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# Differences in clinical characteristics, treatment, and outcomes of sporadic and MEN-1-related insulinomas

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## Abstract

**Introduction:** Although in most cases insulinomas are small, benign, sporadic tumours, they can also be associated with hereditary syndromes, most commonly multiple endocrine neoplasia type 1 (MEN-1). Such a diagnosis significantly affects patient management. The objective was to elucidate the clinical differences between sporadic and MEN-1-linked insulinoma.

**Material and methods:** Comparison of clinical and histopathological characteristics, types of surgery, and outcomes of patients with sporadic and MEN-1-related insulinoma diagnosed between 2015 and 2022.

**Results:** There were 17 cases of insulinomas that underwent MEN-1 genetic testing (10 women and 7 men). In 7 cases, the mutation in the menin gene was confirmed. The median age at the time of diagnosis of sporadic insulinoma related to MEN-1 was 69 years (range 29–87) and 31.5 years (16–47), respectively. Primary hyperparathyroidism (PHP) was found in 6 of 7 patients with MEN-1-related insulinoma, while in none of the patients without MEN-1 mutations. Multifocal pancreatic NETs were found in 3 patients with MEN-1 syndrome, while in all sporadic cases there was a single pancreatic tumour. Two patients with insulinoma related to MEN-1 had a positive familial history of MEN-1-related diseases, while none with sporadic form. Dissemination at diagnosis was found in 4 cases, including 3 patients with insulinoma related to MEN-1-related insulinoma. Patients with sporadic and MEN-1-related insulinoma did not differ in tumour size, Ki-67 proliferation index, and outcome.

**Conclusions:** Of all the features evaluated, only the multifocal nature of pancreatic neuroendocrine tumour (PanNET) lesions and a positive family history differentiated between patients with sporadic and MEN-1-related insulinomas. An age of insulinoma diagnosis of less than 30 years may be a strong indicator of an increased risk of MEN-1 syndrome. **(Endokrynol Pol 2023; 74 (4): 363–371)**

**Key words:** *insulinoma; MEN-1; PanNET; hypoglycaemia; pancreatic tumours*

## Introduction

Pancreatic neuroendocrine tumours (PanNETs) are a rare type of neoplasm with an estimated annual incidence of 0.8/100,000. Most of them are sporadic, but about 10% are related to 4 inherited syndromes: neurofibromatosis type 1 (NF1), Von Hippel-Lindau (VHL), multiple endocrine neoplasia type 1 (MEN-1), and tubular sclerosis (TSC). Among all PanNETs, non-functioning tumours are the most common, whereas insulinomas are the most frequent functioning type followed by gastrinomas, glucagonomas and very rare VIPomas, somatostatinomas, adrenocorticotropic hormone (ACTH), and serotonin producing tumours [1].

Although in most cases insulinomas are small and benign, they pose a risk of severe recurrent hypoglycaemia. Malignant metastatic ones occur in approximately 4% of all insulinomas [2], more often in the case of inherited syndromes, mainly multiple endocrine neoplasia type 1. MEN-1 is an autosomal dominant syndrome related to the mutation of the menin gene (GenBank Accession No. U93236.1), located on chromosome 11q13. Menin directly regulates the expression of the cyclin-dependent kinase inhibitor (CKI) genes CDKN1b (encoding p27) and CDKN2C (encoding p18).

MEN-1 syndrome is characterized by the development of different endocrine and non-endocrine tumours. The typical triad includes parathyroid hyperplasia, gastrointestinal neuroendocrine tumours (NET), and anterior pituitary adenoma. Less common tumours include adrenocortical adenomas, skin tumours, lipomas, and neuroendocrine tumours of the thymus and lungs. Although hyperparathyroidism (hyperPHPT) is usually the first and most common clinical manifestation of MEN-1, the main mortality in MEN-1 syndrome is related to NET, mainly of the duodenum or pancreas. To date, more than 1800 germline and somatic mutations have been reported, including frameshift variants, nonsense, missense, and splice site variants as well as large deletions. They were found in

