Does Mesoappendix Infiltration Predict a Worse Prognosis in Incidental Neuroendocrine Tumors of the Appendix?

A Clinicopathologic and Immunohistochemical Study of 15 Cases

Giulio Rossi, MD,1 Riccardo Valli, MD,1 Federica Bertolini, MD,2 Pamela Sighinolfi, MD,1 Luisa Losi, MD,1 Alberto Cavazza, MD,3 Francesco Rivasi, MD,1 and Gabriele Luppi, MD2

Key Words: Appendix; Carcinoid; Neuroendocrine tumor; Mesoappendix; Ki-67; Classification

Abstract

We conducted a retrospective clinicopathologic and immunohistochemical study of the biologic significance of mesoappendix infiltration in 15 appendiceal neuroendocrine tumors selected from a series of 42 primary tumors. In all cases, the tumor was found incidentally and measured less than 2 cm (mean, 0.84 cm). In 13 cases, it was located in the tip of the appendix and in the midportion in 2. Histologically, none showed relationship with overlying mucosa. Necrosis was absent; mitotic figures were rare. The Ki-67 labeling index was low (1%-2%). In all cases, S-100 protein immunostaining disclosed positive elements with cytoplasmic dendritic processes closely intermingled with neuroendocrine neoplastic cells. All patients (8 males; 7 females; mean age, 38.2 years) underwent simple appendectomy. A right-sided hemicolectomy was performed subsequently in 1 case. After a mean follow-up of 52.6 months (range, 8-143 months), none had died of disease or had recurrent or metastatic disease. Our results confirm that appendiceal neuroendocrine tumors seem to have a different phenotype from those occurring in other gastrointestinal sites. Tumors less than 2 cm, even with mesoappendiceal infiltration, have an excellent prognosis, and simple appendectomy seems to be the appropriate therapeutic approach.

The appendix represents the most common site of origin of gastrointestinal neuroendocrine tumors (so-called carcinoids).1-5 The majority of appendiceal neuroendocrine tumors (NETs) usually are located in the tip of the appendix, where they represent an incidental microscopic finding disclosed during surgical procedures for acute inflammation or other indications.1-6 They are considered malignant with an intrinsic metastatic potential, but they generally pursue a favorable clinical course.1-6 Based on tumor size, level of infiltration, presence of vascular invasion, and whether the tumor is functionally active, the recent World Health Organization (WHO) classification of endocrine tumors recognizes 3 major categories of appendiceal NETs: well-differentiated endocrine tumor (so-called carcinoid), well-differentiated endocrine carcinoma (so-called malignant carcinoid), and mixed exocrine-endocrine carcinoma.7,8 Based on this scheme and regardless of tumor size, the neoplastic infiltration of mesoappendiceal adipose tissue should lead to the diagnosis of well-differentiated endocrine carcinoma.

Despite the finding in several studies that the survival of patients with appendiceal NETs is much better than that reported for NETs in other gastrointestinal sites,1-6,8 controversy persists about the treatment of choice when the tumor shows mesoappendix invasion. To elucidate the prognostic role of this finding, we retrospectively analyzed the clinicopathologic and immunohistochemical features of 15 primary NETs of the appendix found incidentally at surgery and that microscopically displayed clear-cut neoplastic infiltration of the mesoappendix.
Materials and Methods

We identified 42 primary NETs of the appendix in the files of the Section of Pathology, University of Modena and Reggio Emilia, Modena, Italy, diagnosed during the period between January 1991 and April 2002. The surgical specimens were fixed in 5% buffered formalin and embedded in paraffin. Two to ten H&E-stained slides (mean, 3.5 slides) per case were available for the study. All slides were reviewed at a multiheaded microscope by 2 pathologists (G.R. and R.V.). The tumors then were reclassified according to criteria of the WHO classification.7,8

Cases in which the tumor was limited to the appendiceal wall, with a mixed endocrine-exocrine neoplasm, or with a second malignant tumor were excluded from the study. Thus, 15 NETs with mesoappendix infiltration were selected for the study. Surgical pathology reports were reviewed for gross pathologic parameters. Tumor size was determined by measuring the maximum tumor diameter on H&E-stained slides. Clinical data were obtained from the clinical charts and from the referring physicians. The following data were recorded: age at diagnosis, sex, initial clinical symptoms and indication for surgery, tumor location and size, histologic features (including histologic type, presence of necrosis, mitotic activity, labeling index), therapeutic approach, and follow-up (calculated from the date of surgery).

