Morphological Factors Related to Nodal Metastases in Neuroendocrine Tumors of the Appendix

A Multicentric Retrospective Study

Nicole Brighi, MD,[*] Stefano La Rosa, MD,[†] Giulio Rossi, MD,[‡] Federica Grillo, MD,[§] Sara Pusceddu, MD,[¶] Maria Rinzivillo, MD,[||] Francesca Spada, MD,[**] Salvatore Tafuto, MD,[††] Sara Massironi, MD,[‡‡] Antongiulio Faggiano, MD,[§§] Lorenzo Antonuzzo, MD,[¶¶] Donatella Santini, MD,[||||] Fausto Sessa, Prof,[***] Roberta Maragliano, MD,[***] Fabio Gelsomino, MD,[†††] Manuela Albertelli, MD,[‡‡‡] Claudio Vernieri, MD,[¶] Francesco Panzuto, MD,[||] Nicola Fazio, MD,[**] Chiara De Divitiis, MD,[††] Giuseppe Lamberti, MD,[*] Annamaria Colao, Prof,[§§] Gianfranco Delle Fave, Prof,[||] and Davide Campana, MD[§§§]

Objective: The aim of this study was to evaluate clinical and morphological features related to nodal involvement in appendiceal neuroendocrine tumors (NETs), to identify patients who should be referred for oncological radicalization with hemicolectomy.

Background: Appendiceal NETs are usually diagnosed accidentally after appendectomy; the indications for right hemicolectomy are currently based on several parameters (ie, tumor size, grading, proliferative index, localization, mesoappendiceal invasion, lymphovascular infiltration). Available guidelines are based on scarce evidence inferred by small, retrospective, single-institution studies, resulting in discordant recommendations.

Methods: A retrospective analysis of a prospectively collected database was performed. Patients who underwent surgical resection of appendiceal NETs at 11 tertiary Italian centers, from January 1990 to December 2015, were included. Clinical and morphological data were analyzed to identify factors related to nodal involvement.

Results: Four-hundred fifty-seven patients were evaluated, and 435 were finally included and analyzed. Of them, 21 had nodal involvement. Grading G2 [odds ratio (OR) 6.04], lymphovascular infiltration (OR 10.17), size (OR 18.50), and mesoappendiceal invasion (OR 3.63) were related to nodal disease. Receiver operating characteristic curve identified >15.5 mm as the best size cutoff value (area under the curve 0.747). On multivariate analysis, grading G2 (OR 6.98), lymphovascular infiltration (OR 8.63), and size >15.5 mm (OR 35.28) were independently related to nodal involvement.

Conclusions: Tumor size >15.5 mm, grading G2, and presence of lymphovascular infiltration are factors independently related to nodal metastases in appendiceal NETs. Presence of ≥1 of these features should be considered an indication for oncological radicalization. Although these results represent the largest study currently available, prospective validation is needed.

Keywords: Appendiceal, carcinoid, lymph nodes, NET, neuroendocrine neoplasms, Nodal metastases, prognostic factors, surgery

(*Ann Surg* 2020;271:527–533)

Neuroendocrine neoplasms of the appendix have an approximate annual incidence of 0.15 to 0.6/100,000, with a slight female preponderance in Western series. In recent years, the reported incidence has increased and the overall incidence rate is similar among different ethnicities, although some differences have been reported.[1]

Appendiceal neuroendocrine neoplasms are mostly diagnosed incidentally during appendectomy with a rate of approximately 3 to 5/1,000 appendectomies. Neuroendocrine neoplasms, which comprise neuroendocrine tumors (NETs), neuroendocrine carcinomas (NECs), mixed adenoneuroendocrine carcinomas (MANECs), and goblet cell carcinomas, are the largest subgroup of appendiceal neoplasms with approximately 30% to 80% of all neoplasms of this site, followed by adenocarcinomas (36%), sarcomas and gastrointestinal stromal tumors (<1%), and lymphomas (1.7%).[1,2]

According to the most recently updated guidelines[3,4] two surgical procedures should be applied to treat appendiceal NETs: simple appendectomy and right hemicolectomy.

The European Neuroendocrine Tumor Society (ENETS) guidelines recommend tailoring the surgical strategy to the individual situation. In particular, simple appendectomy is considered curative for tumors <1 cm in diameter, whereas right hemicolectomy is advised for NETs >2 cm (T3 stage according to ENETS classification, T2 according to UICC/AJCC classification) because of the increased risk of lymph node metastases, long-term tumor recurrence, and/or distant metastases.

From the [*]Department of Experimental, Diagnostic and Specialty Medicine, S.Orsola-Malpighi University Hospital, Bologna, Italy; [†]Service of Clinical Pathology, Lausanne University Hospital, Institute of Pathology, Lausanne, Switzerland; [‡]Pathology Unit, Azienda Romagna, Ospedale S. Maria delle Croci, Ravenna, Italy; [§]Department of Surgical Science and Integrated Diagnostics (DISC), Pathology Unit, University of Genoa, Genoa, Italy; [¶]Department of Medical Oncology, ENETS Center of Excellence, Fondazione IRCCS Istituto dei Tumori, Milan, Italy; [||]Digestive and Liver Disease Unit, Sant'Andrea Hospital Sapienza University of Rome, Roma, Italy; [**]Unit of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, European Institute of Oncology, IEO, Milan, Italy; [††]Abdominal Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori – IRCCS Naples "Fondazione G. Pascale", Naples, Italy; [‡‡]Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [§§]Department of Clinical Medicine and Surgery, Division of Endocrinology, University Federico II of Naples, Napoli, Italy; [¶¶]S.C. Oncologia Medica 1, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [||||]Department of Diagnostic and Prevention Medicine, S.Orsola-Malpighi University Hospital, Bologna, Italy; [***]Department of Medicine and Surgery, University of Insubria, Varese, Italy; [†††]Department of Oncology and Haematology, Division of Oncology, University Hospital of Modena, Modena, Italy; [‡‡‡]Endocrinology, Department of Internal Medicine and Medical Specialties (DIMI)University of Genoa, Genoa, Italy; and [§§§]Department of Medical and Surgical Sciences, S.Orsola-Malpighi University Hospital, Bologna, Italy.

