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Treatment of Multifocal Neuroendocrine Tumor of the Ileum Whit Lymphatic Metastasis (NETG1) And Low Mutational Burden (TMB).Precision Surgery, Case Report and Review Literature

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Multifocal neuroendocrine tumor; Ileum; Next generation sequencing; Low burden tumor; Lymphadenectomy; Precision surgery

Abbreviations:

SI-NETs: Small Intestinal Neuroendocrine Tumors; TMB: Tumor mutation burden; NET (G1): Grade 1; Mut/Mb: Total number of somatic/acquired mutations per coding area of a tumor genome; mCi: millicurium; TP53: cellular tumor antigen P53 (PTHR11447:SF6); RB1: Retinoblastoma associated protein (PTHR13742:SF17); CDKN1B: Cyclin dependent kinase inhibitor 1B (PTHR10265:SF9); KRAS: GTPase KRAS (PTHR24070:SF388); NRAS: GTPase NRAS (PTHR24070:SF189); MET: Hepatocyte growth factor receptor; HGNC: 7029

1. Abstract

Patient with abdominal pain and diarrhea. CT shows tumor located in the root of the mesentery and partially calcified tumor in the terminal ileum. In addition, there is a cystic in the neck of the pancreas. Elevated blood serotonin. Operative findings: eight tumors in the terminal ileum and a tumor in the root of the mesentery, with retractile fibrosis. Partial resection of the ileum, right hemicolectomy and lymphadenectomy of the lymph nodes close to the ileocolic vessels and right middle colic it´s performed. Tumor has positive expression of chromogranin A and synaptophysin. Mitotic index 1/2 mm2 and K-67 1%. DNA sequencing does not identify copy number changes, translocations, or gene fusions. Tumor mutation load (TMB) is one Mut/Mb. There is microsatellite stability and the mutational signature, is not detected. Tumors (SI-NETs) with TMB above 10 Mut/Mb respond to association with immune checkpoint inhibitors. Pharmacological treatments for (SI-NETs) with low TMB may be ineffective. In addition, prophylactic lymph node dissection (SI-NETs) with low TMB may disrupt the immune surveillance system. For an effective surgical treatment, it is necessary to know beforehand the mutational load of the tumor by liquid biopsy from circulating DNA in plasma. When in follow-up, molecular biomarkers are altered by recurrence with increased mutational burden in the tumor they may be sensitive to immunotherapy. For this reason in the early stage of the tumor, before extensive lymphadenectomy, must be performed sentinel lymph node analysis, to know more precisely the lymphatic diffusion of the metastatic lymph node.

2. Introduction

The incidence of small intestinal neuroendocrine tumors (NETs) is < 4 cases 100,000 persons, with an increase in US prevalence and incidence in recent years [1]. The overall 5-year survival in the Netherlands for these tumors is 75% [2]. Surgery based on surgeons' individual experiences, preferences and traditions is evolving into a discipline based on objective decision making from large-scale data from heterogeneous sources. Precision surgery in oncology has research principles that include, among others, the following knowledge domains: a) surgical technique and oncologic outcomes, b) biochemistry and molecular biology, and c) biostatistics and data processing in surgery [3]. Next-generation genomic sequencing, molecular biomarkers and precision surgery influence surgical management of multifocal neuroendocrine ileum tumor. The report that we present shows the difficulties encountered in making decisions for the diagnosis and treatment of these tumors. This review aims to analyze diagnostic, predictive and molecular biomarkers associated with multifocal ileum neuroendocrine tumors with lymph node metastases.

3. Case Report

The patient was a 72-year-old man who consulted for abdominal pain and diarrhea. Personal history of arterial hypertension to treatment. Physical examination was normal.

Abdominal-pelvic ultrasound showed a 2.8 x 2 cm, hypoechoic nodule in the root of the mesentery. The abdominal-pelvic CT scan showed hepatic steatosis. In the pancreatic neck, there is a cystic image of 2.2 cm. without malignant characteristics. In the mesenteric fat of the right iliac fossa in intimate contact with the terminal ileum and ileocecal valve there is a nodular lesion of 2.6 x 2.1 cm. In the supra umbilical mesenteric fat there is another nodular lesion of 1.9 cm compatible with adenopathy. In Figures 1a, 1b, and 1c, it shows the abdominal-pelvic CT scan performed preoperatively

Diagnostic biomarkers (Table 1).

Colonoscopy showed in the ascending colon, close to the hepatic angle, a polyp of 0.5 x 0.5 cm, compatible with tubular adenoma. The preoperative preparation was performed with, long-acting Sandostatin Lar, evacuating solution and antibiotic prophylaxis. In the postoperative period is administered low molecular weight heparin.