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9 of the 10 exons that encode the MEN-1 gene [3–5]. None of these mutations has unequivocal pathological consequences; however, some are related to a more aggressive course of the disease in affected families [6]. In all cases, the genetic diagnosis of MEN-1 syndrome is clinically important due to the indication for lifelong follow-up, which may reduce disease-related morbidity and mortality. It should be noted that insulinoma develops in 10–15% [7] of patients with MEN-1 syndrome, but 25% to 44% of the genetic variants identified in PanNET are somatic in nature [8]. Currently, no histological criteria have been established for the prediction of insulinoma malignancy, and the presence of metastases differentiates between benign and malignant types. Insulinoma, even malignant, is associated with relatively good overall survival, but even benign tumours can present with a variety of hypoglycaemia-related symptoms increasing mortality and lowering quality of life. Due to the rarity of insulinoma, the differences between sporadic and MEN-1-associated insulinoma are not well described, although comparative studies currently available suggest a similar incidence of malignancy in both groups, while younger age, larger benign pancreatic lesions at the time of resection, and multifocal lesions are more frequent in cases of MEN-1 syndrome [9]. Tumour localization, staging, grading, and somatostatin receptor (SSTR) expression are currently the most important factors that determine the possibilities of treatment [10]. Understanding the differences in the characteristics and course of sporadic and MEN-1-related insulinoma might help to personalize the approach, especially in patients with disseminated disease.

## Study design

The study was designed as a retrospective analysis of differences in demographics, neoplasm characteristics, and clinical course between the sporadic and MEN-1-related insulinomas.

All cases were identified among patients referred to our centre in the years 2013–2021 (since the time of introduction of electronic documentation) due to the clinical presence of insulinoma with a confirmed episode of hypoglycaemia accompanied by insufficient levels of C-peptide or insulin during the 72-hour fasting test. All included patients had performed a *MEN-1* gene analysis.

After identifying eligible patients, consecutive patient records were reviewed for the collection of clinical/outcome data.

The grade of insulinoma was reported according to World Health Classification (WHO) classification criteria for pancreatic neuroendocrine tumours. The mitotic index per 10 high-power fields (G1 < 2, G2 2–20, and G3 > 20) and the Ki-67 proliferation index (G1 < 3%, G2 3-20%, and G3 > 20%) was classified into 3 groups according to WHO criteria. In the case of contradiction between the mitotic rate and Ki-67 assessment, the WHO grade was determined by the highest of both. For patients with multiple pancreatic resections of PanNETs, tumour characteristics from the first resection were used for analysis.

Malignancy was defined by the presence of distant metastases or positive lymph nodes on histopathological examination.

All patients were tested for primary hyperparathyroidism by evaluating the serum concentration of PTH, calcium, and phosphates. In patients with confirmed MEN-1 syndrome, serum PRL and IGF-1 analysis was performed to exclude the risk of functional pituitary adenomas, and most of them (6 out of 7) underwent pituitary magnetic resonance imaging. Preoperative molecular imaging of insulinoma [somatostatin receptor imaging in single-photon emission computed tomography (SPECT)/computed tomography (CT) or positron emission tomography (PET)/CT] was performed to evaluate the expression of the somatostatin receptor.

### *MEN-1 gen analysis*

Genetic examinations were performed on site, in the Krakow University Hospital Genetics Laboratory, as previously described in detail [6]. Briefly, Sanger sequencing included exons coding for exons 2 through 10 and adjacent splicing sites. The analysed sequence was compared with the reference sequence LRG_509t1. In the case of strong clinical suspicion of genetic background without a genetic mutation confirmed in Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) was performed.

### *Follow-up*

According to local practice and guidelines, all patients underwent biochemical and radiological follow-up, usually every 3 to 6 months depending on the clinical course [11]. For this study, the primary outcomes included the appearance of insulinoma recurrence or insulinoma-related progression or death. The type of treatment was determined by a multidisciplinary team consultation. Causes of death were obtained from medical records.

### *Statistical analysis*

Demographic and clinical characteristics were analysed by producing frequency tables for categorical variables and by calculating the median and range for continuous variables.

The duration of follow-up for the group was counted as the range and median value in months, from the time of curative surgery to the last follow-up appointment. IBM SPSS Statistics 26 was used for the statistical analysis.