Reprints requests should be addressed to Dr. Davide Campana.

The authors report no conflicts of interest.

Reprints: Davide Campana, MD, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna - S.Orsola-Malpighi University Hospital, Via Massarenti, 9–40138 Bologna, Italy.

E-mail: davide.campana@unibo.it.

ISSN: 0003-4932/18/27103-0527

DOI: 10.1097/SLA.0000000000002939

In case of a T2 (ENETS) or a T1b (UICC/AJCC) NET (tumors with a size between 1 and 2 cm), lymph node or distant metastases seem unlikely but possible, particularly considering that, relatively young patients with long life expectancy represent the majority of cases. Thus, definitive curative treatment seems much more likely with right hemicolectomy, but this procedure may be burdened by an increased perioperative risk when compared to simple appendectomy. Therefore, the localization of the tumor at the base of the appendix (particularly with R1 resection) or a mesoappendiceal invasion >3 mm is often taken into consideration as additional criteria. In any of these cases, right hemicolectomy is recommended by the most recently updated guidelines, but long-term prospective data are currently lacking. Additional criteria, such as a Ki67 index of ≥3% (NET-G2) or angioinvasion, have been suggested to aid with decision-making, but even less evidence for these criteria has been published.[3] However, ENETS guidelines are based on small case series and scarce evidence.

Considering the many issues still under debate in the management of these tumors and the need for a better prognostic stratification of patients to improve clinical practice, the aim of our study was to evaluate the clinical, pathological, and immunohistochemical features related to nodal involvement in a large multicentric cohort of patients diagnosed with a NET of the appendix after appendectomy, to identify those who should be referred for right hemicolectomy.

METHODS

Study Design

A retrospective analysis of a multicentric prospectively collected database was performed. All consecutive patients who underwent surgical resection of appendiceal NET at 11 tertiary Italian centers, from January 1990 to December 2015, were included and followed up until January 2017. Demographic, clinical, surgical, and pathological data were collected and analyzed to identify predictive factors for nodal involvement in these patients.

All patients or their legal representatives provided written informed consent at the time of surgery for anonymous review of their data for research purpose. This retrospective study was approved by local IRB (Comitato Etico Indipendente, S.Orsola-Malpighi Hospital, Bologna) and was conducted in accordance with the principles of the Declaration of Helsinki (revision of Edinburgh, 2000).

The primary end-point of this study was the identification of prognostic factors related to the presence of nodal involvement in patients with appendiceal NET.

Study Population

All consecutive patients (both male and female; no age restriction) who underwent surgical resection of appendiceal NET at 11 tertiary Italian centers during the study period were included. Patients with NEC G3 (according to WHO 2010 classification) or poorly differentiated endocrine carcinomas (PDEC) (according to WHO 2000), MANEC, goblet cell neoplasm, or no evidence of NET on pathology revision were excluded from the analysis. Cases with incomplete pathology reports were censored.

Data Collection

All data were prospectively collected at the center where the patient had been enrolled. A single computerized data sheet was created and demographic, clinical, surgical, and pathological data were retrospectively analyzed. For each patient, the following data were collected: age, sex, date of diagnosis, age at diagnosis, surgical indication, indication for subsequent hemicolectomy and pathological features, such as tumor size and localization, lympho-vascular invasion, and mesoappendiceal invasion, Ki-67, WHO 2000 or WHO 2010 classification, and TNM staging according to ENETS or UICC/AJCC.

Pathology and Immunohistochemistry Features

The histological specimens were examined by an experienced pathologist at each center. Tumors were classified according to WHO 2010 classification[5] and the ENETS grading system.[6] The Ki-67 proliferation index was expressed as a percentage based on the count of Ki-67-positive cells in 2000 tumor cells in the areas of the highest immunostaining using the Ki67 antibody and Ki-67 (MIB1). Equivocal cases for lymphovascular infiltration were revised by NET-expert pathologists using immunostaining for specific endothelial markers including CD31 or CD34.

Statistical Analysis

Categorical variables were expressed as numbers (percentage). Continuous variables were reported as median and interquartile range (IQR, 25th to 75th percentiles). Categorical variables were compared using Pearson chi square or Fisher exact test, when appropriate. Continuous variables were compared using Mann-Whitney *U* test. Receiver-operating characteristic (ROC) curve was used to identify the best cutoff value for the prediction of nodal involvement according to the size of the tumor.

Two different analyses were performed. In the analysis A, patients with nodal involvement at pathology after hemicolectomy (N+ group; cases) were compared to patients without nodal involvement at pathology after hemicolectomy (N− group; controls).

Furthermore, we hypothesized that patients with occult nodal involvement who did not undergo hemicolectomy, would recur and we arbitrarily set to 10 years the time needed for this to occur. So we assumed that patients without recurrence at the end of a 10-year follow-up would not have had extra-appendiceal disease at diagnosis, whereas patients who showed disease recurrence would have had extra-appendiceal disease at diagnosis. Consequently, in the analysis B, we compared Group 1 (patients from the N+ group and patients with disease recurrence; cases) with Group 2 (patients from the N− group and patients who did not present disease recurrence during 10-year follow-up; controls).