The following is a description of the steps followed during the surgical procedure. Incision by supra-infra umbilical laparotomy. Operative findings: hepatic steatosis; in the root of the mesentery at the level of the ileum-colic vein at the mouth of the superior mesenteric vein, 2.6 cm nodule with fibrosis around the tumor (lymph node) and retraction of the mesentery. On the mesenteric border of the terminal ileum, eight nodular formations what some narrow the lumen of the intestine and infiltrate the visceral serosa. Rest of the small and large bowel apparently normal. Intervention: dissection of the superior mesenteric vein. Localization of the ileocolic vessels. Ligation and section of the ileum-colic vein and artery at its root. Ligation of the right branch of the middle colic artery and vein. Resection: lymphadenectomy with inclusion of the metastatic nodule. Small bowel resection with inclusion of the eight tumor nodules. Right hemicolectomy. Reconstruction: ileocolic end-to-side ileocolic anastomosis with Auto Suture circular Stapler CEEA 28, Purstring 45 and ECHELON™ Covidien GST 60B + Stapler. Closure of the anterior abdominal wall: Maxon loop and stapler for the skin. Perioperative pathology: compatible with neuroendocrine tumor metastasis.

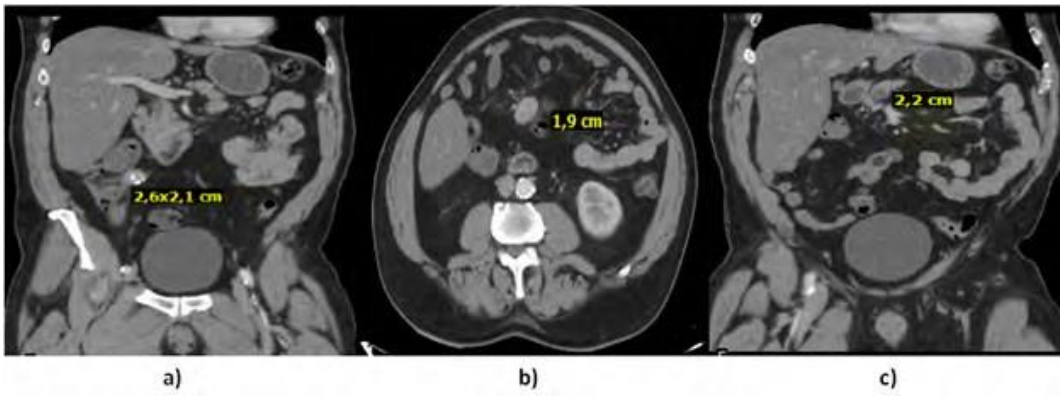

Figure 1: Abdominal-pelvic CT scan. a) Tumor in the right lower quadrant with calcifications, b) Tumor in the root of the mesentery, next to the mesenteric vessels, c) pancreas with cystic image of 2.2 cm.

Table 1: Analytic Preoperative

Blood Serotonin: 1055 ng/mL [70-270].
Diuresis in 24 h: 1,240 L
5-hydroxyindolacetic acid: 4.5 mgr. /24h [0-10]
Serotonin: 232 mcg/mL [0-10]

3.1. Pathologic Description

91cm surgical specimen (terminal ileum and ascending colon) with multifocal neuroendocrine tumor grade 1 (eight nodules), the largest of 2.9 cm. Metastases in two lymph nodes (2/6). Largest is the 1.9 cm. The free borders, pT3N1.

3.2. Macroscopic description

Surgical specimen comprising ileum (75 cm) and colon (16 cm) with appendix of 3.5 x 0.7 cm. A 1.2 cm tumor is located in the terminal ileum extending 1.7 cm deep. In the rest of the ilium, seven separate and scattered lymph nodules of 1.7, 0.9, 0.9, 0.9, 0.9, 0.7, 0.7, 0.7, 0.8, 0.5 and 0.2 cm were identified.. A mesenteric nodule of 1.9 cm and five lymph nodes were isolated from the peripheral fat, studied in rapid biopsy.

