

Dear Ana Donnelly,

Thank you very much for your decision letter and advice on our manuscript (Manuscript # 54184) entitled “Comprehensive treatment of rare multiple endocrine neoplasia type 1: A case report”. We also thank the reviewers for the constructive and positive comments and suggestions. Accordingly, we have revised the manuscript. All amendments are highlighted in red in the revised manuscript. In addition, point-by-point responses to the comments are listed below this letter.

This revised manuscript has been edited and proofread by *Medjaden* Bioscience Limited.

We hope that the revision is acceptable for publication in your journal.

We look forward to hearing from you soon.

With best wishes,

Yours sincerely,

ChenhuiMa/WanxingZhang

First of all, we would like to express our sincere gratitude to the reviewers for their constructive and positive comments.

### Replies to Reviewer 1

#### SPECIFIC COMMENTS TO AUTHORS

Your manuscript is excellent. Keep on studying about this disease.

**Response:** Thank you for your positive comment.

### Replies to Reviewer 2

#### SPECIFIC COMMENTS TO AUTHORS

The case described here is original and describes an intriguing clinical scenario. The management of these diseases is difficult and raises several clinical issues. However, the description of this case is controversial and several clarifications are needed.

Main remarks 1) I suggest removing all the laboratory examinations (paragraph 2.2) from the text and presenting them in a table

**Response:** Thank you for this wonderful suggestion. We have removed all the laboratory examinations and presented them in the new Table 1 as follows:

**Table 1.** Laboratory examination findings from the patient with MEN1 before and after treatment.

Items	Factors	Values at admission	Postoperative Values	Reference range
Thyroid function	Parathyroid hormone	7.96 pmol/L	7.11 pmol/L	1.6-6.9
	Thyroid-stimulating hormone	7.36 $\mu$ IU/L	2.24 $\mu$ IU/L	0.27-4.2
	Total T4	64.68 nmol/L	79.12 nmol/L	78.38-157.4
Pituitary hormone	Prolactin	17.13 ng/mL	25.67 ng/mL	1.61-18.77
Tumor biomarkers	Alpha-fetoprotein	3.83 ng/mL	-	0-25
	Carbohydrate antigen 19-9	15.17U/mL	-	<37

	Carcinoembryonic antigen	3.51 ng/mL	-	<5
Bone metabolism	B-collagen-specific sequence	1.21 ng/mL	-	0.3-0.6
	Total type I collagen amino-terminal extension peptide	204.4 ng/mL	-	20.25-76.31
Diabetes	Fasting insulin	55.29 µU/mL	9.95 µU/mL	2.6-24.9
	Fasting blood glucose	1.79 mmol/L	3.98 mmol/L	3.9-6.1
	Fasting C-peptide	5.06 ng/mL	1.81 ng/mL	1.6-6.9
	Insulin release index	1.31	0.2	<0.3

2) Imaging findings are not clear. I understand from the case description that PET/CT showed a mass in in the pancreatic tail and in the liver. If so, why did you report that the patient "...underwent a pancreatic body/tail resection + pancreatic head mass resection pancreaticoduodenectomy"? I guess that this was done after the result of intraoperative ultrasonography, but it should be explained! Moreover, I think you should call this intervention total pancreatectomy Again I am confused when reading that the surgical margins were clear at the cutting edge of pancreatic body Was this a total pancreatectomy or not?

**Response:** We apologize for the unclear presentation. The patient underwent resection of the tail of the pancreatic body, enucleation of the pancreatic head mass, and ultrasound-guided radiofrequency ablation for liver cancer. In terms of the surgical margins, we wanted to express that although surgical resection seemed clear, multiple tiny nodules around the pancreatic tissues were detected by H&E examination. We have rewritten the surgery and pathology sections accordingly.

“A multiple disciplinary team (MDT) consisting of an endocrinologist, oncologist, pathologist, radiologist, thoracic surgeon, and hepatobiliary surgeon was incorporated to manage this patient. MEN1 with thymic carcinoid, thymoma, parathyroid and insulinomas with intrahepatic metastases was initially diagnosed. Since there were no obvious surgical contraindications,

MDT recommended a radical excision of the lesions to alleviate hypoglycemia symptoms and improve the quality of life for the patient. The patient underwent a resection of the tail of the pancreatic body, enucleation of the pancreatic head mass, and ultrasound-guided radiofrequency ablation for liver cancer. During the operation, a nodule with a diameter of about 0.4-cm was observed in the diaphragmatic surface of the right liver lobe. A mass of about 0.5 cm × 0.5 cm was palpable in the right posterior liver lobe, a tumor with about 2 cm × 2 cm in the falciform ligament about 4 cm from the hepatic margin, a mass of 1.5 cm × 1.0 cm on the pancreatic head, a mass of 5 cm × 3 cm × 3 cm on the pancreatic body, and a mass of 1.5 cm × 1 cm on the pancreatic tail. The intraoperative ultrasonography findings from the liver scan showed the right lobe nodules as cysts, and ultrasound-guided segment IV liver biopsy and radiofrequency ablation were performed.