### *Ethical statement*

The study is in accordance with the principles set out in the Declaration of Helsinki, and the study design was approved by the Bioethics Committee of the Jagiellonian University of Krakow, Poland (Opinion No. 1072.6120.131.2020 28.05.2020). Written informed consent was not required for participation in the study according to national legislation and institutional requirements. Study participants signed an informed consent to genetic analyses performed as a routine examination in insulinoma.

## Results

Through the database search, 19 patients were identified. Among them, in 17 MEN-1 mutation status was determined and they were included in the analysis. There was a higher prevalence of women (10 *vs.* 7 cases). The median age at diagnosis for the entire group was 58 years (range 16–87).

### *MEN-1 gen analysis*

Genetic testing revealed MEN-1 variants among 6 patients; in one additional patient with clinical presentation of MEN-1 syndrome and negative MEN-1 result in Sanger sequencing, MLPA was performed. We

identified 3 missense variants, one inframe insertion, 2 frameshift deletions, and one large deletion. The types of mutations reaffirming MEN-1 syndrome and their clinical significance described so far in the ClinVar and ACMG database are listed in Table 1.

### *Status at diagnosis*

Hyperinsulinaemic hypoglycaemia proven by the 72-h fasting test was diagnosed in the 17 patients, and 16 patients had typical clinical symptoms of hypoglycaemia (Whipple triad) prior to diagnosis. The most commonly reported symptoms included dizziness, irritability, visual disturbances, and impaired concentration, mainly in association with prolonged starvation. In one case, attacks of aggression had occurred that had required psychiatric consultation. The median serum glucose concentration at the end of the 72-h fasting test was 2.04 mmol/L (range 1.0–2.86), while the median insulin concentration was 11.7 uIU/mL (range 3.37–51.0).



**Table 1.** *Genetic and clinical findings in the group of patients with confirmed multiple endocrine neoplasia type 1 (MEN-1) syndrome*

| Patient | Mutation rs number<br>Clinical significance according to ClinVar and ACMG classification | Gender | Age at insulinoma diagnosis | NET grading according to WHO | Insulinoma dissemination | PHPT during follow-up | Family history |
|---|---|---|---|---|---|---|---|
| 1. | c.781C>T (p.Leu261Phe)<br>rs878855198<br>ClinVar: Pathogenic/Likely pathogenic<br>ACMG: Likely pathogenic | F | 43 | G1 | Yes (to the lymph nodes) | Yes | Positive |
| 2. | 1293_1365_1734_?del<br>None<br>No classification in external databases but large deletions inactivate protein function | F | 16 | G2 | Yes (hypoglycaemia recurrence due to the distant metastases) | Yes | Negative |
| 3. | c.1142T>C (p.Leu381Pro)<br>rs1114167471<br>ClinVar: Likely-Pathogenic<br>ACMG: Likely Pathogenic | M | 17 | G1 | No (multifocal PanNETs) | Yes | Negative |
| 4. | c.1134_1134delC (p.Asp423=)<br>None<br>No classification in external databases but frameshift deletion → gained stop codon → inactivated protein function | M | 26 | G1 | No (multifocal PanNETs, hypoglycaemia recurrence due to new PanNET) | Yes | Positive |
| 5. | c.527G>A>T(;)1311G>A (p.Arg176Gln)<br>rs607969<br>ClinVar: Conflicting interpretations of pathogenicity<br>ACMG: Uncertain Significance | F | 69 | G1 | No | No | Negative |
| 6. | c.785_790dupTGCAGC (p.262_263LueGln)<br>rs1555165485<br>ClinVar: Likely pathogenic<br>ACMG: Likely pathogenic | F | 37 | G2 | Yes (to the lymph nodes, infiltration of left adrenal gland multifocal PanNETs) | Yes | Negative |
| 7. | c.945delG (p.Tyr316Profs*57)<br>None<br>No classification in external databases but frameshift deletion → gained stop codon → inactivated protein function | F | 47 | G1 | No | Yes | Negative |

ACMG — American College of Medical Genetics and Genomics; NET — neuroendocrine tumour; WHO — World Health Organization; PHPT — primary hyperparathyroidism; PanNET — pancreatic neuroendocrine tumour. All variants in MEN1 were identified in heterozygous state. Reference sequences: NM_0020244.3, NP_000235.2

In all patients, anatomical [computed tomography (CT) or magnetic resonance imaging (MRI)] and/or molecular imaging [somatostatin receptor imaging or glucagon-like peptide-1 (GLP-1)] was performed after biochemical confirmation of insulinoma, and information on tumour location was available before surgery.