Routine H&E staining was supplemented by immunostaining for pancytokeratins (monoclonal, clone MNF116, 1:100 dilution; DAKO, Glostrup, Denmark), low-molecular-weight cytokeratins (monoclonal, clone CAM5.2, 1:50 dilution; Becton Dickinson, San Jose, CA), high-molecular-weight cytokeratins (monoclonal, clone 34βE12, 1:500 dilution; DAKO), chromogranin A (monoclonal, clone DAK-A3, 1:100 dilution; DAKO), synaptophysin (polyclonal, 1:50 dilution; NeoMarkers, Fremont, CA), CD31 (monoclonal, clone JC70, 1:100 dilution; NeoMarkers), and S-100 protein (polyclonal, 1:50 dilution; DAKO), respectively. Normal appendiceal mucosa and adjacent vessels served as positive internal controls for chromogranin A and synaptophysin and for S-100 protein, respectively. Normal appendiceal mucosa and adjacent vessels served as positive internal controls for pancytokeratins and CAM5.2 and for CD31, respectively. Finally, sections of a basaloid carcinoma of the lung served as the positive control for high-molecular-weight cytokeratins (34βE12). Negative controls were included in each test by replacing the primary antibodies with nonimmune mouse IgG.

Results

The most relevant clinical and pathologic data are summarized in Table I.

Clinical Findings

Briefly, the case series included 15 patients, 8 males and 7 females (male/female ratio, 1.1:1). The mean age at diagnosis was 38.2 years, with a wide range (14-80 years). All patients underwent simple appendectomy, whereas 1 patient underwent a supplementary right-sided hemicolectomy. In this latter case, the histologic examination of the ascending colon, adipose tissue of the adjacent mesentery, and regional lymph nodes did not reveal neoplastic residual or metastatic deposit. Acute abdominal pain was the most common initial symptom, occurring in 12 cases (11 with acute appendicitis and 1 with diverticular disease). In 3 cases, the tumor was found incidentally during surgical intervention for benign ovarian cystadenoma (2 cases) and multiple uterine leiomyomata (1 case). Follow-up was obtained for all patients. None of the patients died of disease, with a follow-up ranging from 8 to 143 months (mean, 52.6 months). All patients except 1 were alive and well at last follow-up. An 80-year-old woman died of cardiac failure (34 months after appendectomy). Carcinoid syndrome related to functioning tumors was not found in any case.

Pathologic Findings

Macroscopically, tumors were solitary, fairly well-circumscribed, and yellowish white. Thirteen tumors were...
located in the tip of the appendix, whereas the remaining 2 were in the midpoint. No case was found at the base of the appendix, and surgical margins of resection were free of tumor in all cases. The tumor size ranged from 0.3 to 1.5 cm, with a mean of 0.85 cm. Histologically, 13 tumors showed solid nests of small monotonous cells arranged in insular, trabecular, or acinar patterns with rosette formations (classic type). One was composed of a monomorphous proliferation of cells forming small tubules with some intraluminal mucin (tubular type). The remaining tumor consisted of a predominantly solid proliferation of monomorphic cells with clear, foamy cytoplasm (classic type, clear cell variant). Mitotic figures were exceedingly rare (<1 mitosis per 10 high-power fields), and necrosis was absent. By definition, clear-cut mesoappendix infiltration was always present \( \text{Image 1} \), and in 5 cases, the tumor also involved the serosal surface. The results of immunohistochemical staining for cytokeratins using a broad-spectrum antibody (clone MNF116) were negative in all 14 classic types, while weak immunostaining was observed in the tubular type. All tumors were completely unstained with the high-molecular-weight cytokeratins (clone 34\( \beta \)E12), whereas weak to moderate cytoplasmic immunostaining was noted for the low-molecular-weight cytokeratins (clone CAM5.2). Results for chromogranin A and synaptophysin were always positive \( \text{Image 2A} \), and S-100 protein highlighted the presence of S-shaped spindle cells with cytoplasmic dendritic processes in all cases \( \text{Image 2B} \). Cytoproliferative activity by Ki-67 (MIB-1) was extremely low (ranging from 1%-2%) \( \text{Image 2C} \). In 8 cases (53%), the tumor was suspected to display vascular invasion at routine light microscopic examination, but CD31 did not disclose features of vascular invasion in any case \( \text{Image 3} \).