After surgery, all diabetes factors with abnormal serum levels were returned to normal levels (Table 1). The pathological findings are shown in Fig. 4. The pancreatic tissues showed CKpan(+), synaptophysin(+), chromogranin A(+), partial CD56(+), p53(-), partial PGP9.5(+), partial SSTR2(+), CD10(-), partial vimentin (+), a mitotic count of 6/10 high power fields (HPFs) and Ki-67 index of about 8%-20%, which confirmed the diagnosis of the pancreas NET grade 2. The liver tissues showed CKpan(+), synaptophysin (+), Chromogranin A(+), partial CD56(+), p53(-), PGP9.5(+), a mitotic count of 4/10 HPFs and Ki-67 index of about 10%-20%, which confirmed the diagnosis of liver NET grade 2. Moreover, there were multiple tiny nodules around the pancreatic tissues, indicating that surgical resection could not completely remove the lesions. NET grade 2 was diagnosed according to the World Health Organization 2010 classifications for gastrointestinal and pancreatic neuroendocrine tumors [4]. Furthermore, whole-exome sequencing revealed a verified pathogenic mutation c.378G>A (p.Trp126\*) in the *MEN1* gene (reference sequence NM\_130799.2). Therefore, the final diagnosis of MEN1 was confirmed.”

3) Please explain why it was decided to operate the patient with such a diffuse disease (in fact surgical resection was not radical) instead of referring the patient for chemotherapy?

**Response:** Surgery was performed due to the following reasons:

1. The patient had undergone thymectomy and prolactinoma resection several years prior. He was admitted to our department because of pancreatic NET at that time. Surgical resection is the preferred treatment for pancreas NET. Liver metastases are common in patients with pancreas NET, with up to 60% of patients having liver metastases at the time of the initial diagnosis. If surgery can remove most of the metastatic tumor volume (>90%), then the primary lesion and liver metastases should be considered for simultaneous or sequential excision. The indications for surgery include well-differentiated G1/G2 tumors, no distant lymph node metastases, no extrahepatic metastases, no diffuse peritoneal metastases, and no right heart dysfunction. According to ENETS guidelines, in MEN1, functional pNETs should be removed, when possible, and there is a dominant lesion (Howe, J. R., N. B. Merchant, C. Conrad, et al. The North American Neuroendocrine Tumor Society Consensus Paper on the Surgical Management of Pancreatic Neuroendocrine Tumors. *Pancreas*, 2020. 49(1):1-33.). Thus, for pancreas NET patients with type I liver metastases who had no surgical contraindications, surgical resection is recommended.
2. The patient had a strong desire to undergo surgery.
3. The surgery could prolong the patient's survival time, control his symptoms, and improve his quality of life.

We have added this information in the revised manuscript.

“A multiple disciplinary team (MDT) consisting of an endocrinologist, oncologist, pathologist, radiologist, thoracic surgeon, and hepatobiliary surgeon was incorporated to manage this patient. MEN1 with thymic carcinoid, thymoma, parathyroid and insulinomas with intrahepatic metastases was initially diagnosed. Since there were no obvious surgical contraindications, MDT recommended a radical excision of the lesions to alleviate hypoglycemia symptoms and improve the quality of life for the patient. The patient underwent a resection of the tail of the pancreatic body, enucleation of the pancreatic head mass, and ultrasound-guided radiofrequency ablation for liver cancer.”

- 4) Please explain why endoscopic ultrasound was not performed to acquire tissue for pre-

operative diagnosis. Patients with MEN neoplasms involving the pancreas are often managed conservatively, particularly if tissue acquisition with EUS-FNA shows well differentiated pancreatic NETs

**Response:** The preoperative diagnosis of the present pancreas NET was relatively clear based on the patient's medical history, insulin levels, Whipple's triad, CT, MRI, and PET/CT findings. The patient stated that if the systemic metastasis was advanced, he would refuse surgery and preferred to receive palliative care. <sup>18</sup>F-FDG PET/CT imaging revealed that the systemic metastasis was in accordance with the principles of surgical treatment and functional pancreas NET treatment. Hence, surgical treatment was performed. PET/CT provided anatomical details to evaluate the pancreatic NET and distant metastases, which helped us select the optimal treatment. Sadly, a biopsy was not performed through EUS-FNA before surgery to confirm the differentiation status of the pancreatic NET.

We have added this information in the revised manuscript.