Analysis of the predictive factors of nodal disease was carried out by univariate and multivariate analysis using logistic regression. Predictive factors were expressed as odds ratio (OR) and 95% confidence interval (95% CI). Forward stepwise method was used to build a multivariate model after inclusion of all variables. All predictive analyses were shown in the results section and tables.

Overall survival (OS) was defined as the length of time from the date of diagnosis of NET to the death of patient or last follow-up visit. Disease-specific survival (DSS) was calculated as the number of months from diagnosis of appendiceal NET to the date of death from NET or to the last follow-up date for patients still alive. DSS and OS distributions were estimated using the Kaplan–Meier algorithm and compared by the log rank test.

The *P* value was considered statistically significant when <0.05. Statistical analysis was performed using SPSS Statistics v. 22 (IBM).

RESULTS

Four hundred fifty-seven (no. 457) consecutive patients undergoing appendectomy and diagnosed with appendiceal NET were evaluated; of them, 9 did not meet inclusion criteria because of diagnosis of goblet cell neoplasia (no. 4), G3 NEC (no. 2), MANEC (no. 2), and appendicitis on pathological revision without evidence of

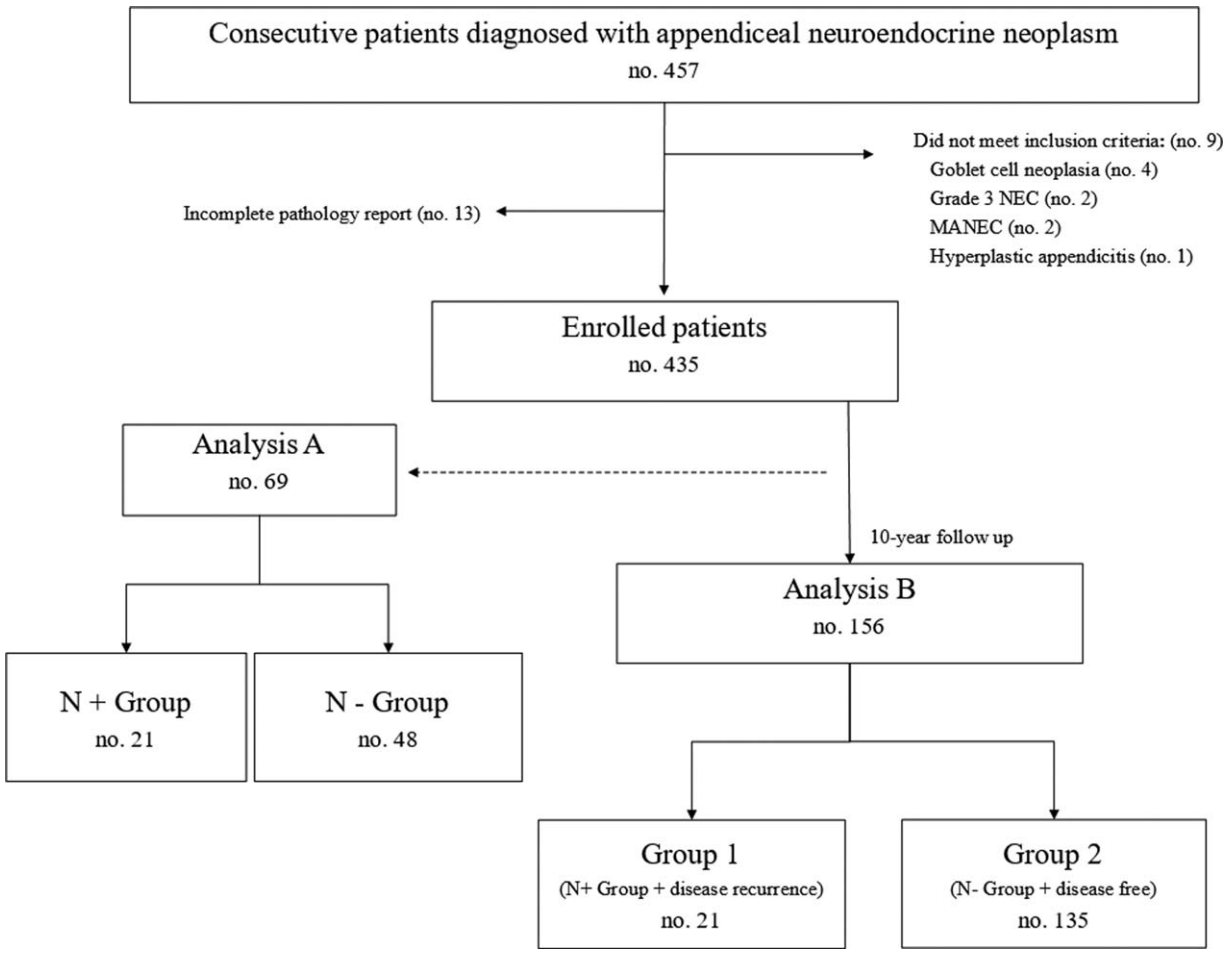

FIGURE 1. Study flow chart. N+ Group: patients with nodal involvement at hemicolectomy; N– Group: patients without nodal involvement at hemicolectomy. Group 1: patients with nodal involvement at hemicolectomy and patients with evidence of disease recurrence during the 10-year follow up; Group 2: patients without nodal involvement at hemicolectomy and disease-free patients in the follow-up period.

tumor (no. 1). Thirteen cases were censored because of missing or incomplete pathological data. Study flow chart is shown in Figure 1.

