

Mixed Neuroendocrine and Non-Neuroendocrine Neoplasm of Pancreas: What Do We Know, What Have We Learnt?

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Keywords

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treatment (chemotherapy + radiotherapy) gains improved
survival in long term.

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Abstract

Pancreatic adeno-mixed neuroendocrine non-endocrine (pMINEN) tumors are extremely rare [Pancreatol. 2021;21(1):224–235]. They are known to have distal metastasis at presentation and have a comparatively lower survival rate than similar staged neuroendocrine (NEN) carcinoma, adenocarcinoma, and small-cell lung tumor from which its treatment patterns are extrapolated. Also, very less is known about its molecular structure and natural courses. There is a dearth of data about pMINEN in the literature, and also there is a lack of large multicentric trials due to which the MINEN tumors do not have a standard universal management protocol. We discuss here the clinical dilemmas that arise during diagnosis and reporting and urge to form a multicentric trial to formulate a focused protocolized approach. We describe here our encounter with a pancreatic head lesion which on immunohistochemical analysis turned out to be a pMINEN with moderately differentiating ductal adenocarcinoma and low-grade NEN tumor. Radical R0 surgery with multimodal

Introduction

Mixed neuroendocrine non-endocrine (MINEN) tumors have recently (2017–2019) been introduced in the WHO classification and are not only rare but also very less is known about their natural history, tumorigenesis, and molecular nature [1–5]. Incidence of pancreatic NET is 1–2% of all pancreatic neoplasm (0.48 per 100,000 population) and it has overall increased to 6.4-fold over the last 40 years [6]. MINEN tumors are even more rare (0.01 per 100,000 population). <30 cases of pancreatic adeno-MINEN (pMINEN) tumors are described in English literature till 2020–21 [1]. Hence, very less is known about its natural course, molecular nature, clinical outcome, and treatment approaches. Recent advances in imaging and genome sequencing can help better understand its true nature, and large studies can help determine a focused management and surveillance protocol.

Case Report

A 45-year-old female had complained of upper abdominal pain, yellowish discoloration of sclera, clay-colored stools, and generalized itching of the body. These symptoms were on and off for 18 months, along with a significant loss of weight. There was no evidence of melena or GI bleed and no history of previous blood transfusion. The patient was not addicted to alcohol/tobacco or any substance.

5 weeks ago, she was hospitalized in another tertiary care institution with a history of acute and severe pain in the epigastric region and right hypochondriac pain along with fever, chills, and jaundice. Abdominal ultrasound suggested that the common bile duct (CBD) was dilated to 12 mm, the gall bladder showed few calculi with no evidence of any lesion noted in the pancreatic region, and the main pancreatic duct (MPD) was dilated. No ascites or lymphadenopathy was present. Her hemoglobin was 9.6 gm/dL, WBCs were 18,500 mm³, platelets were 198,000 mm³. Her total bilirubin was 3.5 mg/dl, direct bilirubin was 2.9 mg/dL, AST/ALT was 128/220 IU/L, alkaline phosphatase was 349 IU/L, total proteins were 6.7 g/dl, and total albumin was 3.4 g/dL. INR was 1.7 and serum creatinine was 1.2 mg/dL.

A diagnosis of acute cholangitis (Tokyo Type 3) was made. ERCP was done and a 7-Fr CBD stent was placed. EUS suggested a 2-cm lesion near CBD and MPD junction which on FNAC turned out to be adenocarcinoma. Her symptoms subsided and LFT normalized over the next 4 weeks. The patient was referred to us for further management at this stage, and a pancreatic protocol CT scan was done which showed an enhancing lesion of around 1.8 cm × 2 cm in the head of the pancreas near the CBD-MPD junction causing obstruction of distal CBD and upstream dilatation. No suspicious lymph nodes or free fluid was noted, and there was no evidence of vascular or locoregional spread. (shown in Fig. 1).

A standard pancreatoduodenectomy was planned. After pancreatoduodenectomy, the patient recovered well and no evidence of postoperative pancreatic fistula was noted. The specimen on histopathological and immunohistochemical (IHC) examination showed features of MINEN neoplasm, neuroendocrine (NEN) component >30%, non-neuroendocrine (nNEN) component – moderately differentiating ductal adenocarcinoma >30% invading up to the duodenal wall up to the muscle layer. There was no evidence of lymphovascular or perineural invasion and the resection margins were free of tumor. All the regional lymph nodes dissected (20) were free of tumor, TNM stage was pT2pN0. IHC staining showed that the tumor cells are synaptophysin+, chromogranin+, and PanCK+, and Ki 67 index was <2% (shown in Fig. 2a–d, f). Hence, the patient was diagnosed as having pMINEN with adenocarcinoma as a nNEN component. The patient is doing well 3 months postoperatively without any evidence of complication and recurrence on follow-up imaging.