### Discussion

The overall 5-year survival rate of patients with appendiceal NETs is favorable, varying from 85% to 100%,\(^1\)\(^-\)\(^6\) whereas metastases are observed rarely and found mostly at the time of first tumor detection.\(^1\)\(^6\)\(^-\)\(^9\)\(^-\)\(^11\) During a retrospective histologic review of gastrointestinal NETs performed at our institution, we noted that appendiceal NETs showed mesoappendix infiltration in 15 (36%) of 42 cases. This finding prompted us to search for the complete clinical data for these patients to better evaluate the possible prognostic role of mesoappendix infiltration in appendiceal NETs.

|| Case No./ Sex/Age (y) | Surgical Indication | Tumor Size (mm) | Histologic Type | Ki-67 LI (%) | Follow-up (mo) | Therapy |
|----------------------|---------------------|----------------|----------------|--------------|--------------|---------|
| 1/F/48               | Uterine leiomyoma   | 15             | Classic        | 2            | Alive and well (143) | Appendectomy and hysterectomy |
| 2/M/32               | Acute appendicitis  | 13             | Classic        | 1            | Alive and well (140) | Appendectomy |
| 3/F/80               | Diverticulosis      | 8              | Classic        | 1            | Alive and well (92) | Appendectomy |
| 4/M/18               | Acute appendicitis  | 5              | Classic        | 1            | Alive and well (80) | Appendectomy and oophorectomy |
| 5/F/17               | Ovarian cystadenoma | 5              | Tubular        | 1            | Alive and well (73) | Appendectomy |
| 6/F/24               | Acute appendicitis  | 4              | Classic        | 1            | Alive and well (46) | Appendectomy |
| 7/F/29               | Acute appendicitis  | 3              | Classic        | 1            | Alive and well (32) | Appendectomy |
| 8/F/34               | Acute appendicitis  | 14             | Classic        | 2            | Alive and well (31) | Appendectomy |
| 9/F/77               | Acute appendicitis  | 5              | Classic        | 2            | Alive and well (10) | Appendectomy |
| 10/F/40              | Acute appendicitis  | 11             | Classic        | 1            | Alive and well (8) | Appendectomy and right-sided hemicolectomy |
| 11/F/47              | Acute appendicitis  | 9              | Classic        | 1            | Alive and well (34) | Appendectomy |
| 12/M/28              | Acute appendicitis  | 6              | Classic        | 1            | Alive and well (24) | Appendectomy and oophorectomy |
| 13/F/47              | Ovarian cystadenoma | 5              | Classic        | 1            | Alive and well (22) | Appendectomy |
| 14/F/14              | Acute appendicitis  | 14             | Classic        | 1            | Alive and well (20) | Appendectomy |
| 15/M/38              | Acute appendicitis  | 10             | Classic        | 1            | Alive and well (20) | Appendectomy |

LI, labeling index.
potential of and recommending the appropriate treatment for this tumor. However, controversy exists concerning the clinical value of microscopic invasion of serosal surface, subserosal lymphatics, and/or mesoappendix for tumors smaller than 2 cm. Several authors suggested that these latter features should be considered reliable parameters of aggressive clinical behavior and, thus, recommended radical surgery (hemicolec-tomy) in patients having at least one of these findings. Moreover, the recent WHO classification of endocrine tumors stated that neoplastic involvement of the mesoappendix and angioinvasion are among the defining diagnostic features of well-differentiated endocrine carcinoma (malignant carcinoid). MacGillivray et al reported a 0.6-cm NET of the appendix with liver metastasis at diagnosis. They also reviewed 414 cases of appendiceal NET reported in the literature and found that both tumors larger than 2 cm and mesoappendiceal invasion were related to metastatic disease. However, metastases were reported in only 17 (4.1%) of 414 cases studied, usually in the form of individual case reports, thus making statistical analysis meaningless and confirming the serendipitous nature of these cases.