“Parathyroid two-phase computed tomography (CT) revealed a strong signal in the upper portion of the left lobes and posterior portion of the right lobes of the thyroid, in addition to the parathyroid glands (Fig. 1a). Pancreatic perfusion CT imaging showed irregular soft tissue densities of the pancreatic body, which was closely associated with the adjacent stomach wall, with a small reduction in blood volume (BV) and a slight increase in flow extraction product (FEP); increased blood flow (BF), BV, mean transit time (MTT) and FEP in nodules of the posterior to the pancreatic body; increased BF and decreased BV in nodules of the pancreatic head; multiple circular low or slightly low-density shadows in liver parenchyma (Fig. 1b). MRI of the liver and pancreas revealed multiple abnormal signals in the liver segment II, segment VIII, and the junction area of liver segments II and IV, as well as occupied lesions in the tail of the pancreatic body (Fig. 2a). Enhanced MRI revealed occupied lesions in the body and tail of the pancreas (Fig 2b). Enhanced MRI revealed that liver segment IV was occupied, considering the possibility of angiomyolipoma. In addition, there were small cysts in segments II and VIII of the liver, and occupied lesions in the tail of the pancreatic body (Fig. 2C).

Positron emission tomography (PET)/CT imaging (Fig. 3) further showed strong  $^{18}\text{F}$ -FDG uptake in the tail of the pancreatic body and segment IV of the liver, indicating the presence of insulinomas with intrahepatic metastases. There were several circular nodules visible in the right and left lungs, showing there was partial increase signals in them. After undergoing complete surgical resection of the thymoma, ring-shaped hypermetabolism became visible around the aortic root, may result from fat intake.”

5) Please include in the discussion a description of the important role that EUS has in this disease when the pancreas is involved

**Response:** We have added the requested discussion in the revised manuscript.

“Endoscopic ultrasound (EUS) can be used to differentiate MEN1-related pancreas NET from sporadic pancreas NET<sup>[10, 11]</sup>. Moreover, preoperative biopsy through EUS/fine needle aspiration (FNA) is useful for determining the differentiation status of pancreas NET<sup>[12-14]</sup>. A well-differentiated grade 1/2 tumor is one of the primary surgical indications for pancreas NET management<sup>[15]</sup>. However, the preoperative diagnosis of pancreas NET is relatively clear based on the patient's medical history, insulin level, Whipple’s triad, CT, MRI, and PET/CT findings. Hence, EUS was not performed, which is a limitation of the present case.”

6) Some references must be added: -Tamagno G, Scherer V, Caimo A, Bergmann SR, Kann PH. Endoscopic Ultrasound Features of Multiple Endocrine Neoplasia Type 1-Related versus Sporadic Pancreatic Neuroendocrine Tumors: A Single-Center Retrospective Study. *Digestion*. 2018;98(2):112–118. -Kappelle WF, Valk GD, Leenders M, et al. Growth rate of small pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1: results from an endoscopic ultrasound based cohort study. *Endoscopy*. 2017;49(1):27–34. -Jenssen, C., Hocke, M., Fusaroli, P., Gilja, O.H., Buscarini, E., Havre, R.F., Ignee, A., Saftoiu, A., Vilmann, P., Burmester, E., Nolsøe, C.P., Nürnberg, D., D'Onofrio, M., Lorentzen, T., Piscaglia, F., Sidhu, P.S., Dietrich, C.F. EFSUMB Guidelines on Interventional Ultrasound (INVUS), Part IV - EUS-guided interventions: General Aspects and EUS-guided Sampling (Short Version)(2016)

Ultraschall in der Medizin, 37 (2), pp. 157-169. -Fusaroli, P., Kypreos, D., Alma Petrini, C.A., Caletti, G. Scientific publications in endoscopic ultrasonography: Changing trends in the third millennium (2011) Journal of Clinical Gastroenterology, 45 (5), pp. 400-404. -Fusaroli, P., Napoleon, B., Gincul, R., Lefort, C., Palazzo, L., Palazzo, M., Kitano, M., Minaga, K., Caletti, G., Lisotti, A. The clinical impact of ultrasound contrast agents in EUS: a systematic review according to the levels of evidence (2016) Gastrointestinal Endoscopy, 84 (4), pp. 587-596.e10.

**Response:** The references mentioned above have been added to the revised discussion section.

“Endoscopic ultrasound (EUS) can be used to differentiate MEN1-related pancreas NET from sporadic pancreas NET<sup>[10, 11]</sup>. Moreover, preoperative biopsy through EUS/fine needle aspiration (FNA) is useful for determining the differentiation status of pancreas NET<sup>[12-14]</sup>. A well-differentiated grade 1/2 tumor is one of the primary surgical indications for pancreas NET management<sup>[15]</sup>. However, the preoperative diagnosis of pancreas NET is relatively clear based on the patient's medical history, insulin level, Whipple’s triad, CT, MRI, and PET/CT findings. Hence, EUS was not performed, which is a limitation of the present case.”

7) Please describe what decisions were made after discovering lung metastases and report further follow up

**Response:** We have added the requested information in the “OUTCOME AND FOLLOW-UP” section.

“After comparing the SPECT/CT scans with the PET/CT from four months prior, these nodules were suspected of being metastatic lesions. However, the nodules were too small to undergo needle biopsy, so the patient was placed under observation without additional treatment. At the last follow-up in January of 2020, the patient appeared healthy without any signs of disease reoccurrence.”



8) A good degree of language improvement is needed

**Response:** This revised manuscript has been edited and proofread by *Medjaden Bioscience Limited*.