Thirteen of the analysed patients underwent imaging of the somatostatin receptor prior to surgery with [$^{99m}$Tc]Tc-EDDA-hydrazinonicotinyl-Tyr3-octreotide ($^{99m}$Tc-EDDA//HYNIC-TOC) or gallium 68-tetraazacyclododecane-tetraacetic acid-octreotate [($^{68}$Ga)Ga-DOTA-TATE]. In 8 cases, a positive result was observed (good or very good SSTR expression (Krenning score 3 or 4)]. In 2 cases, additional GLP-1 scintigraphy was performed and was successful in both cases. Preoperative imaging showed multifocal NET tumours in the pancreas in 2 cases.

### *Surgical procedure undertaken for insulinoma treatment and its outcome*

In all cases, in the perioperative period, to maintain the glucose level, diazoxide was used, in 8 cases accompanied by glucocorticosteroids.

One patient with primary inoperable insulinoma of the head of the pancreas underwent neoadjuvant peptide receptor radionuclide therapy (PRRT) treatment with subsequent tumour shrinkage, allowing surgery. Finally, 16 patients underwent surgery followed by histopathological confirmation of insulinoma. Among them, in 15 cases, surgery was primarily curative, but in 2 cases there was relapse of hypoglycaemia leading to reoperation. As first-line surgery, 3 patients underwent insulinoma enucleation, 11 partial pancreatectomy, and 2 total pancreatectomy. In 2 patients with recurrence of hypoglycaemia (19 and 20 months after insulinoma enucleation), new NET lesions were exclusively found in the pancreas, so these patients finally underwent total pancreatectomy. In one of those cases, liver dissemination was diagnosed 9 months later, and the patient was qualified for systemic treatment (with long-acting somatostatin analogues and PRRT). The median tumour size in histopathology was 15.0 mm (range 1.5–43.0 mm). The median Ki-67 was 3.0% (range 1.0–12.0%). At the time of the analysis, 16 patients were still alive and remain in follow-up at our centre. The median follow-up time was 49.3 months (range 13.8–111.1). One patient died from co-existing pancreatic adenocarcinoma.

### *Comparison of MEN-1 and sporadic insulinoma*

Among 17 patients, the status of the MEN-1 mutation was determined. A positive result was found for germline mutations in the menin gene in 7 cases, among whom 2 analysed insulinoma patients had a positive familial history of NET tumours. There were 5 out of 7 women among patients with the MEN-1 mutation, while, as in the sporadic insulinoma group, women comprised 5 out of 10 cases. The median age at the time of insulinoma diagnosis in cases related to MEN-1 and non-MEN-1 was 69 years (range 29–87) and 31.5 years (range 16–47), respectively.

Primary hyperparathyroidism was diagnosed in 6 of 7 patients with MEN-1-related insulinoma; however, at the time of insulinoma diagnosis primary hyperparathyroidism was found only in 4 of 7 patients.

In 2 patients with MEN-1, during follow-up, nephrolithiasis was diagnosed. None of the patients with sporadic insulinoma developed primary hyperparathyroidism. No pituitary adenoma was found in patients with MEN-1 or non-MEN-1. The median value of prolactin (PRL) was 352 $\mu$IU/L (range 289–1029) and 329 $\mu$IU/L (193.2–529.0) in patients with MEN-1 and not MEN-1, respectively. In all tested cases, IGF-1 concentrations remained in normal age-adjusted ranges.

Preoperative imaging showed multifocal NET tumours in the pancreas in 2 cases of MEN-1 patients (Fig. 1), and histopathology revealed 1 additional case of multifocal disease. The number of NET tumours in the case of multifocal disease was 2 to 7, and its diameter was 1.5 to 35.0 mm. In all sporadic cases, there was a single pancreatic NET tumour (Fig. 2). In one MEN-1 patient, during follow-up, a metachronous NET tumour in the small intestine was diagnosed and radically resected.

