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# Neuroendocrine tumours of the gastrointestinal tract, liver and pancreas: Current management and treatment strategies

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# Neuroendocrine tumours of the gastrointestinal tract, liver and pancreas: Current management and treatment strategies

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# Editorial: Neuroendocrine tumors of the gastrointestinal tract, liver, and pancreas: current management and treatment strategies

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KEYWORDS

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Editorial on the Research Topic
Neuroendocrine tumors of the gastrointestinal tract, liver, and pancreas: current management and treatment strategies

Neuroendocrine neoplasms (NETs) are rare and heterogeneous tumors that are phenotypically similar and originate from the diffuse neuroendocrine cell system. They demonstrate a rising prevalence and incidence which can be partly attributed to the more in-depth understanding of these neoplasms nowadays, but also to the advent and integration of more advanced diagnostic means (1). NETs exhibit slow growth and often an absence of specific symptoms, which is one reason for a belated diagnosis until it is at an advanced stage when overt symptoms could develop. Among sites of origin, gastroenteropancreatic NETs (GEP NETs) represent the commonest subtype, accounting for nearly 60% of all NETs. Among these, small bowel- (SBNEN) and pancreatic-NENs (pNEN) are the most frequent (2–5).

NETs exhibit a variable biologic behavior. They could either be classified as tumors with a “benign” pattern of characteristics without remarkable disease progression and with an excellent prognosis, but there are also tumors that are malignant, associated with an aggressive course, poor prognosis, and a very limited life expectancy.

Therefore the complexity and variability of NETs dictate a wider and better understanding of the current diagnostic and strategic approach and also the determination of the optimal treatment, including an accurate selection of surgical candidates. We are extremely proud and happy for the success of this special issue on NETs as there has been a great response from authors around the world covering, via their accepted publications, aspects of all hot topics. It has been a privilege for me personally to guest edit this special issue with the collaboration of a team of editors including Professor V. Papadopoulos, Professor A. Serrablo, Professor D. Giakoustidis, assistant Professor I. Moschos, and Professor

C. Toumpanakis who is a leading figure London-based Gastroenterologist specialist in managing NETs.

The diagnostic cascade should be initiated once there is a clinical suspicion of a NET. Koffas et al. have provided a very thorough review of diagnostic work-up and levels of advancement in the diagnosis of gastroenteropancreatic neuroendocrine neoplasms. The initial work-up involves the assessment of serum Chromogranin A and, in selected patients, the measurement of gut peptide hormones. The description of the measurement of multiple NEN-related transcripts or the detection of circulating tumor cells has enhanced our current diagnostic armamentarium and appears to perhaps even supersede historical serum markers such as Chromogranin A.

Interestingly, Li et al. present two novel nomogram models based on sex, age, and serum NSE levels to preoperatively predict the histologic grades in GEP-NETs to assist in clinical decision-making. Additionally, Li et al. report on risk factors and predictive score models for early recurrence following curative surgery for patients with poorly differentiated gastrointestinal neuroendocrine neoplasms. As they describe in their study, tumor location, preoperative ALP, and LNR are highlighted as independent factors associated with early recurrence, and the risk-scoring model developed based on these three factors appears to exhibit superior predictive efficiency.

Furthermore, Prisciandaro et al. offer a detailed overview of the current landscape of biomarkers in NETs with high-grade features with a specific focus on those harboring potentially therapeutic targets in the advanced setting.

An interesting parameter of Carcinoid heart disease (CHD) which is a consequence of neuroendocrine tumors releasing 5-hydroxytryptamine (5-HT) into the systemic circulation and affecting right heart valves, causing fibrosis and eventually right heart failure is presented by Shah et al. Its surgical correction with valve replacement surgery improved 5-HIAA levels and also improved liver function and hepatic IVC diameter.

Moving into the surgery section, Mou et al. having analyzed data from 536 patients report a potentially improved survival with primary tumor resection in pancreatic neuroendocrine patients with liver metastases. However, when primary tumor resection was combined with synchronous liver metastasis resection, it was not related to a better survival benefit.

On the other hand, Ye et al. focus on patients with non-functional pancreatic NETs smaller or equal to 2 cm as these patients exhibit different biological behaviors which correlate with different prognostic impacts of surgery. The authors' suggestion is that as long as distant metastasis does not occur and the grade is well–moderately differentiated, these patients will not benefit from surgery regardless of lymph node metastasis. However, when local invasion appears in this group of patients, they advise performing surgery. The same advice also goes for patients with a tumor of poorly differentiated or undifferentiated grade or those with distant metastases as surgery could be of benefit.

Interesting and rare cases are always in demand for discussion, and this is the case with the description of a composite Paraganglioma of the Celiac Trunk in a comprehensive literature review by Tzikos et al.. As we are moving with great speed into enhanced recovery after surgery (ERAS) protocols worldwide, Su et al. give us a detailed overview of what is happening in the world in this field with a bibliometric and visualized study on the global states and hotspots of ERAS research in the last two decades.

Lastly, Opalińska et al. highlight the value of peptide receptor radionuclide therapy as a neoadjuvant treatment in the management of primary inoperable neuroendocrine tumors.

## Author contributions

All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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# Development and Validation of Novel Nomograms Using Serum Tumor Markers for the Prediction of Preoperative Histologic Grades in Gastroenteropancreatic Neuroendocrine Tumors

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**Background:** To develop and validate nomogram models for the preoperatively prediction of the histologic grade of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) to provide appropriate treatments.

**Methods:** A total of 1014 participants, including 211 healthy controls, 293 patients with benign diseases, 299 patients with cancers, and 211 patients with GEP-NETs were included in the final analysis. Their sociodemographic and laboratory information, including serum tumor markers such as AFP, CEA, CA19-9, CA72-4, Cyfra21-1 and NSE were collected. Nomogram models were developed to preoperatively predict histologic grades of GEP-NETs.

**Results:** Among six serum tumor markers, only NSE was found to have a statistically significant association with the histologic grades in GEP-NETs (G1 *vs.* G2: $p < 0.05$; G2 *vs.* G3: $p < 0.001$; G1 *vs.* G3: $p < 0.0001$), which was combined with sex and age to develop the nomogram models. The first nomogram (to differentiate grade 1(G1) and grade 2/3 tumor (G2/G3)) showed a strong association to differentiate with an AUC of 0.747 (95% CI: 0.663-0.832) and 0.735 (95% CI: 0.624-0.847) in the training and validation datasets, respectively. The second nomogram (to differentiate G1/G2 and G3 tumors) showed a strong association to differentiate with an AUC of 0.827 (95% CI: 0.744-0.911) and 0.847 (95% CI: 0.744-0.950) in the training and validation datasets, respectively. The ROC, area under ROC curve (AUC), calibration curve and decision curve analysis (DCA) demonstrated the clinical usefulness of both models.

**Conclusions:** We proposed two novel nomogram models based on sex, age and serum NSE levels to preoperatively predict the histologic grades in GEP-NETs to assist the clinical decision-making.

**Keywords: GEP-NETs, Serum NSE, grade, nomograms, diagnosis**

# INTRODUCTION

Neuroendocrine tumors (NETs) are heterogeneous malignancies arising from the diffuse neuroendocrine system. They can appear in various anatomic locations, but the majority of NETs are restricted to derivatives of the embryological gut, including the gastrointestinal (GI) tract and bronchopulmonary tree. Gastroenteropancreatic neuroendocrine tumors (GEP-NETs), including GI neuroendocrine tumors (GI-NETs) and pancreatic neuroendocrine tumors (pNETs) originate from enterochromaffin cells of the gutislets of Langerhans, respectively (1–5). Although GEP-NETs are rare, they comprise the second most common tumor of the digestive system after colorectal cancer. In the past few decades, the incidence rate of GEP-NETs has been increasing globally, which could be due to increased awareness and improvement in their detection methods (6–8).

According to the 2010 World Health Organization (WHO) grading system, well-differentiated NETs are classified as grade 1 (G1) and grade 2 (G2) tumors, and poorly-differentiated NETs are classified as the grade 3 (G3) tumors, based on the mitotic count and Ki-67 proliferation index (9). Tumor grade is a crucial determinant to guide the GEP-NETs management, but is usually determined on the postoperative specimens, which influencing the physician's decision making in clinical practice (10). Recently, tissue acquision by EUS-guided fine needle aspiration (EUS-FNA) has helped evaluate the preoperative histologic grade, but with controversial accuracy (11, 12).

Various peptide hormones and biogenic amines secreted by GEP-NETs can enter systemic circulation, which could be used as biomarkers in the outpatient setting (13). At present, serum tumor markers are being extensively studied to provide future direction for the diagnosis, prediction and prognosis of the cancers (14). The neuron-specific enolase (NSE) is a cell-specific isozyme of the glycolytic enzyme enolase, which is highly specific for the neurons and peripheral nerve endothelial cells. Malignant neuron hyperplasia in the NETs may lead to an increase in the serum NSE level which can be used for the diagnosis, staging and treatment of these tumors, including GEP-NETs (15, 16). In this study, we have proposed two novel nomogram models to evaluate the role of NSE in the preoperative diagnosis and grade prediction of GEP-NETs.

# MATERIALS AND METHODS

## Study Participants

A total of 1014 participants were included from the First Affiliated Hospital of Nanjing Medical University, China, between January 1, 2012 and December 31, 2019, including 211 healthy controls, 293 patients with benign diseases, 299 patients with cancers, and 211 patients with GEP-NETs were included in the final analysis. And the benign diseases includes gastroenteropancreatic inflammation and polyps. The diagnosis of various diseases was determined by practicing clinicians based on clinical guidelines. Exclusion criteria included: (a) patients with missing data; (b) patients with no histopathology; (c) patients who had already received treatment; (d) the samples showed hemolysis. Ethics committee approval was granted by the First Affiliated Hospital of Nanjing Medical University (Nanjing, China) ethics review board according to the Declaration of Helsinki. (Ethical approval No. 2020-SR- 012). Due to the retrospective nature of the study, informed consent was waived.

## Study Design

We collected the demographic information of the study participants, including sex, age and test results of six serum tumor markers, including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9), cancer antigen 72-4 (CA72-4), cytokeratin 19 fragment 21-1 (Cyfra21-1) and NSE. We compared the distribution of all these serum tumor markers in the study participants, including the HCs, benign disease, cancer and GEP-NETs groups. Next, in the GEP-NETs group, we compared the distribution and differences of serum NSE in different histologic grades as follows: Low grade or Grade 1 [G1] tumors have a mitotic rate of 0 to 1 per 10 high power fields (HPF) and a Ki-67 index of 0% to 2%, the mitotic rate of tumors of intermediate grade (G2) is 2 to 20 per 10 HPF or 3 to 20% of Ki-67 index and the mitotic rate of tumors of high grade (G3) is greater than 20 per 10 HPF or Ki-67 index 20% (9). According to the basic principles of variable selection in clinical prediction modelling, we selected the candidate variables for the model by the univariate logistic regression analysis and clinical knowledge (17). Comprehensively considering the significant levels in the two models (G1 *vs* G2/, G1/2 *vs* G3), variables with significant difference ($p < 0.05$) and clinical significance were chosen. Then, we chose the full model as the final model. The cutoff value of variable was decided according to the maximum Youden index of the ROC curve, which was used to turn into a binary variable.

## Statistical Analysis

Categorical variables are represented by frequency and proportion and continuous variables are represented by mean (standard deviation) and median (minimum and maximum). Because the distribution of the serum tumor markers in this study is right skewed, we transformed them to Normal distribution by taking log10, which made the prediction models more readable. The t-test or Mann–Whitney U test were used to evaluate the differences in the distribution of six tumor markers between the disease groups and the healthy control group. And NSE was compared in different grades of GEP-NETs.

Nomograms are based on the ratio of each regression coefficient to 0 to 100 points in the logarithmic regression conversion. The effect of the variable with the highest β coefficient (absolute value) is assigned 100 points. Add these points to the independent variables to get the total points and convert them into predicted probabilities. The predictive performance of the nomogram was measured by the area under the ROC curve (AUC) and the calibration curve with 1000 bootstrap samples. In addition, we performed the decision curve analysis, which calculates a clinical "net benefit" for the nomograms in comparison to default strategies of treating all or no patients. X-axis is preference, whose unit is High Threshold Probability. The Cost: Benefit Ratio help us see the relationship

between preference and threshold probability easily. Y-axis shows the clinical decision net benefits after the benefits minus the disadvantages (18). R version 3.6.1 (http://www.rproject.org/) for all data analysis.

# RESULTS

## Characteristics of Participants

Data was collected on a total of 1014 individuals during the study period. There were 211 healthy controls, 293 patients with benign diseases, 299 cancer patients, and 211 patients with GEP-NETs in the cohort with male patient accounting for 50.24%, 56.31%, 63.55% and 50.24%, respectively. Other demographic variables and information about the levels of six serum tumor biomarkers for these groups are shown in **Table 1**. The mean ages of the healthy controls, benign diseases, cancer patient and GEP-NETs patients were 54.43, 55.72, 62.7, and 54.36 years, respectively. The serum NSE levels were the highest in the patients with GEP-NETs. **Figure S1** showed the violin plots of six tumor markers in four groups. Three disease groups were compared with the healthy control group, respectively. Among all the tumor markers, NSE was significantly different in GEP-NETs ($p < 0.0001$) and had a smallest overlap with other disease groups. In addition, six tumor markers for distinguishing GEP-NETs from healthy and other disease groups were shown in the **Figures S2** and **S3**, respectively. Among them, serum NSE showed the best diagnostic performance.

## Serum NSE in Different GEP-NETs Grades

In 211 patients with GEP-NETs, the distribution of serum NSE level for different grades of GEP-NETs was significantly different (G1 *vs.* G2: $p < 0.05$; G2 *vs.* G3: $p < 0.001$; G1 *vs.* G3: $p < 0.0001$) (**Figure 1A**). In addition, NSE levels differ significantly between G1 and G2/3, and between G1/2 and G3 ($p < 0.0001$) grades of GEP-NETs (**Figures 1B, C**).

## Nomogram to Differentiate G1 From G2/3 in GEP-NETs

Two hundred eleven patients with GEP-NETs were randomly divided according to the ratio of 7:3. There were 133 people in the training dataset, including 48 people in the G1 group and 85 people in the G2/3 group. Seventy eight individuals were included in the validation dataset, with 34 people in the G1 group and 44 people in the G2/3 group (**Table 2**). The results of the univariate logistic regression analysis showed that sex, age and NSE level was of clinical significance (**Table 1**). The nomogram to differentiate G1 from G2/3 in GEP-NETs was constructed based on the full model (**Figure 2A**). Age was used as a binary variable (<54.5 years and ≥54.5 years) according to the maximum Youden index of the ROC curve. NSE levels were transformed into the Normal distribution by taking log10. The AUC of the model reached 0.747 (95% CI: 0.663-0.832) and 0.735 (95% CI: 0.624-0.847) in the training and validation datasets, respectively (**Figures 2B, C**). The calibration curve showed a high accuracy of the nomogram for predicting tumor pathologic grades both in the training and validation datasets (**Figures 2D, E**). The DCA was used to demonstrate the clinical decision utility of the nomogram. The area under the decision curve in **Figures 2F, G** showed the clinical utility of corresponding strategies. The nomogram (red) showed more area than that the "treat all" (grey) or "treat none" (black) strategies, in both the training and validation datasets.

**TABLE 1** | Characteristics of participants in four groups.

| | | HC (n=211) | Benign (n=293) | Cancer (n=299) | GEP-NETs (n=211) |
|---|---|---|---|---|---|
| Sex | | | | | |
| | Male | 106(50.24%) | 165(56.31%) | 190(63.55%) | 106(50.24%) |
| | Female | 105(49.76%) | 128(43.69%) | 109(36.45%) | 105(49.76%) |
| Age, y | | | | | |
| | Mean (SD) | 54.43(12.53) | 55.72(15.58) | 62.7(10.92) | 54.36(12.64) |
| | Median [Min, Max] | 55.00[20,81] | 56.00[17,97] | 64.00[30,84] | 56.00[17,81] |
| AFP, ng/ml | | | | | |
| | Mean (SD) | 3.50(2.03) | 3.42(9.17) | 7.25(61.81) | 12.01(95.43) |
| | Median [Min, Max] | 3.06[1.09,15.49] | 2.43[0.6,154.90] | 2.70[0.64,1056.00] | 2.40[0.71,1210.00] |
| CEA, ng/ml | | | | | |
| | Mean (SD) | 2.32(1.49) | 2.26(2.69) | 11.01(30.47) | 8.53(69.20) |
| | Median [Min, Max] | 2.05[0.37,13.28] | 1.79[0.2,37.53] | 3.15[0.62,340.8] | 2[0.41,1000] |
| CA199, U/ml | | | | | |
| | Mean (SD) | 13.61(7.96) | 27.49(92.28) | 135.22(255.15) | 40.39(130.11) |
| | Median [Min, Max] | 12.43[0.60,36.41] | 11.12[0.60,1000.00] | 24.38[0.60,1000.00] | 10.86[0.60,1000.00] |
| CA724, U/ml | | | | | |
| | Mean (SD) | 3.14(3.54) | 3.39(17.70) | 6.59(21.33) | 4.52(20.97) |
| | Median [Min, Max] | 2.00[0.31,30.40] | 1.21[0.26,300.00] | 2.02[0.30,300.00] | 1.56[0.20,300.00] |
| Cyfra211, ng/ml | | | | | |
| | Mean (SD) | 2.29(1.01) | 1.7(0.95) | 2.88(2.05) | 2.77(5.38) |
| | Median [Min, Max] | 2.03[0.79 6.30] | 1.54[0.41,6.78] | 2.46[0.67,23.67] | 1.90[0.40,58.89] |
| NSE, ng/ml | | | | | |
| | Mean (SD) | 12.22(1.82) | 14.25(4.36) | 16.17(5.98) | 29.89(55.25) |
| | Median [Min, Max] | 12.15[7.56,16.83] | 13.76[4.64,31.14] | 14.71[7.26,44.11] | 16.14[8.57,467.50] |

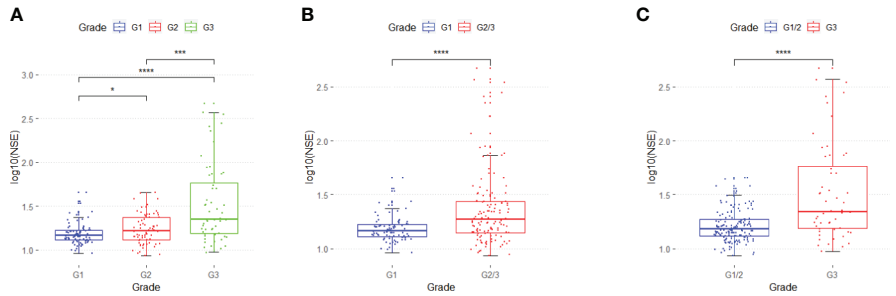


**FIGURE 1** | The distribution of serum NSE in different grades of GEP-NETs. **(A)** The serum NSE levels in G1, G2 and G3. **(B)** The serum NSE levels in G1 and G2/3. **(C)** The serum NSE levels in G1/2 and G3. (*p < 0.05; ***p < 0.01; ****p < 0.0001).

## Nomogram to Differentiate G1/2 From G3 in GEP-NETs

Two hundred eleven patients with GEP-NETs were still randomly divided according to the ratio of 7:3. There were 141 people in the training dataset, including 101 people in the G1/2 group and 40 people in the G3 group. Validation data included 70 people, including 54 and 16 people in G1/2 and G3 groups, respectively (**Table 3**).

Comprehensively considering the results in the two models (G1 *vs* G2/, G1/2 *vs* G3), age, NSE and sex were chosen (**Table S1**). Then, we chose the full model as the final model and the nomogram to differentiate G1/2 from G3 in GEP-NETs was constructed (**Figure 3A**). Age was used as a binary variable (<56.5 years and ≥56.5 years) and NSE levels were taken log10. The AUC reached 0.827 (95% CI: 0.744-0.911) and 0.847 (95% CI: 0.744-0.950) in the training and validation datasets, respectively (**Figures 3B, C**). The calibration curve showed a high accuracy of the nomogram for predicting tumor pathologic grades both datasets (**Figures 3D, E**). The DCA was used to demonstrate the clinical decision utility of the nomogram. The area under the decision curve in **Figures 3F, G** showed the clinical utility of corresponding strategies.

## DISCUSSION

In this study, we evaluated the role of serum NSE levels in the preoperative diagnosis and histologic grade prediction of GEP-NETs. Two novel nomogram models were established to predict the

**TABLE 2** | Characteristics of patients with GEP-NETs in the G1 group and G2/3 group.

| Characteristics | Training Dataset (n=133) | | | Validation Dataset (n=78) | | |
|---|---|---|---|---|---|---|
| | Grade1 (n=48) | Grade2/3 (n=85) | p value | Grade1 (n=34) | Grade2/3 (n=44) | p value |
| Sex | | | 0.348 | | | 0.198 |
| Female | 28 (58.33%) | 41(48.24%) | | 19 (55.88%) | 17 (38.64%) | |
| Male | 20 (41.67%) | 44(51.76%) | | 15 (44.12%) | 27 (61.36%) | |
| Age, y | | | 0.003 | | | 0.013 |
| Mean (SD) | 50. 27(11.43) | 55.93 (12.48) | | 51.59 (13.20) | 57.93 (12.54) | |
| Median [Min, Max] | 50.50 [24, 81] | 58.00 [21, 75] | | 52.00[25, 79] | 61.50 [17, 75] | |
| AFP, ng/ml | | | 0.046 | | | 0.484 |
| Mean (SD) | 2.55 (1.69) | 25.64 (149.83) | | 2.78 (1.44) | 3.12 (2.12) | |
| Median [Min, Max] | 2.20 [0.71,10.90] | 2.64 [0.88, 1210.00] | | 2.50 [1.10, 8.40] | 2.70 [0.85, 13.16] | |
| CEA, ng/ml | | | 0.195 | | | 0.122 |
| Mean (SD) | 2.07 (1.20) | 15.80 (108.29) | | 2.15 (1.43) | 7.88 (17.64) | |
| Median [Min, Max] | 1.85 [0.60, 6.41] | 2.00 [0.41, 1000.00] | | 1.74 [0.61,6.09] | 2.24 [0.70, 89.42] | |
| CA199,U/ml | | | 0.032 | | | 0.566 |
| Mean (SD) | 22.11 (62.17) | 30.91 (91.18) | | 17.54 (23.78) | 92.30 (240.04) | |
| Median [Min, Max] | 7.81 [0.60, 424.2] | 11.5[0.60,688.1] | | 11.66 [0.60, 135.7] | 11.42 [0.600, 1000.00] | |
| CA724,U/ml | | | 0.262 | | | 0.936 |
| Mean (SD) | 2.33 (2.72) | 3.49 (5.39) | | 2.56 (2.66) | 10.39 (45.07) | |
| Median [Min, Max] | 1.45 [0.20, 14.45] | 1.54 [0.45, 37.24] | | 1.67 [0.60, 11.86] | 1.68 [0.25, 300.00] | |
| Cyfra21.1, ng/ml | | | 0.049 | | | 0.386 |
| Mean (SD) | 2.38(2.60) | 2.41 (1.34) | | 1.90 (0.75) | 4.55 (11.22) | |
| Median [Min, Max] | 1.70 [0.75, 15.90] | 2.08 [0.51, 7.26] | | 1.66 [0.70, 3.90] | 1.77 [0.40, 58.89] | |
| NSE, ng/ml | | | <0.001 | | | 0.079 |
| Normal | 15.27 (5.21) | 32.80 (53.75) | | 16.98 (6.39) | 52.15 (91.22) | |
| Abnormal | 13.97[9.20, 35.92] | 19.00 [8.57, 467.5] | | 15.21 [11.4, 45.49] | 17.52 [9.20, 370.00] | |

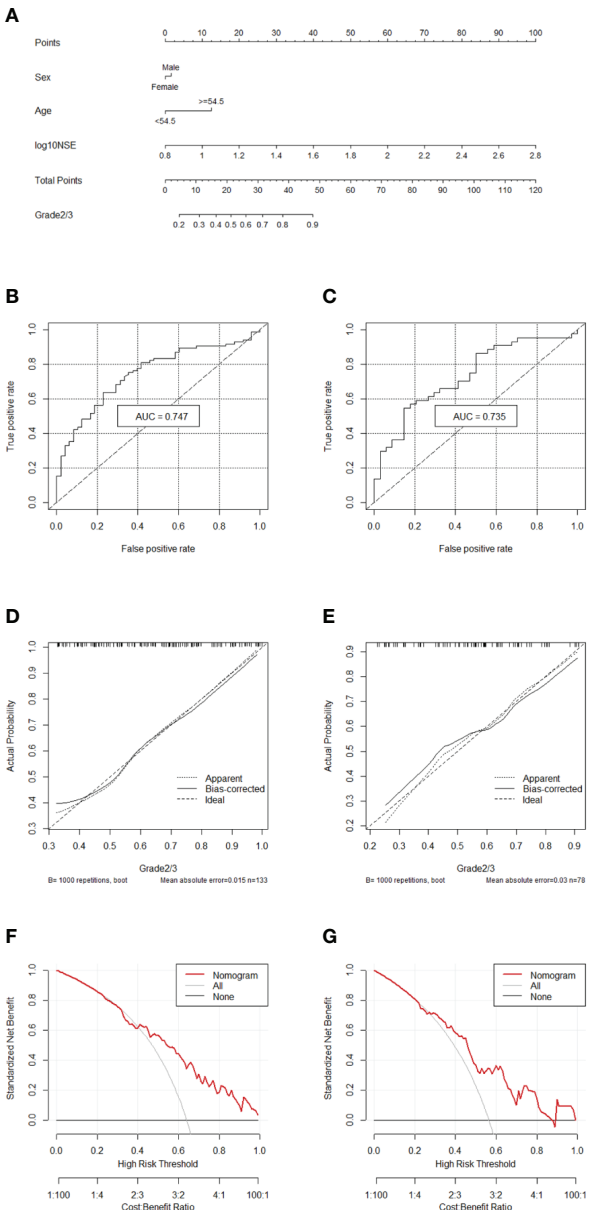


**FIGURE 2** | Nomogram for preoperatively predicting of G2/3 risk and its predictive performance. **(A)** Nomogram to estimate the risk of G2/3 preoperatively in patients with GEP-NETs. The area under the ROC curve [training datset: **(B)** validation dataset: **(C)**, the calibration curve (training datset: **(D)** validation dataset: **(E)**] and decision curve [trainingdata set: **(F)** validation dataset: **(G)**] of the nomogram.

preoperative histologic grades to differentiate G1 and G2/3, and grades G1/2 and G3. The first model differentiated between G1 and G2/3 with an AUC of 0.747 (95% CI: 0.663-0.832) and 0.735 (95% CI: 0.624-0.847) in the training and validation datasets, respectively. The second nomogram differentiated between G1/2 and G3 with AUC of 0.827 (95% CI: 0.744-0.911) and 0.847 (95% CI: 0.744-0.950), respectively. The calibration curve and DCA demonstrated the clinical usefulness of these models.

NSE is localized in the neuronal and neuroendocrine cell cytoplasms and can be used as a circulating marker in GEP-NETs (19). However, NSE alone is not sufficient for the diagnosis of NETs, as only 30% to 50% of NETs secrete NSE (20). In a study involving more than 200 patients with GEP-NETs, the sensitivity and specificity of NSE to distinguish NETs from non-endocrine tumors were only 39-43% and 65-73%, respectively (13). However, an article showed that NSE has specificity for NETs than other tumor markers. In their study, all tumors positive for an accepted neuroendocrine marker also expressed NSE (21). In our study, AUC for NSE to distinguish GEP-NETs from healthy individuals was 0.819 (**Figures S2**). Nevertheless, in

**TABLE 3 |** Characteristics of patients with GEP-NETs in the G1/2 group and G3 group.

| Characteristics | Training Dataset | | | Validation Dataset | | |
|---|---|---|---|---|---|---|
| | (n=141) | | | (n=70) | | |
| | Grade1/2 (n=48) | Grade3 (n=85) | p value | Grade1/2 (n=34) | Grade3 (n=44) | p value |
| Sex | | | 0.212 | | | 0.180 |
| Female | 58 (57.43%) | 15(37.50%) | | 26 (48.15%) | 6 (37.50%) | |
| Male | 43 (42.57%) | 25(62.50%) | | 28 (51.85%) | 10 (62.50%) | |
| Age, y | | | <0.001 | | | 0.002 |
| Mean (SD) | 53.34(13.03) | 62.08 (7.69) | | 48.89 (11.08) | 60.00 (14.56) | |
| Median [Min, Max] | 54.00 [21, 81] | 62.00 [46, 75] | | 50.00 [23, 74] | 65.00 [17, 75] | |
| AFP, ng/ml | | | 0.318 | | | 0.575 |
| Mean (SD) | 9.94 (68.37) | 32.97 (190.89) | | 2.84 (2.04) | 3.61 (2.31) | |
| Median [Min, Max] | 2.30 [0.71,689.7] | 2.32 [0.85, 1210] | | 2.40 [0.75, 14.16] | 2.95 [1.3, 9.8] | |
| CEA, ng/ml | | | 0.071 | | | 0.002 |
| Mean (SD) | 2.41 (1.77) | 34.93 (157.77) | | 1.87 (1.16) | 3.74 (3.33) | |
| Median [Min, Max] | 2 [0.41, 12.88] | 2.45 [0.41, 1000] | | 1.46 [0.56,6.12] | 2.41 [1.2, 12.06] | |
| CA199,U/ml | | | 0.312 | | | 0.144 |
| Mean (SD) | 25.91 (65.15) | 103.53 (255.2) | | 10.72 (8.38) | 74.09 (150.71) | |
| Median [Min, Max] | 11.83 [0.60, 492.30] | 10.97[0.60, 1000.] | | 7.83 [0.60, 39.65] | 18.69 [0.90, 470.30] | |
| CA724,U/ml | | | 0.788 | | | 0.505 |
| Mean (SD) | 2.51 (2.64) | 11.2 (47.12) | | 3.43 (5.41) | 4.15(9.09) | |
| Median [Min, Max] | 1.50 [0.20, 14.45] | 1.80 [0.45, 300.00] | | 1.71 [0.20, 37.19] | 1.08 [0.5, 37.24] | |
| Cyfra21.1, ng/ml | | | 0.077 | | | 0.055 |
| Mean (SD) | 2.28(1.88) | 4.08 (7.80) | | 1.86 (0.91) | 5.65(14.24) | |
| Median [Min, Max] | 1.88 [0.76, 15.9] | 2.27 [0.51, 50.99] | | 1.75 [0.40, 5.06] | 1.76 [0.72, 58.89] | |
| NSE, ng/ml | | | <0.001 | | | 0.003 |
| Normal | 17.67 (7.40) | 65.18 (108.36) | | 16.75 (6.63) | 63.99 (74.58) | |
| Abnormal | 15.63[8.57, 45.49] | 21.95 [9.40, 467.5] | | 14.96 [8.82, 38.00] | 29.89 [10.6, 255.90] | |

distinguishing GEP-NETs from other disease groups, the AUC was 0.657 (**Figures S3**). Despite these results, we believe that NSE still has a potential in the diagnosis of GEP-NETS among the six serological tumor markers.