Discussion

NEN tumors are 95% sporadic in nature and about 5% are associated with hereditary syndromes like MEN 1 (in 70–80%), Von Hippel-Lindau, tuberous sclerosis, and Von Recklinghausen's disease [6, 7]. The lesion is labeled

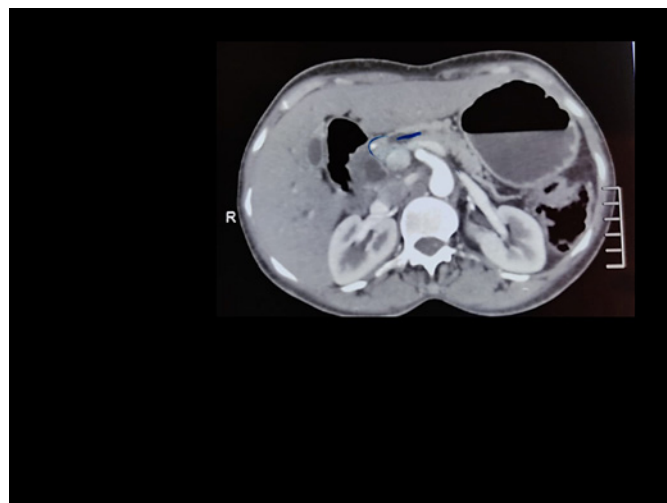


Fig. 1. Pancreatic protocol CT scan showing pancreatic head lesion (marked in semicircular blue) causing dilatation of CBD. Lesion is placed between MPD (marked in linear blue) and CBD.

MINEN when at least 30% of each component (i.e., NEN or nNEN) is found on IHC analysis [5].

Pancreatic neuroendocrine (pNEN) neoplasms mainly arise from cells of NEN origin diffusely spread throughout the organs but are most commonly found in gastrointestinal/pancreatic tissues and bronchopulmonary origin with incidence being 66% and 31%, respectively [8]. Many studies support the fact that there can be a monoclonal cell of NEN and nNEN origin which can progress to MINEN [4, 5]. Few postulations that are formulated and published in the literature are (1) merging of NEN and nNEN components together and growing further as MINEN; (2) MINEN tumor develops from a common pluripotent stem cell and later few cells in the lesion undergo phenotypic differentiation, and (3) MINEN tumor grows from a common monoclonal cell, but during proliferation, some molecular aberrations occur which leads to their differentiation to a different component [4].

NET carries >1.5 times more genetic mutations compared to other carcinomas [4]. The most common aberration was noted in MINEN's NEN and nNEN: *TP53* mutation and loss of heterozygosity. Loss of the *ATRX* gene promotes many pathways for mutation and tumor formation, especially in grade 3 NET. Other genes involved in the aberration are shown in Table 1.

Around 95% of pNEN stains positive for synaptophysin with or without chromogranin A [9]. Expression of *PAX1-9*, *PDX1*, and *ISL1* is found to be of pancreatic origin in around 56–90% of pNEN. This is particularly