The characteristics of the final study population (no. 435) are listed in Table 1. One-hundred forty-two (no. 142) male (32.6%) and 293 female (67.4%) patients with a median age of 29 years (IQR 21–41 years) were included. Indications for appendectomy were acute appendicitis (no. 344; 79.1%), other abdominal surgery (no. 48, 11.0%), other neoplasia (no. 10, 2.3%), primary tumor resection in stage IV disease (no. 2, 0.5%); in 31 cases (7.1%) indication was not reported. In 281 cases (64.6%) tumors were located in the tip, in 46 (10.6%) at the body, in 16 (3.7%) in the base of the appendix, whereas in the remaining 92, (21.1%) site was not reported. Median size of the appendiceal tumor was 7.0 mm (IQR 4.0–11.0). Lymphovascular invasion was present in 51 (11.7%), absent in 286 (65.8%); data were not available in 98 patients (22.5%). Mesoappendiceal invasion was observed in 171 cases (39.3%): among these patients, 88 had an infiltration <3 mm, 46 patients ≥3 mm; data on infiltration depth was not reported in 37 cases. No mesoappendiceal invasion was observed in 217 patients (49.9%); data were not reported in 47 cases (10.8%). According to WHO 2010 classification, 360 patients (82.8%) had G1 NET and 35 (8.0%) had G2 NET. Median Ki-67 was 1.0% (IQR 1.0–1.0). Pathology reports according to WHO 2010 classification were not available in 40 cases (9.2%): of them, 39 cases (9.1%) were well-differentiated endocrine tumor and 1 (0.1%) was a well-differentiated endocrine carcinoma, according to WHO 2000 classification.

Analysis A

Patients' characteristics according to the presence of nodal involvement at pathology after right hemicolectomy are described in Table 2. Among the entire study population, 69 patients (15.9%) underwent hemicolectomy. Of them, 21 patients (30.4%) had nodal involvement at pathology (N+ group), whereas 48 patients (69.6%) did not present any nodal involvement (N– group). Among patients with nodal involvement, 8 (38.1%) were males and 13 females (61.9%); among N– patients, male patients were 16 (33.3%) and female patients were 32 (66.7%) ($P = 0.702$). Median age was 29 years (IQR 24–42) in patients with N+ and 33 years (IQR 20–46) in patients with N– ($P = 0.194$). In patients with N+, median tumor size was 19.0 mm (IQR 15.5–23.5), whereas in patients with N– was 13.5 mm (8.0–17.0) ($P = 0.001$). Among N+ patients, tumor was located in the tip in 7 cases (33.3%), in the body in 3 cases (14.2%), and 1 in the base

TABLE 1. Baseline Characteristics of the Study Population

Characteristic	Patients (no. = 435)
Demographic	
Sex (male), no. (%)	142 (32.6%)
Sex (female), no. (%)	293 (67.4%)
Age, median (IQR), y	29 (21–41)
Surgical indications	
Appendicitis, no. (%)	344 (79.1%)
Other abdominal surgery, no. (%)	48 (11.0%)
Other neoplasia, no. (%)	10 (2.3%)
Debulking, no. (%)	2 (0.5%)
N/A, no. (%)	31 (7.1%)
Pathological features:	
Size, median (IQR), mm	7.0 (4.0–11.0)
Site	
Tip, no. (%)	281 (64.6%)
Body, no. (%)	46 (10.6%)
Base, no. (%)	16 (3.7%)
N/A, no. (%)	92 (21.1%)
Lymphovascular invasion, no. (%)	51 (11.7%)
Mesoappendiceal invasion, no. (%)	171 (39.3%)
Invasion <3 mm	88 (20.2%)
Invasion ≥3 mm	46 (10.6%)
N/A	37 (8.5%)
WHO 2010 classification	
NET G1, no. (%)	360 (82.8%)
NET G2, no. (%)	35 (8.0%)
WHO 2000 classification	
WDET, no. (%)	39 (9.1%)
WDEC, no. (%)	1 (0.1%)
Ki-67, median (IQR), %	1.0 (1.0–1.0)

N/A, not available; WDEC, well-differentiated endocrine carcinoma; WDET, well-differentiated endocrine tumor.

(4.8%); among N– patients, tumor was located in the tip in 23 cases (47.9%), in the body in 7 cases (14.6%), and 1 (2.1%) at the base in one case ($P = 0.674$). Lymphovascular invasion was present in 12 (57.1%) N+ patients and in 14 (29.2%) N– patients ($P = 0.021$). In 16 (76.2%) N+ patients and 28 (58.3%) N– patients, mesoappendiceal invasion was observed ($P = 0.409$). Among 21 N+ patients, 13 (61.9%) had a NET G1 and 8 (38.1%) a NET G2 according to WHO 2010 classification; among N– patients, 34 (70.8%) had a G1 and 11 (22.9%) a G2 ($P = 0.254$). Median Ki-67 was 2.0% (IQR 1.0–5.0) in N+ and 1.0% (IQR 1.0–2.0) in N– ($P = 0.030$).

