Pitfalls in diagnosing a pancreatic neuroendocrine tumor: a case report

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Abstract
Pancreatic neuroendocrine tumors are a rare subset of pancreatic neoplasms. We report the case of a 33-year-old female patient who was admitted to the Diabetes Clinic of Craiova, Romania, due to a two-year history of episodic neuroglycopenic hyperinsulinemic hypoglycemic symptoms, suggestive for insulinoma associated with facial and upper trunk flushing characteristic to carcinoid syndrome. During these episodes, the laboratory investigations showed hypoglycemia (38 mg/dL), hyperinsulinemia (54.72 μU/mL) and normal values of beta-hydroxybutyrate, chromogranin A, serotonin, anti-insulin antibodies and urinary levels of 5-hydroxyindoleacetic acid. Endoscopic ultrasound with SonoVue and 3T MRI revealed an 18.3/16.3 mm hypervascular tissular mass situated in the uncinate process of the pancreatic head in close contact with the superior mesenteric vein without invasion and no other detectable secondary lesions in the pancreas or any other abdominal viscera. Patient underwent enucleation of pancreatic tumor. The histological and immunohistochemical findings indicated a functional well-differentiated pancreatic neuroendocrine tumor, G1 category according to the World Health Organization (WHO) criteria, with uncertain behavior (Ki67 index was 3%), confined to the pancreas, but with tumoral invasion of the delimiting conjunctive capsule. No evidence of tumoral CK19 staining, mitoses and necrosis, angioinvasion or extra-pancreatic invasion was observed. A post-operative nine-month follow-up showed resolution of hypoglycemic symptoms, normalized blood glucose and insulin levels and no evidence of recurrence. Our case report highlights the pitfalls in diagnosing a functional pancreatic neuroendocrine tumor due to atypical symptoms, the difficulty of identification and precise location of the small-size tumor and uncertain histopathological and immunohistochemical behavior.

Keywords: hyperinsulinemic hypoglycemia, pancreatic neuroendocrine tumor, insulinoma, endoscopic ultrasound, enucleation.

\section*{Introduction}
Pancreatic neuroendocrine tumors (pNETs) are a relatively rare subset of pancreatic neoplasms, the annual incidence being estimated to 0.2–0.4/100,000\textsuperscript{[1]}. Functional pNETs are the most common tumor type and frequently include insulinomas, glucagonomas, somatostatinomas, gastrinomas, pNET causing the carcinoid syndrome, pNET secreting growth-hormone-releasing factor or adrenocorticotropic hormone, etc.\textsuperscript{[2]} Usually seen in adults, typically aged 30–60-year-old, pNETs may rarely occur also in children\textsuperscript{[1]}.

pNETs are classified by the World Health Organization (WHO) into three categories: G1 – well-differentiated endocrine tumors, limited to the pancreas, with benign behavior (without angioinvasion or perineural invasion, tumor diameter <2 cm, mitotic index ≤2, Ki67 index ≤2\% or uncertain behavior (angioinvasion or perineural invasion or tumor diameter ≥2 cm or mitotic index >2 or Ki67 index >2\%); G2 – well-differentiated endocrine carcinomas with low-grade malignant behavior and extrapancreatic invasion; and G3 – poorly differentiated endocrine carcinomas, with high-grade malignant behavior\textsuperscript{[3]}.

Insulinomas are the most common pNETs, comprising about 30–40\% of these tumors, with an estimated incidence of 1–3/million population/year\textsuperscript{[1, 4]}. The symptoms are related to excessive release of insulin from the tumor into the bloodstream and are typically represented by autonomic symptoms (palpitations, tremor, sweating, nausea, anxiety, hunger) and neuroglycopenic symptoms (confusion, fatigue, dizziness, headache, diplopia, blurred vision, abnormal behavior, amnesia, seizure, coma)\textsuperscript{[1, 4]}. Up to 85\% of patients present with palpitations, diplopia, blurred vision, or weakness\textsuperscript{[4]}. Hunger may be an important symptom leading to weight gain in about 30\% of the cases\textsuperscript{[4]}. Other symptoms include confusion, abnormal behavior, as well as amnesia. In 12\% of patients, grand mal seizures may occur\textsuperscript{[4]}. 
The diagnosis and classification of insulinomas requires a detailed description of the macroscopic (tumor diameter, extra-pancreatic invasion), microscopic (mitotic index, angioinvasion, perineural invasion) and immunohistochemical characteristics (tumor expression of chromogranin A, synaptophysin and insulin, Ki67 index) [3, 5].