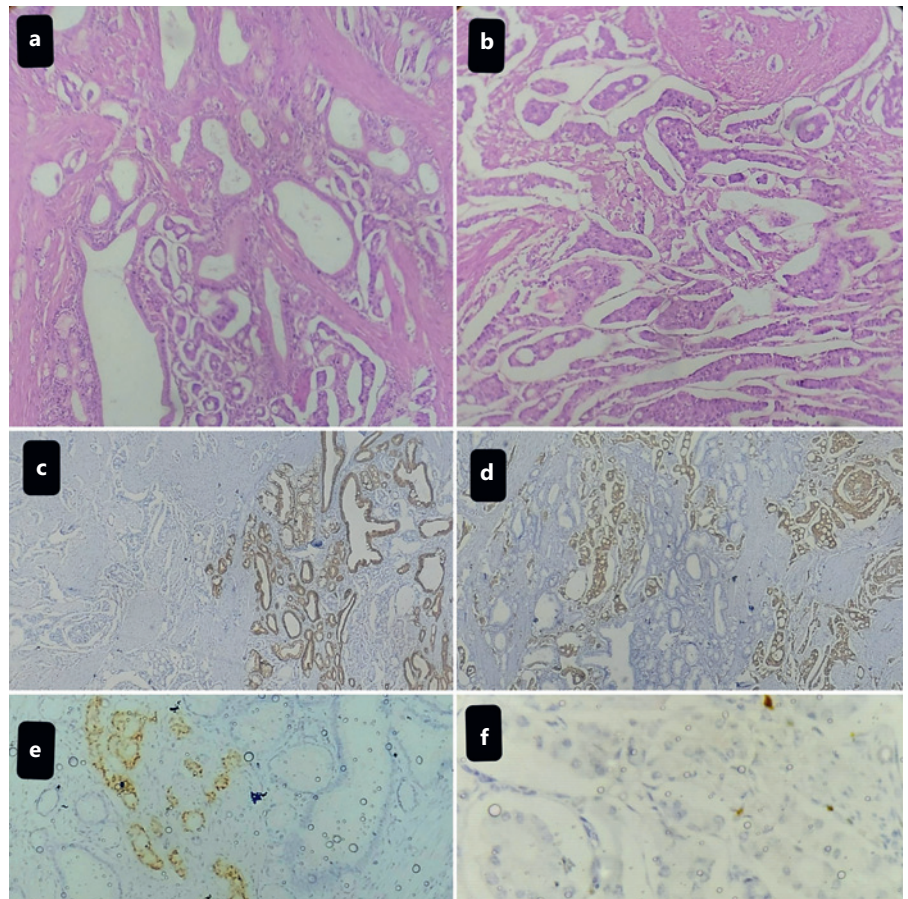


Fig. 2. Histopathology and immunohistochemistry of MINEN. **a** High power ($\times 40$) (H&E); tumor cells arranged in glandular pattern suggestive of adenocarcinoma component. **b** High power ($\times 40$) (H&E); tumor cells arranged in nesting pattern suggestive of NEN component. **c** Low power ($\times 10$); adenocarcinoma component of tumor strongly highlighted by PanCK immunohistochemistry marker. **d** Low power ($\times 10$); NEN component of tumor strongly highlighted by synaptophysin immunohistochemistry marker. **e** High power ($\times 40$); NEN component of tumor highlighted by chromogranin. **f** High power ($\times 40$); Ki 67 index $< 1\%$.

useful for determining the cell origin in an unknown metastatic lesion with an approximate sensitivity of 67–76% and specificity reaching a maximum of 89–98% [5]. The close counterpart to show these similar markers are NEN of rectal/appendiceal origin, but the use of AT-rich sequence-binding protein-2 (SATB2) will prove helpful to identify and differentiate it from pNEN [5]. *Pax8*, *islet 1*, *NESP55*, and *PDX1* are considered highly specific for pNEN [9]. The majority of patients (73.4%) having MINEN would have already developed distant metastasis when they present to us, but the biopsy of the metastatic lesion fails to show both components; hence, it is fair to recommend a second biopsy, and a detailed IHC analysis of the lesion should be undertaken and managed by a multidisciplinary team [7, 10]. Overall survival and outcomes of NET/MINEN of pancreato-biliary origin are poor when compared to NET/MINEN of intestinal or broncho-pulmonary origin [4, 5, 11].

PI3K-AKT-mTOR pathway dysregulation can trigger and progress NEN; its aberration is known to develop chemoresistance. This makes it important to plan future chemotherapy protocols [6, 9]. *MEN1* inactivation and

DAXX/ATR mutations are markers of better prognosis [9]. Microsatellite instability is also emerging as a potential prognosticating driver for pMINEN [7].

Ki-67 is expressed in the G1, S1, G2, and M phases of the cell cycle, and it functions as a surfactant by dispersing mitotic chromosomes. Its expression of $> 55\%$ is associated with very aggressive growth, poor response to chemotherapy, and poor overall survival (25 months vs. 43 months $p = 0.002$) [2, 5, 12]. Also a lower expression of somatostatin receptor 2A (*SSTR2A*) in metastatic sites and highly progressive NEN suggests *SSTR2A* downregulation can be a sign of poor prognosis [5]. Similarly, truncation of *SSTR5* is suggested to be considered a worse prognostic indicator [5]. CK19 is considered an independent prognostic factor, and in a meta-analysis of CK19, negative cases showed a 5-year survival of 100%, while it was 47% in CK19-positive NEN [13]. Also, *INSM1* and c-KIT are associated with aggressive biology and worse prognosis not only in pNEN but also in gastrointestinal NEN [5]. Similarly, in the NEN carcinoma, higher percentages of Ki-67, MIB-1, and mitotic indexes have shown to be a driving factor of poor prognosis [2].