TABLE 2. Patients' Characteristics According to Nodal Involvement at Right Hemicolectomy

Characteristic	N+ (no. 21)	N– (no. 48)	P^*
Sex			0.702
Male, no. (%)	8 (38.1%)	16 (33.3%)	
Female, no. (%)	13 (61.9%)	32 (66.7%)	
Age median (IQR), y	29 (24–42)	33 (20–46)	0.194
Size, median (IQR), mm	19.0 (15.5–23.5)	13.5 (8.0–17.0)	0.001
Site			0.674
Tip, no. (%)	7 (33.3%)	23 (47.9%)	
Body, no. (%)	3 (14.2%)	7 (14.6%)	
Base, no. (%)	1 (4.8%)	1 (2.1%)	
N/A, no. (%)	10 (47.6%)	17 (35.4%)	
Lymphovascular invasion, no. (%)	12 (57.1%)	14 (29.2%)	0.021
Mesoappendiceal invasion, no. (%)	16 (76.2%)	28 (58.3%)	0.409
WHO 2010 classification			0.254
NET G1, no. (%)	13 (61.9%)	34 (70.8%)	
NET G2, no. (%)	8 (38.1%)	11 (22.9%)	
Ki-67, median (IQR), %	2.0 (1.0–5.0)	1.0 (1.0–2.0)	0.030

N/A, not available.
*P value from the comparison between negative and positive LN involvement.

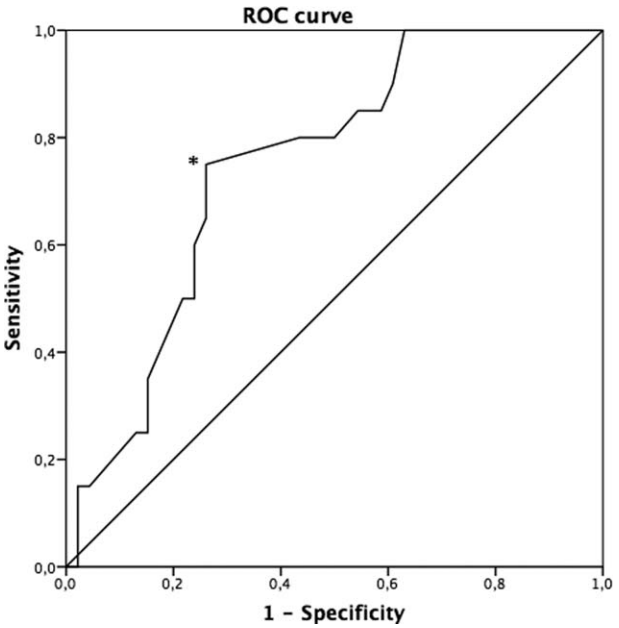

FIGURE 2. ROC curve for the best cutoff value of tumor size for the identification of nodal involvement at pathology. Area under ROC curve (AUROC): 0.747; 95% confidence interval: 0.627–0.867; standard error: 0.061; cutoff value: 15.5 mm.

The ROC curve of the tumor size in predicting patients who had N+ is shown in Figure 2. The size was quite accurate (area under the curve ± standard error, 0.747 ± 0.061; $P < 0.002$), and the best cutoff value for nodal involvement was 15.5 mm. Fifteen (71.4%) N+ patients had an appendiceal NET >15.5 mm, whereas only 12 (25.0%) N– patients presented a tumor >15.5 mm.

Factors related to nodal involvement are reported in Table 3. On univariate analysis, lymphovascular invasion (OR 4.11; $P = 0.025$) and size >15.5 mm (OR 8.50; $P = 0.001$) were related to nodal involvement; male sex, NET G2, and mesoappendiceal invasion were not related to nodal involvement at univariate analysis. On multivariate analysis, size >15.5 mm (OR 10.33; $P = 0.015$) was the only variable independently related to nodal involvement after hemicolectomy.

TABLE 3. Prognostic Factors Related to Nodal Involvement at Univariate and Multivariate Analysis in Patients Undergoing Right Hemicolectomy

	Univariate Analysis			Multivariate Analysis		
Characteristic	OR	95% CI	P	OR	95% CI	P
Male (sex)	1.23	0.42–3.57	0.703	—	—	—
NET G2	1.90	0.62–5.79	0.257	—	—	—
Lymphovascular invasion	4.11	1.20–14.13	0.025	—	—	—
Mesoappendiceal invasion	1.71	0.47–6.21	0.412	—	—	—
Size >15.5 mm	8.50	2.54–28.43	0.001	10.33	1.57–67.97	0.015

TABLE 4. Patients' Characteristics According to Analysis B

Characteristic	Group 1 (no. 21)	Group 2 (no. 135)	P^*
Sex			0.770
Male, no. (%)	8 (38.1%)	47 (34.8%)	
Female, no. (%)	13 (61.9%)	88 (65.2%)	
Age median (IQR), y	30 (21–36)	29 (24–42)	0.519
Size, median (IQR), mm	19.0 (15.5–23.5)	8.0 (3.0–14.0)	<0.001
Size >15.5 mm, no. (%)	15 (71.4%)	18 (13.3%)	<0.001
Site			0.160
Tip, no. (%)	7 (33.3%)	95 (70.4%)	
Body, no. (%)	3 (14.3%)	13 (9.6%)	
Base, no. (%)	1 (4.8%)	3 (2.2%)	
N/A, no. (%)	10 (47.6%)	24 (17.8%)	
Lymphovascular invasion, no. (%)	12 (57.1%)	21 (15.6%)	<0.001
Mesoappendiceal invasion, no. (%)	16 (76.2%)	65 (48.2%)	0.021
WHO 2010 classification			<0.001
NET G1, no. (%)	13 (61.9%)	108 (80.0%)	
NET G2, no. (%)	8 (38.1%)	11 (0.9%)	
Ki-67, median (IQR), %	1.0 (0.5–1.0)	2.0 (1.0–5.0)	<0.001

N/A, not available.
*P value from the comparison of the two groups.