By contrast, but in agreement with other series, we found that simple appendectomy was the adequate treatment for small appendiceal NETs, even when mesoappendix or serosal invasion occurred. Radical surgery clearly is necessary in tumors measuring more than 2 cm in diameter or clearly involving the base of the appendix and in cases with lymph node metastases or peculiar histologic types (mucinous or goblet cell carcinoid and mixed exocrine-endocrine tumors). In support of this fact, the only 2 cases with appendiceal NETs greater than 2 cm that we retrieved from our archival files had mesoappendix infiltration and

![Image 2](image2.png)

metastatic disease at diagnosis. By the way, the high percentage of appendiceal NETs smaller than 2 cm and invading mesoappendix at diagnosis but associated with benign clinical behavior, as in our series, is not entirely surprising. Only a few previous studies elucidated the frequency and clinical significance of the aforementioned features, and the present study, to our knowledge, is the first studying the predictive value of mesoappendix invasion in selected appendiceal NETs smaller than 2 cm.

In a series of 21 appendiceal NETs, Dunn reported invasion of the mesoappendix and lymphatic permeation in 33% and in almost half of cases, respectively. In addition, Prommegger et al found mesoappendix infiltration in 27 (57%) of 47 appendiceal NETs, and the tumor was suspected to have vascular invasion in 30 cases (64%). However, Prommegger et al failed to confirm blood vessel invasion by means of immunohistochemical analysis with CD31. It is, therefore, reasonable that the high frequency of angioinvasion reported so far in NETs of the appendix might actually be related to tissue artifacts rather than to a true neoplastic vascular involvement. In equivocal cases, simple immunostaining using specific endothelial markers may be necessary to decide whether suspected vascular invasion is real.

In our series, all tumors consisted of incidentally discovered lesions smaller than 2 cm and invading mesoappendix at diagnosis but associated with benign clinical behavior, as in our series, is not entirely surprising. Only a few previous studies elucidated the frequency and clinical significance of the aforementioned features, and the present study, to our knowledge, is the first studying the predictive value of mesoappendix invasion in selected appendiceal NETs smaller than 2 cm.

From a histopathogenetic viewpoint, NETs of the appendix differ from other gastrointestinal NETs, with a neuroectodermal origin from the subepithelial neuroendocrine cells located mainly in the tip of the appendix rather than from intraepithelial neuroendocrine cells. In agreement with this hypothesis, in all of our cases, the neoplastic cells lacked anatomic relationship with the overlying mucosa, as previously documented by Wilander and Scheibenpflug, and showed weak to moderate expression for only a restricted subset of low-molecular-weight cytokeratins. Moreover, as observed by different authors, all cases in the present series displayed scattered, interstitial S-100–positive elements with cytoplasmic ramifications closely admixed with the neuroendocrine neoplastic cells, similar to those usually found in pheochromocytoma and/or paraganglioma (so-called sustentacular cells). This characteristic immunophenotype seems to separate NETs arising in the appendix from similar tumors occurring in other gastrointestinal sites.

The findings of our clinicopathologic analysis are in agreement with previously reported data and support the contention that mesoappendix invasion does not seem to have a prognostic role in NETs of the appendix. Consequently, in our opinion, the use of this parameter to distinguish well-differentiated endocrine carcinoma from well-differentiated endocrine tumor, as stated by the WHO classification scheme, is questionable. Despite the limitations of this study in regard to the retrospective design and the relatively small number of cases, the excellent clinical behavior of these tumors in comparison with NETs of other gastrointestinal sites might be explained by their different biologic characteristics and histogenesis.

From the 1Department of Pathologic Anatomy and Forensic Medicine, Section of Pathology; 2Department of Oncology and Hematology, Section of Oncology, University of Modena and Reggio Emilia; and 3Operative Unit of Pathology, S Maria Nuova Hospital, AUSL Reggio Emilia, Italy.

Address reprint requests to Dr Rossi: Dept of Pathologic Anatomy and Forensic Medicine, Section of Pathology, University of Modena and Reggio Emilia, via del Pozzo, 71, 41100-Modena, Italy.
References