Regarding malignancy, most insulinomas are classified as well-differentiated endocrine tumors (G1) but occasionally they belong to G2 or G3 grade [4, 5]. Malignant insulinomas account for only about 5–10% of all cases [5], tumor size ≥2 cm associated with Ki67 index >2% and chromosomal instability being predictive factors of malignancy [6, 7].

Aim

In our case report, we emphasize the pitfalls in diagnosing a functional pNET. Thus, we present the case of a female patient with small size pNET, with uncertain malignant behavior, who presented hypoglycemic hyperinsulinemic symptoms and flushing. Our case study also highlights the usefulness of preoperative imaging, especially endoscopic ultrasound (EUS) for identification and precise localization of small-size pancreatic tumors.

Case report

A 33-year-old female patient was admitted to the Diabetes Clinic of Craiova, Romania, due to recurrent episodes of confusion, blurred vision, severe dizziness associated with pruriginous facial and upper trunk flushing for more than two years. The clinical symptoms occurred 2–3 times/day and all remitted after ingestion of sugar containing food. Antihistamines have failed to induce flash remission. The patient also had weight gain (20 kg in the last two years). She did not complain of anxiety, palpitations, diaphoresis or diarrhea and abdominal pain. The patient had no significant family history or prior medical history.

Physical exam revealed an elevated body mass index (BMI) 32.1 kg/m² and waist (84 cm), normal blood pressure (135/80 mmHg) and heart rate (78 bpm) and no heart murmurs.

During the first day of hospitalization, she had experienced two episodes (fasting and one-hour postprandial) of severe dizziness, cognitive and visual impairment and facial flushing, but without an increase in blood pressure and heart rate. Laboratory data, carried out when these episodes occurred, confirmed hyperinsulinemic hypoglycemia: fasting glycemia was 33 mg/dL, fasting insulinemia was 21.36 μU/mL (normal range: 3–17 μU/mL), fasting β-hydroxybutyrate levels were 0 mmol/L (normal range: <0.6 mmol/L) and, respectively, one-hour postprandial glycemia was 38 mg/dL, one-hour postprandial insulinemia was 54.72 μU/mL and one-hour postprandial β-hydroxybutyrate levels were 0.1 mmol/L. During these hypoglycemic episodes, serum levels of chromogranin A, serotonin, anti-insulin antibodies and urinary levels of 5-hydroxyindoleacetic acid (5-HIAA) were in normal range.

Kidney and liver function, fundoscopy, chest X-ray, ultrasound of abdomen, pelvis and thyroid were also normal.

Clinical and biochemical evidence of hyperinsulinemic hypoglycemia led to imaging investigations searching for pNET.

Computed tomography (CT) scanning of the abdomen and pelvis showed no pathological changes in the areas examined.

Endoscopic ultrasound (EUS) was the imaging of choice that showed the presence of tumor (Figure 1), indicating a 18.3/16.3 mm tissue mass located in the uncinate process of the pancreatic head in close contact with the superior mesenteric vein without invasion and no other detectable secondary lesions in the pancreas or any other abdominal viscera. EUS detected a single peri-pancreatic lymph node of 5 mm. EUS-guided fine-needle aspiration of the pancreatic mass and lymph node was performed and the analysis of cytological specimen suggested benignity.

Figure 1 – Endoscopic Doppler ultrasonography indicating a 18.3/16.3 mm tumor in the uncinate process of the pancreatic head adjacent to the superior mesenteric vein and without invasion.

3 Tesla magnetic resonance imaging (3T MRI) showed that the tumor was well defined, homogeneous, with low signal in T1-weighted out-of-phase (Figure 2a) and in-phase (Figure 2b) sequences, high signal in T2-weighted (Figure 3a) and fat suppression (Figure 3b) sequences.

EUS with microbubble contrast agent (SonoVue), 4.8 mL as the contrast enhancing substance, showed high contrast enhancement in the early arterial phase compared to surrounding pancreatic parenchyma, with discrete washout in late venous phase characteristic to hypervascular lesions (Figure 4, a–c).

The patient was scheduled for elective surgery aiming to complete removal of the tumor. A 2 cm, well-bounded tumor was safely excised with a small amount of surrounding pancreatic tissue from the pancreatic uncinate process, avoiding injury of the main pancreatic duct and the superior mesenteric vein, which were nearest to the tumor (Figure 5, a and b). Visual and palpatory examination of pancreas did not reveal any other pancreatic lesion.

The pancreatic samples were embedded in paraffin after being fixed for 72 hours in 10% neutral formalin solution. Four μm-thick histological cups, sectioned using a microtome, were stained with Hematoxylin–Eosin (HE).
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Figure 2 – 3T MRI showing a tumor with low signal in T1-weighted out-of-phase (a) and in-phase (b).

