Peer Review File

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Reviewer A

Interesting set of two case reports of non-functional pNETs that eventually started secreting proinsulin. I have encountered more than a few such cases and agree that they are challenging to treat.

There are several important principles to highlight, some of which have been published and some may not have been:

1. In my experience, malignant insulinomas tend to be characterized by high levels of proinsulin rather than insulin

Reply: We agree with this comment. This is true in the 2 cases we presented (Case 1 with proinsulin of 352 pmol/L, insulin 12.5 mcU/mL; Case 2 with proinsulin 1831.3 pmol/L, insulin 26.0 mcU/mL). We have included a study that compares malignant and benign insulinomas showing malignant insulinomas have higher levels of pro-insulin, insulin, C-peptide compared to benign insulinomas. In that same study, the proinsulin levels are several times greater the upper limit of normal compared to insulin levels in malignant insulinoma.

Changes in the text: Added "Malignant insulinomas have higher levels of pro-insulin, insulin, C-peptide and are associated with worse survival compared to benign insulinomas (12). Malignant insulinomas tend to have increased relative expression of proinsulin compared to insulin (12)." (Lines 46-49)

2. Diazoxide tends to be relatively ineffective in malignant insulinoma compared to benign

Reply: We agree with this comment and available data suggests that malignant insulinomas are harder in general to treat due to greater burden of disease, necessitating a multimodal treatment approach.

Changes in the text: Added "Malignant insulinomas are less responsive to therapies including diazoxide compared to benign insulinomas and require a multimodal approach (20)." (Lines 184-186)

3. Octreotide can sometimes worsen hyperglycemia by inhibiting the counter-regulatory hormone glucagon.

Reply: We agree with this comment and have included some reported cases of octreotide-induced hypoglycemia in insulinoma patients with pathophysiologic rationale.

Changes in the text: Added "There have been reports of octreotide worsening hypoglycemia in insulinoma, which is thought to be secondary to increased relative suppression of glucagon compared to insulin (24, 25). This paradoxical effect is thought to be worse in patients with insulinomas which lack SSTR-2 and SSTR-5 (26)." (Lines 193-197)

Please comment on these above points.

Specific comments:
1. line 90-91: I assume you mean octreotide 100mcg tid, not 100mg monthly?
Reply: We thank the reviewer for pointing this out and this has been corrected to "subcutaneous octreotide 100 mcg three times per day."
Changes in the text: Changed prior text to "During a prolonged one-month admission, his treatment included intravenous (IV) dextrose solution, diazoxide 100 mg four times per day, prednisone 10 mg twice per day, and subcutaneous octreotide 100 mcg three times per day." (Lines 83-86)

2. lines 152-155: don't understand why the purpose of the Octreoscan was to find a lesion within the pancreatic mass to cryoablate. This doesn't make any sense to me. Presumably, there was transformation of the cancer as a whole, and even if there was a focal insulin-producing lesion, there's no reason to suspect it would be detected on Octreoscan
Reply: We appreciate the comment. Given the location of the tumor, a more distal embolization would place the patient at high risk for pancreatitis/necrosis and other complications. The intention of the scan was to potentially identify a smaller functional portion of the tumor for further targeting from a radiotherapy perspective. These were recommendations from the Interventional Radiology service at the time.
Changes in the text: None

3. Line 175-177: Agree that it is doubtful that a new insulinoma developed. No reason to even speculate on that. Nonfunctional NETs can become functional: this is not uncommon
Reply: We agree with the suggestion and have removed this from the text.
Changes in the text: Deleted "A new small primary insulinoma could not be fully excluded, though we considered it highly unlikely given the degree of medical treatment needed to treat hypoglycemia." (Lines 178-180)

4. It seems to me that other interventions could have been tried, such as everolimus which often works fairly well to reverse hypoglycemia. In patient #2, could also have considered radiating the primary tumor.

Reply: We appreciate the following comments. Everolimus was considered as future adjunctive treatment in both cases, but the patients' conditions worsened before it was able to be utilized. In patient 2, radiation was considered but deferred in the setting of significant episodes of gastrointestinal bleeding as mentioned in lines 150-152.

