S, Abdo EE, Machado MA, Herman P, Montagnini

AL, Pinotti H. Insulinoma: diagnostic strategies and surgical treatment. A 22-year experience. Hepatogastroenterology 2001;48(39):854-8.

13. Mirallié E, Pattou F, Malvaux P, Filoche B, Godchaux JM, Maunoury V, Palazzo L, Lefebvre J, Huglo D, Paris JC, Carnaille B, Proye C. Value of endoscopic ultrasonography and somatostatin receptor scintigraphy in the preoperative localization of insulinomas and gastrinomas. Experience of 54 cases. Gastroenterol Clin Biol. 2002;26(4):360-6.

14. Jyotsna VP, Rangel N, Pal S, Seith A, Sahni P, Ammini AC. Insulinoma: Diagnosis and surgical treatment. Retrospective analysis of 31 cases. Indian J Gastroenterol. 2006;25(5):244-7.

15. Sotoudehmanesh R, Hedayat A, Shirazian N, Shahraeeni S, Ainechi S, Zeinali F, Kolahdoozan S. Endoscopic ultrasonography (EUS) in the localization of insulinoma. Endocrine 2007;31:238-241.

16. Joseph AJ, Kapoor N, Simon EG, Chacko A, Thomas EM, Eapen A, Abraham DT, Jacob PM, Paul T, Rajaratnam S, Thomas N. Endoscopic Ultrasonography - A Sensitive Tool in the Preoperative Localization of Insulinoma. Endocr Pract 2013;20:1-16

17. Finlayson E, Clark OH. Surgical treatment of insulinomas. Surg Clin N Am 2004;84:775–785.

18. Sima II, Cherana G, Popescu AL, Rusu E, Costache RS, Jinga V, Spiroiu C, Ranetti AE, Jinga M. Neuroendocrine tumors of the pancreas: preoperative topographic diagnosis. European Journal of Internal Medicine 2011;22:S109-S109.

19. Spiroiu C, Mazilu A, Jinga M, Mihai AM, Ranetti AE. Diagnostic difficulties in insulinomas. The importance of endoscopic ultrasonography. Acta Endocrinologica 2006;2(1):95-107.

20. Andronesi D, Andronesi A, Tonea A, Andrei S, Herlea V, Lupescu I, Ionescu-Târgovişte C, Coculescu M, Fica S, Ionescu M, Gheorghe C, Popescu I. Insulinomul pancreatic - analiza unei serii clinice de 30 de cazuri. Chirurgia 2009; 104:675-685.

21. Guettier JM, Gorden P. Insulin secretion and insulin-producing tumors. Expert Rev Endocrinol Metab. 2010;5(2):217–227.

22. Mukherjee E, Carroll R, Matfin G. Endocrine and metabolic emergencies:hypoglycemia. Therapeutic Advances in Endocrinology and Metabolism 2011;2(2):81-93.

23. Douillard C, Mention K, Dobbelaere D, Wemeau JL, Saudubray JM, Vantyghem MC. Hypoglycemia related to inherited metabolic diseases in adults. Orphanet Journal of Rare Diseases 2012;7:26.

24. Mohamed Z, Arya VB, Hussain K. Hyperinsulinaemic Hypoglycemia: Genetic Mechanisms, Diagnosis and Management. Journal of Clinical Research in Pediatric Endocrinology 2012;4(4):169-181.

25. Angelescu C, Gheorghe C, Gheorghe L, Lupescu I, Herlea V, Ionescu M. Pancreatic neuroendocrine tumor (Quiz). J Gastrointestinal and Liver Diseases 2006;15:403-405.

26. Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K. The North American Neuroendocrine Tumor Society Consensus Guideline for the Diagnosis and Management of Neuroendocrine Tumors Pheochromocytoma, Paraganglioma, and Medullary Thyroid Cancer. Pancreas 2010;39(6):775-783.

27. Halfdanarson TR, Rubin J, Farnell MB, Grant CS, Petersen GM. Pancreatic endocrine neoplasms: epidemiology and prognosis of pancreatic endocrine tumors. Endocrine-Related Cancer 2008;(15):409–427.

28. Pearse AG. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. The Royal Society 2010; 170(1018):71-80.

29. Jinga M, Nicolaie T, Spiroiu C, Dumitrescu M, Vasilescu F, Campeanu I, Danaila E. The role of endoscopic ultrasound in diagnosis and preoperative localization of pancreatic inulinomas. Dig Endosc 2004;16(2):A71.

30. Hodul PJ, Strosberg JR, Kvols LK. Aggressive Surgical Resection in the Management of Pancreatic Neuroendocrine Tumors: When Is It Indicated?. Cancer Control. 2008;15(4):314-221.

31. Poiană C. De la carcinoid la cancer – Nosologie, topografie şi epidemiologie. Medic.ro 2011;71(1-2): 4-8.