Figure 3 – 3T MRI showing a tumor with high signal in T2-weighted (a) and fat suppression (b) sequences.

Figure 4 – EUS with SonoVue 4.8, pre-contrast (a), post-contrast in the arterial phase (b) and venous phase (c) indicating a hypervascular pancreatic tumor.

Figure 5 – Intraoperative appearance of the tumor (a) and resected specimen, which was sectioned in half to show the homogenous pattern (b).

For the immunohistochemical study, the histological sections were incubated in a thermostat at 37°C for 24 hours, and then they were dewaxed and hydrated. Next, the sections were boiled in a sodium citrate solution, in order to perform the antigen unmasking. In order to block the activity of the endogenous peroxidase, the sections were placed, at room temperature (RT), in 3% hydrogen peroxide for 30 minutes, followed by a 10 minutes washing in distilled water and a five minutes wash in 1% phosphate-buffered saline solution. Afterwards, the sections were pass through 2% skim milk for 30 minutes, which led to the blocking of non-specific sites. The histological sections were incubated overnight (18 hours) with primary antibodies at 4°C. A secondary antibody was applied the next day followed by 3–5 minutes washings in phosphate-buffered saline (PBS) solution. After applying Streptavidin–HRP (horseradish
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peroxidase) for 30 minutes, the sections were washed three times in PBS solution.

For positive and differential diagnosis, the following immunohistochemistry markers were used:

- Chromogranin A: monoclonal, 1:100 dilution, clone DAK-A3 (Dako);
- Synaptophysin: monoclonal, 1:50 dilution, clone DAK-SYNAP (Dako);
- Insulin: polyclonal, 1:100 dilution (Dako);
- Somatostatin: polyclonal, 1:200 dilution (Dako);
- Carcinoembryonic antigen (CEA): monoclonal, 1:50 dilution, clone II-7 (Dako);
- Vimentin: monoclonal, 1:100 dilution, clone V9 (Dako);
- CD31: monoclonal, 1:20 dilution, clone JC70A, (Dako);
- Ki67: monoclonal, 1:100 dilution, clone MIB-1 (Dako);
- CD34: monoclonal, 1:100 dilution, clone EP373Y (Dako);
- Cytokeratins (CKs):
  - CK5/6, monoclonal, 1:50 dilution, clone D5/16 B4 (Dako);
  - CK7, monoclonal, 1:100 dilution, clone CV-TL 12/30 (Dako);
  - CK19: monoclonal, 1:50 dilution, clone RCK 108 (Dako);
  - MNF-116: monoclonal, 1:100 dilution, clone MNF-116 (Dako).

Histological analysis revealed cell proliferation with trabecular pattern, which exceeded the delimiting conjunctive capsule. There was no cytological atypia, mitotic activity in the tumoral cells and angioinvasion or perineural invasion identified (Figure 6, a and b), supporting the diagnosis of pancreatic neuroendocrine tumor.

Several immunohistochemical stainings were carried out in order to establish the positive diagnosis of pNET. For highlighting the neuroendocrine origin of the tumor, chromogranin A and synaptophysin staining were performed, showing a positive reaction for both chromogranin A (Figure 7a) and synaptophysin (Figure 7b), with a diffuse, granular, cytoplasmic pattern of staining. We further investigated the functional character of the tumor, which was positive for insulin and negative for somatostatin and CEA. Regarding the reaction to insulin, it was not a homogenous one, areas with an intense reaction alternating with areas with a poor reaction and with cells with a negative reaction to insulin (Figure 8a). The vimentin and CD31 were negative in tumoral cells and positive in blood vessels indicating a low tumor cells proliferation and no angioinvasion. The Ki67 index was between 3% and 10% (Figure 8b).
We also analyzed the presence of certain cytokeratins (CK5/6, CK7, CK19 and MNF-116) in the tumor cells. The tumor was negative for CK5/6, CK7 (Figure 9, a and b), CK19 (Figure 10, a and b) and slightly positive for MNF-116 (Figure 11).

Using the CD34 antibody, a marker of angiogenesis, we observed a high number of new blood vessels, compared to the normal pancreas surrounding the tumor (Figure 12, a and b).

The procedures followed in our case, were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.
Ten days after the surgical intervention, the patient was discharged asymptomatic and with normal glycemic profiles. Nine months postoperative, the patient is still free of all the previous symptoms, with normal glycemic values and glycated hemoglobin increased to 6.2% and insulin levels were 7.8 μU/mL. Currently (February 2015), she is pregnant and pregnancy had a normal evolution to date.