Analysis B

Eighty-seven patients completed a 10-year follow-up and none of them presented disease recurrence. As shown in the study flow chart (Fig. 1), patients with nodal involvement at pathology examination after right hemicolectomy (no. 21) and patients with disease recurrence during follow-up (no. 0) were grouped together and compared to patients with N– (no. 48) grouped with patients with no evidence of disease recurrence after 10-year follow-up (no. 87). Patients' characteristics according to analysis B are described in Table 4. Among Group 1 patients, 8 (38.1%) were males and 13 females (61.9%) and among Group 2, male patients were 47 (34.8%) and female patients were 88 (65.2%); no difference was observed ($P = 0.770$). Median age was 30 years (IQR 21–36) in Group 1 patients and 29 years (IQR 24–42) in Group 2 patients ($P = 0.519$). In Group 1 patients, median tumor size was 19.0 mm (IQR 15.5–23.5), whereas in Group 2 patients, median tumor size was 8.0 mm (IQR 3.0–14.0) ($P < 0.001$). Fifteen N+ patients (71.4%) had an appendiceal NET >15.5 mm, whereas 18 Group 2 patients (13.3%) presented a tumor >15.5 mm ($P < 0.001$). Among Group 1 patients, tumor was located in the tip in 7 cases (33.3%), in the body in 3 cases (14.3%) and 1 at the base (4.8%); among Group 2 patients tumor was located in the tip in 95 cases (70.4%), in the body in 13 cases, (9.6%) and 3 (2.2%) at the base ($P = 0.160$). Lymphovascular invasion was present in 12 (57.1%) Group 1 patients and in 21 (15.6%) Group 2 patients ($P < 0.001$). In 16 Group 1 patients (76.2%) and 65 (48.2%) Group 2 patients, mesoappendiceal invasion was observed ($P = 0.021$). Among 21 Group 1 patients, 13 (61.9%) had a NET G1 and 8 (38.1%) a NET G2 according to WHO 2010 classification; among Group 2, 108 (80.0%) had a G1 and 11 (0.9%) a G2 ($P < 0.001$). Median Ki-67 was 1.0% (IQR 0.5–1.0) in Group 1 and 2.0% (IQR 1.0–5.0) in Group 2 ($P < 0.001$).

Factors related to nodal involvement at follow-up are reported in Table 5. On univariate analysis, G2 NET (OR 6.04; $P < 0.001$), lymphovascular invasion (OR 10.17; $P < 0.001$) size >15.5 mm (OR 18.50; $P < 0.001$), and mesoappendiceal invasion (OR 3.63; $P = 0.028$) were identified as predictive factors for nodal disease; on the contrary, male sex was not related. On multivariate analysis, G2 NET (OR 6.98; $P = 0.030$), lymphovascular invasion (OR 8.63; $P = 0.008$), and size >15.5 mm (OR 35.28; $P < 0.001$) were independently related to nodal disease within the follow-up period.

Survival Analysis

A total of 11 (4.0%) of 273 patients died during follow-up. Four patients of 11 died for NET (1.5%), and 7 patients died for other disease not related with NET. Mean OS was 275 months (95% CI: 263–287 months). Mean DSS was 286 months (95% CI: 279–293 months). Differences in DSS were observed when stratified according to: grading (mean DSS of NET G1 vs NET G2: 291 vs 64 months; $P < 0.001$), lymphovascular invasion (mean DSS in patients with vs without lymphovascular invasion: 291 vs 166 months; $P < 0.001$) and size >15.5 mm ($P < 0.001$).

Patients with nodal involvement after hemicolectomy had a mean DSS of 78 months (95% CI: 65–92 months), whereas patients without nodal involvement after hemicolectomy had a median DSS of 141 months (95% CI: 129–154 months; $P = 0.102$) (Fig. 3).

DISCUSSION

In this study, we evaluated retrospectively clinical, pathological, and immunohistochemical features related to nodal involvement in a large multicentric cohort of patients diagnosed with a NET of the appendix, to identify those who should be referred for hemicolectomy. We observed that tumor size, grading, and lymphovascular invasion are independent predictive factors for nodal involvement.

Appendiceal NETs are most frequently diagnosed incidentally after appendicectomy for suspected or manifest acute appendicitis. The management of these relatively indolent neoplasms is controversial. Size is considered the most relevant prognostic factor in the majority of studies, thus guiding the therapeutic approach. Current ENETS and North American Neuroendocrine Tumor Society (NANETS) guidelines recommend that appendiceal NETs >2 cm should be treated with right hemicolectomy because the risk of lymph node metastases increases with the size of the tumor.[3,4] However, as these tumors are rare, management recommendations have been inferred from the results of small, retrospective, single-institution studies and long-term prospective data are currently not available. The clinical management of tumors <2 cm is even more controversial. Many different factors other than size, such as the localization within the appendix, Ki 67 index or grading,

TABLE 5. Prognostic Factors Related to Nodal Involvement at Follow-up

	Univariate Analysis			Multivariate Analysis		
Characteristic	OR	95% CI	*P*	OR	95% CI	*P*
Male (sex)	1.15	0.45–2.98	0.770	—	—	—
NET G2	6.04	2.06–17.74	<0.001	6.98	1.21–40.45	0.030
Lymphovascular invasion	10.17	3.23–32.01	<0.001	8.63	1.77–42.53	0.008
Mesoappendiceal invasion	3.63	1.15–11.48	0.028	—	—	—
Size >15.5 mm	18.50	5.99–57.16	<0.001	35.28	6.13–203.12	<0.001