3.3. Microscopic description

Multifocal tumor (8 nodules) composed of a uniform population of cells with round nuclei and granular "salt and pepper" chromatin. The cells grow in nests, rosettes, and trabeculae. They express chromogranin A and synaptophysin, are negative for P-53, and have a Ki-67 proliferative index of 1%. Vascular invasion and has a mitotic index of 1/2mm2 is identified. The rest of the tumors appear as mucous or submucosal nests or with deep infiltration of the muscular wall and sub-serosa wall. The tumor close to the ileocecal valve shows fibrosis and calcifications. Metastasis in two of the six isolated lymphatic nodes. Free margins and normal appendix

Molecular Biomarkers. A targeted sequencing analysis (NSGDNA) of the small bowel neuroendocrine multifocal tumor has been performed with the (ONC4585 gene panel), from NIMGenetics, Madrid (Spain), annex 1. The analysis has been performed from paraffin-embedded tumor sample, after amplification with the Oncomine Comprehensive Plus library (ThermoFisher), oriented to the genomic analysis of 500 genes in tumor samples, in order to detect biomarkers with prognostic or predictive value of response (ONC4585).

After evaluation by the oncology department, the therapeutic decision was not to perform complementary treatment. The data used to support this decision were, small bowel neuroendocrine tumor, grade 1, with low mitotic index, low proliferative activity and low mutational load, with R0 surgical resection, after CT and octreotide scintigraphy.

4. Results

Postoperative evolution was satisfactory. Discharged from the hospital 5 days after surgery. The postoperative analysis performed one month after surgery showed normalization of serotonin in blood and urine, with a moderate elevation of chromogranin in blood, although there is no preoperative data for comparison (Table 2).

Biomarkers and prognostic genes analyzed by immunohistochemistry are shown below (Table 3).

Full body scintigraphy with Tc99m-EDDA/HYNIC-Tyr-octreotide (Tektrotyd), acquired tomographic images at 4 hours after intravenous administration of 20 mCi Tc99m-Tektroyd (Curium Pharma lot 600590832). A nonspecific increased uptake in the hooked process of the pancreas and a diffuse uptake of slight intensity in the body of the pancreas. An abdominopelvic CT scan reevaluated gamma uptake, showing a thrombus of terminal morphology, affecting the portal branches and partial thrombosis of the superior mesenteric vein. Cystic lesion in the neck of the pancreas with lobulated morphology, with no evidence of malignancy. The dose of anticoagulation was increased and two months later an echo-Doppler showed complete resolution of the thrombotic process.

According to the sequencing analysis, no clinically relevant point mutations or insertion-deletion polymorphisms no copy number changes and no translocations/fusions found in the genes analyzed.

Tumor Mutational Burden (TMB), Microsatellite Instability (MSI) and mutational signature are also, analyzed. The mutational load is one Mut/Mb, the MSI is negative (MSS pattern) and the mutational signature, is not detected. The mutational burden is one Mut/Mb, microsatellite instability is negative (MSS pattern) and the mutational signature is not detected.

Table 2: Postoperative Analysis

Blood Serotonin : 241 ng/mL [70-270]
Urine 5-hydroxyindolacetic acid /24h: 3,2,mgr/24h [0-10]
Urine Serotonin /24 h: 232 mcg/24h [50 -250]
Blood Chromogranin 11 nmol /L [< 4 nmol /L]

Table 3: Multifocal neuroendocrine tumor of the ileum. Grade 1.

Immunohistochemistry	Tissue
Cromogranina A	+
Synaptophysin	+
P-53	-
Ki-67	1%
mitotic index	1/2 mm^2

5. Discussion

The first decision during surgery was whether to, resect the tumor or not. Neither the diagnostic biomarkers nor the imaging methods used have not detected tumor extension. Therefore, manual intestinal palpation at the time of surgery seems essential today.

The following data support the resection decision: in order to improve the patient's symptoms and quality of life, conservative resection of the bowel, lymph nodes and areas of fibrosis in the mesentery is possible. In addition, surgical bowel resection does not represent a greater risk than the disease itself. Finally, to avoid obstructions, bleeding or perforations, it is possible to resect 90% of the tumor burden.

According to the classification of (WH0), the multifocal ileum neuroendocrine tumor of the ileum shown is a well-differentiated tumor, with a mitotic index < 2 mm2 and K-67 proliferation index < 3. The pathology classifies the tumor as NET G1 (Table 4) [4].

The 8th edition of the AJCC-TNM classification has created a new N2 classification to better stratify ilium lymph node metastases. N1 if there are fewer than 12 regional lymph nodes and N2 if there are more than 12 or if the mesenteric mass is > 2 cm). For neuroendocrine tumor of the ileum, N2 is a marker of liver metastases, and is not, an independent prognostic factor [5]. The pathology classifies the tumor as pT3N1 [6].

Metastatic small intestinal neuroendocrine tumors frequently harbor driver mutations in genes such as TP53, RB1, CDKN1B, KRAS, NRAS and MET. All tumors have microsatellite stability and show low TMB. The Ki67 proliferation index is significantly associated with the presence of driver mutations ($p = 0.015$) [7].