In this study, serum NSE was found to be effective for GEP-NETs grade classification. GEP-NETs are heterogeneous in terms of origin, biological behavior with a malignant potential (5, 22). In the past few decades, various classification systems based on the embryological origin or morphological differences have been proposed for GEP-NETs (23, 24). The World Health Organisation (WHO) 2010 classified GEP-NETs in well-differentiated (G1 and G2) tumors, while poorly differentiated neuroendocrine carcinoma (NEC) are considered equivalent to G3 tumors (25). Different grades of GEP-NETs have different clinical severity and prognosis with different treatment approach (26, 27). In addition, different histologic grades have different prognosis based in the origin. In midgut GEP-NETs, the 5-year OS rates for G1, G2, and G3 tumors are 79%, 74%, and 40%, respectively (28). In pNETs, the 5-year OS of G1, G2, and G3 is 75%, 62%, and 7%, respectively (29). Therefore, it is crucial to predict the histologic grade of GEP-NETs preoperatively to help clinicians take decisive management actions effectively. A previous study proposed a combined nomogram model based on the radiomics signature and clinical-stage to distinguish G1 and G2/3 in pNETs for the treatment. In their study, parenchyma-sparing resections for G1 and a comprehensive treatment strategy including radical surgical resection with systematic chemotherapy was needed for patients with G2/3 to improve the survival (30). However, another study showed that the treatment strategies between G2 and G3 in pNETs should not be the same (31). These patients should receive surgical treatment in patients with limited metastatic disease, if technically feasible. Besides, targeted therapy with everolimus or sunitinib and somatostatin analogs (octreotide) is also used for advanced pNETs G1/2. Therefore, it is crucial to differentiate between G1/2 and G3 among pNETs than between G1 and G2/3 pNETs (31). Here, we developed two nomograms, one was used to distinguish G1 and G2/3 and the other was to distinguish G1/2 and G3.

A study showed that the average values of serum NSE for G1, G2, and G3 were 13, 17 and 21 μg/L in p-NETs, respectively (32). This was consistent with our results of NSE with significant differences in three grades of GEP-NETs. In our study, the nomogram differentiating G1/2 from G3 had a larger AUC than the nomogram differentiating G1 from G2/3. These results are consistent with the previous reports of advantages of NSE in diagnosing NETs with poor differentiation. In addition, elevation in NSE levels also reflected the overall survival of patients with GEP-NETs. Elevated serum NSE indicated the active disease, suggesting that the elevated NSE levels at the time of intial diagnosis is associated with poor prognosis (33). Therefore, the NSE can be used as a reliable diagnostic and prognostic markers in patients with GEP-NETs. We would also like to note some limitations of our study: (I) Relatively small sample size because it was a single-center study; (II) The information about the CgA was unavailable; (III) Inability to perform external validation of the data, and the conclusion in this study requires a larger multicenter validation analysis in future.

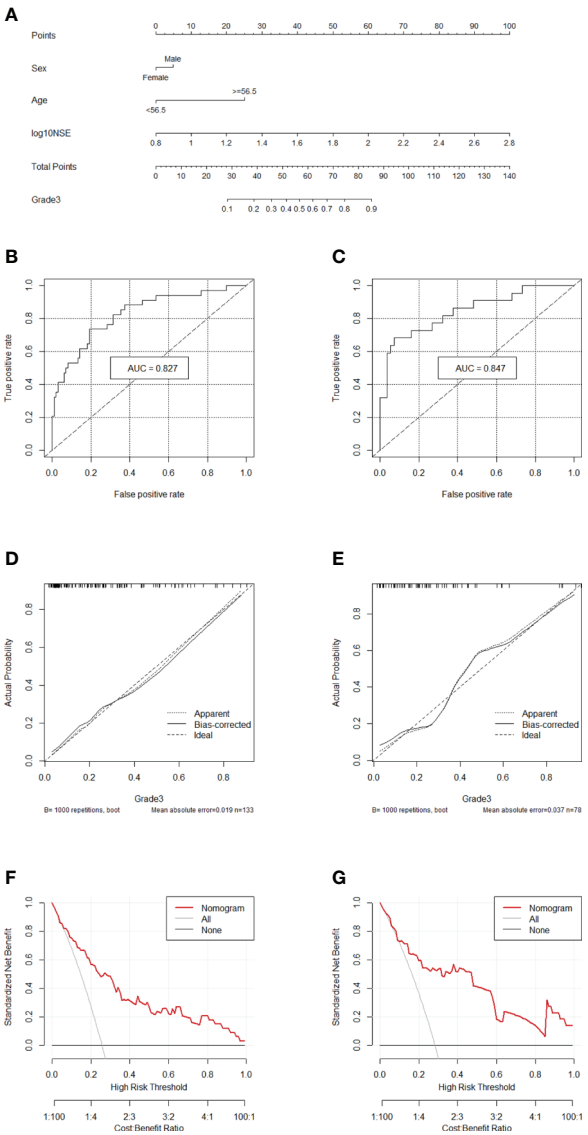


**FIGURE 3** | Nomogram for preoperatively predicting of G3 risk and its predictive performance. **(A)**, Nomogram to estimate the risk of G3 preoperatively in patients with GEP-NETs. The area under the ROC curve (training datset: **(B)**; validation dataset: **(C)**, the calibration curve (training datset: **(D)**; validation dataset: **(E)** and decision curve [trainingdata set: **(F)**; validation dataset: **(G)**] of the nomogram.

In summary, we reassessed the role of serum NSE in the diagnosis and prediction of preoperative histologic grades in GEP-NETs. We developed two novel nomogram models based on sex, age and serum NSE levels, which can be used as a non-invasive and accurate assessment tool for GEP-NETs patients during preoperative period to help clinicians tailor treatment plans accordingly.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Nanjing Medical University (Nanjing, China) ethics review board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.681149/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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# Risk Factors and Predictive Score Model for Early Recurrence After Curative Surgery in Patients With Poorly Differentiated Gastrointestinal Neuroendocrine Neoplasms

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**Purpose:** Studies on early recurrence in gastrointestinal neuroendocrine carcinoma (NEC) and mixed adenoneuroendocrine carcinoma (MANEC) are lacking and risk factors related to early recurrence are not clear. We evaluated risk factors for early recurrence in such patients and developed a predictive scoring model.

**Methods:** Patients undergoing curative surgery for GI-NEC or MANEC between January 2010 and January 2019 were included. Early recurrence was defined as recurrence within 12 months after surgery. Risk factors for early recurrence were identified using logistic regression.

**Results:** Of the 80 included patients, 27 developed early recurrence and 53 had no early recurrence. Independent risk factors associated with early recurrence included tumor location in the midgut/hindgut [odds ratio (OR) = 5.077, 95% confidence interval (CI) 1.058–24.352, $p = 0.042$], alkaline phosphatase (ALP) >80 (OR = 5.331, 95% CI 1.557–18.258, $p = 0.008$), and lymph node ratio (LNR) >0.25 (OR = 6.578, 95% CI 1.971–21.951, $p = 0.002$). Risk scores were assigned to tumor location (foregut, 0; midgut/hindgut, 1), ALP (≤80, 0; >80, 1), and LNR (≤0.25, 0; >0.25, 1). Patients with a high risk (score 2–3) for early recurrence had significantly shorter disease-free survival and overall survival than those with low- (score 0) and intermediate risks (score 1) (both $p < 0.001$). The novel scoring model had superior predictive efficiency for early recurrence over TNM staging (area under the curve 0.795 vs. 0.614, $p = 0.003$).

**Conclusion:** Tumor location, preoperative ALP, and LNR were independent factors associated with early recurrence after curative surgery for GI-NEC or MANEC. The risk scoring model developed based on these three factors shows superior predictive efficiency.

**Keywords: neuroendocrine neoplasms, gastrointestinal, early recurrence, predictive model, risk factors**

# INTRODUCTION

Neuroendocrine neoplasms (NENs), formerly known as "carcinoids," are highly heterogeneous neoplasms originating from sensory and secretory neuroendocrine cells (1). The incidence of NENs has increased over the past few decades, from 1.09 per 100,000 individuals in 1973 to 6.98 per 100,000 in 2012 (2). NENs can occur in various locations of the body, such as the lung, pancreas, gastrointestinal tract, and thymus, with the gastrointestinal tract being the most common affected site (3). The 2010 World Health Organization (WHO) classification of tumors of the digestive system categorizes NENs according to the degree of tumor cell differentiation: well or moderately differentiated neuroendocrine tumor (NET), poorly differentiated neuroendocrine neoplasms (PDNEN) including neuroendocrine carcinoma (NEC) and mixed adenoneuroendocrine carcinoma (MANEC) (4).

Surgical resection remains the mainstay of treatment for patients with gastrointestinal PDNEN (GI- PDNEN) (5). Adjuvant systemic chemotherapy is often also required for patients with a high degree of malignancy. Previous studies have reported that the 1-year progression-free survival of GI-PDNEN patients varies from 52 to 58%, indicating that a considerable number of patients will develop early recurrence within 12 months after surgery, despite treatment initiation with various adjuvant chemotherapy regimens (6–8). Early recurrence is closely related to poor prognosis; therefore, early screening of GI-PDNEN patients who are at high risk of early recurrence is essential for their improved prognosis. However, thus far, the risk factors associated with early recurrence of GI-PDNEN are not clearly analyzed.

Therefore, we focus on the early recurrence of patients with GI-PDNEN and aimed to evaluate the associated risk factors. Furthermore, we also aimed to establish a prediction model for early recurrence of such patients, which may help clinicians to screen GI-PDNEN patients according to the risk of early recurrence and guide clinical treatment.

# MATERIALS AND METHODS

## Patient Selection

This retrospective study has been approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (NO. 2021-0181). Two hundred and sixty-one patients with primary gastrointestinal NENs diagnosed at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology from January 2010 to January 2019 were included in this study. The exclusion criteria were as follows: (1) patients who did not receive complete resection of NENs ($n = 32$); (2) patients diagnosed with NET ($n = 119$); (3) patients with a history of other malignant neoplasms ($n = 3$); (4) patients who received preoperative radiotherapy and chemotherapy ($n = 4$); (5) patients lost to follow-up ($n = 9$); and (6) patients who died of other reasons other than GI-PDNEN within 12 months postoperation ($n = 7$; three died of cerebrovascular disease, two died of heart disease, one died of chronic obstructive pulmonary disease, and one died of a car accident); (7) patients with distant metastasis at first visit ($n = 6$). Informed consent was obtained from all patients included in this study.

## Pathological Diagnosis

GI-NEC and GI-MANEC were histopathologically defined according to the WHO 2010 classification (4). The sections contained typical morphological findings and neuroendocrine markers including chromogranin A and synaptophysin as observed on immunohistochemical staining. GI-NEC was poorly differentiated with mitotic count >20/10 high-power field (HPF) and/or Ki-67 index >20%. If the grade of Ki-67 index was not in agreement with the grade of the mitotic rate, the parameter with the highest grade was used for classification. GI-MANEC was referred to as a carcinoma with at least 30% of neuroendocrine or non-neuroendocrine neoplasms. The pathological TNM stage of GI-PDNEN was re-evaluated according to the 8th Edition of TNM Classification issued by the American Joint Committee on Cancer (AJCC) (9).

## Definitions and Data Collection

Recurrence was diagnosed according to radiologic findings or biopsies with suspicious lesions. Patients with early recurrence, defined as recurrence within 12 months after surgery, were included in the early recurrence group, while patients with recurrence after 12 months or no recurrence were included in the non-early recurrence group.

Preoperative complete blood counts and alkaline phosphatase (ALP) levels were measured within seven days before surgery. The platelet-to-lymphocyte ratio (PLR) was defined as the ratio of the number of platelets to the number of lymphocytes. The neutrophil-to-lymphocyte ratio (NLR) was calculated by diving the neutrophil count by the lymphocyte count. The lymph node ratio (LNR) was calculated by dividing the positive lymph node value by the total number of examined nodes. Other clinicopathological data of GI-PDNEN patients, including gender, age, tumor location, tumor size, Ki-67 index, TNM stage, and adjuvant therapy, were retrospectively collected. Location of the primary tumor was classified as foregut (esophagus, stomach, and proximal duodenum; excluding pancreas), midgut (distal duodenum, appendix, and proximal colon), and hindgut (colon and rectum).

## Patient Follow-Up

Postoperative follow-up was regularly conducted for every patient through outpatient visits, telephone calls, letters, or the Internet. The investigations items included clinical symptoms, detection of biochemical indexes, routine imaging (CT / MRI)

**Abbreviations:** NENs, Neuroendocrine neoplasms; GI-PDNEN, Gastrointestinal neuroendocrine carcinoma and mixed adenoneuroendocrine carcinoma; WHO, World Health Organization; NET, Neuroendocrine tumor; NEC, neuroendocrine carcinoma; MANEC, mixed adenoneuroendocrine carcinoma; HPF, High-power fields; AJCC, American Joint Committee on Cancer; ALP, Alkaline phosphatase; PLR, Platelet-to-lymphocyte ratio; NLR, Neutrophil-to-lymphocyte ratio; LNR, The lymph node ratio; DFS, Disease-free survival; OS, Overall survival; IQR, Interquartile region; ROC, Receiver operating curve; OR, Odds ratio; CI, Confidence interval; EP, Etoposide plus cisplatin; FOLFOX, fluorouracil /leucovorin/oxaliplatin combinations.

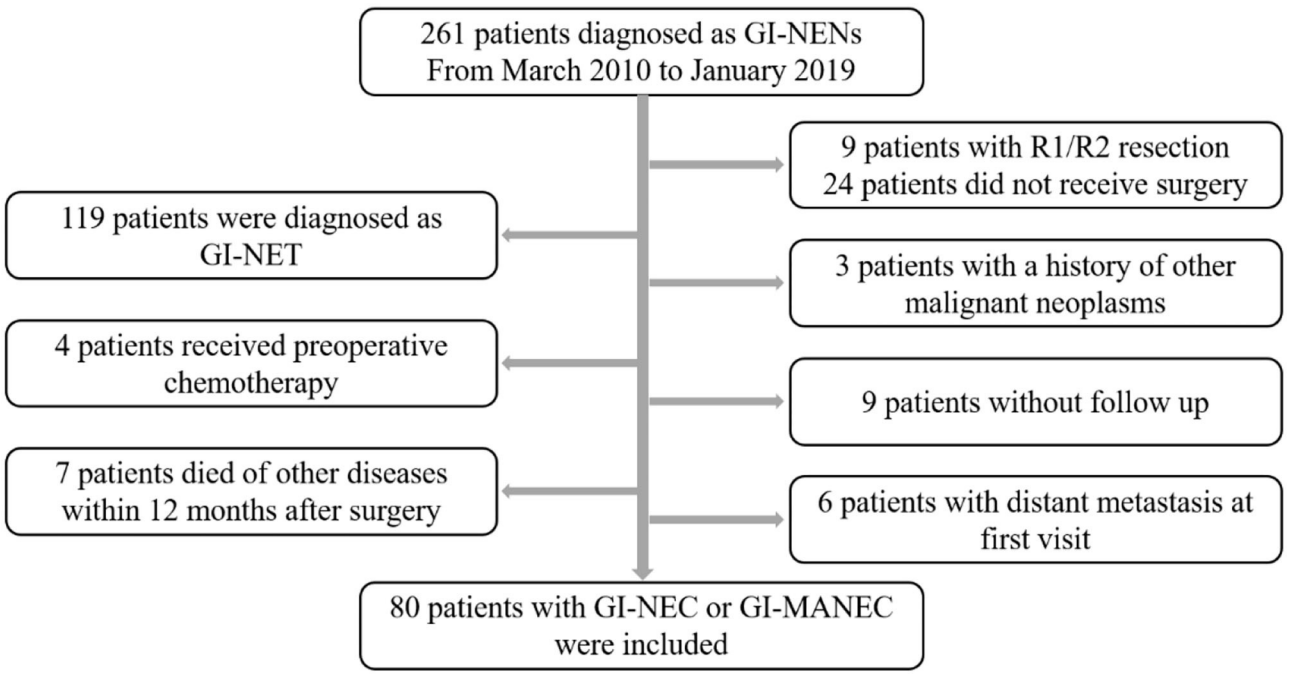


**FIGURE 1 |** Study scenario of the patients undergoing surgical resection for GI-PDNEN.

and endoscopy. Dates of follow-up and recurrence, and vital status data were collected. The last follow-up in the present study was carried out in January 2020. The primary endpoint of this study was early recurrence, disease-free survival (DFS), and overall survival (OS). DFS was defined as the time from surgery to relapse, death, or last follow-up. OS was defined as the time from surgery to death from any cause or last follow-up.

## Statistical Analysis

Categorical variables were expressed as frequency and percentage, while continuous variables were presented as mean ± standard deviation or median with interquartile region (IQR). Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Continuous variables were compared using Student's *t*-test or the Mann-Whitney *U*-test. The cutoff values for PLR, NLR, ALP, and LNR on early recurrence were determined using receiver operating curve (ROC) analysis. The Kaplan-Meier method was used to calculate the cumulative survival rate and generate survival curves, which were compared using the log-rank test. Differences in the clinicopathological characteristics of patients between the early recurrence and non-early recurrence groups were investigated. Risk factors for early recurrence after surgery were identified by univariate and multivariate logistic regression analyses using the forward stepwise (likelihood ratio) method. The impact of various clinicopathological factors on early recurrence was assessed through odds ratio (OR) and 95% confidence interval (CI). A two-tailed *p*-value of $<0.05$ was considered to indicate statistical significance. All statistical analyses were performed using SPSS version 25.0 for Windows (IBM, Armonk, New York, USA).

# RESULTS

## Demographic Characteristics

A total of 80 patients were included in this study (**Figure 1**), of which 55 patients were male (68.8%) and 25 were female (30.2%). The median patient age was 60 years (range 38–85). None of the patients had clinical symptoms related to hormones. Most of the tumors were located in the foregut ($n = 66$, 82.5%), followed by hindgut ($n = 9$, 11.2%), and 5 (6.2%) were located in the midgut. The mean tumor size was 4.69 ± 2.70 cm. Among the 80 patients, 52 (65.0%) were diagnosed with GI-NEC and 28 (35.0%) with GI-MANEC. According to AJCC staging, five cases (6.3%) were stage I, 10 (12.5%) were stage II, and 65 (81.3%) were stage III. Forty-one patients received adjuvant chemotherapy. Thirty-five patients received platinum-containing regimens including etoposide plus cisplatin (EP) or fluorouracil/leucovorin/oxaliplatin combinations (FOLFOX), while the remaining six patients received a platinum-free regimen. Because all patients in this study had non-functional tumors, none of the patients received treatment with somatostatin analogs after surgery. The demographic characteristics of the patients are shown in **Table 1** and **Supplementary Table 1**.

## Follow-Up Results and Survival Analysis

At the last follow-up, the median survival time of the whole cohort was 46 months (range 5–85). During the follow-up

**TABLE 1 |** Demographic characteristics of all the patients.

| | All patients | Early recurrence | |
|---|---|---|---|
| | | No (%) | Yes (%) |
| **Sex** | | | |
| Male | 55 (68.8%) | 35 (66.0%) | 20 (74.1%) |
| Female | 25 (31.2%) | 18 (34.0%) | 7 (25.9%) |
| **Age (years)** | | | |
| <60 | 43 (53.8%) | 27 (50.9%) | 16 (59.3%) |
| ≥60 | 37 (46.2%) | 26 (49.1%) | 11 (40.7%) |
| **Location** | | | |
| Foregut | 66 (82.5%) | 49 (92.5%) | 17 (63.0%) |
| Midgut | 5 (6.2%) | 3 (5.7%) | 2 (7.4%) |
| Hindgut | 9 (11.2%) | 1 (1.9%) | 8 (29.6%) |
| **Preoperative factors** | | | |
| **PLR** | | | |
| ≤174 | 59 (73.8%) | 43 (81.1%) | 16 (59.3%) |
| >174 | 21 (26.2%) | 10 (18.9%) | 11 (40.7%) |
| **NLR** | | | |
| ≤2.26 | 41 (51.2%) | 31 (58.5%) | 10 (37.0%) |
| >2.26 | 39 (48.8%) | 22 (41.5%) | 17 (63.0%) |
| **ALP** | | | |
| ≤80 | 55 (68.8%) | 42 (79.2%) | 13 (48.1%) |
| >80 | 25 (31.2%) | 11 (20.8%) | 14 (51.9%) |
| Tumor size (cm) | 4.7 ± 2.7 | 4.8 ± 2.7 | 4.5 ± 2.7 |
| **WHO 2010** | | | |
| NEC | 52 (65.0%) | 32 (60.4%) | 20 (74.1%) |
| MANEC | 28 (35.0%) | 21 (39.6%) | 7 (25.9%) |
| **pT stage** | | | |
| T1-T2 | 16 (20.0%) | 13 (24.5%) | 3 (11.1%) |
| T3-T4 | 64 (80.0%) | 40 (75.5%) | 24 (88.9%) |
| **Lymph node metastasis** | | | |
| No | 25 (31.2%) | 20 (37.7%) | 5 (18.5%) |
| Yes | 55 (68.8%) | 33 (62.3%) | 22 (81.5%) |
| **LNR** | | | |
| ≤0.25 | 54 (67.5%) | 44 (83.0%) | 10 (37.0%) |
| >0.25 | 26 (32.5%) | 9 (17.0%) | 17 (63.0%) |
| **TNM stage** | | | |
| I-II | 18 (22.5%) | 16 (30.2%) | 2 (7.4%) |
| III | 62 (77.5%) | 37 (69.8%) | 25 (92.6%) |
| **Ki-67** | | | |
| ≥80% | 46 (57.5%) | 30 (56.6%) | 16 (59.3%) |
| <80% | 34 (42.5%) | 23 (43.4%) | 11 (40.7%) |
| **Adjuvant chemotherapy** | | | |
| No | 39 (48.8%) | 26 (49.1%) | 13 (48.1%) |
| Yes | 41 (51.2%) | 27 (50.9%) | 14 (51.9%) |
| **Adjuvant chemotherapy regimens** | | | |
| No | 39 (48.8%) | 26 (49.1%) | 13 (48.1%) |
| Platinum-containing regimens | 35 (43.8%) | 26 (49.1%) | 9 (33.3%) |
| Other regimens | 6 (7.5%) | 1 (1.9%) | 5 (18.5%) |

period, 44 patients (55.0%) had disease recurrence, of which early recurrence occurred in 27 patients, while the other 17 patients developed late recurrence. Among the whole cohort, the most common site of recurrence was the liver ($n = 35$), followed by local lymph nodes ($n = 4$), and three patients had metastases of other sites including the lung, bone, and brain. During follow-up, 35 patients died, of which 30 died due to GI-PDNEN and the remaining five died due to other causes such as cardiovascular and cerebrovascular diseases or accidents.

The 1- and 3-year DFS rates of the whole cohort were 61.1 and 39.8%, respectively, and the one- and 3-year OS rates were 79.8 and 52.0%, respectively. The median OS of patients in the non-early recurrence group was not reached, while that of the patients in the early recurrence group was 12 months ($p < 0.001$). The OS of patients with no recurrence was superior to that of patients with early recurrence or recurrence after 12 months ($p < 0.001$) (**Supplementary Figure 1**).