Table 1. Genes involved in NEN and nNEN [4, 7, 9]

Genes common to NEN	Genes common to nNEN	Genes common to both
ATRX RB1	ARID1A PIK3CA CTNNB1 MYC	TP53 Loss of heterozygosity KRAS BRAF APC PI3KCA MUTYH CHECK2 BRCA2

Ki-67 is used to determine and guide treatment in WHO classification. But, the very foundation of using the Ki-67 index and its ability to correctly guide prognosis is often questioned due to the following issues [7, 9]:

1. Ki-67 may show a reduced rate if the time to fix the tissue sample is more.
2. Ki-67 may be reported falsely high due to its expression in intratumoral lymphocytes, endothelial cells, pyknotic nuclei, apoptotic debris.
3. Differences in interlaboratory values, observer variations, and nonstandardization of staining methods can create a reporting bias.

Lack of knowledge or nonspecific use of terms before WHO definition may also be one of the reasons for its paucity of literature [1]. As an arbitrary principle, MINEN is labeled when we find >30% component each of NEN and nNEN in the IHC analysis which seems to be clonally related [4, 5, 14]. While the term *collision tumor* is used when NEN and nNEN arise from the same tissue/organ and is seen in juxtaposition to one another, the term *composite tumor* is used where they are seen intermingled with other neoplasms and may get accidentally diagnosed [5, 7, 14]. A new classification system is demanded especially in immunohistochemically highly aggressive tumor components. This should be defined as MINEN irrespective of the amount seen (i.e., even <30%) as it can direct its natural course, management, and surveillance. One study suggests using around a 10% cut-off value to report it as MINEN [1, 7, 14, 15].

MINEN has both NEN and nNEN components. The natural course is difficult to predict, but a working general principle to consider can be guided by the most aggressive cell population in MINEN and keeping the other component under surveillance, which can be challenging at times [5, 15]. But few studies are in favor of considering poorly differentiated/high-grade NEN component to be the main deciding factor for its aggressive behavior and outcome. Some studies suggest

treating high-graded MINEN similarly to poorly differentiated adenocarcinoma [1, 2, 7, 10]. But these treatment protocols lack the support of randomized large trials and hence should be cautiously used and scrutinized from time to time. [7, 11]

Advancement in molecular pathology and use of whole-genome sequencing as well as use of more specific biological targeted therapy is on the horizon. As mentioned a previous study, use of dabrafenib/vemurafenib along with trametinib (*BRAF-MEK* inhibitor) has shown a promising response in lesions of colorectal NEN positive for *BRAF V600E* mutation. Currently, our knowledge in this regard is limited and large-scale studies are required [16]. Cisplatin/5-FU with etoposide is used in most cases which are based on treatment protocols for adenocarcinoma or small-cell lung cancer [2, 3]. Use of epirubicin, cyclophosphamide, and vincristine is also proposed as second-line therapy [3].

The best survival was seen in patients who were at early stage of disease, underwent R0 resection, and who had no lymph nodal or distant metastasis. [2, 17] Also the poor degree of differentiation of the nNEN carries the worst prognosis (viz. poorly differentiated adenocarcinoma) compared to well-differentiated carcinoma. In a study by Hang YC et al. [2], they found that the overall survival in a poorly differentiated adenocarcinoma component is 20 months and that of a moderately differentiated component is 40 months ($p = 0.001$). In a meta-analysis, the MINEN lesions' median progression-free survival was 5–6 months and median overall survival was 12–18 months, which is similar to advanced carcinomas. [7, 11] In another study with MINEN patients, the overall survival was 44.5 months and median recurrence-free survival was 12.5 months. Observation to be noted in this study was the use of multimodal treatment strategy (chemotherapy ± radiotherapy) versus surgery alone that prolonged the overall survival from 75 months in the former group to 18.9 months in the latter group [18].

Conclusion

We report a rarely studied form of pMINEN with an adenocarcinoma component. Also, very less is known regarding its natural course, molecular biology, and overall outcome which many times creates difficulty in achieving the maximum outcome. Attempts are made to study the whole-genome sequence of MINEN which can better guide and help formulate a definite and specific treatment protocol for MINEN. High clinical suspicion, prompt IHC analysis, early detection of radical surgical resection (R0), multimodal treatment, and lack of lymph nodal and distant metastasis have proven to drive maximum recurrence-free and overall survival rate in MINEN patients.

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. Also consent was obtained to publish photos and hide identity. This case was reviewed by the Unit Head and Head of Department of Surgery and Pathology, and it was determined that as this is a case report, it does not require any further approval.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

As per ICMJE Criteria for Authorship: Vijay W. Dhakre designed the article and also contributed to conception and literature search. Sneha T. Galande contributed to analysis and literature search. Varsha Patil contributed to literature search and procured case details and photographs. Nikita C. Shah contributed to literature search and the pathology part of discussion. Chatan Rathod contributed to article design and literature search. Kaiumarz Sethna contributed to literature search, discussion analysis, and final proof reading. Anjali Amrapurkar contributed to literature search, pathology literature search, and final proof reading.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.