No clinically relevant point mutations or insertion-deletion polymorphisms in the genes analyzed, in the case presented have been identified. There is an absence of microsatellite instability and low TMB, with an average of one variant per Mb.

The lack of somatic single nucleotide variants in metastatic multifocal ileum tumors supports the independent clonal origin of the different tumors, suggesting the contribution of a local priming factor to tumor development [8].

Eight morphologically identical tumors cluster within a segment of the terminal ileum around a mesenteric root lymph node metastasis. There are no gene-driven alterations detected by sequencing. However, since only has been sequenced, the most distal tumor we cannot determine the clonal origin of the rest of the tumors.

Interact intrinsically with their environment by secretion of serotonin of the enterochromaffin cell type establish synaptic connections with the enteric nervous system and with receptors that sense nutrients from the luminal contents and the microbiome. In the enteroendocrine cells type of the intestine the apical release of 5-hydroxytryptamine (5-HT), positively activates the microbiota and vice versa, inducing its proliferation. These local factors together with flow and pressure modifications in patients with a competent ileocecal valve may contribute to multifocal tumor development. However, the underlying oncogenic mechanism is unknown. Therefore, there is a lack of pharmacological targets to support biologic therapy.

Moreover, chemotherapy treatment is of little benefit in treating well-differentiated intestinal neuroendocrine tumors, because no regimen has shown has demonstrated objective tumor response rates. [10].

The number of somatic mutations by mega-base of DNA presented to the immune system determines the TMB. It is used as an indicator of neo-antigen load with which it correlates positively. Neuroendocrine tumors of the small intestine have a low TMB [11]. Tumors with high TMB carry a large number of tumor neo-antigens. These not recognized as their own, provoking the activation of T lymphocytes in the tumor microenvironment.

Tumors that respond to immune checkpoint inhibitors have a higher level of immune infiltrates and/or an interferon (IFN) signature indicative of an inflammatory T-cell phenotype, [12]. This has led to the use of immunotherapy in these tumors. However, current standard therapies consistently offer objective response rates of less than 20% and trials with immune checkpoint inhibitors to date have failed to improve outcomes.

In addition, the low tumor mutational burden and tumor microenvironment contrast sharply with tumors with low immune infiltration. These tumors have no inflammation contributing T cells to the milieu. This makes immune checkpoint inhibitors fail to improve outcomes in this tumor subtype [11].

The use of TMB as a predictive biomarker of recurrence and the detection of serotonin levels in the follow-up of this patient may make it possible to use this therapy in the case of tumor recurrence and if there is an increase in mutational burden.

Multiple metastases in the same patient may originate from one or more primary tumors. Therefore, it is necessary to identify and remove all primary tumors that have metastatic potential.

Another question raised is whether lymphadenectomy of the nodes should be extensive. To improve overall survival it is necessary to resect 8 to 12 resected lymph nodes, [13].Is performed the lymphadenectomy along the mesenteric vessels, up to the inferior border of the pancreas, including performing a systemic lymph node dissection up to the retro-pancreatic area [14].

The immune system detects and eliminates cells in the different processes of carcinogenesis through the immune-surveillance system (cancer cycle. immunity) [15]. Tumor antigen capture and maturation of dendritic cells occurs in the first stage Lymph node there is stimulation of the immune system mediated by CD8 cytotoxic T lymphocytes. A third stage of lymphocyte migration to the tumor microenvironment and finally elimination of the neoplastic cells by immune effector mechanisms.

The ileum neuroendocrine tumor with lymph node metastases and low mutational load is likely to have an impaired immune-surveillance system. Genes of the immune system in the tumor microenvironment can be inhibited and cause resistant tumor cell clones to escape immune surveillance and spread locally or distantly.

In early stages, elective lymphadenectomy is the treatment of choice, although extensive lymphadenectomy is sometimes proposed. [16].

If it is acceptable to use immunotherapy in these tumors, it is desirable not to perform prophylactic dissection and to maintain the integrity of the immune system, especially in tumors with low BMT, which are generally not initially responsive to immunotherapy.

Therefore, in these early stage patients, it is necessary to perform a lymphadenectomy with navigation of the sentinel node, which in this case would be the node accompanying the ileocolic vessels [17].

Table 4: The World Health Organization (WHO) 2022 Classification of Neuroendocrine Neoplasms

Neuroendocrine Neoplasm Well-differentiated	Classification	Mitotic Rate (Mitoses/2 mm^2)	Ki-67 Index
	NET, grade 1	<2	<3%
	NET, grade 2	2–20	3–20%
	NET, grade 3	>20	>20%

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