**TABLE 2 |** Univariate analysis of the risk factors associated with early recurrence for GI-PDNEN.

| | OR (95% CI) | *P*-value |
|---|---|---|
| Sex (female) | 0.681 (0.243–1.909) | 0.465 |
| Age (>60 years) | 0.714 (0.280–1.824) | 0.481 |
| Location (midgut/hindgut) | 7.206 (1.995–26.023) | 0.003 |
| PLR (>174) | 2.956 (1.054–8.288) | 0.039 |
| NLR (>2.26) | 2.395 (0.923–6.214) | 0.072 |
| ALP (>80) | 4.112 (1.505–11.236) | 0.006 |
| WHO2010 (MANEC) | 0.533 (0.192–1.482) | 0.228 |
| Tumor size (>5) | 0.695 (0.257–1.880) | 0.473 |
| pT stage (T3-4) | 2.600 (0.672–10.065) | 0.166 |
| Lymph node metastasis | 2.667 (0.871–8.162) | 0.086 |
| LNR (>0.25) | 8.311 (2.879–23.996) | <0.001 |
| TNM stage (III) | 5.405 (1.141–25.597) | 0.033 |
| Ki-67 (≥80%) | 0.897 (0.250–2.297) | 0.82 |
| Adjuvant chemotherapy | 1.037 (0.410–2.621) | 0.939 |

**TABLE 3 |** Multivariate analysis of the risk factors associated with early recurrence for GI-PDNEN.

| | *β* | Wald | OR | *P*-value |
|---|---|---|---|---|
| Location (midgut/hindgut) | 1.625 | 4.124 | 5.077 (1.058–24.352) | 0.042 |
| ALP (>80) | 1.674 | 7.1 | 5.331 (1.557–18.258) | 0.008 |
| LNR (>0.25) | 1.884 | 9.388 | 6.578 (1.971–21.951) | 0.002 |

## Univariate and Multivariate Analyses of Postoperative Early Recurrence in Patients With GI-PDNEN

The median values of PLR, NLR, ALP, and LNR in the whole cohort were 139 (IQR, 120–177), 2.29 (IQR, 1.73–3.32), 69 (IQR, 60–88), and 0.13 (IQR, 0–0.32), respectively. According to ROC analysis, the optimal cutoff values of PLR, NLR, ALP, and LNR for early recurrence were 174, 2.26, 80, and 0.25, respectively. All factors were divided into two variables according to category, cutoff values, and mean or median values. Univariate analysis revealed that tumor location, PLR, preoperative ALP, LNR, and TNM stage are related to early recurrence of GI-PDNEN ($p <$

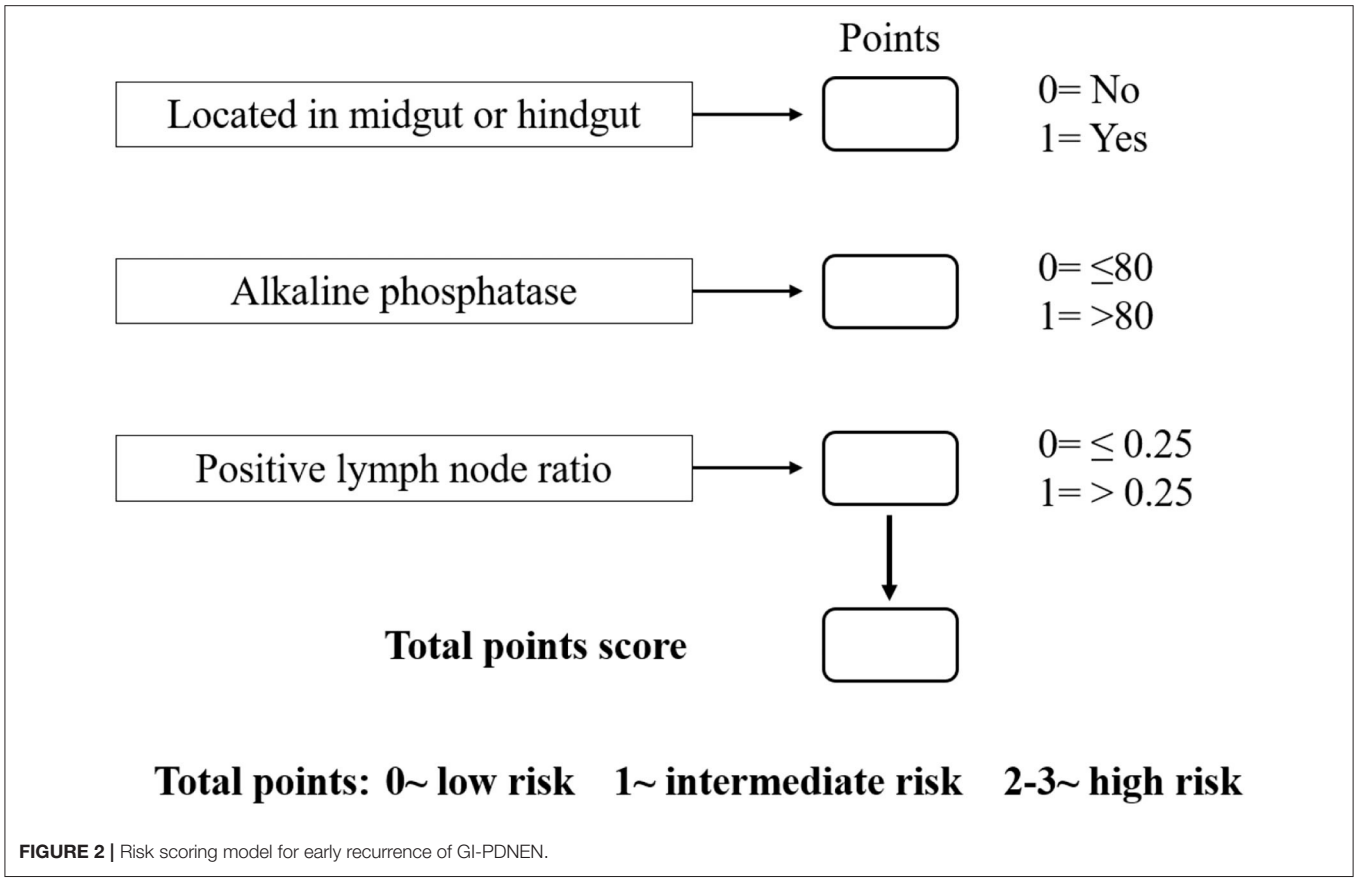


**FIGURE 2 |** Risk scoring model for early recurrence of GI-PDNEN.

0.05) (**Table 2**). Including these four factors in the multivariate analysis, using the forward stepwise (likelihood ratio) method, showed that tumor location, ALP, and LNR were independent factors influencing postoperative early recurrence in patients with GI-PDNEN (**Table 3**). Patients with tumors located in the midgut/hindgut ($\beta = 1.625$; OR = 5.077, 95% CI 1.058–24.352, $p = 0.042$), ALP >80 ($\beta = 1.674$; OR = 5.331, 95% CI 1.557–18.258, $p = 0.008$), and LNR >0.25 ($\beta = 1.884$; OR = 6.578, 95% CI 1.971–21.951, $p = 0.002$) were associated with a higher risk of postoperative early recurrence. In the multivariate analysis, the risk of early recurrence for patients with GI-PDNENs was

$$P = \frac{\exp(1.625 \times \text{location} + 1.674 \times \text{ALP} + 1.884 \times \text{LNR} - 3.892)}{1 + \exp(1.625 \times \text{location} + 1.674 \times \text{ALP} + 1.884 \times \text{LNR} - 3.892)}.$$

## Establishment and Validation of a Risk Predictive Scoring Model for Early Recurrence

Based on the $\beta$ values of the three aforementioned factors identified in multivariate analysis, a risk predictive scoring model for early recurrence was established (**Figure 2**). The ratio of the $\beta$ values of location, ALP, and LNR was 0.863, 0.889, and 1.000 respectively. For the convenience of clinical application, the risk scores were assigned to tumor location (foregut, 0; midgut/hindgut, 1), ALP (≤80, 0; >80, 1), and LNR (≤0.25, 0; >0.25, 1). According to the total points scored, patients with GI-PDNEN were stratified into three groups: 0 point as low risk of postoperative early recurrence, 1 point as intermediate risk, and ≥2 points as high risk. The recurrence rate within 12 months after surgery in the high-risk group was significantly higher than that in the intermediate- and low-risk groups ($p < 0.001$) (**Table 4**). To validate the prediction efficiency of this model, a comparison with the TNM stage on early recurrence was conducted. The corresponding ROC analysis showed that the AUC of the prediction scoring model was significantly higher than that of the TNM stage (0.795 vs. 0.614, $p = 0.003$). Additionally, the DFS and OS of patients with low risk were significantly superior to those of intermediate- or high-risk groups (**Figure 3**). The 1- and 3-year DFS and OS rates of patients according to risk groups are shown in **Table 4**.

## DISCUSSION

It has been well-documented that early recurrence after surgery leads to dismal prognosis (10–12). Because there is no consensus on the optimal threshold for differentiating early and late recurrence of GI-PDNEN, early recurrence was defined as recurrence within the 1st year after surgery, which is in line with that used in previous studies (10–12). With a relatively large sample size from one single-center institution in China, we demonstrated that the rate of early recurrence after curative

surgery in GI-PDNEN was 30.7% and the median overall survival was 12 months. We also investigated the risk of early recurrence after curative surgery and scored this risk according to preoperative and postoperative clinicopathological factors.

Knowledge about the factors influencing early recurrence of GI-PDNEN remains scarce. Preoperative inflammatory and biochemical markers such as PLR, NLR, and ALP have been reported to play an important role in the prognosis of patients with GI-PDNEN (13–15). In the present study, we analyzed the relationship between these factors and early recurrence and found that preoperative ALP is a more meaningful indicator to predict the early recurrence of GI-PDNEN than inflammatory makers. GI-PDNEN patients with preoperative ALP >80 are at an increased risk of early recurrence. Similarly, Lamarca et al. elaborated that preoperative ALP ≥83 is a risk factor for the overall survival of GI-NEC patients (14). ALP is an enzyme that can dephosphorylate multiple substrates. Serum ALP is mainly derived from the liver and bone tissue. Therefore, in patients with GI-PDNENs with elevated levels of serum ALP, attention should be paid to the risk of metastasis to liver or bone. In addition, our study also demonstrated that an increased LNR, rather than lymph node metastasis (LNM), is related to an increased risk of early recurrence, which means that the risk of early recurrence in some patients with lymph node metastasis may be overestimated. Therefore, the determination of the LNR may, to some extent, decrease the possibility of risk migration compared with lymph node metastasis. Nevertheless, because the value of the LNR is related to the total number of lymph nodes dissected, this finding should be carefully analyzed and further studies are required to verify our results. Additionally, tumor location was also an independent factor for the early recurrence of GI-PDNEN in this study.

Predictive models, which may provide a personalized assessment of the prognosis using patient-specific characteristics, have been increasingly incorporated into clinical practice in the field of GI-NEN (16–18). In this study, with the aforementioned three independent risk factors, we established a scoring model to predict the risk of postoperative early recurrence in GI-PDNEN patients. To the best of our knowledge, this is the first predictive scoring model for early recurrence of GI-PDNEN. According to the total score points, patients with GI-PDNEN were stratified into three groups for the risk of early recurrence: low risk, intermediate risk, and high risk. The risk of postoperative early recurrence in the high-risk group (score, 2–3) was significantly higher than that in the low-risk (score, 0) or intermediate-risk (score, 1) groups, which indicates that intensive follow-up and active adjuvant therapy may be required for these patients. Furthermore, we compared the DFS and OS of the three groups of patients, and the scoring model also showed a good stratification for the prognosis of GI-PDNEN patients. Additionally, the novel scoring model has a superior predictive efficiency for early recurrence over TNM staging according to

**TABLE 4 |** Recurrence patterns and 1-, 3- DFS/OS of different groups.

| | Risk of early recurrence | | | *P*-value |
|---|---|---|---|---|
| | **Low** | **Intermediate** | **High** | |
| Early recurrence | | | | <0.001 |
| Yes | 5 | 8 | 14 | |
| No | 30 | 22 | 1 | |
| Adjuvant chemotherapy | | | | <0.001 |
| Early recurrence | 2 | 3 | 9 | |
| No early recurrence | 12 | 14 | 1 | |
| Recurrence patterns | | | | |
| Liver | 5 | 6 | 11 | |
| Local recurrence | 0 | 1 | 1 | |
| Other locations | 0 | 1 | 2 | |
| DFS | | | | <0.001 |
| 1-year | 85.7% | 59.3% | 6.7% | |
| 3-year | 60.2% | 31.5% | 6.7% | |
| OS | | | | <0.001 |
| 1-year | 94.3% | 86.7% | 53.3% | |
| 3-year | 81.5% | 35.0% | 15.2% | |

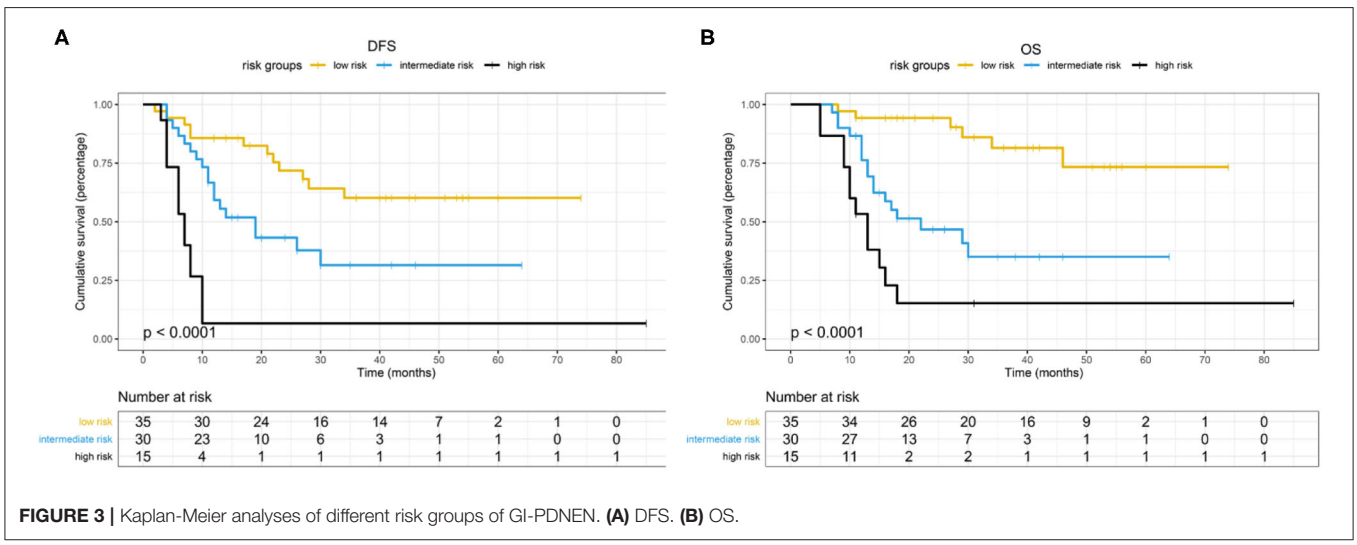


**FIGURE 3 |** Kaplan-Meier analyses of different risk groups of GI-PDNEN. **(A)** DFS. **(B)** OS.

ROC analysis. Because it is a convenient, cost-effective, and reliable model, the novel scoring model may assist in clinical treatment and postoperative follow-up. Furthermore, it may also influence clinical trial design with respect to patient stratification.

Currently, adjuvant chemotherapy of GI-PDNEN patients remains controversial. Platinum-containing chemotherapy regimens, especially the etoposide and cisplatin (EP) regimen, are the most commonly used (19). Given the heterogeneous nature of GI-PDNEN, the efficacy of adjuvant chemotherapy varies. A few studies with small sample sizes have reported that for esophageal or gastric PDNEN patients, improved survival was observed for patients receiving adjuvant chemotherapy after surgery as compared to surgery alone (6, 20). However, adjuvant chemotherapy did not benefit the three groups of patients in this study. The difference may be owing to the small number of patients receiving each chemotherapy regimen, which might result in bias. In addition, the patients in this study received different adjuvant treatments, which may also weaken the influence of adjuvant therapy on the prognosis. Therefore, the optimal adjuvant chemotherapy regimen for GI-PDNEN still requires further research.

There are some limitations in this study. First, this study was a single-center retrospective study; hence, there might be an intrinsic selection bias. Additionally, although the time span of this study was as long as 9 years, the sample size was relatively small, which may be due to the low incidence of GI-PDNEN and may have decreased the robustness of the study. The definition of early recurrence in GI-PDNEN patients is still not very clear and external validation was not performed in our study, further multicenter studies with large sample sizes are still required to confirm the findings. Finally, due to the high heterogeneity of GI-PDNEN, its prognosis mainly depends on its biological behavior. In-depth understanding of its molecular mechanism is of great significance for improving the prognosis of patients, and this is also the direction that needs to be studied in the future.

## CONCLUSION

In conclusion, tumor location, preoperative ALP, and LNR are independent factors influencing early recurrence of GI-PDNENs. The novel scoring model has superior predictive efficiency for early recurrence of GI-PDNENs and may assist in clinical treatment.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

This retrospective study was approved by the Ethics Committee of Union Hospital, Tongji Medical School, Huazhong University of Science and Technology (no. 2021-0181). Informed consent was obtained from all patients included in this study.

## AUTHOR CONTRIBUTIONS

KT contributed to the conception and administrative support of the study. CheL and PZ contributed to the study design. XS and WL contributed to the collection and assembly of the data. PZ and WL contributed to the quality control of data. XT, XC, and WY contributed to the data analysis and interpretation. CheL and ChoL prepared the manuscript. ZW and KT edited the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2021.703138/full#supplementary-material

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# Value of Peptide Receptor Radionuclide Therapy as Neoadjuvant Treatment in the Management of Primary Inoperable Neuroendocrine Tumors

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**Introduction:** Neuroendocrine neoplasms including neuroendocrine tumors (NETs) are often diagnosed as primary disseminated or inoperable. In those cases, systemic extensive therapy is necessary, but radical treatment is unlikely. As described in the literature, in some selected cases, peptide receptor radionuclide therapy (PRRT) may be used as a first-line/neoadjuvant therapy that allows further successful surgery. Such treatment may enable a reduction of total tumor burden or allow a radical treatment which improves the final outcomes.

**Aim:** This study aims to assess whether neoadjuvant PRRT could be a treatment option for patients with initially unresectable NETs.

**Methods:** Among the group of 114 patients treated with PRRT between the years 2005 and 2020, in 32 cases, it was the first-line therapy, mainly due to massive disease burden at the time of diagnosis. Among them, nine patients received PRRT as the first-line treatment due to the primary inoperable tumors with the intention of preoperative reduction of the tumor size in order to allow for a surgical treatment.

**Results:** Neoadjuvant PRRT enabled surgery in four out of nine (45%) patients. Finally, in two out of four cases, the goal (radical surgery) has been achieved.

**Conclusion:** PRRT may be considered not only as a palliative but also as a neoadjuvant therapy in advanced, somatostatin-positive NETs that were initially inoperable.

**Keywords: inoperable neuroendocrine tumors, PRRT, neoadjuvant therapy, NEN, NET (neuroendocrine tumors)**

## INTRODUCTION

Well-differentiated neuroendocrine tumors (NET G1, G2, and G3 according to WHO 2019 classification) are a widely heterogeneous group of malignancies regarding their place of origin, clinical presentation, hormone secretion, tumor growth, and metastases spread rate. A common feature of most NETs is overexpression of somatostatin receptors on their surface, which became the

molecular basis for a theranostic approach using somatostatin analogs in the diagnosis and therapy of NETs.

However, in the presence of a localized, non-metastatic disease, surgery is the most effective treatment procedure which can enable complete recovery. In the case of advanced NETs not eligible for surgical treatment, several different antitumor therapeutic options may be used, but a chance for radical treatment is very low (1, 2). Among them, long-acting somatostatin analogs are the first-line treatment in the vast majority of NETs. Nevertheless, in some clinical settings, initial therapy with peptide receptor radionuclide therapy (PRRT) may bring benefits before further treatment. Several clinical trials proved PRRT to be one of the most effective therapeutic options in terms of objective responses in disseminated NET treatment. It has been demonstrated to be effective not only in improving progression-free survival (PFS) but also overall survival (OS) in those patients (3–5). Moreover, in selected cases, it may enable the resection of primarily inoperable tumors (6–8). For that reason, the rationale for the use of PRRT as first-line treatment may be especially valuable in case of extensive disease burden at the time of diagnosis, hormonal syndromes resistant to somatostatin analogs, or a chance for subsequent curative surgery. However, the overall outcome in the abovementioned clinical situations remains completely different.

The purpose of this study was to evaluate if PRRT used as neoadjuvant therapy in patients with NETs may enable radical surgery.

## MATERIALS AND METHODS

Among a group of 114 patients treated in our center with PRRT between the years 2005 and 2020, 32 of them received PRRT as first-line therapy. Nine of them were qualified for PRRT with the intention of preoperative reduction of the tumor size, which could lead to potential subsequent radical surgery. The "unresectable primary tumor" was defined as extensive large vessel infiltration by neoplastic tissue or tumor invasion to adjacent organs, visualized on preoperative CT scans. All patients referred for preoperative PRRT were consulted by a multidisciplinary team including an oncological surgeon and a radiologist.

In this group, all patients had a histopathological diagnosis of well-differentiated NET according to the European Neuroendocrine Tumor Society–World Health Organization 2010 and 2017 grading system before PRRT, depending on the time of diagnosis. In eight patients, foregut tumors were present [in two in the lungs and in six in the pancreas (pNET)], and one patient was diagnosed with a midgut tumor (small intestine). In two patients, lesions were hormonally active (one insulinoma, one glucagonoma), and in another two, there was a suspicion of single liver metastasis detected in somatostatin receptor imaging (SRI) or computed tomography (CT) scans. Before and after PRRT, all of them were in generally good condition (Karnofsky index over 70%).

All patients qualified for PRRT had a positive result (Krenning scores 3 and 4) of SRI [(99mTc)Tc-octreotide SPECT/CT or (68Ga) Ga-DOTA-TATE PET/CT]. Cytoreductive chemotherapy or long-acting somatostatin analog was not used before PRRT in seven cases. Two patients received chemotherapy prior to PRRT with no response.

In all patients, 3 to 5 cycles of PRRT were applied. [90Y]Y-DOTA-TATE [mean cumulative dose 13.4 GBq ( ± 1.44)] and [90Y]Y/[177Lu]Lu-DOTA-TATE (cumulative dose 14.8 GBq) were applied in eight patients and one patient, respectively. To reduce the radiation dose to the kidneys, as recommended, an infusion of amino acids (arginine and 2.5% lysine) was administered.

The type of radiopharmaceutical used for PRRT depended on PRRT type availability in consecutive years. Routine blood count, liver function, and kidney function were assessed before each therapy cycle and at follow-up visits.

CT was performed 1–3 months prior to PRRT and 4–6 months after PRRT. Multidetector row spiral CT of 2 mm slice thickness and reconstruction increment were used after the administration of non-ionic contrast media. Further follow-up examinations were performed according to the applicable guidelines and the individual clinical course of the disease.

Diameter, volume, and the mean attenuation reduction of each lesion were calculated by CT image processing software.

Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, where the partial response to the therapy is described as ≥30% decrease of the sum of the longest diameters of target lesions, whereas progression is a ≥20% increase of it (**Table 1**). The Choi criteria define objective response as a ≥10% decrease in the sum of tumor diameters or ≥15% decrease in the tumor density on contrast-enhanced CT scan (**Table 1**).

Response categories were assessed on a subsequent CT scan until the disease progression.

### Statistics

Percentage changes in tumor diameter, volume, and density of tumor mass 1–3 months before and 4–6 months after PRRT were counted as well as the response to PRRT in RECIST 1.1 scale and Choi criteria. Additionally, the percentage of patients who underwent surgery (including complete surgical excision of the tumor) was assessed.

Finally, PFS and OS were calculated. PFS was defined as the time from the first PRRT to radiological or clinical disease progression or death from any cause.

## RESULTS

The group of nine patients (six males and three females) were eligible to the analysis. The mean age of the patients equaled 53.78 years ( ± 14.86, range: 28–78 years).

After the PRRT, the median tumor diameter changed by −1.6 cm (range from −3.7 to 0.3 cm). The median tumor volume decreased by 105.0 $cm^3$ (range from −186.2 to 34.7 $cm^3$), whereas attenuation decreased by 9.1 HU (range from −17.6 to 17.9 HU). There was no significant difference in the reduction of the tumor diameter, volume, and attenuation between pNET and other (not pNET) lesions (**Table 2**).

According to RECIST 1.1 criteria, stabilization of the disease (SD) and partial response (PR) were observed in six and one patient, respectively, and progressive disease (PD) was seen in two patients. In two patients, liver metastases described in the initial SRI were not

**TABLE 1** | Definition of radiological responses to therapy according to RECIST 1.1 and Choi criteria.

| | RECIST 1.0/1.1 | Choi |
|---|---|---|
| **Measurement** | **Largest diameter** | **Largest diameter + attenuation** |
| **Complete response (CR)** | Disappearance of all target lesions | Disappearance of all target lesions |
| **Partial response (PR)** | At least a 30% decrease in the sum of the greatest unidimensional diameters of target lesions | Decrease in tumor size ≥10% or decrease in tumor density ≥15% on CT |
| **Disease progression (PD)** | An increase of at least 20% in the sum of the diameters of target lesions | Increase in tumor size ≥10% and does not meet PR criteria by tumor density |
| **Disease stabilization (SD)** | Does not meet the criteria for CR, PR, or PD | |

**TABLE 2** | Changes in median (range) of diameter, volume, and attenuation of tumor before and after PRRT in pNET and not pNET patients.

| | Median difference of tumor diameter before and after PRRT, cm (range) | Median difference of tumor volume before and after PRRT, $cm^3$ (range) | Median difference of tumor attenuation before and after PRRT, HU (range) | Statistical significance |
|---|---|---|---|---|
| pNET | –0.4 (–3.70 to 0.30) | –7.8 (–186.20 to 34.72) | 1.0 (–17.60 to 17.90) | NS |
| Not pNET | 0.0 (–1.47 to 0.00) | –0.1 (–125.94 to –0.1) | –4.2 (–4.40 to 6.10) | NS |

*pNET, pancreatic NET; not pNET, not pancreatic NET; NS, not significant.*

found after PRRT on the follow-up SRI scans, but in one of them, a new SRI negative lesion was detected on CT examination.

According to the Choi criteria counted in eight patients, SD was observed in three, PR in three, and PD in two cases. The correspondence of those two scales was low and the evaluation of the PRRT results was comparable only in 50% of the cases (four patients) (**Table 3**).

The median time of follow-up was 56.9 months (range from 7.8 to 117.7 months). PRRT did not cause clinically important myelotoxicity or nephrotoxicity (CTCAE version 5.0 grades 3 and 4).

Among the whole group of patients, surgery was performed in four cases (45%), but a radical procedure was possible only in two of them (22%). The main cause of renouncement or ineffectiveness of surgery was an infiltration of the large vessels by neoplastic tissue, visualized on CT scans or found during the operation. No surgical complications which could be related to PRRT administration were observed. There was no perioperative mortality.

Two patients who underwent radical surgery are free from disease as of now, one of them for 27.13 months and another for 117.43 months. Both remain in the follow-up group. The assessment of radiological response to PRRT in patient no. 9 (treated radically) differed on the RECIST 1.1 and Choi scales, being SD and PR, respectively (**Table 3**).

In patient no. 8, based on medical documentation, the tumor mass significantly decreased after PRRT, which then enabled surgical intervention. Unfortunately, the CT scan done after PRRT completion was not available.

Among other two patients who underwent incomplete surgery, PFS equaled 8.2 and 72.9 months.

In the group of patients who did not qualify for surgery, the median PFS was 21.5 months (range from 5.6 to 70.1). The median OS for the whole group was 56.9 months (range from 7.6 to 117.7) (**Table 4**). No significant difference in survival time was observed in patients stratified according to primary localization of NET (pNET *vs.* non-pNET).

## DISCUSSION

Neoadjuvant therapy is an initial therapy which may be given to shrink the neoplastic tumor and enable further surgical intervention. It is widely used in different types of cancers including breast, pancreatic, and others, but not common in NETs due to usually large tumor burden at diagnosis.

According to current ENETS guidelines, various systemic therapies are available for locally advanced, metastatic, and progressive gastroenteropancreatic neuroendocrine tumors (GEP-NETs) (1). Long-acting somatostatin analog therapy is applied as a first-line treatment in the presence of somatostatin receptor (SSTR) expression at molecular imaging. The second- or third-line therapy regimens include chemotherapy with capecitabine and temozolomide (CAPTEM), PRRT, protein kinase inhibitors, streptozocin-based chemotherapy, or locoregional therapies, usually liver-directed (2, 3). PRRT is effective independently of the type of beta minus emitter (Y-90/Lu-177) or somatostatin analog (TATE/TOC) being used (9, 10). Moreover, PRRT efficacy is high although the schemes of therapy and the use of specific radionuclide differ between centers. The direct effectiveness of PRRT in comparison with other types of therapy regimens is planned to be evaluated on the basis of ongoing or future clinical trials including comparison of PRRT to everolimus in progressing G1 and G2 GEP-NETs (COMPETE, ClinicalTrials.gov Identifier: NCT03049189) or to everolimus, FOLFOX, and CAPTEM in aggressive G2 and G3 GEP-NETs (COMPOSE, ClinicalTrials.gov Identifier: NCT04919226).

Among other treatment possibilities, temozolomide has shown antitumor activity in pNETs either as monotherapy or in combination with capecitabine (CAPTEM) or bevacizumab. The objective response rates ranged from 33% (11) to 70% (12), with the highest response rates in studies using CAPTEM. However, the use of CAPTEM regimen in patients with localized pNEN stratified by grade and neoadjuvant or

**TABLE 3 |** Presentation and radiological and clinical outcomes of the patients.

| No. | Gender | Place of primary tumor | Metastases to the liver | Type of PRRT | Change of tumor diameter after PRRT | % change of tumor volume after PRRT | Response to PRRT in RECIST criteria | % change of tumor attenuation after PRRT | Response to PRRT in CHOI criteria | Surgery | R 0 | Time to progression after PRRT | Follow-up (months) | Status at last follow-up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | Lung | No | 90Y | 0% | –0.6% | SD | 10.7% | SD | N | N | 62.5 | 62.5 | Dead |
| 2 | M | Pancreas | No | 90Y | –48% | –80.7% | PD (new liver lesion) | 3.5% | PD (new liver lesion) | Y | N | 3.1 | 51.8 | Dead |
| 3 | F | Pancreas | No | 90Y | –1% | 12% | SD | –25% | PR | N | N | 59.5 | 117.8 | Dead |
| 4 | M | Pancreas | No | 90Y | 5% | 5% | SD | 30% | PD | N | N | 0.7 | 93.4 | Dead |
| 5 | M | Small intestine | No | 90Y | –21% | –60% | SD | –10% | PR | Y | N | 65.0 | 105.6 | Dead |
| 6 | M | Lung | No | 90Y | 0% | –3% | SD | –9.0% | SD | N | N | 2.3 | 7.6 | Dead |
| 7 | M | Pancreas | No | 90Y | –30% | –83% | PR | 19% | PD | N | N | 16.3 | 48.1 | Dead |
| 8 | F | Pancreas | Yes | 90Y | n/a | n/a | PD | n/a | n/a | Y (hemi-hepatectomy) | Y | 3.8 | 116.2 | Alive |
| 9 | M | Pancreas | Yes | 177Lu/90Y | –8% | –18% | SD | –11% | PR | Y | Y | 26.4 | 26.4 | Alive |

*PD, disease progression; SD, disease stabilization; PR, partial response; R0, surgical resection assessed as radical in histopathology report; Y, yes; N, no; n/a, not available.*

**TABLE 4 |** Long-term outcome of the patients who underwent PRRT as neoadjuvant therapy.

| Features | All patients (*n* = 9) |
|---|---|
| **Disease progression up to 6 months after PRRT** | 2 |
| **Time to progression, months; median (range)** | 21.5 (5.8–64.7) |
| **Overall survival, months; median (range)** | 56.9 (7.6–116.7) |
| **Surgeries after PRRT, *n*** | 4 |
| **Radical surgeries after PRRT, *n*** | 2 |

adjuvant therapy in comparison to somatostatin analog was associated with poorer OS (13), which raises doubts about the potential use of CAPTEM as first-line therapy even with the intention of using it as neoadjuvant therapy. In one of the studies, neoadjuvant CAPTEM regimen with or without radiation has been successfully applied in six pNETs with borderline resectable disease. All patients had radiological evidence of tumor regression after neoadjuvant treatment (two PR and four SD stabilization), and all of them could undergo successful resection of the primary tumor with negative margins in four out of six patients (14).