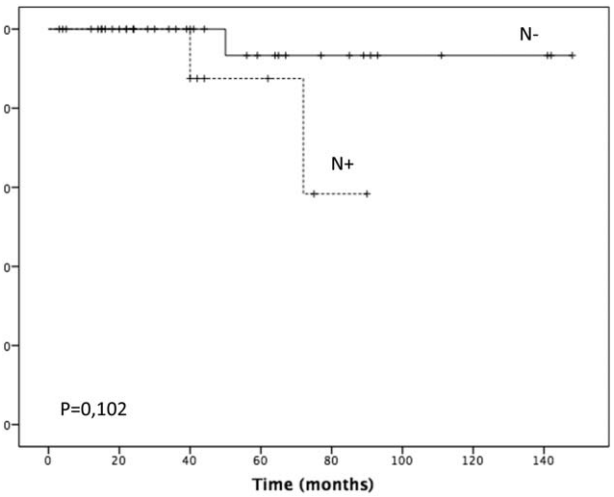

FIGURE 3. Kaplan-Meier estimates of DSS in patients with appendiceal NEN according to presence of nodal metastases.

lymphovascular or mesoappendiceal invasion, are usually taken into account in clinical practice as additional criteria for the choice of the therapeutic approach. However, even less evidence for these criteria is available.

Our results confirm the relevance of tumor size as a prognostic factor and as a pivotal parameter for the management of appendiceal NETs. However, differently from what reported in current ENETS guidelines, considering the size of 2 cm as cutoff value for clinical decisions, our analysis showed a different dimensional cutoff to identify the patients to refer for right hemicolectomy. Similarly to what reported by Anderson and Wilson,[7] a size >15 mm proved to be a better value to predict nodal involvement after hemicolectomy and in patients during 10-year follow-up. These data could allow a better prognostic stratification of patients at the moment of the diagnosis.

Guidelines and several studies[3,4,8–10] suggest the use of additional parameters to guide the decision-making for the management of NET sized 1–2 cm, such as WHO grading (G2), lymphovascular invasion, and mesoappendiceal infiltration >3 mm.

In our series, when evaluated in the group of patients undergoing follow-up, grading G2 and lymphovascular infiltration proved to be independently related to the presence of nodal involvement both at univariate and multivariate analysis. Although grading has been proved to be a prognostic factor performing well in GEP NETs, studies indicating its real significance in this setting are lacking. In a retrospective analysis of 138 cases, Volante et al[11] reported that the outcome of patients affected by appendiceal NETs was influenced by neither mitotic nor proliferative index. However, current guidelines suggest the use of WHO grading as an additional parameter to take into account in the management of appendiceal NETs <2 cm.

Lymphovascular infiltration (found in 11.7% of our series) has been considered a factor indicating aggressiveness since the study of Capella et al in 1995.[12] According to Rossi et al,[13] angioinvasion found at pathology in appendiceal NET specimen is frequently a result of artifacts during the preparation of samples. In our study only NET-expert pathologists were involved and they were also asked to revise unclear cases, to avoid this possible bias.

According to the most recently updated guidelines, mesoappendiceal infiltration >3 mm represents an indication to right hemicolectomy.[3,4] Tumoral invasion of the mesoappendix is observed in up to 20% of adults and up to 40% in children, but studies report how this parameter is often underreported.[14,15] In our series, this parameter was observed in 39.3% of patients, possibly because of the high expertise of pathologists involved. The depth of invasion beyond 3 mm has been suggested to reflect the aggressiveness of the disease. ENETS TNM classification uses this parameter to distinguish T2 from T3 tumors even in case of tumors <2 cm, meaning that even small tumors with deep mesoappendiceal invasion (ie, beyond 3 mm) carry a higher risk of metastases. A more aggressive surgical approach and an appropriate follow-up strategy is suggested by guidelines, although prospective long-term data are not available at the moment.[3] Data on the prognostic role of mesoappendiceal invasion are supported by scarce evidence,[11,16,17] whereas many authors did not find a prognostic relevance of this factor, similarly to what we have observed.[13,18,19] In particular, Rossi et al[13] reported how simple appendectomy can be considered the adequate treatment for small appendiceal NETs, even when mesoappendix or serosal invasion occurs. However, the previously cited analysis by Volante et al[11] showed a significant association of extramural extension (including the mesoappendix) with adverse clinical outcomes in these patients.

In our series, mesoappendiceal invasion resulted neither related to nodal involvement at hemicolectomy nor at long-term follow-up at multivariate analysis.

We did not find any correlation between localization of tumor within the appendix and nodal involvement. However, according to guidelines and several studies, location at the base of appendix is considered an additional parameter to take into account for referral of the patient for radicalization with right hemicolectomy.[3,4] Although there is no clear correlation with outcome, several studies report how incomplete resection resulting in recurrence and metastases may likely occur more frequently with an appendiceal NET located next to or at the base of the appendix.[19–21]

One of the limitations of our study concerns the study design. To achieve a methodologically correct study, all patients with a diagnosis of appendiceal NET should have undergone right hemicolectomy, to homogeneously evaluate nodal involvement and to start a follow-up program. This approach would not be considered ethical and it would not be applicable. We hypothesized that patients with nodal involvement who did not undergo hemicolectomy would present a disease recurrence within a 10-year follow-up. Accordingly, all patients who completed a 10-year follow-up and did not develop disease recurrence have been considered free of nodal involvement at the time of appendectomy. We also hypothesized that a 10-year follow-up was sufficient to observe a potential recurrence, to enlarge the cohort of patients on which evaluate the presence of nodal involvement with an ethically acceptable method, although appendiceal NETs have a very indolent nature and a slow growth. One of the main limitations of our study is therefore having used a somehow arbitrary 10-year follow-up cut-off, which could not be a long enough interval to detect a recurrence in a disease with this peculiar disease history.