**Discussion**

The imaging, histological and immunohistochemical findings support the diagnosis of functional well-differentiated pNET with uncertain behavior confined to the pancreas, G1 category according to the WHO criteria [3]. The clinical and biochemical data suggest that the pNET type is insulinoma [4].

Patients with insulinoma, a pNET that secretes insulin, typically present hypoglycemic symptoms, especially neuroglycopenic symptoms and also symptoms due to sympathetic overdrive, which occur usually fasting [2, 8, 9].

The particularities of this case are the association of neuroglycopenic hyperinsulimemic hypoglycemic symptoms, suggestive for insulinoma with facial and upper trunk flushing characteristic to carcinoid syndrome and the absence of autonomic symptoms during the hypoglycemic episodes.

The presence of facial and upper trunk flushing with normal serum levels of serotonin and 5-HIAA are uncommon [6]. The flush, non-responsive to antihistamines therapy, may be justified by the tumor secretion of small serotonin quantities, which did not lead to increased serum serotonin levels or by secretion of other mediators such as kinins, prostaglandins, responsible for carcinoid syndrome [2]. Moreover, Liu et al. reported normal serum serotonin and urinary 5-HIAA levels in a series of 13 cases with primary pancreatic carcinoid tumors [10].

Although, in our case, the skin flush raises the suspicion of carcinoid tumor, the association of normal serum chromogranin A levels with positive immunohistochemical staining of chromogranin A in tumoral cells consist in a pattern characteristic to benign insulinoma [11, 12].

The absence of hypoglycemic autonomic symptoms could be explained by the high frequency of hypoglycemic episodes, which may induce autonomic failure by blunting counter regulatory responses [13].

In our case study, the tumor had 2 cm in diameter and was sporadic which is consistent with previous studies [1, 14]. The small pNETs size makes them difficult to detect using conventional imaging [15]. Sensitivity for transabdominal ultrasound and for CT scanning in detecting
small pancreatic tumor ranged from 9% to 63% and from 16% to 72%, respectively [16]. Several studies indicated that EUS is useful in localizing pNETs, especially insulinomas, which are small tumors, with a reported sensitivity between 80% and 90% [15, 17–19]. The tumor was revealed in our case by EUS and 3T MRI, the CT scan failing to identify it.

The prognosis of pNET is generally good, especially if surgical resection is performed [20, 21]. Typical and sufficient surgical resection is enucleation if the tumor has no relationship with the main pancreatic duct and is not situated deep in the pancreatic parenchyma. Injury or impossibility to avoid the pancreatic duct implies a more complex pancreatic surgery ranging from central pancreatectomy to distal pancreatectomy or even duodenopancreatectomy [4].

Outcome of surgical resection of insulinomas are generally satisfactory, with no mortality and good functional results [22], because enucleation is the best possible way to preserve pancreatic parenchyma. However, the technique must be very meticulous and careful to avoid postoperative complication as pancreatitis, pancreatic fistula or hemorrhagic complications. The recurrence rate is very low (3%), and it is more likely associated with pNET grade G2 [22]. This is another argument for a good prognosis in our case, but nevertheless, a postoperative period follow-up is mandatory.

Multiple studies have shown that metastatic tumor, local invasion, high tumor mitotic rate, tumor necrosis, high Ki67 index, positive tumor staining for CK19 and CD10, overexpression of angiogenesis markers, chromosomal instability are markers that predict poor prognosis in patients with pNET [7, 21, 23–26], with a higher malignant potential in non-functional pNET [14, 26]. The analysis of several cytokeratins showed that the tumor was negative for CK5/6, CK7, CK19 and slightly positive for MNF116 unlike a non-functional pNET analyses in a recent study [26], in which CK19 and MNF-116 were intensely positive.

In our case, the benign character of the tumor is equivocal due to the local tumoral invasion, the tumor cells exceeding the delimiting conjunctive capsule. Moreover, the Ki67 index suggests an uncertain pNET behavior.

However, the negative tumor staining for CK19, the absence of mitoses and tumor necrosis, lack of angi-invasion or extra-pancreatic invasion and the evolution after surgical intervention are factors that plead for the benign nature of the tumor and a favorable prognosis.

Conclusions

We present this rare case of pNET, the particularity of the case consisting in the atypical combination of hypoglycemic symptoms and carcinoid syndrome symptoms, as well as the histopathological aspect of the tumor. Endoscopic ultrasound and immunohistochemical investigations were essential to localize this small pNET and to establish therapeutic management. The postoperative evolution of our case fits the classical pattern described, with lack of hypoglycemic symptoms and normal insulin levels.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Adina Mitrea, Bogdan Silviu Ungureanu, Ioana-Andreea Gheonea and Valeriu Surlin had an equal contribution to the paper, equal to that of the first author.

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