Throughout the 15 years of PRRT treatment in our center, we used both Y-90 and Lu-177 separately or as a tandem therapy combining Y-90 and Lu-177 with an activity ratio of 1:1. In all types of PRRT schemes, positive results were observed after the use of PRRT as first- or second-line therapy. In very few cases, PRRT was administered in an attempt to reduce the baseline tumor size with an intention of further radical surgical treatment. This approach offers hope for complete recovery which is not likely achievable with other forms of systemic treatment. Until now, there are only a few publications summarizing the use of PRRT as neoadjuvant therapy in NET patients, and a significant number of them relate to small groups of patients and case reports. The publication describing the largest group of patients who underwent neoadjuvant PRRT shows an encouraging rate of successful surgeries even in 31% of patients (9 out of 29 cases) (15). In our material, the rate of successful surgeries after PRRT was slightly lower (22%) in comparison with the abovementioned publication, but the rate of complete recoveries still appears inspiring enough to consider such treatment in selected cases. It is worth emphasizing that among our patients, there was one case who presented single liver metastasis on CT/SRI scans prior to PRRT with no evidence of hepatic lesions on both CT and SRI scans after PRRT treatment. Similar results, including the cure of liver metastases by PRRT, were described in the past by a few authors (16–19). Those observations also encourage considering PRRT as neoadjuvant therapy even in the presence of a single liver metastases, especially if they are poorly available for locoregional treatment.

The second potential advantage of the use of PRRT at the beginning of treatment is a significant decrease of total tumor burden. This fact was clearly demonstrated mainly for 177Lu-DOTA-TATE therapy in one randomized trial (NETTER-1 trial) (3) and several non-randomized trials (20). According to a meta-analysis done on patients with disseminated pancreatic NETs, the pooled median PFS after PRRT was 25.7 months (95% CI: 18.9–32.4 months) and was better than in patients treated with everolimus [PFS 14.7 months (95% CI: 11.2–18.1 months)] (21), which is recommended as second-line treatment in disseminated pNETs. In our group, median PFS (in a corresponding group of patients who did not undergo surgery) was 21.5 months (range 5.6–70.1). The results obtained in our group are significantly better, which probably results from the selection of patients with a chance of radical surgery, i.e., with a relatively small disease burden, without multiple metastases. The PFS increase additionally encourages the use of PRRT at the beginning of treatment, especially if there is initially high tumor burden and when prolongation of PFS (less probable to achieve with the use of other systemic therapy) may be considered as an added benefit.

The results of PRRT assessed as disease regression, stabilization, or progression depend on the radiological method used for the evaluation of response to therapy. The most common methods used for that purpose are RECIST, Southwest Oncology Group (SWOG), or Choi criteria. RECIST scale (1.0 and more common nowadays 1.1) is already a radiological gold standard for the assessment of tumor response to cytoreductive treatment for different malignancies. However, this scale is hardly efficient in the validation of neoplasms with relatively slow growth (22). The main weakness of this scale is that it measures only the longest diameters of all selected target lesions, while the linear diameter does not vary sufficiently to correct estimation of total lesion

volume. For this reason, scales such as the Choi criteria, which try to also take into account changes in the radiological density of lesions (reduction of attenuation, weaker contrast enhancement as the effect of neoplastic tissue necrosis), were created. Those scales are considered to be more useful in tumors with relatively slower growth. In our material, the response of seven out of nine patients (assessed as a PR or SD) to the treatment fits into one of those scales: seven out of nine in the RECIST scale and five out of eight into the Choi criteria. However, the same type of response in both scales was seen in four cases showing relatively poor compatibility (50%) of both of those rating systems. Another side of the imperfection of those scales is seen in the example of a patient with a pancreatic NET producing insulin. After [90Y]Y/[177Lu] Lu-DOTA-TATE treatment, the tumor diameter decreased by 8%, its volume by 18% and attenuation decreased by 11%. It allowed us to assess the response to therapy as SD and PR according to RECIST and CHOI criteria respectively (**Figures 1**, **2**). However, the reduction of tumor size and the decrease of tumor vascular involvement enabled curative surgery, confirming that neither of those radiological tools is highly effective in the preliminary assessment of PRRT efficacy nor does it predict a clinical outcome (patient was radically operated) (**Figures 1**, **2**). It implies that it is very difficult to indicate, before qualification for PRRT, whether or not the patient will respond to the therapy and what the maximal tumor size change will be and whether PRRT may be considered as a neoadjuvant therapy. Moreover, in our work, the reduction of tumor volume was significant in many cases, but in two cases, we observed disease progression which means that PRRT did not always bring about the expected outcome. Finally, we also counted the percentage of tumor size shrinkage after PPRT, and we found that the response to therapy in both groups (pNETs *vs.* non-pNETs) was similar. Unfortunately, the cardinality of the group studied in our work was too small to draw unequivocal conclusions as to whether the use of different PRRT types and schemes brings about the same results. Among many radiological and clinical features, only negative results of [18F]FDG PET/CT examination, histopathological grading (23), and to some extent the good SSTR expression on SRI (24) are widely known indicators of prognosis in NET patients. Nevertheless, none of those parameters are confirmed as factors influencing PRRT to be a neoadjuvant therapy.

It should also be considered that the use of different radionuclides (Y-90 and Lu-177 or mixed Y-90/Lu-177 having different radiation lengths and energies) may have an impact on the final outcome of the treatment. However, there are currently no studies comparing the different types of radionuclides used for PRRT.

Although we have not found an association between clinical and radiological features which could be helpful in proper patient selection for neoadjuvant PRRT, it is worth noting the possibility of the multigenomic blood mRNA biomarker (NETest) and PRRT predictive quotient (PPQ) use. PPQ had been evaluated as a predictor of PRRT response in 97%. NETest accurately monitors PRRT response and is an effective surrogate marker of PRRT radiological response (25). Perhaps, it will be possible to use those parameters, facilitating the selection of patients who have a greater chance for radical surgery after neoadjuvant therapy.

Despite the lack of serious adverse events in our cohort, PRRT may be associated with the risk of short- and long-term side effects. Most side effects are connected directly with myelosuppression reversible and rather dose-limiting, but the problem of long-term complications remains crucial due to the expected long-time survival in radically treated patients. The most important long-term complications include myelodysplastic syndrome, acute myeloid leukemia, or bone marrow aplasia with the median latency period at diagnosis about 41 months (26). The prevalence of those severe, delayed adverse hematological events is estimated at 1.4%–4% (27, 28). In case of PRRT radiopharmaceuticals labeled with 90Y, kidney-

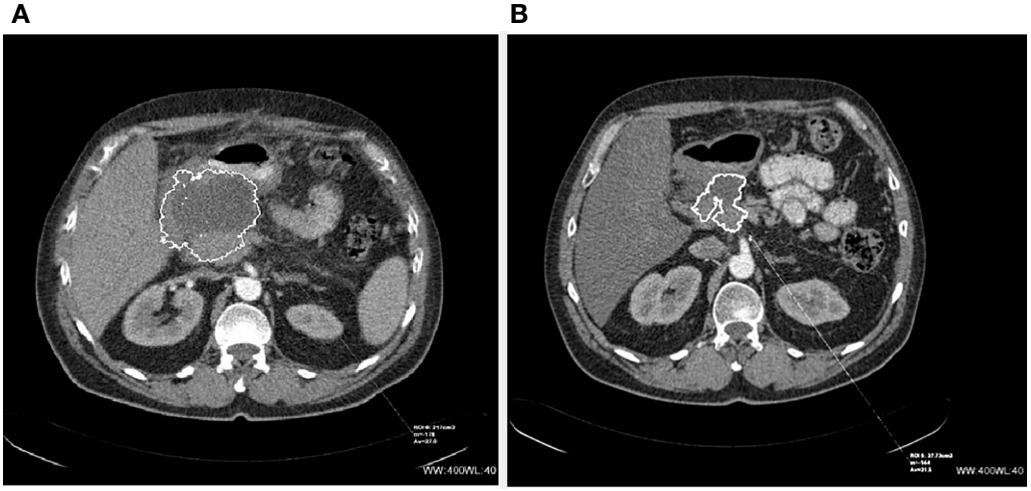


**FIGURE 1** | CT scans of inoperable (before and after PRRT) NET of the pancreas: **(A)** before PRRT and **(B)** 4 months after 4 cycles of 90Y-DOTA-TATE.

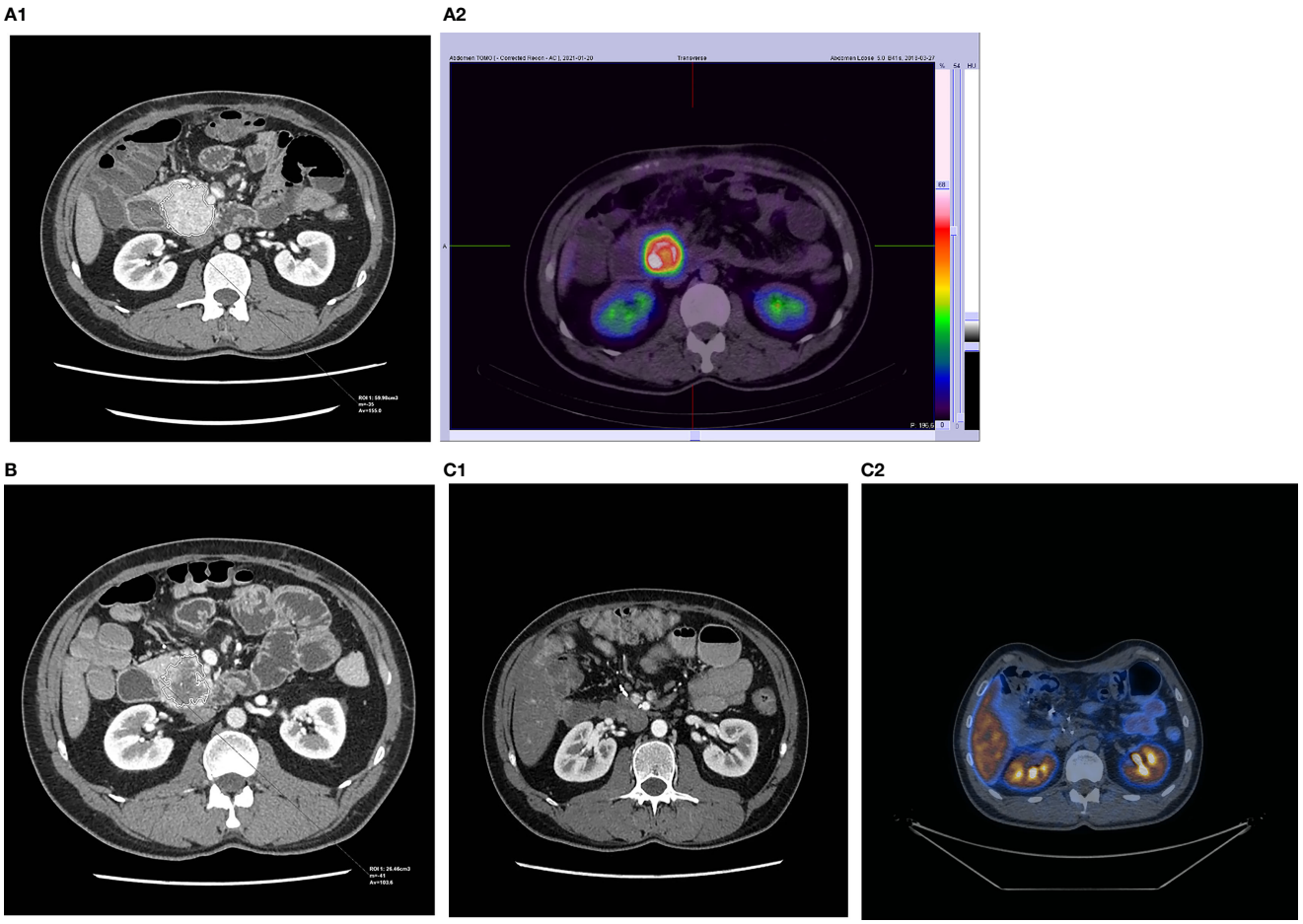


**FIGURE 2** | CT and SRI scans of a patient with successfully operated NET of the pancreas: **(A1, 2)** before PRRT, **(B)** 3 months after 4 cycles of 177Lu/90Y-DOTA-TATE (only CT), and **(C1, 2)** after complete tumor removal.

related toxicity should also be considered (29). Some of those toxicities may be limited by proper dosimetry.

## CONCLUSIONS

- In some cases of SSTR-positive NETs, PRRT used as a first-line treatment may cause significant tumor size reduction, which enables radical surgical intervention. In other cases (in majority of the patients), the benefits include reduction of total tumor burden and long-term stabilization of the disease according to RECIST criteria.
- To date, there are no clinical or radiological features (except high tumor burden) that give a fully unambiguous answer to the question of whether PRRT may allow for radical surgical treatment.
- All PRRT regimens can be considered as a useful therapy for somatostatin receptor-positive NETs, including the application of PRRT as a neoadjuvant therapy in primary inoperable tumors. Currently, there are no data indicating which PRRT regimen (177Lu, 90Y/177Lu, 90Y; TATE/TOC) and schemes could be most effective.
- PRRT was clinically well tolerated and did not interfere with the subsequent surgical or oncological treatment.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The study protocol was approved by the Local Ethics Committee of the Jagiellonian University in Krakow (reference number: 1072.6120.32.2021). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MO: data collection, imaging review, data analysis, manuscript drafting, manuscript editing and approval, and study coordination. AS-S: data collection, imaging review, data analysis, manuscript drafting, and manuscript editing and approval. AG: data collection, imaging review, manuscript drafting, and manuscript editing and approval. HO: data collection and manuscript editing and approval. AH-D: data collection, imaging review, data analysis, manuscript drafting, and manuscript editing and approval. All authors contributed to the article and approved the submitted version.

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REVIEW
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# Biomarker Landscape in Neuroendocrine Tumors With High-Grade Features: Current Knowledge and Future Perspective

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Neuroendocrine tumors (NETs) are classified based on morphology and are graded based on their proliferation rate as either well-differentiated low-grade (G1) to intermediate (G2–G3) or poorly differentiated high-grade neuroendocrine carcinomas (NEC G3). Recently, in gastroenteropancreatic (GEP) NETs, a new subgroup of well-differentiated high-grade tumors (NET G3) has been divided from NEC by WHO due to its different clinical–pathologic features. Although several mutational analyses have been performed, a molecular classification of NET is an unmet need in particular for G3, which tends to be more aggressive and have less benefit to the available therapies. Specifically, new possible prognostic and, above all, predictive factors are highly awaited, giving the basis for new treatments. Alteration of *KRAS*, *TP53*, and *RB1* is mainly reported, but also druggable alterations, including *BRAF* and high microsatellite instability (MSI-H), have been documented in subsets of patients. In addition, PD-L1 demonstrated to be highly expressed in G3 NETs, probably becoming a new biomarker for G3 neuroendocrine neoplasm (NEN) discrimination and a predictive one for immunotherapy response. In this review, we describe the current knowledge available on a high-grade NET molecular landscape with a specific focus on those harboring potentially therapeutic targets in the advanced setting.

**Keywords: neuroendocrine tumors, neuroendocrine carcinoma (NEC), next-generation sequencing (NGS), PD-L1, high microsatellite instability (MSI-H)**

## INTRODUCTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of rare malignant cancers that arise from diffuse neuroendocrine cells. In recent years, the incidence and prevalence of NENs have steadily risen, with a 6.4-fold increase in age-adjusted incidence rate from 1.09 cases per 100,000 in 1973 to 6.98 per 100,000 in 2012 in the United States (1). About 62%–67% of all NEN cases are of

gastroenteropancreatic (GEP) origin, 22%–27% of cases have a thoracic origin (lung and thymus NEN), and 10% of the primary tumor remains unknown (2–4). According to the 2019 WHO classification, GEP NENs are classified into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs) based on both morphological features and proliferation rate (Ki-67 and/or mitotic index) (5). Recently, the NET category G3 was distinguished from the others. It is characterized by well-differentiated neoplasms but with a Ki-67 proliferative index >20%, which is typical of NECs. The need to recognize this new subgroup arose from the observation of a more favorable clinical trend and a different response to medical therapies of this subgroup of patients compared with patients with poorly differentiated tumors. Specifically, as we recently demonstrated, well-differentiated morphology constitutes an independent prognostic factor for GEP NEN with Ki-67 of between 20% and 55% (NET G3 and NEC with Ki-67 20%–55%), while the 55% cutoff of Ki-67 is an independent prognostic factor for poorly differentiated GEP NENs (6). Ki-67 of the neuroendocrine component appears to be the main prognostic factor also for mixed neuroendocrine non-NENs (MiNEN), and lung large cell NECs (LCNECs) (7, 8). Different from NETs, GEP NECs encompass poorly differentiated G3 neoplasms with Ki-67 proliferation index >20% and/or mitotic index >20 per 10 high-power fields (5). They are characterized by a proliferation of tumor cells with irregular nuclei and high mitotic features, with limited immunohistochemical staining for neuroendocrine markers, often displaying faint or focal staining for chromogranin A and diffuse synaptophysin expression (9). Of note, up to 40% of NECs may contain elements of non-neuroendocrine histology (9, 10). While well-differentiated NETs tend to have a relatively indolent behavior, with an excellent prognosis for NETs G1 (Ki-67 < 3%) and good to intermediate for NETs G2 (Ki-67 3–20%), NETs G3 and NECs display an aggressive disease course leading to poor survival outcomes with median overall survival (OS) ranging from 7.5 to 15 months (6, 11). NENs of the lung, on the other hand, according to the latest WHO classification of thoracic tumors (5th edition 2021), remain classified into four histological variants according to necrosis amount and mitotic count: typical and atypical carcinoid, LCNEC, and small cell lung cancer (SCLC) (12). According to the unifying nomenclature proposed by the International Agency for Research on Cancer (IARC) and the WHO Classification of Tumours Group, carcinoids are NETs with low mitoses number and absent or focal necrosis, contrary to LCNECs and SCLCs, which are NECs with extensive necrosis and high mitosis number. Therefore, high-grade NENs of the lung and thymus include SCLC and LCNECs by definition (12). Although several next-generation sequencing (NGS) analyses have been performed, one of the main unmet needs is the lack of a molecular classification of NETs, in particular for high-grade tumors, which tend to be more aggressive and have less benefit from the scantily available therapies. Chemotherapy with platinum compounds plus etoposide still represents the gold standard of first-line treatment, whereas the use of other chemotherapeutic agents [such as irinotecan, fluoropyrimidines, and temozolomide (TMZ)] in further lines of treatment is mostly supported by non-randomized or retrospective evidences (13). Nevertheless, recent progress in tumor genomic profiling has shed some light on the complex molecular scenario of high-grade NETs, identifying a wide range of genomic alterations (mutations, translocations, or amplifications) that could play both a prognostic role, conferring a much aggressive behavior to the tumor, and a predictive one, identifying tumors that may be suitable to biologic agents, allowing a deeper treatment personalization. In this review, we will describe all the available data on the landscape of molecular alteration in NENs with high-grade features (NETs G3 and NECs) particularly focusing on their future clinical and therapeutic role.

## GENOMIC ALTERATIONS

Personalized oncology, defined as the use of molecular profiling to drive treatment strategies for a single patient, is currently a reality in many cancers. In the last decades, the discovery of several oncogenic drive mutations in different malignancies, i.e., *Epidermal Growth Factor Receptor*, and *BRAF* mutations, led to the development of a huge number of targeted drugs with a totally different mechanism of action compared with chemotherapy, which is still, however, commonly used. As far as NENs are concerned, excluding well-established hereditary genetic syndromes caused by germline mutations and commonly associated with well-differentiated NETs, only a few data exist on tissue somatic gene alterations as markers of prognosis or predictive of treatment benefit in high-grade NETs. However, NGS data are expected to emerge rapidly in this field. In the first reports, all the genomic abnormalities observed seemed to be similar to those of the corresponding exocrine neoplasm of the same site (14, 15). Nonetheless, additional mutations specifically related to NETs were also described. Several studies showed that *TP53*, *Kirsten rat sarcoma* (*KRAS*), and *Retinoblastoma 1* (*RB1*) mutations were highly represented in NECs and represent markers of poor differentiation (16–21). On the contrary, several gene mutations may characterize well-differentiated NETs, as observed with *Menin 1* (*MEN1*), *Death Domain Associated Protein* (*DAXX*), and *alpha-thalassemia/mental retardation*, *X-linked* (*ATRX*) mutations in well-differentiated pancreatic NETs (22). Based on this, along with morphological differentiation and proliferation rate, NETs and NECs can be classified and differentiated according to their molecular profile (10). In GEP NETs, the presence of *TP53*, *KRAS*, and *RB1* mutations may also help in differentiating pancreatic NECs from NETs G3 and in predicting the response to platinum-based chemotherapy in the first ones (23). Molecular classification can be also hypothesized in lung NENs according to their genomic alterations (24). Mutations in *TP53* and *RB1* are present in all classes of lungs NENs (typical and atypical carcinoids, SCLCs, and LCNECs) but significantly enriched in NECs (24). Specifically, when mutations and copy number changes were combined, MEN1 alterations were almost exclusive to carcinoids, whereas alterations of the *TP53* and

*RB1* cell cycle regulation genes and *Phosphatidylinositol-4,5-Bisphosphate 3-Kinase* (*PI3K*)/*AKT*/*Mechanistic Target of Rapamycin Kinase* (*mTOR*) pathway genes were significantly enriched in carcinomas (25). Recently, Simbolo et al., based on transcriptomic and genomic data, separated atypical carcinoids and LCNECs into three different and clinically relevant molecular diseases (26). Furthermore, in LCNECs, two mutually exclusive genomic subtypes have been identified: one profile shows concurrent *TP53* and *RB1* mutations similarly to SCLC, whereas the other subtype is predominantly RB1 wild-type and displays concurrent biallelic *TP53* and *Serine/Threonine Kinase 11 (STK11)/Kelch Like ECH Associated Protein 1 (KEAP1)* alterations, similarly to non-SCLC instead (27, 28). Besides a potentially new molecular classification, deep sequencing would be helpful also to predict patient outcomes. Indeed, *RB1* mutation and *Telomerase Reverse Transcriptase (TERT)* gain are shown to be independent unfavorable prognostic markers in all lung NENs, *MEN1* mutation was associated with poor prognosis in atypical carcinoids, and *Histone-Lysine N-Methyltransferase 2D* mutation was associated with longer survival in SCLCs (25, 26). Likewise, to those genes described before, chromatin-modifying genes, in particular, *AT-Rich Interaction Domain 1A (ARID1A)*, could also play a major role in atypical carcinoids and LCNECs (24, 25).

In addition to those previously described, mutations of other genes have been also described in NECs (**Table 1**) (16, 29). In a recent NGS dataset analysis, Chen *et al.* found that about 20.8% of patients with colorectal NECs harbored *BRAF* V600E mutation (20). This may represent a potential target for tyrosine kinase inhibitors (TKIs), such as dabrafenib and trametinib, as it happens in colorectal and lung cancers (29, 30, 103). Another novel potential therapeutic target is *Delta-like protein 3* (DLL3) (31), an inhibitory ligand of the Notch receptor pathway, which is highly expressed in lung NECs (about 80% of SCLCs and 65% of LCNECs) (32, 33), GEP NECs (34), and renal NECs (35). In a recent retrospective analysis, Liverani et al. demonstrated that Dll3 expression assessed *via* immunohistochemistry (IHC) was present in GEP NEC and absent in GEP NET G3, representing a valuable histological marker, for the diagnosis of NECs. In addition, Dll3 expression was also correlated with *RB1*-loss ($p < 0.001$), negative $^{68}$Ga-PET/CT scan ($p = 0.001$), and a worse OS (34). A correlation between Dll3 expression and *RB1*-loss was also observed in SCLC but not in LCNEC (27, 31). Dll3 has been recently studied as a potential target for a novel antibody-drug conjugate called rovalpituzumab tesirine. Despite early-phase trials showing encouraging single-agent antitumor activity, rovalpituzumab tesirine failed, unfortunately, to demonstrate OS superiority in SCLC over placebo as maintenance after platinum-based therapy (36) and over topotecan in second-line setting (37) in phase III trials. Nonetheless, there were several trials investigating the role of novel Dll3 inhibitors in SCLCs, LCNECs, and NECs (**Table 2**).

Furthermore, the role of the homologous recombination repair of the double-stranded DNA pathway in the pathogenesis of NENs has been also recently suggested (42). Recent studies have shown, indeed, that pancreatic NENs can be associated with germline pathogenic variants in genes involved in DNA damage repair, such as *MutY DNA Glycosylase*, *Checkpoint Kinase 2*, and above all *BRCA* (22, 38, 39). Two case reports described patients with prostate NEC, a highly aggressive histologic subtype of prostate cancer, one with germline and the second with somatic *BRCA* mutation, confirming platinum and *Poly[ADP-ribose] polymerase 1* (*PARP*) inhibitor sensibility similar to that of malignancies that frequently present this type of alteration (40, 41). Interestingly, in one of these cases, a novel reversion mutation that restores Brca 1/2 function was described, which might be the reason for primary resistance to *PAPR* inhibitors (41). In addition, the role of *Schlafen* (*SLFN*) 11 was also recently explored in SCLC. Besides its known antiviral properties, several preclinical and clinical studies have been shown its ability to sensitize cancer cells to DNA damaging agents such as chemotherapy and *PARP* inhibitors (42–45). In the MA 11.07 trial, 100 SCLC patients with 1–2 prior lines of therapy were treated with TMZ with either veliparib or placebo. Although the primary endpoint was not met in this trial, patients receiving the combination of TMZ plus veliparib had an almost 3-fold higher response rate as compared with the temozolomide plus placebo arm (39% vs. 19%). Median OS was 8.2 months in the temozolomide plus veliparib arm and 7.0 months in the temozolomide plus placebo arm ($p = 0.50$). However, a significantly longer progression-free survival (PFS) and OS were observed in patients receiving TMZ/veliparib combination who had detectable Slfn11 by IHC (44).

## GENE REARRANGEMENTS

The advances in the genomic profiling of solid tumors shed a light on the contribution of gene translocations, fusions, and amplifications in cancer initiation and progression. In addition to this, recently, gene rearrangements demonstrated also their potential role as prognostic and predictive markers or, most important, as therapeutic targets with the aim of personalizing the treatment algorithm (104). Nevertheless, the frequency of likely oncogenic recurrent gene fusions across the different cancer types is globally low, about 2%–3%, thus limiting the investigation on the singular genomic alterations (105, 106). In the setting of high-grade NENs, the deeper understanding of the molecular scenario recently provided interesting insights into their genomic landscape. With the limitation of the high clinical and molecular heterogeneity of NET G3/NEC, concerning gene fusions or amplifications, a few potential targets have been identified, with frequent tissue-specific features, and are under study (22, 107–110).