In all these cases, the surgical strategy was planned on the basis of the location of the lesion on anatomical and molecular imaging and the possibility of a precise removal of NET. The total number of patients treated with tumour enucleation or distal pancreatic tail resection was 6, but there was only one MEN-1 case. Due to the development of multiple pancreatic tumours in MEN-1 patients, 4 cases underwent partial or total pancreatectomy. Pancreas-sparing procedures were performed more frequently in younger patients (below 40 years old). Among MEN-1 patients, NET tumours were found in 3 of 7 cases in the pancreatic head, 3 cases were multifocal, and in one case located in the pancreatic tail. In non-MEN-1-related insulinomas, tumours were located in the head of the pancreas in 5 of 10 cases, and 5 patients developed insulinoma in the distal part of the pancreas. In all cases, the diagnosis of well-differentiated neuroendocrine tumour (NET) was established on histopathological examination. In MEN-1-related and non-MEN1-related cases, surgery was finally curative in 6 of 7 and 8 of 9 cases, respectively.

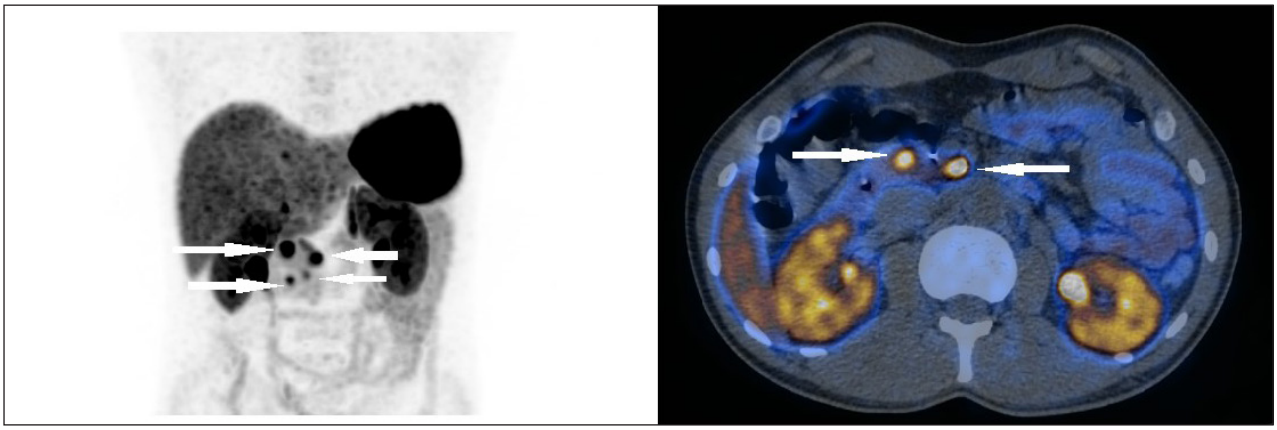

**Figure 1.** *Gallium 68-tetraazacyclododecane-tetraacetic acid-octreotate ([$^{68}$Ga]Ga-DOTA-TATE) positron emission tomography/computed tomography (PET/CT) showing multifocal neuroendocrine tumours in pancreatic body (arrows) in a patient with recurrent insulinoma related to multiple endocrine neoplasia type 1 (MEN-1) syndrome*