Changes in the text: Added "Some of the additional treatment modalities mentioned in this section were considered for our patients, but overall clinical stability and goals of care precluded usage." (Lines 221-223)

Reviewer B

The authors describe two interesting cases of metastatic non-functioning pancreatic neuroendocrine tumors (PanNETs) that subsequently transformed into metastatic, functional insulinoma. Both cases are characterized by refractory hypoglycemia despite multimodal therapies, including cytotoxic chemotherapy, medical suppression of insulin secretion, and dextrose supplementation.

Comments/recommendations for the authors are as follows:

1) During the description of medical therapy for the pt'srefractory hypoglycemia (Page 3, Line 90-91), "octreotide 100 mg monthly" is listed. However, this is not a viable dose for the LAR formulation (which is 10-40 mg IM q 4 weeks), and monthly dosing is clearly insufficient for the short acting formulation. Dose clarification is needed.

Reply: We thank the reviewer for pointing this out and this has been corrected to "subcutaneous octreotide 100 mcg three times per day."

Changes in the text: Changed prior text to "During a prolonged one-month admission, his treatment included intravenous (IV) dextrose solution, diazoxide 100 mg four times per day, prednisone 10 mg twice per day, and subcutaneous octreotide 100 mcg three times per day." (Lines 83-86)

2) In both cases, the diagnosis of insulinoma is made based on biochemical data (including elevated insulin, proinsulin, and C-peptide concentrations). However, it is critical that the blood glucose level be given, as blood glucose must be low (i.e., < 55 mg/dL) for these labs to be interpretable. Kindly include blood glucose level at time of the above laboratory studies (page 3, lines 97-99; page 4, lines 140-142).

Reply: We agree with the suggestion and have included the data requested.

Changes in the text: Changed prior text to "Relevant laboratory studies drawn during episode of hypoglycemia include glucose of 19 mg/dL…" (Lines 91-92). Modified prior text to "Relevant

laboratory studies drawn during episode of hypoglycemia include glucose 50 mg/dL...” (Lines 132-133)

3) To add to the above, the interpretation for proinsulin, insulin, and C-peptide is different in the setting of hypoglycemia, as the euglycemic reference range does not apply. Inappropriate elevation of these labs in the setting of hypoglycemia (i.e., blood glucose < 55 mg/dL) include the following cutoffs: insulin >= 3 mcU/mL, proinsulin >= 5 pmol/L, and C-peptide >= 0.2 nmol/L). Kindly adjust the "normal range" accordingly. Also, these levels are all elevated, so "inappropriately normal" insulin (page 3, line 100) does not apply.

Reply: We agree with the suggestion and have changed the text accordingly.

Changes in the text: Changed prior text to “Relevant laboratory studies drawn during episode of hypoglycemia include glucose of 19 mg/dL, proinsulin 352.9 pmol/L (normal range in setting of hypoglycemia: ≥ 5 pmol/L), insulin 12.5 mcU/mL (normal range in setting of hypoglycemia: ≥ 3 mcU/mL), C-peptide 3.81 ng/mL (normal range in setting of hypoglycemia: ≥ 0.2 ng/mL), and negative insulin autoantibody. Diagnosis of insulinoma was made based on inappropriately elevated insulin, proinsulin, and C-peptide in the setting of hypoglycemia, known hepatic PanNET metastases, and clinical history of prolonged symptomatic hypoglycemia improved with intravenous dextrose.” (Lines 91-100). Modified prior text to “Relevant laboratory studies drawn during episode of hypoglycemia include glucose 50 mg/dL, proinsulin 1831.3 pmol/L (normal range in setting of hypoglycemia: ≥ 5 pmol/L), insulin 26.0 mcU/mL (normal range in setting of hypoglycemia: ≥3 mcU/mL), C-peptide 6.03 ng/mL (normal range in setting of hypoglycemia: ≥0.2 ng/mL), negative sulfonylurea screen, and negative insulin autoantibody. Diagnosis of insulinoma was made based on inappropriately elevated proinsulin, insulin, and C-peptide in the setting of hypoglycemia, and the clinical history of prolonged symptomatic hypoglycemia improved with intravenous dextrose.” (Lines 132-140)

4) The hypoglycemia evaluation generally includes sulfonylurea screen and insulin antibody testing; if available, these should be included as well.

Reply: We agree with the suggestion and have included most of the recommended data for cases 1 and 2. Unfortunately a sulfonylurea screen was not obtained during the episode of hypoglycemia for case 1, but we have included all other recommended data.