### Anaplastic Lymphoma Kinase

Anaplastic lymphoma kinase (*ALK*) gene encodes for the Alk protein, which is a receptor tyrosine kinase belonging to the insulin receptor superfamily that activates a downstream signaling pathway involved in cell survival, proliferation, and oncogenesis. A gene rearrangement involving the fusion of *ALK* with another gene, generating a novel driver oncogene, was first identified in anaplastic large cell lymphoma and afterward in

**TABLE 1 |** Potential novel biomarkers in high-grade NET and relative therapeutic agent.

| Molecular Target | Disease | Clinical Correlations | Targeted Therapies | Ref |
|---|---|---|---|---|
| **TP53** | NECs | Marker of poor differentiation | None | (10, 16–21, 23–28) |
| **KRAS** | NECs | Marker of poor differentiation | None | (10, 16–21, 23) |
| **RB1** | NECs | Marker of poor differentiation | None | (10, 16–21, 23–29) |
| | Lung NETs | Worse prognosis | | |
| **MEN1** | GEP NETs | Marker of well differentiation | None | (22, 24–28) |
| | Carcinoids | Diagnostic marker | | |
| | | Worse prognosis (AC) | | |
| **DAXX** | GEP NETs | Marker of well differentiation | None | (22) |
| **ATRX** | GEP NETs | Marker of well differentiation | None | (22) |
| **ARID1A** | Lung NETs | Pathogenetic role | None | (24, 25) |
| | | Enhancing mutational burden | | |
| **BRAF** | Colorectal NECs | Response to BRAF-MEK inhibition | BRAF-MEK inhibitors | (20–30) |
| **DLL3** | GEP NECs | Marker of poor differentiation | Rovalpituzumab tesirine | (27, 31–37) |
| | | Negative 68Ga-PET | | |
| | | Worse prognosis | | |
| | SCLC | Correlated to RB1-loss | | |
| | LCNEC | None | | |
| | Renal NECs | None | | |
| **BRCA** | Pancreatic NETs | Response to platinum-based regimes | PARP inhibitors | (22, 38–41) |
| | Prostatic NECs | Response to PARP inhibitors | | |
| **SLFN11** | SCLC | Response to platinum-based regimes | PARP inhibitors | (42–45) |
| | | Response to PARP inhibitors | | |
| **ALK** | SCLCLCNEC | Worse prognosis | ALK inhibitors | (46–55) |
| **NTRK** | GEP NECs | Response to NTRK inhibitors | Entrectinib, larotrectinib, taletrectinib | (56–61) |
| | SCLC | | | |
| | LCNEC | | | |
| **PD-L1** | GEP NECs | Marker of poor differentiation | Immune checkpoint inhibitors | (16, 62–70) |
| | | Worse prognosis | | |
| | | Response to immunotherapy | | |
| | SCLC | Response to immunotherapy | | |
| | LCNEC | Response to immunotherapy | | |
| **H-MSI** | Gastric/colorectal NECs | Response to immunotherapy | Immune checkpoint inhibitors | (71–75) |
| **TMB** | GEP NECs | Response to immunotherapy | Immune checkpoint inhibitors | (21, 76–84) |
| | SCLC | | | |
| | LCNEC | | | |
| **miRNAs** | GEP NETs | Diagnostic markers | None | (85–102) |
| | Lung NETs | Prognostic markers | | |

*NEC, neuroendocrine carcinoma; GEP, gastroenteropancreatic; NETs, neuroendocrine tumors; SCLC, small cell lung cancer; LCNEC, large cell neuroendocrine carcinoma; AC, atypical carcinoid; KRAS, Kirsten rat sarcoma; RB1, Retinoblastoma 1; MEN1, Menin 1; DAXX, Death Domain Associated Protein; ATRX, alpha-thalassemia/mental retardation, X-linked; ARID1A, AT-Rich Interaction Domain 1A; DLL3, Delta-like protein 3; PARP, Poly[ADP-ribose] polymerase 1; SLFN11, Schlafen 11; ALK, Anaplastic Lymphoma Kinase; NTRK, Neurotrophic receptor tyrosine kinase; PD-L1, programmed cell death protein ligand 1; H-MSI, high microsatellite instability; TMB, tumor mutational burden; miRNA, microRNA.*

**TABLE 2 |** Ongoing molecular-driven clinical trial involving high-grade NETs.

| Drug(s) | Target | NCT Number | Patient Population | Phase |
|---|---|---|---|---|
| **Pembrolizumab** | H-MSI | NCT02628067 | Solid tumors including NETs | II |
| **INCB099318** | H-MSI (cohort 2) | NCT04272034 | Solid tumors including NETs | I |
| **BI 764532** | DLL3 | NCT04429087 | SCLC, LCNEC, NEC | Ib |
| **Entrectinib** | NTRK 1, 2, 3/ALK/ROS1 | NCT02568267 | Solid tumors including NETs | II |
| **Pralsetinib (BLU-667)** | RET | NCT03037385 | Solid tumors including NETs | Ib/II |
| **Selpercatinib (LOXO-292)** | RET | NCT03157128 | Solid tumors including NETs | I/II |
| **Encorafenib + binimetinib** | BRAF V600 | NCT03864042 | Solid tumors including NETs | I |
| **Avapritinib** | CKIT/PDGFRA | NCT04771520 | Solid tumors including NETs | II |

*Data taken from clinicalTrials.com.*

*H-MSI, high microsatellite instability; DLL3, Delta-like protein 3; NTRK, Neurotrophic receptor tyrosine kinase; ALK, Anaplastic Lymphoma Kinase; NETs, neuroendocrine tumors; SCLC, small cell lung cancer; NEC, neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma.*

other tumors, i.e., lung cancer (in about 5% of cases), and it represents nowadays a key biomarker for targeted treatments, with a much improved clinical outcome (46, 111). In the setting of lung NENs, including typical and atypical carcinoids, SCLCs, and LCNECs, the occurrence of *ALK* fusions is extremely rare, with few cases reported (**Table 1**) (47–52). With the available literature data, the incidence of *ALK* fusions in high-grade lung NENs appeared lower than in NSCLC, <3% versus 3%–5%. In a

dataset of 108 patients with lung NENs, *ALK* fusions were reported in 0.9% of cases (53). In these cases, no associations with a particular histological type were observed, and the main fusion partner was *EMAP Like 4*, as in NSCLC. Rarer partners have been reported, such as *Kinesin Family Member 5B* with no impact on the clinical and therapeutic outcomes (48, 54). Interestingly, most NENs with *ALK* translocation were characterized by high-grade and advanced stage with disseminated lesions, even to the brain, with features that closely correlate with a poor prognosis. Therefore, the rearrangement of *ALK* in lung NEC may represent a specific molecular subtype endowed with more aggressive behavior (47). The diagnostic assessment should include either fluorescence *in situ* hybridization (FISH), reverse transcription PCR, or NGS to confirm the evidence of Alk expression by IHC, especially in cases with focal or heterogeneous expression. In fact, in a high-sensitivity Alk immunostaining on 227 lung NEC tissue microarrays dataset, it was shown that focal positivity with heterogeneous intensity did not correlate with *ALK* rearrangement/amplification in FISH or somatic mutation. Therefore, the aberrant expression of Alk could represent a potential pitfall in the molecular diagnosis of lung NECs, and its relevance relies particularly on the potential therapeutic implication of targeted treatment with Alk inhibitors (55). Due to their practice-changing results on NSCLC, crizotinib, ceritinib, and alectinib were investigated also in lung NECs harboring *ALK* fusion, showing significant disease responses with manageable tolerability in several cases (about 7 partial responses on the 13 cases collected in a literature-based case series review) (47, 49, 51, 54). Nevertheless, the low level of evidence, due to the rarity of the disease and the low frequency of this alteration, limits the clinical implication of *ALK* rearrangement in lung NECs. The greatest burden of data on *ALK* fusions has been collected for lung NECs, given the relevant role in the therapeutic management of NSCLC patients, whereas for non-lung NECs, the evidence of *ALK* fusions/amplifications is scarce, with reports of complete lack of expression in pancreatic NETs (0/46 cases) (46).

## Neurotrophic Receptor Tyrosine Kinase

The neurotrophic receptor tyrosine kinase (*NTRK*) is a tyrosine kinase receptor family including NTRK 1, 2, and 3, which encode the tropomyosin receptor kinase receptors, Trka, Trkb, and Trkc, respectively, involved in normal development, survival, and functionality of the nervous system. The Trk receptor, thanks to the binding with its ligand, homodimerizes and activates a downstream signaling cascade that modulates the activity of several key pathways including *RAS/MAPK* and *mTOR/AKT*. In solid tumors, *NTRK* translocations may occur, resulting in constitutively active protein fusions that display an oncogenic action (112). *NTRK* fusions are a rare finding in the most frequent tumors, although they are enriched in selected low-frequency cancers, such as secretory breast carcinoma, mammary analog secretory carcinoma, and congenital infantile fibrosarcoma, where *NTRK* fusion represents a defining diagnostic parameter (113, 114). In a large dataset of 2,417 NET patients, a total number of 6 cases (0.3%) of *NTRK* fusions were identified, including both intra- and inter-chromosomal translocations, and frequent or unique fusion partners, with no specific characteristics for organ of origin (lung, pancreas, uterus, and unknown primary) although with a peculiar selection for high-grade tumors as NECs or LCNECs (56). The relevance of *NTRK* fusions, aside from their low prevalence in solid tumors, is the potential therapeutic implication since *NTRK* rearrangements have emerged as a powerful actionable driver for targeted therapy. Recently, the selective inhibitors entrectinib and larotrectinib showed practice-changing results in the treatment of tumors with *NTRK* fusions, leading to the agnostic approval of the Food and Drug Administration (FDA) in advanced adult or pediatric tumors bearing this alteration (57–59). For NETs, the evidence collected on this topic is limited. In detail, a patient with metastatic well-differentiated NET, likely originating from the small intestine, bearing an ETS Variant Transcription Factor 6-NTRK3 fusion, was treated with entrectinib in the STARTRK2 trial with a rapid and meaningful tumor response preceded by initial tumor growth and necrosis (60). Moreover, 12 patients with NENs were treated with taletrectinib, a *ROS1/NTRK* inhibitor in a phase I study, reporting 1 partial response and 7 stable diseases according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria, with a manageable toxicity profile (61). Although limited, these results appear promising for further investigations besides being impaired by the double rarity of the cases, that is, NETs that represent rare cancers and NTRK fusions that are a low-frequency molecular alteration (**Table 1**).

## Human Epidermal Growth Factor Receptor 2

*Human epidermal growth factor receptor 2* (*HER2* or *ERBB2*) is a member of the epidermal growth factor receptor family, involved in the regulation of tumor cell proliferation, apoptosis, adhesion, migration, and differentiation (115). *HER2* plays a central role in several tumors with evidence of amplification or overexpression in 7%–34% of all cancers, namely, breast, colon, bladder, ovarian, endometrial, lung, uterine cervix, head and neck, esophageal, and gastric cancers (116). It also represents a key target for the definition of the therapeutic algorithm in many cancer diseases, with numerous approved targeted agents that are able to provide a significant advantage on the clinical outcome (117, 118).

In NENs, the prognostic and predictive role of the amplification/overexpression of *HER2* has not been defined due to its rarity. Most data have been provided in NECs of breast and gastric primitivity, in concordance with the non-NENs (**Table 1**). In particular, breast NEC is a rare subset of breast cancer, accounting for 2%–5% of cases, even though neuroendocrine differentiation is observed in up to 20% of breast tumors, and it belongs mainly to the luminal subtype, with a low rate of Her2 positivity (119). The real impact of the amplification/overexpression of *HER2* on the prognosis of breast NENs is not clear, but an anti-Her2-targeted approach could be considered, even though solid evidence has not been collected (120).

Concerning gastric cancer, case series studies have been performed on this topic, 51 gastric NECs (15 pure and 36 associated with adenocarcinoma and/or dysplasia) were analyzed, and *HER2* amplification was reported in 3 NECs (6%) and 7 (19%) mixed tumors. However, none of them displayed Her2 expression in IHC (121). Consistently, in the other three studies, Her2 expression in IHC was found to be negative, or *HER2* copy number analysis did not show amplification in 31 primitive gastric NECs overall (122–124). Therefore, the available evidence suggests that *HER2* may not represent a valid therapeutic target, although this could be influenced by intratumoral heterogeneity, and further studies should be warranted on this topic. Finally, a study encompassing an expression profiling analysis in LCNECs reported that two cases displayed overexpression of Her2 at IHC, suggesting a potential role as a treatment target to be further investigated (125).

## IMMUNE RESPONSE BIOMARKERS

Recently, the introduction of immunotherapy dramatically changed the natural history of several cancer subtypes, like melanoma, lung cancer, and kidney cancer. Nonetheless, in some cases, the benefit of this treatment is confined only to a small portion of patients who show predictive biomarkers such as programmed cell death protein 1/ligand 1 (PD-1/PD-L1) or deficient mismatch repair (dMMR)/high microsatellite instability (MSI-H) status. In NENs, an increasing number of clinical trials with immunotherapy have been conducted (62). In March 2017, based on the results of the JAVELIN Merkel 200 trials, avelumab became the first FDA-approved agent for the treatment of metastatic Merkel cell carcinoma, a rare but aggressive NEC of the skin, and represented a new therapeutic option to improve patients' survival (126, 127). Two years later, following the results of the IMpower133 trial, atezolizumab combined with chemotherapy was approved by the FDA for first-line treatment of extensive-stage SCLC. In this trial, the combination of chemotherapy and immunotherapy improved PFS and OS, with median PFS 5.2 versus 4.3 months (hazard ratio [HR] 0.77; 95% CI: 0.62–0.96; p = 0.02) and median OS 12.3 versus 10.3 months (HR 0.70; 95% CI: 0.54–0.91; p = 0.007), compared with chemotherapy alone (128). More recently, in phase II studies, the significant activity of spartalizumab in thoracic NENs (129) and also with the combination of ipilimumab plus nivolumab (objective response rate (ORR) 44%) in patients with non-pancreatic high-grade NENs (130).

With the exception of these few cases, unfortunately, there is a relatively low efficacy of immunotherapy in the unselected population of NENs, especially in GEP-NET. Therefore, one of the biggest challenges is to find those biomarkers that will allow to select those patients who will have a higher probability to benefit from this kind of treatment. Due to the heterogeneity of NENs and their rarity, as well as the fact that different primary tumor sites have different microenvironments, exploration in this field is indeed quite difficult. However, there is increasing evidence of the role that PD-1/PD-L1, tumor mutational burden (TMB), and dMMR/H-MSI status may also have in NENs (**Table 1**).

### Targeting PD-1/PDL-1 Pathway

PD-L1, an immune inhibitory protein, is often upregulated in tumor cells by interferon-gamma secreted from effector T cells when tumor antigens are recognized. By interacting with PD-1, PD-L1 can suppress many immune cell functions, especially T-cell activation favoring tumor cell immune escape. Expression levels of PD-L1 assessment *via* IHC on tumor cells are one of the predictive factors for patients treated with immunotherapy. Several retrospective studies demonstrated that PD-L1 expression is a frequent occurrence in high-grade GEP-NENs (62). Kim et al. firstly reported a 21.9% (7/32) PD-L1 expression rate in patients with metastatic GEP-NET, which was significantly associated (p = 0.008) with high-grade classification (63). Similar to this, PD-L1 positivity was found by Cavalcanti et al. in approximately 28% (16/57) of cases, and again, PD-L1 expression in both tumor and infiltrating immune cells was significantly higher in poorly differentiated NENs (p = 0.001), and its expression rates increased with the tumor aggressiveness. These findings may be related to possibly acquired resistance to immune surveillance by the upregulation of PD-L1 and the inhibition of peritumoral and intratumoral infiltrating lymphocytes limiting T cell-mediated tumor aggression (64). This may explain the higher PD-L1 expression rates observed in later case series restricted to high-grade GEP NETs. PD-L1 positivity of 48.8% was observed by Yang et al. in 43 gastric NECs (65), while 24.1% was described by Busico et al. in tumor-infiltrating lymphocytes (TILs) of 54 GEP high-grade NENs (16). In both studies, the high expression of PD-L1 was associated with poor OS. An increase of PD-L1 expression along the GEP-NENs grading stages was also reported in a retrospective study performed in our institution (66). In addition, we demonstrated that the transition from G1/G2 NETs to G3 NETs and G3 NECs is associated with profound changes in the tumor and stromal profile for inflammatory and immune-related markers and point to more frequent activation of adaptive immunity in NECs and a strong immune escape mechanism. Moreover, a subset of NECs has microenvironment features consistent with spontaneous activation of adaptive immunity (co-expression of CD3, CD4, CD8, PD-1, and PD-L1). Recently, we further evaluated the tumor microenvironment of high-grade NENs, by expanding the immune profiling to myeloid markers and identifying two prognostic subpopulations of tumors likely compatible with the "hot/cold tumor" idea: high-grade NENs characterized by a prevalent immune infiltrate cells had better survival (67). According to this, it was suggested that microenvironment-related immune and inflammatory markers can improve prognostic prediction in GEP-NENs when combined with the known prognostic factors, and they may predict potential responsiveness to immunotherapy of GEP NECs (66, 67). Furthermore, Bosch et al. demonstrated that high TILs and PD-1 expression are significantly associated with shorter survival and higher grading in GEP NENs. In addition, high expression of PD-L1 in tumor cells was associated with high rates of PD-1-positive lymphocytes and a significantly higher number of TILs. According to this, the authors suggested that in high TIL tumors, a higher number of PD-1-positive lymphocytes

is present; thereby, tumor cells with the higher PD-L1 expression may be more able to escape from the immune response by upregulation of this pathway (68).

In summary, according to previous data, PD-L1 expression may be a useful biomarker first to discriminate GEP high-grade NENs, and then, it may potentially be a prognostic and, above all, predictive biomarker for response to immune checkpoint inhibitors (ICIs).

When considering high-grade lung NENs, PD-L1 positive rates tend to vary immensely across different studies. A reason for this wide range may be related to the use of different clones of anti-PD-L1 antibody for IHC along with variable cutoffs. But in those studies in which FDA-approved anti-PD-L1 antibodies and their relative cutoffs were used, expression rates tend to be low (69, 70). Interestingly, substantial PD-L1 expression occurs on stroma cells, including TILs, in SCLCs with favorable clinical outcomes. Overall, this relatively low PD-L1 expression along with the deficient expression of major histocompatibility complex class I molecules, which prevents tumor cells from presenting neoantigens to CD8+ T cells in the lymph nodes and inhibiting cytotoxic T lymphocytes, may be one of the main reasons why the efficacy of ICIs in SCLCs is not as good as that in NSCLCs (69).

## High Microsatellite Instability

H-MSI phenotype is another well-known biomarker that is under investigation in many neoplastic diseases. MMR proteins represent a complex system involved in DNA repair mechanisms, which ensure genomic integrity and remove DNA errors. Deficiency in MMR proteins (MLH1, MSH2, MSH6, and PMS2), commonly assessed by IHC, leads to an accumulation of DNA replication errors and mutations as well as expansion or contraction of microsatellite regions (131). The resulting hyper-mutated phenotype strongly enhances the formation of neo-antigens, making cancer cells more recognizable by the host immune system. Additionally, dMMR/H-MSI tumors have prominent lymphocyte infiltrates (132) and are more likely to express PD-L1 (133), which may predict response and durable clinical benefit to PD-1 blockade. For all these reasons, dMMR/H-MSI tumors are responsive to immunotherapy. Recently, FDA approval was granted for use of the anti-PD-1 antibody pembrolizumab for the treatment of metastatic non-hematologic cancers that are characterized by this alteration. Usually, dMMR is related to Lynch syndrome, which is caused by germline mutations of MMR proteins, leading to a 50%–70% lifetime risk of colorectal cancer, 40%–60% risk of endometrial cancer, and increased risks of several other malignancies (134). Despite this, dMMR/H-MSI can be also observed in sporadic cancer. Data on H-MSI in NENs are limited. Recent studies demonstrated that the presence of H-MSI phenotype on subsets of gastrointestinal (GI) NECs and MiNEN of the stomach and colorectum with an incidence rate up to 15%; it was mostly subsequent to MHL1 promoter methylation and with a more favorable prognosis (71, 72). In contrast, defects in DNA MMR proteins are rare in pancreatic NETs, small intestinal NETs (73, 74), and NECs of the endometrium (75) and cervix (75). These data suggest the prevalence of H-MSI in relatively low NETs; it is site-dependent and closely related to those organ sites in which H-MSI status is usually observed in the exocrine neoplastic counterparts, such as colorectal, gastric, and endometrial adenocarcinomas. Nevertheless, given the potential prognostic role and the clinical benefit of immunotherapy, dMMR/H-MSI testing must be encouraged as well as testing of other malignancies like colorectal cancer.

## Tumor Mutational Burden

In addition to the previous two TMBs is another recently discovered biomarker. It is broadly defined as the number of somatic mutations per megabase of interrogated genomic sequence. TMB is believed to be a key driver in the generation of immunogenic neopeptides displayed on major histocompatibility complexes on the tumor cell surface that influences patient response to ICIs (76). In a phase II study in patients with previously treated, unresectable, or metastatic solid tumors (KEYNOTE-158), TMB-high status (≥10 mut/Mb) was associated with a clinically meaningful improvement in the efficacy of pembrolizumab (77). According to this, the FDA approved pembrolizumab monotherapy for the subgroup of solid-tumor patients with TMB ≥10 mut/Mb who are treatment-refractory and lack satisfactory alternative treatment options.

TMB of NETs has not been fully studied yet. In a study of 4,125 patients with various GI cancer types, TMB levels have been analyzed. Among those, pancreatic NETs were found to have one of the lowest TMB (5.8 mut/Mb) (78). More recently, in another retrospective study, Shao et al. assessed TMB in 2,559 patients with different tumors. SCLC was found to have the highest median TMB (8.6 mut/Mb) and the highest rate of TMB-high (cutoff ≥10 mut/Mb, 40%), which is, interestingly, followed by the NETs (29.3%). However, this remarkable rate was driven by the patients with LCNEC in which TMB high rate was 45.6%. On the contrary, in the small bowel, colon, and rectal NETs grouped with LCNECs, the rate was lower (5.9%, 11.8%, and 0%, respectively). Despite this, no differences in OS were seen between TMB high and low tumors (79). High TMB and elevated TMB-high rates in SCLC were described in several other studies (80–83). Furthermore, the role of TMB as a predictive biomarker in extensive-stage SCLC was also explored in patients who were treated with nivolumab alone or combined with ipilimumab after the failure of at least one prior chemotherapy regimen (CheckMate032 trial). In these populations, ORR by treatment arm increased in patients whose tumors showed high versus medium versus low TMB levels. In addition, in patients with high TMB tumors, dual ICI treatment was associated with an impressive ORR of 46.2% and an estimated 1-year OS rate of 62.4% (84). Lastly, in another recent report, Hoffman-Censits et al. demonstrated that over 26% of small cell bladder cancer had high TMB, in particular TMB > 10 mutations/Mb, and 3% had TMB > 20 mut/Mb, with a median of 6.2 mut/Mb (21).

## MicroRNA

MicroRNAs (miRNAs) are small, non-coding RNAs with a length of 21–25 nucleotides and participate in gene regulation on the post-transcriptional level (135, 136). The role of miRNAs in cancerogenesis is now well-established, and several studies demonstrated the correlation between specific miRNA and different cancer subtypes (85). According to this, miRNA expression profiles are potentially exploited as practical supportive markers for differential NEN diagnosis and prognosis and provide adequate information on proper patient care and management (85–90). When considering pancreatic NETs, the expression of specific miRNAs is able to discriminate them from normal pancreas and other pathologic conditions such as pancreatic ductal adenocarcinoma and acinar pancreatic tumors (87, 91, 92). Specifically, the expressions of miR-144/451 cluster, miRNA-21, and MiR-193b were observed in insulinomas compared with normal pancreatic tissue, while miR-103 and miR-107 overexpression and miR-155 underexpression distinguish pancreatic NETs from acinar cell carcinomas (87, 91, 93). In addition to this, different miRNA expressions discriminate different clinical behaviors and prognoses of pancreatic NETs (88). Indeed, the overexpression of miR-21, miR-642, and miR-196a was found to be positively correlated with the Ki-67 proliferation index, whereas miR-210 correlated with the presence of liver metastases (93, 94). Additionally, miR-196a expression was significantly associated with stage, mitotic count, and decreased OS and disease-free survival (95). The pattern of miRNA expression was also explored in small bowel NETs (91, 96). MiR-7-5p, miR-182, miR-183, and miR-96-5p were found to be upregulated in NETs of the small bowel compared with normal tissue (91). In addition, the last three, along with the downregulation of miR-129-5p and miR-133a, were found to be overexpressed in the metastatic lesions compared with primary tumors (91, 97). Considering the prognostic role, high levels of circulating miR-21-5p and miR-22-3p and low levels of miR-150-5p were associated with shorter OS (98). Specific miRNA expressions were also reported in other GEP-NENs such as gastrin-induced miR-222 overexpression in hypergastrinemic patients and type 1 gastric NETs, which may be associated with tumor development by decreasing p27 expression (99); low levels of miR-96 and high levels of miR-133a expression in appendiceal carcinoids (91); underexpression of miR-186 in colorectal NETs (100); and overexpression of miR-885-5p in rectal carcinoids (101). Lastly, in a recent study, Cavalcanti et al. reported that 8 miRNAs were expressed in all GEP-NETs grades (miR-10b-5p, miR-130b-3p, miR-192-5p, miR-194-5p, miR-210-3p, miR-214-3p, miR-7-5p, and miR-96-5p), but their expression level was different between differentiation grades. Among these, miR-96-5p were found to have increased expression levels from G1 to G3, and this may be probably related to the downregulation of FoxO1 gene by this miRNA (85).

The role of miRNAs as a diagnostic, prognostic, and chemoresistance tool was also explored in lung NENs. Recently, Yoshimoto et al. collected formalin-fixed paraffin-embedded samples of lung and GEP NETs, lung and GI adenocarcinomas, olfactory neuroblastomas, schwannomas, and related normal tissue for the analysis of their miRNA expression. After a very complex hierarchical clustering analysis, they found that lung and GI-NETs had a similar pattern of miRNA expression, suggesting a common origin between them, which was different from adenocarcinomas, SCLCs, and normal tissue. They also showed a distinct miRNA expression profile of SCLCs from lung carcinoids (89), and this may be useful to distinguish between low- and high-grade lung NENs. In addition, Rapa et al. showed that lung carcinoids have distinct miRNA expression profiles as compared with high-grade NECs, explaining that specific miRNAs might have potential implications as diagnostic tools or clinical biomarkers (102). As described for GEP-NENs, specific miRNA expression may also be used as prognostic markers (88). Specifically, overexpression of miR-92a2* and miR-7 and low levels of miR-150, miR-886-3p, miR-192, miR-200c, and miR-205 were described to be correlated to OS and PFS of SCLCs. MiR-92a2* and miR-7, along with mir-147 and miR-574-5p, were found to be associated with chemoresistance too (88, 90). A correlation with survival was also observed in typical and atypical carcinoids and LCNECs with upregulation of mir let-7d, miR-19, miR576-5p, miR-340*, and miR-1286, while overexpression miR-21 and low levels of miR-409-3p, miR-409-5p, and miR-431-5p correlated with the presence of lymph node metastases (88).

## CONCLUSIONS

Emerging evidence suggests an important role for biomarker identification and also NENs, in particular those with high-grade features. High-grade NENs can express different biomarkers (PD-L1, H-MSI status, miRNA expression patterns, and other alterations). A comprehensive exploration of biomarkers is still lacking as well as a molecular-driven clinical trial involving patients with NENs apart from the phase I/II multi-disease trial (**Table 2**). So considering that many of those biomarkers can be the target for new generations of drugs, with a subsequent significant clinical benefit, greater effort should be focused on spreading routine molecular analysis also in this setting of patients, like what usually happens with other malignancies. This may be important firstly for the patients themselves, giving the chance to obtain additional treatments with expanded access programs or nominal use, and secondly, because it may the basis for future clinical trials specific for this group of patients that may significantly change the currently untailored chemotherapy-based treatment strategies.

## AUTHOR CONTRIBUTIONS

Conceptualization: MP, MMi, and SP. Methodology: MP, FM, NP, MN, and SC. Investigation: MP, AM, AR, GG, and FC. Resources: GR, FP, EC, and MMa. Data curation: MP, AM, AR, and FC. Writing—original draft preparation: MP, AM, AR, and FC. Writing—review and editing: MP, FM, NP, MN, SC, SP, GC, GS, AM, GC, and MB. Visualization: GR, FP, EC, and MMa. Supervision: MB, FB, MMi, and SP. All authors have read and agreed to the published version of the manuscript.

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# Composite Paraganglioma of the Celiac Trunk: A Case Report and a Comprehensive Review of the Literature

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**Introduction:** Composite paragangliomas consist of two components, paraganglioma and ganglioneuroma, representing a rare subgroup of paragangliomas. The purpose of the study is to describe a case of composite paraganglioma of the celiac trunk and a brief review of the existing literature.

**Case Presentation:** A 64-year-old female patient with a history of epigastric abdominal pain and a 51 mm-diameter tumor found in a Computerized Tomography of the abdomen was admitted to our surgical department for further evaluation and treatment. After a brief preoperative surgical assessment, the patient underwent a mini-laparotomy for the excision of this tumor. After having the results of the pathology report, a comprehensive review of the international literature was carried out by applying the appropriate search terms.

**Results:** As it was found intraoperatively, the tumor was located at the cephalad aspect of the common hepatic artery, over the portal vein and the inferior vena cava. A negative-margin resection was achieved and the tumor was sent for pathology analysis. The final pathology report revealed a composite paraganglioma, with α paraganglioma and a ganglioneuroma component. Seventeen cases of extra-adrenal composite paraganglioma have been reported in the international literature so far. This case was the first one found in the area of the celiac trunk.

**Conclusions:** Composite paragangliomas comprise rare and potentially malignant tumors with variable prognosis. Establishing their diagnosis promptly is of vital significance. Due to the first-described location of the composite paraganglioma in our case, the differential diagnosis of tumors in this area should also include composite paragangliomas.

**Keywords: composite paraganglioma, ganglioneuroma, celiac trunk, case, neuroendocrine tumors**

## INTRODUCTION

Pheochromocytomas are rare chromaffin catecholamine-secreting tumors, usually located within the adrenal glands. However, when these tumors arise outside of the adrenal glands, they are defined as paragangliomas. Paragangliomas may occur anywhere along the path of the autonomic ganglia, from the base of the skull to the urinary bladder. Composite paragangliomas, a subtype of paragangliomas, usually consist of the paraganglioma and the ganglioneuroma component. Composite paragangliomas are estimated to be around 3% of the adrenal paragangliomas, being very rare especially outside the adrenal glands (1). They are reported to be found usually in the adrenal glands and less frequently in the mediastinum, in the Zuckerkandl organ at the abdominal aortic bifurcation, in the retroperitoneum, in the urinary bladder, and the central nervous system (2, 3). Herein, we present a case of composite paraganglioma located on the right of the celiac trunk, over the common hepatic artery.