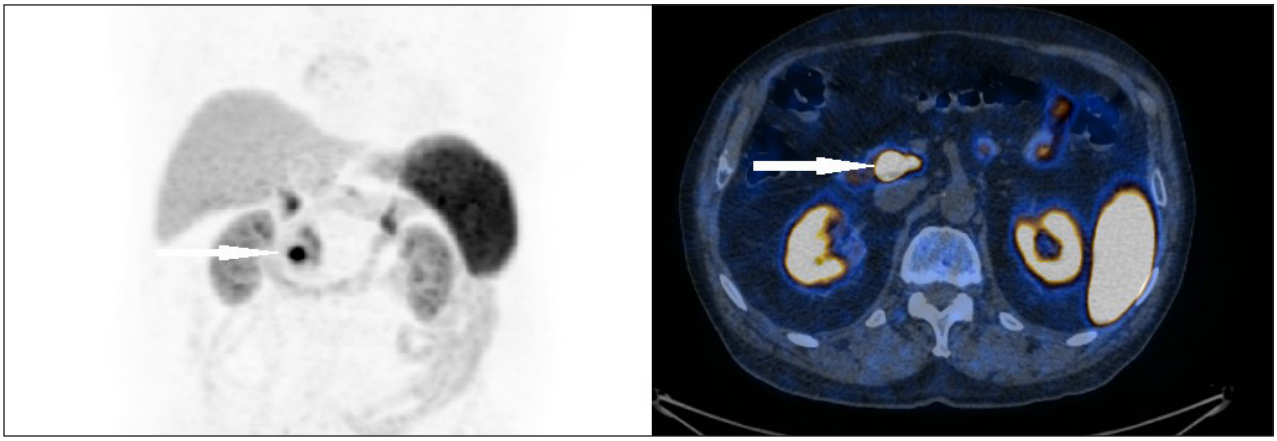

**Figure 2.** *Gallium 68-tetraazacyclododecane-tetraacetic acid-octreotate ([$^{68}$Ga]Ga-DOTA-TATE) positron emission tomography/computed tomography (PET/CT) revealing a single neuroendocrine tumour (NET) (insulinoma) of the head of the pancreas (arrow) in a patient with non-MEN-related insulinoma*

The median tumour size in cases related to MEN-1 and non-MEN-1 was in both cases 15 mm, range 1.5 to 35.0 and 10.0 to 43.0, respectively.

The median Ki-67 for insulinoma related to MEN-1 and non-MEN-related insulinoma was 2.5% (range 1.0–8.0) and 2.0% (range 1.0–12.0), respectively. The median Ki-67 value for all patients (4 cases) with insulinoma dissemination was 6.9% (range 2.0–12.0).

Preoperative dissemination of insulinoma was found in 2 of 7 patients with MEN-1 and in one of 10 patients with insulinoma not related to MEN-1. In the postoperative specimen, local lymph node involvement was found in an additional one case of insulinoma not related to MEN-1. One of the disseminated patients had metastases in the left adrenal gland, which was surgically cured. Three patients had dissemination to the liver. In all cases, the good expression of SSTR was confirmed on molecular imaging and patients were treated with long-acting somatostatin analogues with subsequent PRRT obtaining a good clinical result (reduction of hypoglycaemia episodes and doses of diazoxide). In one case after PRRT, the primary unresectable tumour with a single metastasis to the liver became resectable and the patient underwent curative surgery [12].

Currently, all 10 patients with non-MEN-1-related patients are alive, but one patient with MEN-1-related insulinoma has died from coexisting pancreatic cancer. A comparison of the most important clinical features of MEN-1-related and sporadic insulinomas is provided in Table 2 and 3.

## Discussion

In the present study, we analysed the clinical features of insulinoma in patients with MEN-1- and non-MEN-1-related PanNETs. Of all the characteristics we evaluated,

**Table 2.** *Baseline characteristics of the entire study cohort and in the division of multiple endocrine neoplasia type 1 (MEN-1) and non-MEN-related insulinoma*

| | All cases (n = 17) | Non-MEN-1-related insulinoma (n = 10) | MEN-1-related insulinoma (n = 7) |
|---|---|---|---|
| Age [years] | 58 (range 16–87) | 69 (range 29–87) | 31.5 (range 16–47) |
| Male/female | 0.7/1.0 | 1.0/1.0 | 0.4/1.0 |
| Symptomatic hypoglycaemia at diagnosis | 16 | 10 | 6 |
| Dissemination at diagnosis (n) | 3 | 1 | 2 |
| Surgical management (n) | 16 | 9 | 7 |
| Size of the lesions [mm] | 15 (range 1.5–43.0) | 15 (range 1.5–35.0) | 15 (range 10.0–43.0) |
| Multifocal NET lesion in pancreas | 3 | 0 | 3 |
| Ki-67, median % (range) | 3.0 (1.0–12.0) | 2.0 (1.0–12.0) | 2.5 (1.0–8.0) |
| Insulinoma grading, G1/G2 | 1.0/0.55 | 1.0/0.7 | 1.0/0.4 |
| Presence of metachronous NET | 1 | 0 | 1 |
| Recurrence of symptoms after pancreas spearing surgery (n) | 2 | 0 | 2 |
| PRRT (n) | 2 | 1 | 1 |
| Long-acting somatostatin analogues (n) | 2 | 1 | 1 |