Regarding survival analysis, we observed an important difference in DSS in patients with nodal involvement compared to those without nodal metastases (74 vs 141 months). These results, although not statistically significant, reveal for the first time the potential effect of nodal metastases on the prognosis of these patients.

In conclusion, even with the above-mentioned limitations, this is the largest multicentric study not based on registries on appendiceal NETs. According to the reported results, primary tumor size >15.5 mm, evidence of lympho-vascular infiltration and grading G2 according to WHO 2010 resulted independently related to nodal involvement in patients with appendiceal NETs. Patients presenting one or more of these features should be considered for radicalization with right hemicolectomy. In our series, mesoappendiceal invasion, localization of tumor within appendix or demographic data did not

prove to be valid prognostic factors, differently to what suggested by the guidelines.

To validate these data, a prospective study should be conducted. However, considering the small number of cases due to the relatively low incidence of this disease, a similar study would require a wide participation of many tertiary centers to increase the sample size, thus greatly raising costs and risk of biases.

Nevertheless, there is growing attention on the importance of registries standardizing and aggregating data from case series and case reports in the setting of rare diseases. In fact, in these populations, due to small sample sizes and logistic limitations, the conduction of more structured studies- such as randomized controlled trials- is unlikely. A meticulous standardized collection and critical analysis of the data collected by these registries, could instead represent a way to guide decision-making and improve the clinical management of these patients.[22]

REFERENCES

1. Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol*. 2017;3:1335–1342.
2. Mullen JT, Savarese DMF. Carcinoid tumors of the appendix: a population-based study. *J Surg Oncol*. 2011;104:41–44.
3. Pape UF, Niederle B, Costa F, all other Vienna Consensus Conference participants. ENETS Consensus Guidelines for Neuroendocrine Neoplasms of the Appendix. *Neuroendocrinology.* 2016;103:144–152.
4. Boudreaux JP, Klimstra DS, Hassan MM, et al. The NANETS consensus guidelines for the diagnosis and management of neuroendocrine tumors: well-differentiated neuroendocrine tumors of the Jejunum, Ileum, Appendix, and Cecum. *Pancreas*. 2010;39:753–766.
5. Bosman FT, Carneiro F, Hruban RH, et al., World Health Organization Classification of Tumours. *Pathology and Genetics. Tumors of the Digestive System.* Lyon: IARC Press; 2010.
6. Rindi G, Klöppel G, Couvelard A, et al. TNM staging of midgut and hindgut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch.* 2007;451:757–762.
7. Anderson JR, Wilson BG. Carcinoid tumours of the appendix. *Br J Surg*. 1985;72:545–546.
8. Groth SS, Virnig BA, Al-Refaie WB, et al. Appendiceal carcinoid tumors: predictors of lymph node metastasis and the impact of right hemicolectomy on survival. *J Surg Oncol*. 2011;103:39–45.
9. McGory ML, Maggard MA, Kang H, et al. Malignancies of the appendix: beyond case series reports. *Dis Colon Rectum*. 2005;48:2264–2271.
10. Landry CS, Woodall C, Scoggins. et al. Analysis of 900 appendiceal carcinoid tumors for a proposed predictive staging system. *Arch Surg*. 2008;143:664–670.
11. Volante M, Daniele L, Asioli S. Tumor staging but not grading is associated with adverse clinical outcome in neuroendocrine tumors of the appendix: a retrospective clinical pathologic analysis of 138 cases. *Am J Surg Pathol*. 2013;37:606–612.
12. Capella C, Heitz PU, Höfler H, et al. Revised classification of neuroendocrine tumours of the lung, pancreas and gut. *Virchows Arch*. 1995;425:547–560.
13. Rossi G, Valli R, Bertolini F, et al. Does mesoappendix infiltration predict a worse prognosis in incidental neuroendocrine tumors of the appendix? A clinicopathologic and immunohistochemical study of 15 cases. *Am J Clin Pathol*. 2003;120:706–711.
14. Prommegger R, Obrist P, Ensinger C, et al. Retrospective evaluation of carcinoid tumors of the appendix in children. *World J Surg*. 2002;26:1489–1492.
15. Parkes SE, Muir KR, Sheyyab M, et al. Carcinoid tumours of the appendix in children 1957-1986: Incidence, treatment and outcome. *Br J Surg*. 1993;80:502–504.
16. Safioleas MC, Mulakakis KG, Kontzoglou K, et al. Carcinoid tumors of the Appendix. Prognostic Factors and Evaluation of Indications for right Hemicolectomy. *Hepatogastroenterology.* 2005;52:123–127.
17. Syracuse DC, Perzin KH, Price JB, et al. Carcinoid tumors of the appendix. Mesoappendiceal extension and nodal metastases. *Ann Surg*. 1979;190:58–63.
18. Hemminki K, Li X. Incidence trends and risk factors of carcinoid tumors: a nationwide epidemiologic study from Sweden. *Cancer.* 2001;92:2204.
19. Moertel CG, Weiland LH, Nagorney DM, et al. Carcinoid tumor of the appendix: treatment and prognosis. *N Engl J Med.* 1987;317:1699–1701.
20. Pape UF, Perren A, Niederle B, et al. ENETS consensus guidelines for the management of patients with neuroendocrine neoplasms from the jejuno-ileum and the appendix including goblet cell carcinomas. *Neuroendocrinology.* 2012;95:135156.
21. Moertel CG, Weiland LH, Telander RL. Carcinoid tumor of the appendix in the first two decades of life. *J Pediatr Surg*. 1990;25:1073–1075.
22. Frieden TR. Evidence for health decision making—beyond randomized controlled trials. *N Eng J Med*. 2017;377:465–475.