## CASE PRESENTATION

A 64-year-old female patient was referred to our outpatient department for surgical evaluation due to paroxysmal epigastric abdominal pain, with mild deterioration after movement and exercise. The patient's medical history referred that she underwent total thyroidectomy, with central and left lateral compartment dissection due to thyroid papillary cancer with nodal metastasis (pT3b(m)N1Mx based on The American Joint Committee on Cancer staging) one year before her admission to our clinic. Moreover, she had already visited a Gastroenterologist, who suggested her undergoing endoscopy of the upper gastrointestinal (GI) system and a Computerized Tomography (CT) scan. The report of the upper GI endoscopy referred that mild esophagitis and gastritis were present while the CT scan revealed a 51 mm-diameter solid mass at the site of hepatogastric ligament applying pressure on the abdominal aorta. Our findings during the initial physical examination and laboratory test were normal (**Table 1**). Next, to identify the nature of this mass, we suggested that she should undergo an endoscopic ultrasound during which biopsies from the bulging mass would be received and sent for pathology and immunohistochemical analysis. The differential diagnosis of pathology report suggested a ganglioneuroma or a neuroendocrine neoplasm and the further immunohistochemical analysis reported that the mass included neoplasmatic cells being positive to S100 protein and also cells with positive immunostaining for neurofibers (NF), two findings which both were indicative of ganglioneuroma. Under the probable diagnosis of a ganglioneuroma, we decided to evaluate its activity regarding the secretion of catecholamines or other neurotransmitters. As a result, an analysis for creatinine, total catecholamines, metanephrines, and vanillylmandelic acid of a 24-h urine collection was performed, without revealing any abnormality (**Table 1**). In addition, the patient underwent screening for mutations in the ret proto-oncogene from the patient's genomic deoxyribonucleic acid (DNA) for any genetic disorders to be found, which did not detect any mutation in

**TABLE 1** | Patient's laboratory results.

| Test | Value | Normal values |
|---|---|---|
| *Complete blood count* Hematocrit | 40.2% | 42.0–54.0% |
| Hemoglobin | 13.9 gr/dl | 13.0–18 gr/dl |
| Red blood cell count | 4.56 M/ml | 4.5–5.5 M/ml |
| Mean Corpuscular Volume (MCV) | 88.2 fl | 78.0–98.0 fl |
| Mean Corpuscular Hemoglobin (MCH) | 30.5 pg | 27.0–31.0 pg |
| Mean Corpuscular Hemoglobin Concentration (MCHC) | 34.6 gr/dl | 32.0–36.0 gr/dl |
| Red Blood Cell Distribution Width (RDW-CV) | 14.3% | 11.5–14.0% |
| White Blood Cell Count | 6.45 K/μL | 4.0–11.0 K/μL |
| Neutrophils | 75.7% | 40.0–70.0% |
| Platelet Count | 262 K/μL | 142–450 K/μL |
| *Biochemical Tests* Serum glucose | 96.5 mg/dl | 70–105 mg/dl |
| Serum Urea | 28.56 mg/dl | 19.00–44.00 mg/dl |
| Serum Creatinine | 0.72 mg/dl | 0.72–1.25 mg/dl |
| Aspartate Transaminase (AST) | 20.4 U/L | 5.0–34.0 U/L |
| Alanine Transaminase (ALT) | 20.7 U/L | 00.0–55.0 U/L |
| Gamma-glutamyl Transferase | 8.4 U/L | 12.0–64.0 U/L |
| Alkaline Phosphatase (ALP) | 63.4 U/L | 40–150 U/L |
| Lactate Dehydrogenase (LDH) | 190.3 U/L | 125–220 U/L |
| C Reactive Protein (CRP) | 0.039 | <0.5 mg/dl |
| Serum Potassium | 4.23 mmol/L | 3.4–5.1 mmol/L |
| Serum Sodium | 143.3 mmol/L | 136.0–145.0 mmol/L |
| *Hormonal Assay* Triiodothyronine (T3) | 1.18 ng/ml | 0.6–1.6 ng/ml |
| Thyroxine (T4) | 6.53 μg/dl | 4.87–11.72 μg/dl |
| Thyroid Stimulating Hormone (TSH) | 1.43 μIU/ml | 0.35–4.94 μIU/ml |
| Serum Free Metanephrine | 0.31 | <0.50 nmol/L |
| Serum Free Normetanephrine | 0.56 | <0.90 nmol/L |
| *Serology* Surface antigen of the hepatitis B virus (HBsAg) | 0.11 S/CO | Negative < 1.00 S/CO |
| Hepatitis C Virus test | 0.09 S/CO | Negative < 1.00 S/CO |
| HIV Ag/Ab | 0.11 S/CO | Negative < 1.00 S/CO |
| *Coagulation Tests* activated Partial Thromboplastin Time (aPTT) | 31.6 sec | 25.0–45.0 sec |
| Prothrombin Time (PT) | 11.55 sec | 12.00–14.00 sec |
| International Normalized Ratio (I.N.R.) | 0.87 | 1.00–1.50 |
| *24-h urine collection* Creatinine | 768 mg/24 h | 600–1,800 mg/24 h |
| Total Catecholamines | 213 μg/24 h | 65–515 μg/24 h |
| Adrenaline | 11.3 μg/24 h | 0.0–20.0 μg/24 h |
| Noradrenaline | 62.7 μg/24 h | 15.0–80.0 μg/24 h |
| Total Metanephrines | 0.87 μg/ mg of creatinine | <1.20 μg / mg of creatinine |
| Vanillylmandelic Acid | 3.4 mg/24 h | <11.0 mg / 24 h |

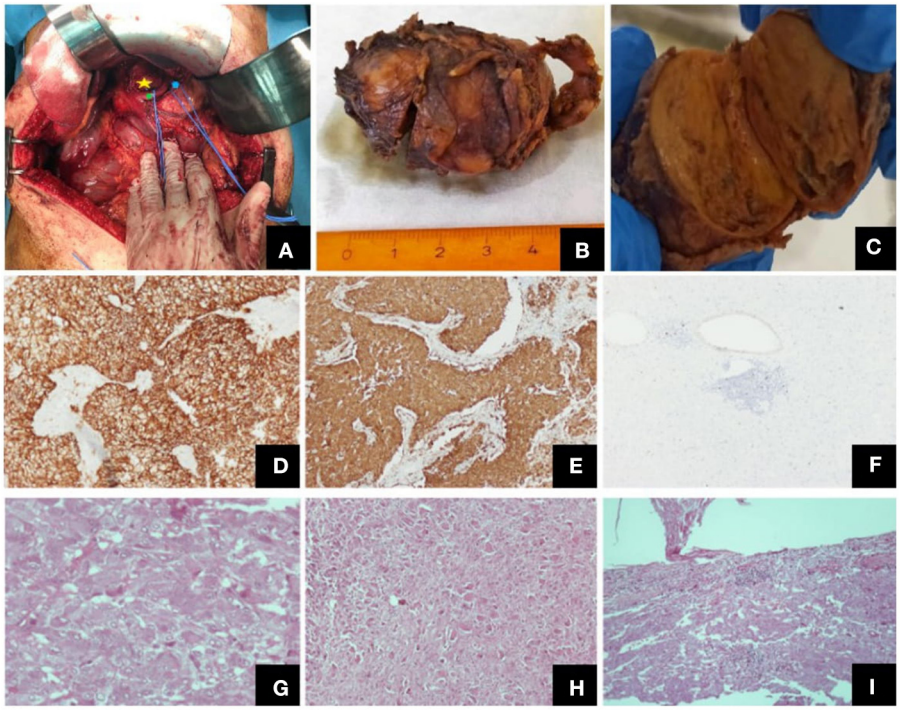


**FIGURE 1 |** Different views of the tumor (intraoperatively, macroscopically after excision) and its histological features. **(A)** Intraoperative view of the tumor (yellow star: the tumor, green point: common hepatic artery, blue point: left gastric artery), **(B)** Macroscopic view of the excised tumor, **(C)** Tumor on vertical cross-section, where both the paraganglioma and the ganglioneuroma components were identified, Representative images of histological features: **(D)** CD56, ×100, **(E)** synapt (×100), **(F)** Ki67 labeling index (×40), **(G)** paraganglioma, H + E, (×400), **(H)** ganglioneuroma, H + E, (×100), **(I)** tumor invasion of the capsule, H + E, (×100).

exons 5, 8, 10, 11, 13, 14, 15, and 16. Moreover, it was of great importance, a more comprehensive radiological assessment to be held, due to mass' special location. Thus, the patient underwent chest CT scan and abdominal CT Angiography scan with 3D reconstruction of the celiac trunk which reported again the known tumor of about 51 mm diameter, located between the aorta and the inferior vena cava in the hepatogastric space. Based on all the before mentioned data, we decided, after consensus, to suggest the patient undergoing surgical excision of the tumor due to its potential for malignancy. The patient's decision was congruent with our suggestion and she underwent a laparotomy and surgical excision of the mass. Intraoperatively, the tumor was found at the cephalad aspect of the common hepatic artery, over the portal vein, and the inferior vena cava. A negative-margin resection was achieved and the tumor was sent for pathology analysis. In addition, all the nodal tissue around the celiac trunk was excised after skeletonizing the vessels (**Figure 1**). The postoperative period was uncomplicated and the patient was discharged the 2nd postoperative day. The final pathology report revealed that the tumor included two masses, a sub round one enveloped by capsule and sized 6.0 × 4.2 × 3.7 cm, and a second smaller one attached to the first's outer surface. It was presented with neoplastic features and consisted of large, irregularly shaped cells. The number of nuclei was >5/10 per visual field, while in some areas they formed "zellballen balls," and the cytoviscosity was considered as moderate to maximum. In addition, in some areas the ganglion cell population did not mix with the upper cellular findings, but appeared to be of neurogenic origin, as evidenced by immunohistochemical staining for protein S100 and CD56. Furthermore, in some areas a capsule was identified, and it was disrupted by the neoplastic cells. In these areas, neoplasmatic embolisms were identified inside the thin-walled vessels. However, the wall of the vessels was not found to be positive for immunohistochemical staining regarding CD34 and D2-40. Moreover, the neoplasmatic cells were positive for CD56, synaptophysin, and chromogranin and their nuclei presented also positive for ATRX staining, while p53 protein was identified only in <5% of them. However, the cells were negative for neurofibrins, inhibin, Melan A and HMB-45. In conclusion, the superior morphological and immunohistochemical findings were consistent with a composite paraganglioma and to a small limited extent, a ganglioneuroma. In addition, the particular histological features of the paraganglioma (diffuse growth, capsular and vascular infiltration, Ki67 cell proliferation index >2%, tumor size >5 cm) classify the neoplasm as a high metastatic potential one, according to the GAPP classification system. Two months after the procedure, the subsequent imaging evaluation of the patient, with chest and abdominal CT scan and ultrasound of the upper abdomen, did not reveal any pathological findings, except for a new-described small cyst at the head of the pancreas possibly due to chronic pancreatic inflammation. The laboratory tests were normal as well.

## DISCUSSION

Pheochromocytomas are tumors which in their majority originate from the chromaffin cells of the adrenal medulla (85–90%) and less frequently from the extra-adrenal sympathetic nerve tissue, mainly paraspinal or paraaortic, called paragangliomas (4). They can be either sporadic or familial (5). The usual location for paragangliomas is primarily in the head, the cervix, or the mediastinum (6), usually secreting catecholamines. Similar to common paragangliomas, the composite paragangliomas are primarily functional, secreting catecholamines such as adrenaline, noradrenaline, and dopamine or corticotrophin-releasing hormone (CRH) as well (7). Based on this fact, common symptoms are severe headache, nausea, palpitations, sweating, fatigue, permanent or paroxysmal hypertension, or even orthostatic hypotension. Moreover, pallor, redness of the face, weight loss, and hyperglycemia are common signs as well (8). However, in our case, since the composite paraganglioma was not an active one, the patient did not experience any of the relating with catecholamines-secretion symptoms, but only a feeling of abdominal tenderness and pain especially right after exercise.

Ten percent of all the pheochromocytomas have a chance of malignancy, with extra-adrenal gland localization advocating a particularly high rate of malignancy and metastatic disease (9). On the other hand, ganglioneuromas are considered to be the most common form of neuroblastoma, mainly in young adults (10). They are primarily retroperitoneal and tend to be asymptomatic until they become large enough to give symptoms by pressing nearby structures (11). However, some ganglioneuromas may be also functional and secrete peptides, such as VIP and somatostatin, causing diarrhea, hypertension, and sweating.

Regarding composite paragangliomas, they are considered to be rare tumors. In about 70% of them, paraganglioma coexists with a ganglioneuroma component. They affect patients around 40 to 60 years old, with equal distribution across males and females (12). The size of composite paragangliomas ranges from 1 to 35 cm (13). In our case, the size of the tumor was considered to be an average one, about 6 × 4.2 × 3.7 cm (the volume was about 80 cc).

Fewer than 70 cases have been reported in the medical literature, most of which are located in the adrenal glands, while the extra-adrenal composite tumors have been reported only occasionally (14). In particular, only 17 extra-adrenal cases have been described in the literature so far (**Table 2**). From them 5 were found in the urinary bladder, 6 in the retroperitoneum, 1 in the neck, 1 in the duodenum, 1 in the pancreas, 1 in the filum terminale, 1 in the caude equine and 1 case of spinal and pelvic bone metastatic lesions. The mean age of all these cases was 58.8 years old, whereas 10 of them were females and 6 were males (In one case the gender was not reported). Thus, to our knowledge, in this study we describe the first case demonstrating a composite paraganglioma-ganglioneuroma located in the area near the celiac trunk, cephalad to the common hepatic artery.

Composite paragangliomas are often associated with familial neoplasm syndromes, such as neurofibromatosis type 1 (NF1) or multiple endocrine neoplasia type 2 (MEN2) (15). In multiple endocrine neoplasia type 2 (MEN2), an autosomal-dominant cancer syndrome with major components of medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism, about 50% of the patients develop pheochromocytomas, located, almost always, in the adrenal medulla. Moreover, extra-adrenal pheochromocytomas (paragangliomas) are unusual (about 3% of the cases), while the co-existence of a composite paraganglioma-ganglioneuroma with any kind of MEN2-familial syndromes is quite rare (5). In our case, any association of this particular composite tumor was not confirmed, nor any kind of familial syndrome, based on the results of the genetic screening. However, it is worth mentioning that the patient has already undergone total thyroidectomy due to papillary, not medullary, metastatic cancer. In addition, in the postoperative imaging follow-up, a mass in the area of the head of the pancreas was found, constituting a point of concern, although it is currently attributed to a benign cystic lesion.

Imaging studies for the diagnosis of paragangliomas include CT or Magnetic Resonance Imaging (MRI) scan, while scintigraphy may be an alternative utility when these imaging tests fail to localize the tumor. Abdominal CT scan has an accuracy of about 85–95% for detecting tumors with a threshold size of 1 cm (16). Most of the pheochromocytomas reveal CT attenuation of more than 10 Hounsfield Units (HU) but sometimes it is quite difficult to differentiate a pheochromocytoma from another adenoma or adrenal metastasis (17). On the other hand, MRI is reported to have a sensitivity of about 100% in detecting adrenal pheochromocytomas, and in about 70% of T2-weighted images, the tumors appear hyperintense due to their water content or internal hemorrhage (18). Furthermore, scanning with $^{123}$iodine-labeled metaiodobenzylguanidine (MIBG) may be helpful for cases in which CT or MRI are inconclusive, even though the pheochromocytoma has already been proven biochemically. Its specificity is reported to be between 82 and 92% and its sensitivity ranges widely, from 53 up to 94% (19).

In this case, we would like to emphasize the difficulty of establishing the diagnosis of paraganglioma preoperatively. The laboratory tests were indicative and the ultrasound imaging revealed a mass, without any special characteristic indicative of its origin. Moreover, an ultrasound-guided biopsy of this tumor suggested the diagnosis of a ganglioneuroma and not a paraganglioma. In addition, images obtained by CT scan were not characteristic of a paraganglioma, while CT-angiography just helped us identify the relations of the tumor with the celiac artery and the other structure of this area. As a result, the definite diagnosis of the composite paraganglioma was established only after the final pathology analysis of the excised tissue, and besides, it is very common to have the precise diagnosis for this kind of tumors only after the pathology examination have been completed (20).

The prognosis of composite paragangliomas varies and depends on the existence of malignancy. For non-malignant disease, the 5-year survival rate is more than 95%. However, in patients with malignancy, the 5-year survival rate is <50%

**TABLE 2 |** Extra-adrenal composite paragangliomas referred in the literature.

| Case | Title | First author | Year of publication | Location of the composite paraganglioma | Age (years) | Gender | Size (mm) | Symptoms | Activity (preoperatively) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Composite Paraganglioma-Ganglioneuroma of the Urinary Bladder: A Clinicopathologic, Immunohistochemical, and Ultrastructural Study of a Case and Review of the Literature | King-Yin Lam | 1998 | Urinary bladder | 81 | Female | NR* | Whole-stream painless hematuria | NR* |
| 2 | Pigmented composite paraganglioma-ganglioneuroma of the urinary bladder | Pavel Dundr | 2003 | Urinary bladder | 70 | Female | 65(diameter) | NR* | NR* |
| 3 | Composite paraganglioma-ganglioneuroma of the urinary bladder | Hiroyuki Usuda | 2005 | Urinary bladder | 73 | Male | 40 × 30 × 25 | Dysuria | Elevated serum catecholamine and elevated VMA** and catecholamine in 24-hour urine collection |
| 4 | Composite paraganglioma-ganglioneuroma of the urinary bladder: a rare neoplasm causing hemodynamic crisis at tumor resection | C-H Chen | 2009 | Urinary bladder | 64 | Male | 50 × 40 × 30 (Residual tumor: 18 × 11 × 30) | Gross painless hematuria | Not measured |
| 5 | Composite Paraganglioma and Neuroblastoma of the Urinary Bladder: A Rare Histopathological Entity | Evan Lacefield | 2015 | Urinary bladder | 45 | Male | 44(diameter) | Flank, abdominal pain and dysuria | Elevated serum normetanephrine, urine VMA**, urine norepinephrine and chromogranin A |
| 6 | Composite Paraganglioma: Pioneering in the Head and Neck | Santiago Delgado | 2019 | Neck | 50 | Female | 59 × 12 × 5 | Incidental finding of an enhancing mass in the right carotid space, (8 months after the first diagnosis) neck pain, anxiety, and episodes of dizziness, | 24-hour urine catecholamine levels, including epinephrine, norepinephrine and dopamine were measured to be within normal range |
| 7 | Composite paraganglioma-ganglioneuroma in the retroperitoneum | Shoji Hirasaki | 2009 | Retroperitoneum | 63 | Female | 65 × 50 × 30 | Left femoral shaft fracture and left leg edema | Serum adrenaline, noradrenaline and dopamine were measured to be within normal range |
| 8 | Composite paraganglioma with ganglioneuroma in the retroperitoneal space | Hideaki Ito | 2010 | Retroperitoneum | 31 | Female | 60 × 50 × 46 | Referral for evaluation of pulmonary embolism after she had a scheduled Cesarean section at 37 weeks of pregnancy. Incidental finding of the mass after CT | NR* |
| 9 | Adrenal and Extra-Adrenal Non-functioning Composite Pheochromocytoma/Paraganglioma with Immunohistochemical Ectopic Hormone Expression: Comparison of Two Cases | Jing Gong | 2010 | Retroperitoneum | 50 | Male | 45 × 40 × 25 | Dull back pain for 3 months | Serum catecholamine levels and consecutive 2-day measurements of 24-hour urine catecholamine levels were measured to be within normal range |

*(Continued)*

**TABLE 2 |** Continued

| Case | Title | First author | Year of publication | Location of the composite paraganglioma | Age (years) | Gender | Size (mm) | Symptoms | Activity (preoperatively) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | Composite paraganglioma and ganglioneuroma in the retroperitoneum: a case report | Yuji Ohtsuki | 2012 | Retroperitoneum | 68 | Female | 30 × 22 × 20 | Abdominal pain for 4 months | NR* |
| 11 | Retroperitoneal composite pheochromocytoma-ganglioneuroma: a case report and review of literature. | Jinchen Hu | 2013 | Retroperitoneum | 52 | Female | 60 × 50 × 40 | Watery diarrhea and febricity for one day, palpitation and debilitation for 6 hours | Not measured |
| 12 | Composite paraganglioma-ganglioneuroma concomitant with adrenal metastasis of medullary thyroid carcinoma in a patient with multiple endocrine neoplasia type 2B: A case report | Mutsushi Yamasaki | 2016 | Retroperitoneum | 59 | Male | 30(diameter) | Multiple endocrine neoplasia type 2B (MEN2B) | Elevated 24-hour urinary metanephrine and VMA** |
| 13 | Extra-adrenal Composite Paraganglioma with Ganglioneuroma Component Presenting as a Pancreatic Mass | Frediano Inzani | 2009 | Pancreas | 57 | Female | 30 × 30 × 25 | Hypertension | Not measured |
| 14 | Paraganglioneuroma of the duodenum: an evolutionary hybrid? | T Cooney | 1977 | Duodenum | 65 | Female | 10(diameter) | Incidental finding of the mass during necropsy | Not measured |
| 15 | Ganglioneuromatous paraganglioma of the cauda equina—a pathological case study | Peter Pytel | 2005 | Cauda equina | 74 | Female | 18(diameter) | Back and leg pain without any weakness or other neurological deficits | NR* |
| 16 | Composite ganglioneuroma-paraganglioma of the filum terminale | Ganesh M. Shankar | 2010 | Filum terminale | 47 | Male | 26 × 17 × 12 | 8-week history of worsening lower-back pain, intermittent tingling sensation in the inguinal area and painful bowel movements. Hypertension. | NR* |
| 17 | Recurrent multiple spinal paragangliomas as a manifestation of a metastatic composite paraganglioma-ganglioneuroblastoma | Jens Gempt | 2013 | Spinal and pelvic bone metastatic lesions | 51 | NR* | Not resected, only biopsies obtained from the bone marrow | Low back pain, radicular and progressive ataxia | NR* |

*NR, Not Reported.*
**VMA, Vanillylmandelic Acid.*

**TABLE 3 |** The grading system for adrenal pheochromocytoma and paraganglioma (GAPP score).

| GAPP parameters | Points scored |
|---|---|
| *Histological pattern* | |
| Zellballen | 0 |
| Large and irregular cell nest | 1 |
| Pseudorosette (even focal) | 1 |
| *Comedo-type necrosis* | |
| Absence | 0 |
| Presence | 2 |
| *Cellularity* | |
| Low (<150 cells/U) | 0 |
| Moderate (150–250 cells/U) | 1 |
| High (>250 cells/U) | 2 |
| *Ki67 labeling index (%)* | |
| <1 | 0 |
| 1–3 | 1 |
| >3 | 3 |
| *Vascular or capsular invasion* | |
| Absence | 0 |
| Presence | 1 |
| *Catecholamine type* | |
| Non-functioning | 0 |
| Adrenergic type | 0 |
| Noradrenergic | 1 |
| **Total maximum score** | **10** |

(21). Dhir et al. reported that the likelihood of malignant disease is greater among younger patients, having a larger-sized tumor or being diagnosed with paraganglioma, as well as in patients with mutations in succinate dehydrogenase complex (SDHD) gene (22). Metastatic lesions are almost always derived from the neural component. Pheochromocytoma metastases, as a single entity or in conjunction with the malignant neural component, were found in some uncommon cases in which liver metastatic lesions were reported deriving from a composite paraganglioma (23). Fortunately, in our case, a locally advanced tumor according to imaging examination was not confirmed. However, based on the histopathological and immunohistochemical characteristics, this composite paraganglioma of our patient is considered to have high malignancy potential, based on the GAPP score classification (**Table 3**). According to the detailed pathology report, the excised tumor had some foci of "zellballen and pseudorosette-forming" pattern, moderate to high cellularity, vascular and capsular invasion, Ki-67 immunoreactivity more than 1%, and also coagulation necrosis. In addition, the immunohistochemical analysis reported that the tumor was found to be positive in protein S100, which is also associated with a worse prognosis. As a result, this tumor scored 6–7 points and was classified as a tumor with moderate to low differentiation and of high metastatic risk (24). This is the reason why a very strict active surveillance of the patients is mandatory. Three months postoperatively, imaging examinations with CT scan and ultrasound were completed and no recurrent or metastatic disease has been documented so far.

In conclusion, composite paragangliomas comprise rare and potentially malignant tumors with variable prognosis. Establishing their diagnosis promptly is of vital significance. Based on our case, due to the first-described location of a composite paraganglioma near the celiac artery, the differential diagnosis of the tumors found in this area should include composite paragangliomas as well.

## AUTHOR CONTRIBUTIONS

GT and AMe were the chief investigators, wrote the manuscript, and collected the majority of the data. AC and AT conducted the histopathological and immunohistochemical analysis. IP and SA were the surgeons who conducted the excision of the tumor. II collected some additional data for the study. TP wrote and corrected the manuscript for its scientific basis. AMi was the director of the Department of Surgery and provided his permission for this study. All authors have read and approved the final manuscript.

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# The Role of Primary Tumor Resection in Patients With Pancreatic Neuroendocrine Tumors With Liver Metastases

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**Background:** Liver metastases (LMs) are common in advanced pancreatic neuroendocrine tumor (PNET) patients. Currently, the benefit of primary tumor resection (PTR) in the setting of PNET patients with liver metastases is still controversial in several guidelines.

**Methods:** Data were extracted from the Surveillance, Epidemiology and End Results (SEER) database to evaluate this issue. The main index of interest in our study was overall survival time.

**Results:** Information on 536 PNET patients with liver metastases from the SEER database was identified. A total of 214 patients (PTR group) received primary tumor resection, and more than half of them (132 patients) had synchronous LM resection. The other 322 PNET patients (non-PTR group) with liver metastases did not receive primary tumor resection. A significant survival benefit was gained from PTR when compared with non-PTR patients, both in OS (72.93 ± 2.7 *vs*. 36.80 ± 2.22 months) and 3- or 5-year survival rates (75.1% *vs*. 28.9% and 67.9% *vs*. 22.3%, respectively). No difference was found between PTR alone and PTR with synchronous LM resection. From univariate and multivariate analyses, younger age (<65 years) and good or moderate tumor differentiation may be more important when considering primary tumor resection. However, we found that all grades of tumor differentiation could result in a better overall survival time after primary tumor resection.

**Conclusion:** Our study suggested that primary tumor resection in pancreatic neuroendocrine patients with liver metastases could result in a longer survival time. Primary tumor resection with synchronous liver metastasis resection was not related to a better survival benefit. This treatment strategy may routinely be taken into consideration in these patients.

**Keywords: primary tumor resection, pancreatic neuroendocrine tumors, liver metastases, tumor differentiation, overall survival (OS)**

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**Abbreviations:** LM, liver metastases; PNETs, pancreatic neuroendocrine tumors; PTR, primary tumor resection; SEER, Surveillance, Epidemiology and End Results; PNELM, pancreatic neuroendocrine tumors liver metastases; OS, overall survival; NCCN, National Comprehensive Cancer Network; SI-NETs, small intestinal neuroendocrine tumors; ENETS, European Neuroendocrine Tumor Society; NANETS, North American Neuroendocrine Tumor Society; PRRT, peptide receptor radionuclide therapy; PFS, progression-free survival.

# INTRODUCTION

Pancreatic neuroendocrine tumors (PNETs) are a heterogeneous group of neoplasms representing approximately 1% of all pancreatic cancers by incidence and 10% of pancreatic cancers by prevalence (1). Surgical resection remains the primary and potentially curative treatment approach for PNETs. However, most patients have metastatic disease at diagnosis that often occurs first in the liver, and approximately 28-77% of patients develop liver metastases (LM) in their lifetime (2, 3). Management of pancreatic neuroendocrine tumor liver metastases (PNELMs) may depend on whether the liver disease is resectable.

For patients with limited liver metastases, surgical resection of both the primary tumor and hepatic disease in a staged or synchronous fashion is recommended. The role and benefit of primary site resection (PTR) in patients with unresectable liver metastases are still controversial. A recent systematic review and meta-analysis showed that palliative resection of primary PNETs in patients with unresectable metastatic liver disease can increase overall survival time (OS), but there was a bias toward patients with better performance status, less advanced disease, or a tumor located in the body or tail of the pancreas (4). Similar findings were demonstrated in another meta-analysis, but the limitations of the included studies do not allow firm conclusions (5). Until now, there has been no adequate robust evidence for whether a primary tumor should be resected in the presence of unresectable liver metastases. Moreover, additional pancreatic resection morbidity, the relatively indolent behavior, and the lower symptomatic presentation of nonfunctional PNETs should be taken into consideration.

Therefore, we designed this study to investigate whether primary tumor resection has a survival benefit in patients with pancreatic neuroendocrine tumors with liver metastases, even if the liver metastases are unresectable.

# MATERIALS AND METHODS

## Patient Collection and Data Source

We used SEER*Stat software version 8.3.8 to retrieve the data for our study from the Surveillance, Epidemiology, and End Research (SEER) database (SEER Research Data, 18 Registries, Nov 2019 Sub 2000–2017). The primary sites for tumors of the pancreas were based on the column of site and morphology, which was labeled C25.0 to C25.9. The patients were enrolled according to the International Classification of Disease for Oncology, third edition (ICD-O-3) histology/behavior codes: pancreatic endocrine tumor, malignant (8150/3), insulinoma, malignant (8152/3), glucagonoma, malignant (8153/3), vipoma, malignant (8155/3), somatostatinoma, malignant (8156/3), enterochromaffin-like cell tumor, malignant (8242/3), goblet cell carcinoid (8243/3), neuroendocrine carcinoma, NOS (8246/3), and atypical carcinoid tumor (8249/3). Patient demographics included sex, age at diagnosis, year of diagnosis, grade, tumor size, surgery for primary site (derived from column RX Summ—Surg Prim Site (1998+)), surgery for distant sites (derived from column RX Summ—Surg Oth Reg/Dis (2003+)), survival months, vital status and SEER cause-specific death classification.