NET — neuroendocrine tumour; PRRT — peptide receptor radionuclide therapy

**Table 3.** *Staging of insulinoma, data of the entire study cohort, and in division of insulinoma related to multiple endocrine neoplasia type 1 (MEN-1) and non-MEN-1*

| | All cases (n = 17) | Non-MEN-1-related insulinoma (n = 10) | MEN-1-related insulinoma (n = 7) |
|---|---|---|---|
| Number of patients with a single pancreatic lesion | 14 | 10 | 4 |
| Number of patients with multiple NET lesions in pancreas (pTXm, N0, M0) | 3 | 0 | 3 |
| Number of patients with lymph node metastases (pTX, N1, M0) | 1 | 1 | 0 |
| Number of patients with distant metastases (pTX, NX, M1) | 3 | 1 | 2 |

only the multilocality of pancreatic lesions and a recent positive family history were differentiating factors for patients with the presence of germline menin mutations. Younger age (especially younger than 30 years) of insulinoma diagnosis could be an indicator of the increased risk of MEN-1 syndrome. We did not find differences in tumour size, grade, and Ki67% index between MEN-1- and non-MEN-1-related insulinomas.

Although in a case of sporadic insulinoma, the diagnosis is usually associated with a relatively good prognosis [13], the diagnosis of insulinoma associated with some hereditary syndromes, including MEN-1 syndrome, is clinically burdening [14] due to the risk of other cancers, the need for lifetime follow-up, and its heredity. However, a previously published analysis of 311 patients with sporadic insulinoma and MEN-1-associated insulinoma did not show statistically significant differences in overall survival [9]. Currently, no direct translation of the MEN-1 genotype to phenotype is identified [15], and the clinical differentiation of sporadic and MEN-1 related insulinomas would be of great practical clinical value.

Sporadic insulinomas can occur at any age, but the highest incidence is observed in the fifth decade of life [16]. Conversely, insulinomas related to MEN-1 syndrome are diagnosed at a younger age (often before 40 years old), and as much as 25% of cases can occur by the age of 22 years [17]. In our group, the mean age of insulinoma diagnosis in the MEN-1 group was markedly lower; however, 3 patients in that group were older than 40 years, while 3 patients with non-MEN-1-related insulinoma were younger than 40. These results in-

dicate that although a younger age of occurrence of insulinoma increases the risk of MEN-1 syndrome, age should not be taken as an independent indicator of the risk of menin mutation, especially in people over 30 years of age.

The imaging diagnosis of insulinoma can be challenging, especially if the pancreatic lesion cannot be detected by CT or MRI. In such cases, molecular nuclear medicine imaging has been shown to be of value [18]. Among them, GLP1-R was shown to be a superior method, but its availability is very limited. Imaging of NETs, based on somatostatin analogues, is feasible, and the use of PET/CT with [$^{68}$Ga] Ga-somatostatin analogues should be the first-line option, due to its higher sensitivity and better spatial resolution compared to SPECT/CT techniques, giving a sensitivity of 46–50% to 50–86%, respectively [19]. Using them for initial staging significantly facilitates the planning of the operation extension [20]. This high sensitivity was observed in our cohort; nuclear medicine techniques allowed the location of NET tumours in 85% of the examined patients. Among all imaging techniques, EUS achieves up to 100% sensitivity in the diagnosis of pancreatic NEN; however, endoscopic ultrasonography (EUS) is an invasive technique with limited ability to detect metastases, and for that reason it should be complemented by nuclear (SSTR or GLP-R) or anatomic (CT or MRI) imaging techniques [21, 22].

Malignant insulinomas are relatively rare and are found in approximately 5% to 12% of all insulinoma cases reported in the literature [23], but their prevalence is higher, up to 14% in genetically determined syndromes, which was also observed in our cohort.