Changes in the text: Changed prior text to “Relevant laboratory studies drawn during episode of hypoglycemia include glucose of 19 mg/dL, proinsulin 352.9 pmol/L (normal range in setting of hypoglycemia: ≥ 5 pmol/L), insulin 12.5 mcU/mL (normal range in setting of hypoglycemia: ≥ 3 mcU/mL), C-peptide 3.81 ng/mL (normal range in setting of hypoglycemia: ≥ 0.2 ng/mL), and negative insulin autoantibody.” (Lines 91-96). Modified prior text to “Relevant laboratory studies drawn during episode of hypoglycemia include glucose 50 mg/dL, proinsulin 1831.3 pmol/L

(normal range in setting of hypoglycemia: ≥ 5 pmol/L), insulin 26.0 mcU/mL (normal range in setting of hypoglycemia: ≥3 mcU/mL), C-peptide 6.03 ng/mL (normal range in setting of hypoglycemia: ≥0.2 ng/mL), negative sulfonylurea screen, and negative insulin autoantibody." (Lines 132-137)

Reviewer C

1.Are there any implications on surveillance that should be considered, particularly for cases that occur beyond 10 years? Authors can consider commenting.

Reply: We believe that routine oncologic and primary care follow up would be sufficient surveillance. We have modified our text to emphasize that this transformation can occur at any point.

Changes in the text: Changed prior text to "Clinical practitioners should be aware of the possibility of gain-of-function transformation at any point in patients with metastatic PanNETs." (Lines 180-181)

2. In speaking about treatments, would suggest that authors comment on the expected responses from therapeutic modalities noted.

Reply: Metastatic insulinomas are quite difficult to treat and prior published reports note that a multimodal approach is utilized. The clinical responses are quite variable, but generally err on being ineffective and if effective, only for a short time. We include a report of a dramatic response to everolimus, but that has yet to be realized in a larger cohort. This highlights a broader need for better therapeutics in this condition.

Changes in the text: Added "The treatment modalities discussed for metastatic insulinoma have variable clinical efficacy and often have a short-lived therapeutic response. While there have been reports of significant response to certain therapeutics such as everolimus (42), there is no single optimal therapy and our preferred approach is the multimodal one discussed above." (Lines 223-226)

3. these cases may represent old cases, prior to the era of more widely accept NGS, if any information on molecular profiling is available it would be of interest to readers to comment. Additionally, please comment on if there is a role or possible utility, of NGS (genotype-> phenotype correlations) for PNETs and with further understanding the transformation to insulinomas.

Reply: Unfortunately, there is no next-generation-sequencing data available for these cases.

We have added mutational data of non-functional PanNETs and insulinomas, and unique mutations to each respective cohort. There are no studies to our knowledge looking at the mutational profile of non-functional PanNETs which have transformed into functional PanNETs.

We believe this would be an interesting point of study, though difficult given the very limited sample size. Given the unique mutations of epigenetic modifiers in insulinomas, we hypothesize that non-functional PanNETs with mutations in epigenetic modifiers would be at higher risk for transformation.

Changes in the text:

Added "In terms of genetics and compared to insulinomas, non-functional PanNETs have mutations in alpha-thalassemia/mental retardation X-linked (ATRX) and death domain-associated protein (DAXX) and mammalian target of rapamycin pathway (mTOR) genes (15, 16). Insulinomas have unique mutations in epigenetic regulators including Yin Yang 1 (YY1), H3 histone family 3A (H3F3A), lysine-specific demethylase 6A (KDM6A), and ATR serine/threonine kinase (ATR) (15-17). Given the unique mutations of epigenetic modifiers in insulinomas, we hypothesize that non-functional PanNETs with mutations in epigenetic modifiers would be at higher risk for transformation. However, there have been no studies to our knowledge looking at the mutational profile of non-functional PanNETs which have transformed into functional PanNETs." (Lines 160-170)

This case report presents again a unique case of PNETS transformation into an insulinoma. similar case presentations have been published before regarding transformation for nonfunctional PNETs to functional ones (keen, EDM, 2020) and (Nahmia EDM, 2015), one of which is referenced in current manuscript. This case presentation is unique in that the transformations occurs far longer than previously reported and adds to the very small body of literature presentably available.

I Would recommend this case report for publication with comments addressed.