The selection criteria were as follows: (1) patients who had one primary cancer only and pancreatic NETs was the first; and (2) patients who had liver metastasis only at the time of diagnosis without other known sites of metastasis. The exclusion criteria were as follows: (1) incomplete follow-up information; (2) unknown cause of death or death attributed to causes other than this cancer; and (3) unknown characteristics.

## Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 21.0. Patients' baseline characteristics, tumor characteristics, and treatments were compared by the Mann–Whitney *U* test or Pearson chi-squared test. Data are presented as percentages or mean values. We used Kaplan–Meier curves to analyze the overall survival time (OS), and the differences between groups were compared by the log-rank test. Univariate and multivariate analyses were performed using Cox proportional hazards models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. A $p$-value less than 0.05 was defined as statistically significant.

# RESULTS

## Baseline Characteristics of Patients and Tumors

A total of 536 patients were included based on our inclusion criteria (**Figure 1**). All of these patients were diagnosed with pancreatic neuroendocrine tumors and had liver metastasis at diagnosis. The baseline characteristics are summarized in **Table 1**. There were 323 men and 213 women in this population, and the median age at diagnosis was 58 years. The number of patients with pancreatic head tumors (171 patients, 31.9%) was similar to that at the pancreatic tail (190 patients, 35.4%). The mean tumor size was 54.44 ± 31.57 mm, and 59.9% of tumors were more than 4 cm. All patients were pathologically diagnosed after resection surgery of biopsy, and the most common pathological type was neuroendocrine carcinoma, comprising 54.5% of these populations. Moreover, five tumors were functional PNETs, including two patients with insulinoma, two patients with gastrinoma, and one with glucagonoma. Based on the degree of differentiation, tumors were divided into four grades (grade I: well differentiated; grade II: moderately differentiated, grade III: poorly differentiated, grade IV: undifferentiated). Approximately 76.3% of patients were well and moderately differentiated. All patients had liver metastases at diagnosis without other known sites (such as lung, brain, bone) of metastases.

## Primary Tumor Resection and Survival Time

A total of 39.9% of patients (214 of 536 patients) received primary tumor resection, except for 8 patients who were recommended for surgery but not performed. The rest of the patients were not recommended for surgery. Surgical procedures included partial pancreatectomy (consisting of partial pancreatectomy and local excision of tumors), pancreaticoduodenectomy (with or without distal/partial gastrectomy), and total pancreatectomy. The median

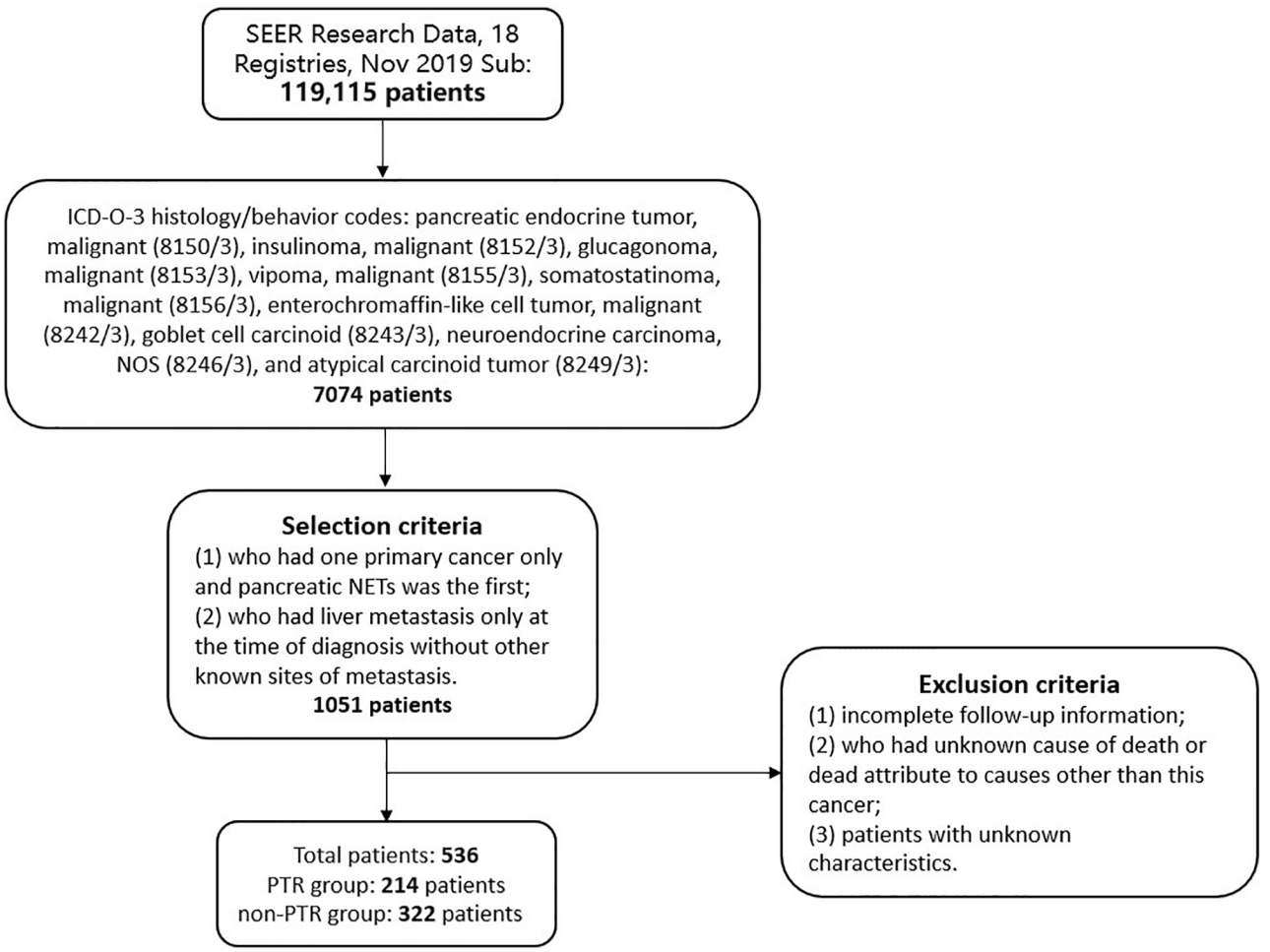


**FIGURE 1** | Flow chart of patients selection.

follow-up time was 43 months (1–95 months). The mean OS of all patients was 53.54 ± 2.03 months. Significant differences existed between PTR patients and non-PTR patients, and the OS of these two groups was 72.93 ± 2.70 and 36.80 ± 2.22 months, respectively ($p = 0.000$). The 3- and 5-year survival rates of PTR patients were 75.1% and 67.9%, respectively, while the same indexes of non-PTR patients were 28.9% and 22.3%, respectively. Additionally, we found no significant difference in the PTR group with or without LM resection. (PTR patients with LM resection vs. without LM resection: 71.48 ± 3.46 vs. 73.47 ± 4.01, $p = 0.528$) (**Figure 2**).

Based on tumor differentiation, all four grade groups showed that PTR significantly improved survival time (**Table 2**), especially in the grade III group (poor differentiation). The OS of the PTR patients was nearly 5-fold that of the non-PTR patients (64.58 ± 7.90 vs. 12.95 ± 2.53). In PTR patients, worse tumor differentiation was associated with decreased OS. The same results were shown when dividing patients based on tumor size into three groups (tumor size ≤2 cm, 2 cm < tumor size ≤4 cm, tumor size >4 cm). All 14 patients who received primary tumor resection with a tumor size less than 2 cm survived at the end of follow-up. Different surgical procedures also led to different outcomes. Patients who received partial pancreatectomy had better OS than the other two groups, which may be related to higher tumor differentiation, smaller tumor size, and lower additional mortality associated with the surgical procedure (**Figures 3**–**5**).

From univariate and multivariate analyses, we found that age over 65 years (HR: 1.493, 95% CI: 1.137–1.962), poorly differentiated or undifferentiated tumors (HR: 4.102, 95% CI: 2.942-5.721; HR: 3.338, 95% CI: 2.043–5.455, respectively) and primary tumor resection (HR: 3.771, 95% CI: 2.702–5.263) were independent risk factors related to overall survival time (**Table 3**).

## DISCUSSION

Current National Comprehensive Cancer Network (NCCN) guidelines for neuroendocrine tumors of the pancreas support resection of the primary site and metastases if complete resection is possible, and both staged and synchronous resection are recommended (6). However, the role of primary tumor resection

**TABLE 1** | Baseline characteristics of pancreatic neuroendocrine tumors with liver metastases.

| | All patients (*n* = 536) | Primary tumor resection (*n* = 214) | Nonprimary tumor resection (*n* = 322) | *p*-value |
|---|---|---|---|---|
| Age | 57.99 ± 13.77 | 60.61 ± 13.01 | 54.05 ± 13.87 | 0.000 |
| Sex | | | | 0.073 |
| Male | 323 (60.3%) | 119 (55.6%) | 204 (63.4%) | |
| Female | 213 (39.7%) | 95 (44.4%) | 118 (36.6%) | |
| Primary site | | | | 0.159 |
| Head | 171 (31.9%) | 59 (27.6%) | 112 (34.8%) | |
| Body | 55 (10.3%) | 23 (10.7%) | 32 (9.9%) | |
| Tail | 190 (35.4) | 89 (41.6) | 101 (31.4%) | |
| Neck | 12 (2.2) | 4 (1.9%) | 8 (2.5%) | |
| Overlap lesions | 60 (11.2%) | 19 (8.9%) | 41 (12.7%) | |
| NOS | 48 (9.0%) | 20 (9.3%) | 28 (8.7%) | |
| Histology | | | | 0.047 |
| Neuroendocrine carcinoma | 292 (54.5%) | 110 (51.4%) | 182 (56.5%) | |
| Carcinoid tumor | 172 (32.1%) | 66 (30.9%) | 106 (32.9%) | |
| Atypical carcinoid tumor | 54 (10.1%) | 27 (12.6%) | 27 (8.5%) | |
| Neuroendocrine tumor | 13 (2.4%) | 9 (4.2%) | 4 (1.2%) | |
| Insulinoma | 2 (0.4%) | 0 (0%) | 2 (0.6%) | |
| Gastrinoma | 2 (0.4%) | 2 (0.9%) | 0 (0%) | |
| Glucagonoma | 1 (0.1%) | 0 (0%) | 1 (0.3%) | |
| Tumor differentiation | | | | 0.001 |
| I. Well differentiation | 248 (46.3%) | 109 (51.0%) | 139 (43.1%) | |
| II. Moderately differentiation | 159 (29.7%) | 73 (34.1%) | 86 (26.7%) | |
| III. Poorly differentiation | 98 (18.3%) | 26 (12.1%) | 72 (22.4%) | |
| IV. Undifferentiation | 31 (5.7%) | 6 (2.8%) | 25 (7.8%) | |
| Tumor size (mm) | 54.44 ± 31.57 | 52.41 ± 28.66 | 57.5 ± 35.34 | 0.337 |
| ≤2 cm | 35 (6.5%) | 14 (6.5%) | 21 (6.5%) | |
| 2–4 cm | 180 (33.6%) | 75 (35.1%) | 105 (32.6%) | |
| >4 cm | 321 (59.9%) | 125 (58.4%) | 196 (60.9%) | |
| Surgical procedure | | | | |
| None | | 0 | 322 | 0.000 |
| Partial pancreatectomy | | 104 | 0 | |
| Pancreaduodenectomy | | 88 | 0 | |
| Total pancreatectomy | | 22 | 0 | |

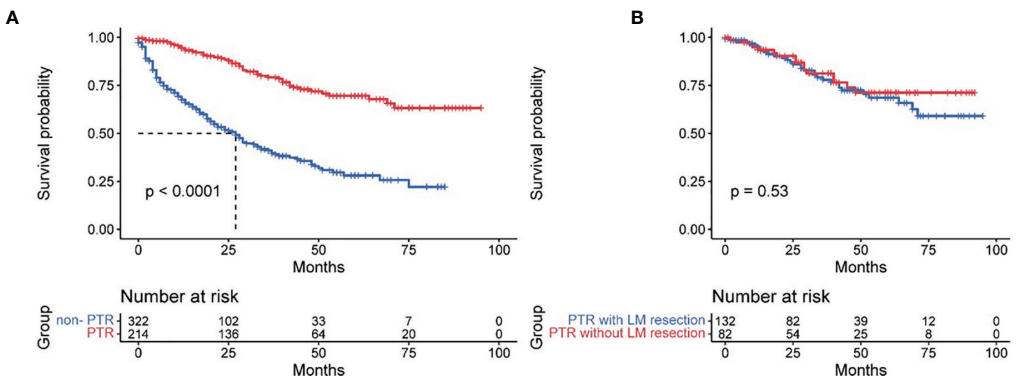


**FIGURE 2** | Effect of primary tumor resection **(A)** and with/without liver metastases resection **(B)** in pancreatic neuroendocrine tumor patients with liver metastases.

for PNET patients with unresectable liver metastases is still controversial. For small intestinal neuroendocrine tumors (SI-NETs), palliative PTR may prevent or solve complications such as bowel obstruction or intestinal ischemia associated with primary tumors. Thus, primary tumor resection of intestinal NETs is strongly recommended even in the presence of liver or lymph node metastases (7). In contrast, a systematic review meta-analysis of midgut neuroendocrine tumor patients with unresectable metastatic liver disease suggested that PTR had a significant role in improving OS with a low perioperative risk of mortality (8). In the setting of unresectable PNELM, neither the European Neuroendocrine Tumor Society (ENETS) nor the North American Neuroendocrine Tumor Society (NANETS) guidelines recommend routine palliative primary resection (9, 10).

Our findings show that primary tumor resection in pancreatic neuroendocrine tumor patients with liver metastases is

**TABLE 2** | Overall survival time in different groups.

| | Primary tumor resection (*n* = 214) | Nonprimary tumor resection (*n* = 322) | *p*-value |
|---|---|---|---|
| Primary tumor resection | 72.93 ± 2.70 | 36.80 ± 2.22 | 0.000 |
| Liver metastases resection | | | |
| Yes | 71.48 ± 3.46 | | |
| No | 73.47 ± 4.01 | | |
| Tumor differentiation | | | |
| I: Well differentiation | 77.250 ± 3.44 | 46.717 ± 3.49 | 0.000 |
| II: Moderately differentiation | 69.67 ± 4.71 | 46.5 ± 4.32 | 0.001 |
| III: Poorly differentiation | 61.58 ± 7.90 | 12.95 ± 2.53 | 0.000 |
| IV: Undifferentiation | 31.17 ± 7.46 | 16.31 ± 4.10 | 0.067 |
| Tumor size (mm) | | | |
| ≤2 cm | All alive | 28.86 ± 6.41 | |
| 2–4 cm | 66.37 ± 4.44 | 32.10 ± 3.68 | 0.000 |
| >4 cm | 72.45 ± 3.62 | 39.58 ± 2.86 | 0.000 |
| Surgical procedure | | | |
| Partial pancreatectomy | 74.24 ± 3.42 | | |
| Pancreaduodenectomy | 70.72 ± 4.34 | | |
| Total pancreatectomy | 60.35 ± 6.70 | | |

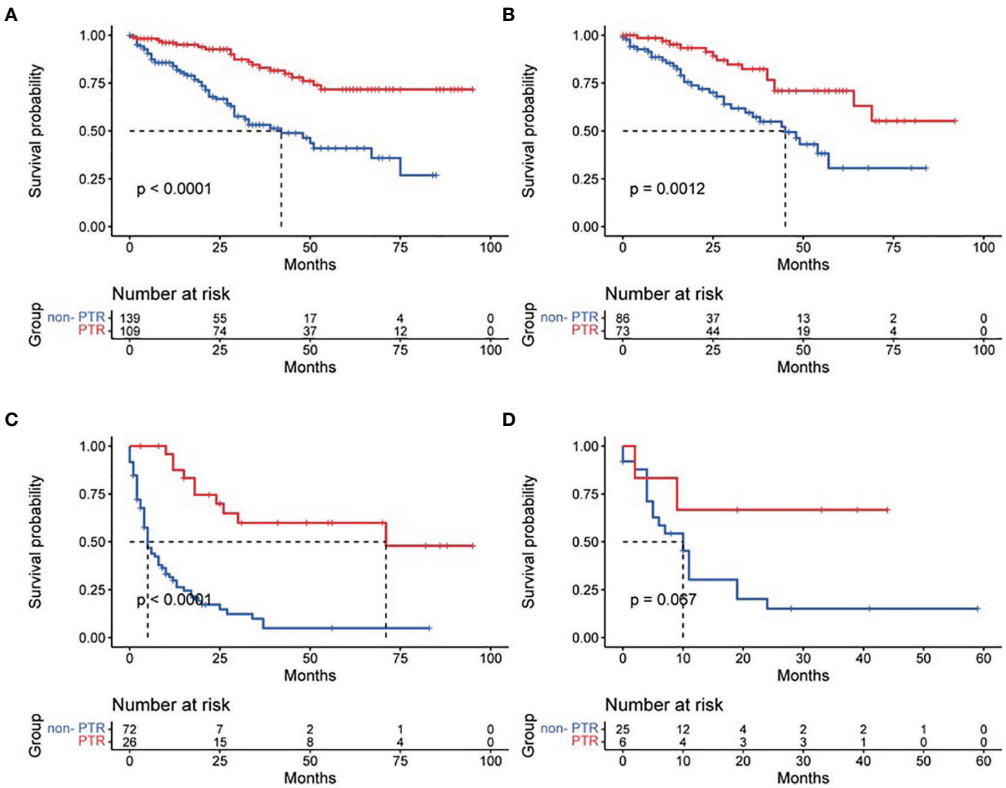


**FIGURE 3** | Effect of primary tumor resection in patients with different tumor differentiation: **(A)** well-differentiated patients; **(B)** moderately differentiated patients; **(C)** poorly differentiated patients; and **(D)** undifferentiated patients.

significantly associated with improved survival time. Furthermore, we evaluated potential risk factors related to OS. We found that age less than 65 years and well-differentiated or moderately differentiated tumor grade were associated with prolonged survival. Younger age and well-differentiated tumors may be important selected factors when considering primary tumor resection. Younger patients may have a better physical status to tolerate more aggressive treatment and fewer comorbidities. Citterio reported improved survival times in primary tumors resected from well-differentiated pancreatic NETs (median survival times were 138 and 37 months, respectively) (11). Furthermore, according to our findings, all differentiation grades

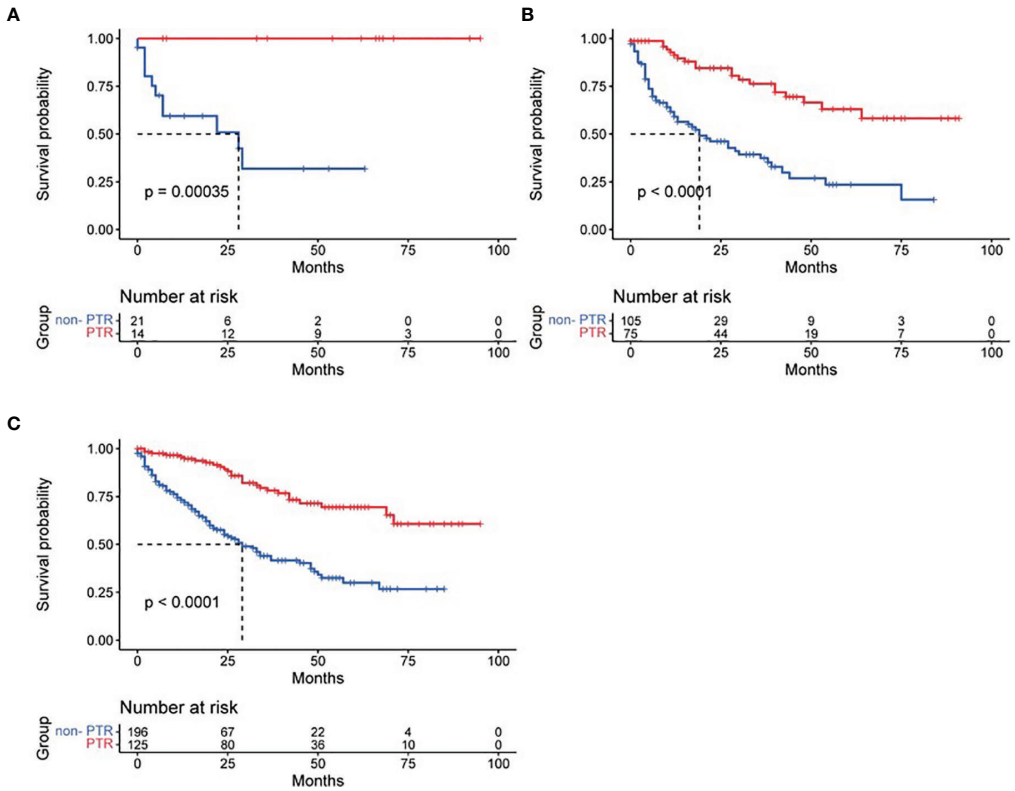


**FIGURE 4** | Effect of primary tumor resection in patients with different tumor sizes: **(A)** tumor size ≤2 cm; **(B)** tumor size 2–4 cm; **(C)** tumor size >4 cm.

had significantly better OS after PTR, especially in patients with poorly differentiated tumors, and PTR increased survival by nearly 5-fold.

Other factors, such as sex, tumor location, tumor size, and surgical procedures, were not significantly independent factors. Both the ENATS and NANETS guidelines proposed tumor location as a surgical selection factor. For nonfunctional PNETs, primary tumors located in the head of the pancreas are related to higher odds of specific symptoms, such as jaundice or duodenal occlusion, and these complications could be solved by endoscopic or surgical bypasses (9). In addition, distal pancreatectomy has lower morbidity than pancreaticoduodenectomy (Whipple procedure). Thus, primary lesions located in the body or tail may be more favorable for resection and derive better quality of life and outcomes (9, 10). A previous study supported the positive survival benefit of PTR in PNET patients of the body and tail with unresectable liver metastases when compared with non-PTR individuals (median survival time: 111 vs. 52 months) (12). We evaluated whether different surgical procedures had different outcomes, and the results showed that partial pancreatectomy and pancreaticoduodenectomy had similar OS. Moreover, univariate analysis also showed that both tumor location and surgical procedure were not significant independent risk factors. Tumor location and surgical procedures may not be limiting conditions in deciding whether to perform primary tumor resection with liver metastases.

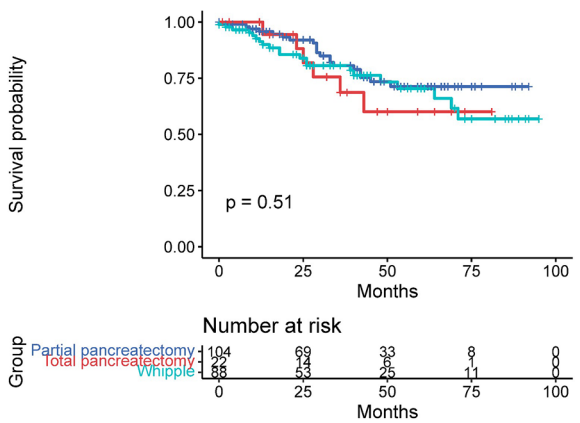


**FIGURE 5** | Overall survival time in patients who received different surgical procedures.

Peptide receptor radionuclide therapy (PRRT) in somatostatin-positive NETs is a replacement treatment strategy in patients who are not suitable for radical resection, and PRRT could result in disease stabilization, partial remission, or reduction of tumor mass (13). A lower tumor burden and smaller lesions may allow a high dose of concentration and a higher chance of tumor response (14);

**TABLE 3 |** Possible variables at univariate and multivariate analyses in pancreatic neuroendocrine patients with liver metastases.

| Risk factors | Category | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | Hazard ratio (95% CI) | *p*-value | Hazard ratio (95% CI) | *p*-value |
| Age | ≤65 years | 1 | | 1 | |
| | >65 years | 1.73 (1.319–2.268) | 0.000 | 1.493 (1.137–1.962) | 0.004 |
| Sex | Female | 1 | | | |
| | Male | 0.869 (0.660–1.145) | 0.319 | | |
| Primary tumor location | Head | 1 | | | |
| | Body | 1.111 (0.664–1.860) | 0.688 | | |
| | Tail | 0.807 (0.420–1.553) | 0.522 | | |
| | Neck | 0.792 (0.472–1.329) | 0.378 | | |
| | Overlapping lesions | 0.879 (0.326–2.369) | 0.799 | | |
| | NOS | 0.986 (0.540–1.798) | 0.963 | | |
| Tumor size | ≤2 cm | 1 | | | |
| | 2–4 cm | 0.759 (0.409–1.407) | 0.381 | | |
| | >4 cm | 1.242 (0.937–1.647) | 0.132 | | |
| Tumor differentiation | Well differentiation | 1 | | 1 | |
| | Moderately differentiation | 1.055 (0.734–1.517) | 0.773 | 1.004 (0.698–1.445) | 0.983 |
| | Poorly differentiation | 4.024 (2.895–5.595) | 0.000 | 4.102 (2.942–5.721) | 0.000 |
| | Undifferentiation | 4.093 (2.510–6.673) | 0.000 | 3.338 (2.043–5.455) | 0.000 |
| Primary tumor resection | Yes | 1 | | 1 | |
| | No | 3.88 (2.800–5.396) | 0.000 | 3.771 (2.702–5.263) | 0.000 |
| Surgical procedure | Partial pancreatectomy | 1 | | | |
| | Pancreaticoduodenectomy | 0.651 (0.261–1.622) | 0.357 | | |
| | Total pancreatectomy | 0.881 (0.355–2.184) | 0.784 | | |

thus, palliative or debulking surgery may increase the response to PRRT. Based on this hypothesis, a previous study in the setting of G1-G2 PNETs with diffuse liver metastases suggested that PTR prior to PRRT results in better progression-free survival (PFS) (70 vs. 30 months, $p = 0.02$) and OS (112 vs. 65 months, $p = 0.011$) (15). Another recent study also found that PTR before PRRT provides a significant survival benefit in patients with stage IV neuroendocrine neoplasms, and both PFS and OS improved (134 vs. 67 months, $p < 0.001$ and 18 vs. 14 months, $p = 0.012$, respectively) (16). These results provide us with a novel strategy for the combination of primary tumor resection and PRRT for advanced pancreatic neuroendocrine tumors with distant metastases.

Due to the relatively low incidence and heterogeneity of pancreatic neuroendocrine tumors, it is difficult to design randomized trials to provide strong evidence for standard treatment strategies. Our study also had some limitations due to its retrospective nature and selection bias. First, the tumor differentiation grade from the SEER database is different from the current guidelines, which are based on mitoses in a high power field and the Ki-67 index. Second, we do not have information about adjuvant therapies and postoperative therapies, which may influence the survival analysis in all patients. Third, the tumor burden of liver metastases (tumor location and number of lesions) may be a confounding variable. Fourth, the SEER database did not include tumor margin information.

Although several limitations exist in our study, we still suggest the significant role of primary tumor resection in pancreatic neuroendocrine tumors with liver metastases for improving survival time. Although all patients who receive resectable primary tumors may be potentially beneficial, younger patients and well- or moderately differentiated primary PNETs should be preferentially considered.

## CONCLUSIONS

Primary tumor resection is associated with longer survival in pancreatic neuroendocrine tumor patients with liver metastases, but additional synchronous liver metastasis resection was not related to better overall survival time. The combination of primary tumor resection and other treatment strategies (e.g., peptide receptor radionuclide therapy) may result in a better outcome.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## AUTHOR CONTRIBUTIONS

N-WK and X-BL designed the study. Y-HC and C-LT acquired the data. Z-YW analyzed and interpreted the data. YM wrote the paper. N-WK critically revised the manuscript for important intellectual content. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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# The Global States and Hotspots of ERAS Research From 2000 to 2020: A Bibliometric and Visualized Study

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**Background:** Enhanced recovery after surgery (ERAS) protocol has been implemented in surgeries for more than 20 years, this study investigated the global states and hotspots of ERAS research.

**Methods:** Based on the Web of Science database, a bibliometric and visualized study of original ERAS research from 2000 to 2020 was performed, including the trends of publications and citations; distribution of countries, authors, institutions, sources; study design, level of evidence, served surgeries and surgical disciplines. Hotspots were revealed by research interests and keywords.

**Results:** Within the field of original ERAS research, there was a rising trend in annual publications and citations. The USA was the greatest contributor. Kehlet, H, University of Copenhagen were the most influential author and institution, respectively. British Journal of Surgery and Annals of Surgery were the most cited journals. Though there were more prospective designs, more than half of the studies presented level IV evidence and had fewer citations and citation densities compared to that of level II and level III. ERAS protocol was overwhelmingly implemented in colorectal surgeries. Most studies focused on elements of ERAS, the top three research interests were "length of stay," "pain management," and "complications." In recent years, bariatric surgery, compliance with ERAS, and feasibility in the elderly were new hotspots.