Taking into account the risk of dissemination, tumour size should not be underestimated. Previously published studies suggested that non-secreting PanNET smaller than 2 cm rarely develops metastases [24], and aggressive surgical management is not associated with better survival [25].

These findings are reflected in the current NET guidelines; however, in the case of symptomatic tumours, including insulinomas, tumour resection is recommended regardless of its size [10, 26]. In our cohort, only tumours over 30 mm were associated with dissemination, which seems to be in line with previous data. Accurate location of insulinomas in the case of multifocal PanNETs is challenging but necessary in the planning of individual surgical procedures (tissue-sparing). Only GLP-1R imaging and EUS-guided fine needle biopsy may allow for the correct location of insulinomas. However, in the cases of multiple PanNETs related to MEN-1, the extent of surgery considering only removal of insulinomas or all visible PanNETs is still controversial and should take into account the size and location of other PanNETs [21]. In our material, in the 2 cases of MEN-1 patients who underwent successful primary insulinoma enucleation, insulinoma recurrence was observed. It may indicate that a more extensive surgical approach should be considered as the first-line option in MEN-1 patients; however, this consideration needs further investigation, and such a small study group does not allow us to draw such far-reaching conclusions.

PTH-induced hypercalcaemia (primary hyperparathyroidism, PHPT) is the most common manifestation of MEN-1 syndrome. It commonly occurs before the age of 20 years and affects more than 95% of MEN-1 patients older than 40 years [27]. In our material, none of the patients had a diagnosis of PHPT before the onset of endogenous hypoglycaemia, probably due to the asymptomatic course of hyperparathyroidism. However, 4 of 7 of our MEN-1 patients had hypercalcaemia in laboratory tests at the time of insulinoma diagnosis. This is less than expected according to the literature; however, 2 more patients with MEN-1 developed primary hyperparathyroidism during follow-up.

Low sensitivity to MEN-1 syndrome risk refers to a positive family history of MEN-1 syndrome components. In our cohort of MEN-1-associated insulinomas, only 2 patients had a positive family history. At this point, it is worth stressing the possibility of the presence of phenocopies in families with MEN-1 syndrome, which can make the clinical diagnosis of these syndromes difficult. Phenocopies can mimic MEN-1 syndrome in 2 ways: by presenting a single sporadic endocrine tumour in a member of the MEN1 family or by the coexistence of 2 endocrine pathologies of different aetiology. Phenocopies are estimated to be present in as much as 5% of MEN-1 families [28].

According to the literature, and as observed in our study, multiple NETs in the pancreas [29], insulinoma recurrence, and metachronous NETs are most frequently found in patients with MEN-1.

Pituitary adenomas occur in 20–40% of patients with MEN-1 syndrome, up to 50% of which may not function [30]. Among our patients, 5 patients with MEN-1-related insulinoma had increased prolactin values at diagnosis, but none of them had a pituitary tumour confirmed on MRI, so the presence of pituitary adenoma was not a differentiating factor between patients with MEN-1- and non-MEN-1-related insulinoma. None of the patients in our cohort had accompanying NET of the lung or thymus, as observed in up to 10% of patients with MEN-1 syndrome [31–32]. Finally, a similarly good prognosis was observed in both groups, although insulinoma recurrence was observed only in

the MEN-1 patients. The main limitations of the study result from its retrospective nature, the small size of each study group, and the relatively short follow-up time to compare the long-term outcome of sporadic and MEN-1-related tumours. However, the rarity of insulinomas and the presence of many clinical uncertainties in optimizing its management prompted us to present our experience.

## Conclusions

Of all the features we evaluated, only the multilocality of the pancreatic NET lesions and a recent positive family history were differentiating factors for patients with insulinoma and the presence of mutation in the *MEN1* gene.

The age of diagnosis of insulinoma below 30 years could be a strong indicator of the increased risk of MEN-1 syndrome.

### *Author contributions*

M.O. and A.G.J. contributed to the conception of the work; A.K., A.B., K.M.S., and E.R. extracted data; M.O. and A.G.J. drafted the manuscript and contributed to the acquisition, analyses, and interpretation; M.O., A.G.J., A.S.S., and A.H.D. critically revised the manuscript and contributed to interpretation. All authors gave final approval and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy. All authors have read and agreed to the published version of the manuscript.

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