**Conclusion:** Revealing the global states and hotspots can help researchers better understand the trends in ERAS research. The USA was the greatest contributor to ERAS research. Kehlet, H, was the most influential author in the field. Bariatric surgery, compliance with ERAS, and feasibility in the elderly represent the new trend of ERAS research. Most of the ERAS research had a low evidence levels, studies with high-level evidence are still required in this field.

**Keywords: ERAS, fast-track, bibliometric, visualized study, surgery, hotspots**

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## INTRODUCTION

In 2001, the Enhanced Recovery After Surgery (ERAS) Study Group was established in Europe, then they published the first consensus for colonic resection in 2005 (1). Before that, the concept of fast-track surgery had been used for several years. The earliest attempt of accelerated recovery after surgery can be traced back to 1990, Krohn BG et al. reported the experience of rapid recovery for

open heart operations for the first time (2). In 1994, Engelman RM (3) described the first fast-track recovery protocol for patients undergoing cardiopulmonary bypass surgery, followed by similar research on multimodal management protocols for colonic surgery (4) and open sigmoidectomy (5). Though the ERAS concept has been widely adopted around the world, and the ERAS Group wanted to emphasize the importance of quality rather than speed of recovery (6), many researchers still use the concept of "fast-track surgery." Meanwhile, as an important part of ERAS protocol, fast-track anesthesia has been promoted greatly by the progress of ERAS. To our knowledge, few studies have systematically analyzed the global states and hotspots of original ERAS research, we performed a 20-year bibliometric and visualized study to help researchers better understand the trends in ERAS research.

# METHODS

## Literature Search

This study was based on the Web of Science (WOS) database. The following search strategy was used: "TS=enhanced recovery after surgery" or "TS=fast-track surgery," all subdatabases were retrieved, including Web of Science Core Collection, SciELO Citation Index, KCI-Korean Journal Database, MEDLINE®, BIOSIS Previews, and Russian Science Citation Index. The publication year was restricted from 2000 to 2020, regardless of language.

## Article Screening

Literature were screened online on the WOS website, articles that contained "enhanced recovery" or "fast track" in the title or abstract were filtered preliminarily. The further exclusion criteria were as follows: only original research were included, review (including systemic review, meta-analysis, and pooled analysis), guidelines, case reports, expert experiences, consensus, meeting abstract, editorial materials, letters and responses, commentaries, corrections, trial protocols, position papers, animal studies, suggestions, special articles, book chapters, highlights, journal abstracts were excluded. Besides, fast-track diagnostics, fast-track referrals systems, and articles that did not focus on ERAS were excluded. Finally, 2,117 articles were included for bibliometric analysis (**Figure 1**). Articles were imported to literature management software Endnote X9 (Clarivate Analytics, Philadelphia, PA, USA), Microsoft Excel 2019 (Microsoft Corp. Redmond, WA, USA), Vosviewer 1.6.16 (Leiden University, Leiden, The Netherlands), and Citespace 5.7 (Drexel University, Philadelphia, PA, USA) for analysis.

## Bibliometric Analysis

The annual number of publications and citations was counted. The citation density was defined as the citation per year (total citations/years since published). The country distribution was determined by corresponding authors, if there were more than one corresponding author, the last corresponding author was selected.

The level of evidence was graded following the Oxford Center for Evidence-Based Medicine (OCEM) 2011 Levels of Evidence system (7). Two authors looked through the full article texts and graded the level independently, divergences were resolved by discussion. Comparison of citations and citation density was performed among different levels of evidence. Names of surgery in each article were distracted and divided into different surgical disciplines. Research interests were summarized in the full texts.

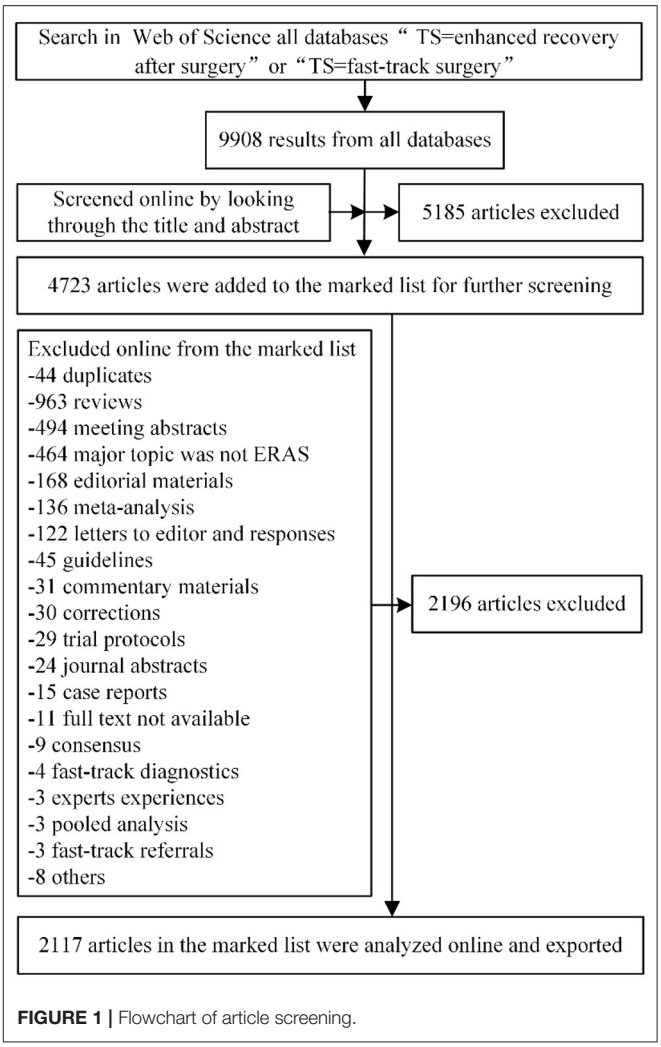



**FIGURE 1 |** Flowchart of article screening.

## Visualization

Vosviewer and Citespace were used for visualized analysis, including bibliographic coupling analysis of authors, institutions, and journals; co-cited analysis of cited authors in the reference lists; co-occurrence analysis of author keywords. For author keywords, an overlay visualization map weighed by average published year was shown in Vosviewer, burst detection was further performed by Citespace to reveal the hotspots by years.

## Statistical Analysis

SPSS 24.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. The distribution of continuous variables was checked by using the sing-sample Kolmogorov-Smirnov

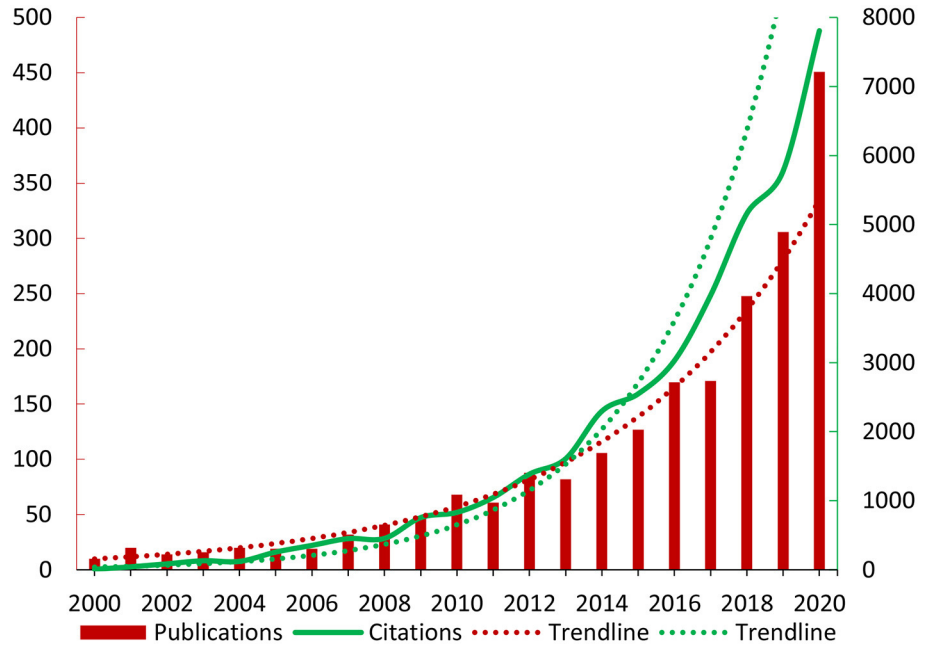



**FIGURE 2 |** The trend in publications and citations.

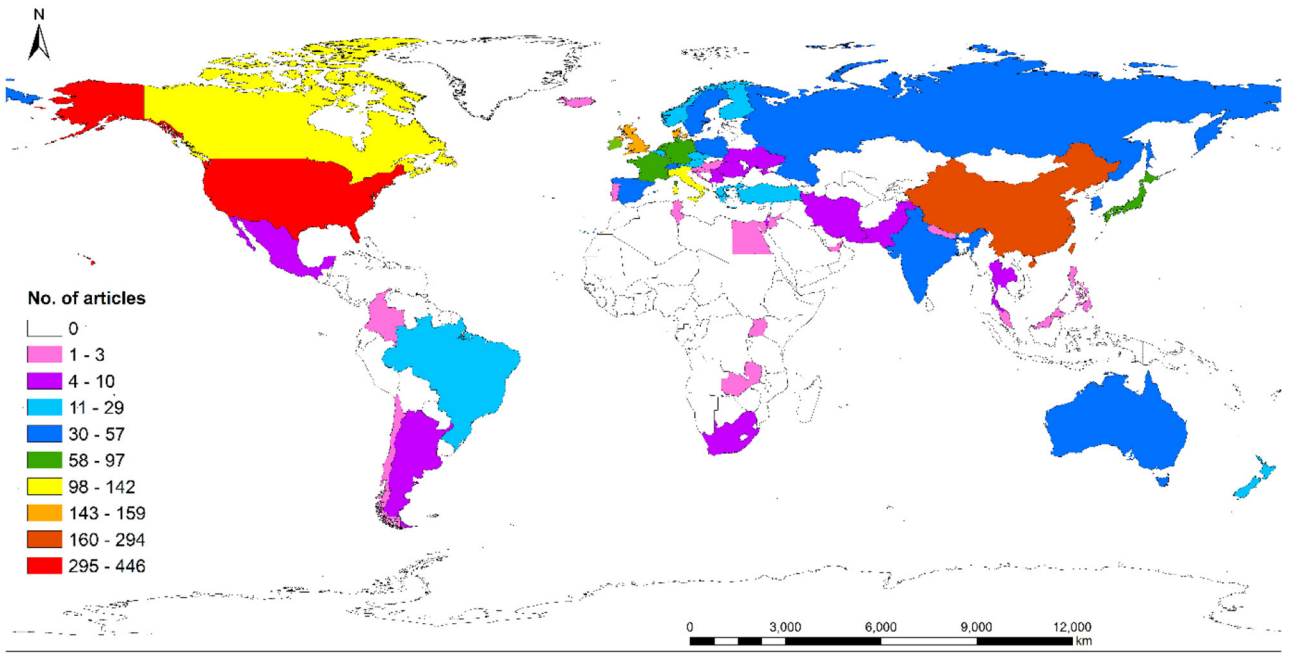



**FIGURE 3 |** Global distribution of ERAS research.

test, non-normally distributed data were presented as median (interquartile range, *IQR*). The homogeneity tests showed uneven variance among multiple samples, thus the analysis of variance was not suitable, comparison between multiple variables was performed by the Kruskal-Wallis H test, which was used for multiple comparisons among independent variables. The Kolmogorov-Smirnov test showed that the years and publications, citations are non-normal distributed data, so the correlation between the year and the publications; year and citations was tested by the Spearman test. The Kappa consistency

**TABLE 1 |** Top 10 countries with the greatest number of publications.

| Country | Publications | Citations | Citations per item | h-index |
|---|---|---|---|---|
| USA | 446 | 6,940 | 15.56 | 42 |
| China | 293 | 2,460 | 8.39 | 26 |
| England | 159 | 4,341 | 27.30 | 34 |
| Denmark | 143 | 5,752 | 40.22 | 38 |
| Italy | 112 | 1,483 | 13.24 | 23 |
| Canada | 98 | 2,083 | 21.25 | 29 |
| Germany | 85 | 2,045 | 24.06 | 25 |
| Japan | 78 | 705 | 9.04 | 15 |
| Netherlands | 74 | 3,213 | 43.42 | 28 |
| France | 75 | 523 | 6.97 | 13 |

test was used to determine the consistency between the two authors in the grading of the level of evidence. The significance level was defined as 0.05.

# RESULTS

## Publications and Citations

Of the 2117 articles, 1720 were from Web of Science Core Collection Database, 351 were from MEDLINE® Database, 19 were from Russian Science Citation Index Database, 12 were from BIOSIS Previews Database, 9 were from SciELO Citation Index Database, and 6 were from KCI-Korean Journal Database. The majority of the articles were written in English (90.55%), followed by Chinese (2.78%), Russian (1.51%), German (1.36%), and Spanish (1.18%), the remaining 15 Languages each counted <1%. By March 18, 2021, the 2117 articles had a total h-index of 83, total citation of 38,114 (29,243 without self-citations). The citations of each article ranged from 0 to 546 times, the median citation was 6 (1, 21), the median citation density was 1.67 (0.36, 3.96).

From 2000 to 2020, there was an annually rising trend of publications and citations (**Figure 2**). The number of citations has been increasing quickly since 2014. Spearman correlation test revealed a strong positive correlation between year and citations ($\rho = 0.99$, $P < 0.001$); year and publications ($\rho = 0.97$, $P < 0.001$).

## Country Distribution

The 2,117 articles were from 56 countries (**Figure 3**), the USA contributed the greatest number of articles, with 446 articles (21.07%) and 6,940 citations, the h-index was 42. The second-largest contributor was China, with 293 articles (13.84%) and 2,460 citations, the h-index was 26. The third was England, with 159 articles (7.51%) and 4,341 citations, the h-index was 34. The top 10 countries with the greatest number of publications were listed in **Table 1**.

## Authors and Institutions

The author with the greatest number of publications and citations was Kehlet, Henrik, with 98 articles and 5,275 citations, the h-index was 38. The visualized analysis showed that the total link strength was 91,772. The second was Demartines, Nicolas, with 34 articles and 1,106 citations, the h-index was 15, the total link strength was 114,763. Followed by Huebner, Martin, with 34 articles and 826 citations, the h-index was 15, the total link strength was 46,188 (**Figure 4A**). The top 10 authors with the greatest number of publications were listed in **Table 2**.

Co-citation analysis of cited authors showed that Kehlet, Henrik was the top-cited author in the reference lists, who was cited by 1,541 times and the total link strength was 22,638. Followed by Gustafsson, Ulf O, cited by 508 times and the total link strength was 8,980. The third was Basse, Line Hollesen, cited by 414 articles and total link strength was 7,083 (**Figure 4B**). The top 10 most cited authors were listed in **Table 3**.

The institution with the greatest number of publications and citations was the University of Copenhagen, with 117 articles and 5,939 citations, and an h-index of 38, the total link strength was 63,857. Followed by the Rigshospitalet, with 94 articles and 3,823 citations, and an h-index of 34, the total link strength was 30,753 (**Figure 4C**). The top 10 institutions with the greatest number of publications were listed in **Table 4**. The top 10 articles with the greatest number of citations were shown in **Table 5**.

## Journals

These articles were published in 597 journals, the journal with the greatest number of publications was Surgical Endoscopy and Other Interventional Techniques, with 54 articles, followed by the World Journal of Surgery, and Colorectal Disease. When weighted by citations, the visualized analysis showed that the British Journal of Surgery and Annals of Surgery had the greatest number of citations, followed by Acta Orthopaedica (**Figure 4D**). The top 10 most often published journals were listed in **Table 6**.

## Study Design and Level of Evidence

There were 692 articles (32.68%) with prospective study design, 549 articles (25.93%) with retrospective design (including retrospective analysis of prospectively collected data), and 22 articles with combined design (1.03%), study designs were not mentioned in the remain 854 articles. In terms of levels of evidence, 407 articles presented level II evidence (19.23%), 473 articles presented level III evidence (22.34%), while more than half of them presented level IV evidence (55.69%). The remaining 58 articles could not be graded according to the OCEM system. The agreement between the two authors was excellent ($kappa = 0.97$, $P < 0.001$). Among them, 1,769 articles (83.56%) were therapeutic analysis, 155 articles were prognostic analysis, 101 articles were surveys, 31 articles were cost-effective analysis, 55 articles were other designs.

The Kruskal-Wallis H test showed that there was a significant difference in citations between level II and level IV studies ($Z = 3.37$, $P = 0.001$); level III and level IV studies ($Z = 3.70$, $P < 0.001$). While there was no significant difference in citations between level II and level III studies ($Z = -0.11$, $P = 0.913$) (**Figure 5A**). In terms of citation density, there was a significant difference between level II and level IV studies ($Z = 2.34$, $P = 0.019$); level III and level IV studies ($Z = 4.89$, $P < 0.001$), while there was no significant difference between level II and level III studies ($Z = -1.94$, $P = 0.052$) (**Figure 5B**).

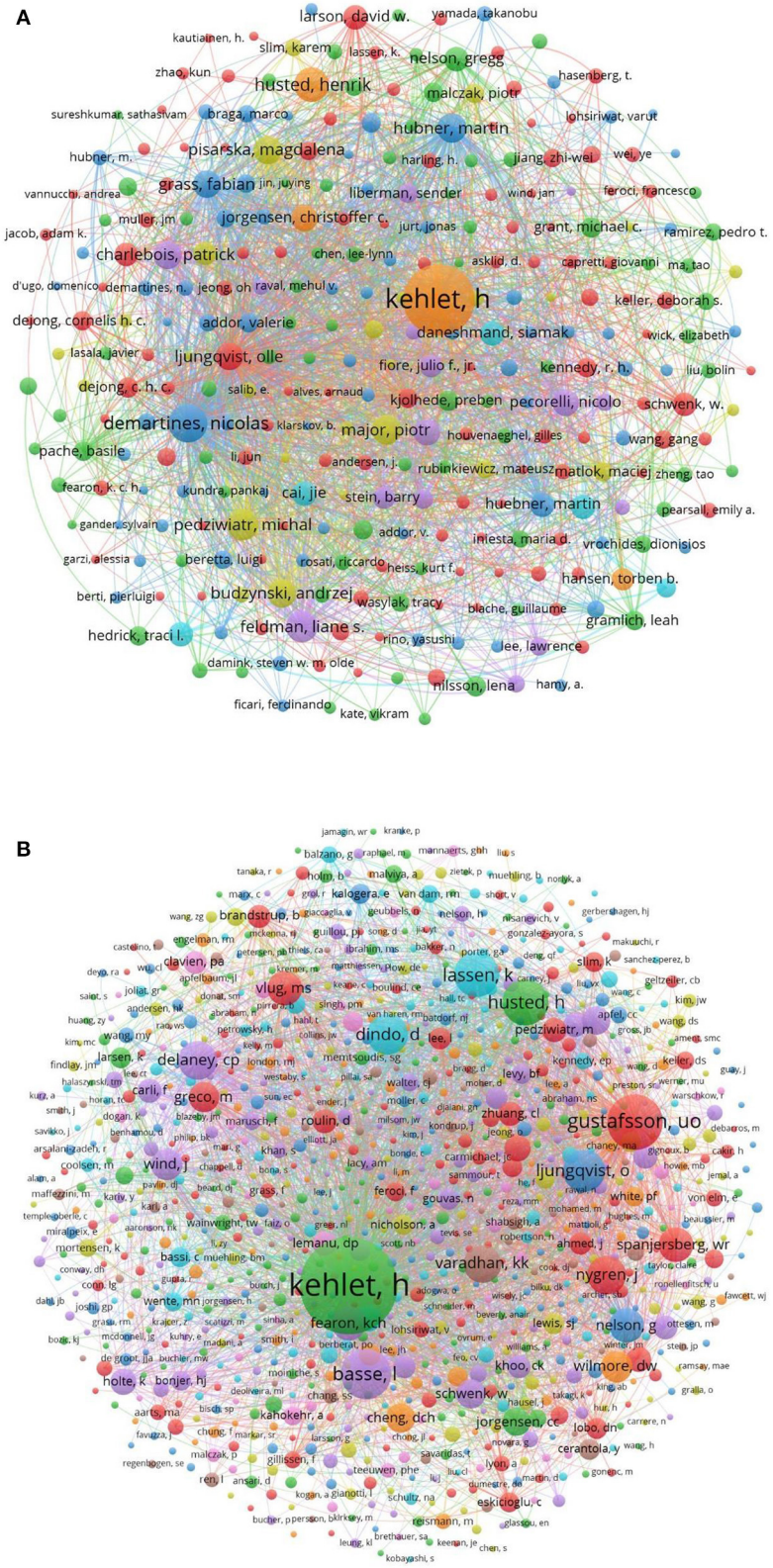



**FIGURE 4 |** *Continued*

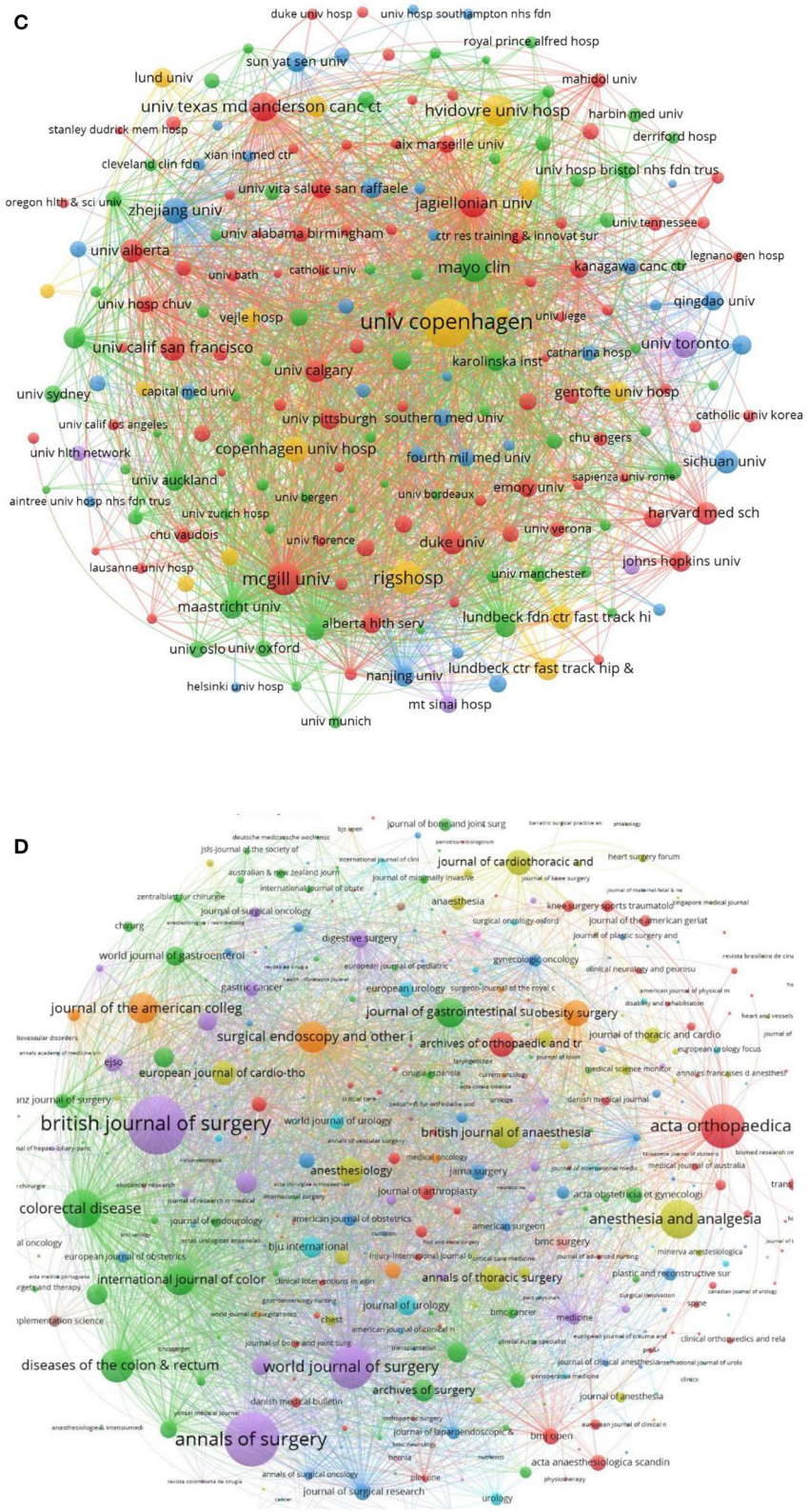



**FIGURE 4 |** Visualized analysis of authors, cited authors, institutions, journals. **(A)** Bibliographic coupling analysis of authors. **(B)** Co-citation analysis of cited authors. **(C)** Bibliographic coupling analysis of institutions. **(D)** Journals.

**TABLE 2 |** Top 10 authors with the greatest number of publications.

| Author | Affiliation | Publications | Citations | h-index |
|---|---|---|---|---|
| Kehlet, Henrik | University of Copenhagen | 98 | 5,275 | 38 |
| Demartines, Nicolas | University of Lausanne | 34 | 1,106 | 15 |
| Huebner, Martin | University of Lausanne | 34 | 826 | 15 |
| Husted, Henrik | University of Copenhagen | 28 | 1,573 | 19 |
| Jorgensen, Christoffer Calov | University of Copenhagen | 27 | 656 | 15 |
| Feldman, Liane S. | McGill University | 22 | 391 | 11 |
| Ljungqvist, Olle | Orebro University | 21 | 2,114 | 17 |
| Carli, Francesco | McGill University | 18 | 538 | 13 |
| Pedziwiatr, Michal | Jagiellonian University | 20 | 459 | 14 |
| Schwenk, Wolfgang | Klin Allgemein | 20 | 536 | 13 |

**TABLE 3 |** Top 10 most-cited authors in the reference lists.

| Author | Affiliation | Cited times | Total link strength |
|---|---|---|---|
| Kehlet, Henrik | University of Copenhagen | 1,541 | 3,331 |
| Gustafsson, Ulf O | University of Lausanne | 508 | 1,402 |
| Basse, Line Hollesen | Novo Nordisk | 414 | 1,452 |
| Husted, Henrik | University of Copenhagen | 346 | 541 |
| Lassen, Kristoffer | National Hospital Norway | 329 | 1,051 |
| Ljungqvist, Olle | McGill University | 287 | 737 |
| Dindo, Daniel | Hirslanden Med Ctr | 271 | 718 |
| Delaney, Conor P. | Dana-Farber Cancer Institute | 249 | 1,032 |
| Nygren, Jonas | Karolinska Institutet | 243 | 923 |
| Varadhan, Krishna K. | Nottingham University Hospital NHS Trust | 235 | 821 |

**TABLE 4 |** Top 10 institutions with the greatest number of publications.

| Institution | Country | Publications | Citations | h-index |
|---|---|---|---|---|
| University of Copenhagen | Denmark | 117 | 5,939 | 38 |
| Rigshospitalet | Denmark | 94 | 3,823 | 34 |
| University of Lausanne | Switzerland | 43 | 956 | 17 |
| University of Texas system | USA | 40 | 868 | 15 |
| Lundbeckfonden | Denmark | 36 | 879 | 18 |
| Aarhus University | Denmark | 34 | 881 | 17 |
| McGill University | Canada | 32 | 633 | 14 |
| University of California System | USA | 32 | 562 | 14 |
| Mayo Clinic | USA | 27 | 663 | 15 |
| Humboldt University of Berlin | Germany | 26 | 637 | 15 |

**TABLE 5 |** The top 10 most cited articles.

| Author/year | Title | Citations |
|---|---|---|
| Basse et al. (8) | A clinical pathway to accelerate recovery after colonic resection | 545 |
| Vlug et al. (9) | Laparoscopy in Combination with Fast Track Multimodal Management is the Best Perioperative Strategy in Patients Undergoing Colonic Surgery A Randomized Clinical Trial (LAFA-study) | 508 |
| Gustafsson et al. (10) | Adherence to the Enhanced Recovery After Surgery Protocol and Outcomes After Colorectal Cancer Surgery | 409 |
| Basse et al. (11) | Functional recovery after open vs. laparoscopic colonic resection - A randomized, blinded study | 340 |
| Husted et al. (12) | Predictors of length of stay and patient satisfaction after hip and knee replacement surgery - Fast-track experience in 712 patients | 332 |
| Maessen et al. (13) | A protocol is not enough to implement an enhanced recovery programme for colorectal resection | 317 |
| Basse et al. (14) | Colonic surgery with accelerated rehabilitation or conventional care | 313 |
| Currie et al. (15) | The Impact of Enhanced Recovery Protocol Compliance on Elective Colorectal Cancer Resection Results From an International Registry | 297 |
| King et al. (16) | Randomized clinical trial comparing laparoscopic and open surgery for colorectal cancer within an enhanced recovery programme | 267 |
| Delaney (17) | "Fast track" postoperative management protocol for patients with high co-morbidity undergoing complex abdominal and pelvic colorectal surgery | 261 |

## Research Interests and Surgeries

The ERAS was most often implemented in colorectal surgeries, with 583 articles, followed by hip and knee arthroplasty (202 articles), and cardiac surgery (106 articles). The top three most focused elements were "length of stay," "pain management," and "complications." The top 10 surgeries and most focused elements were shown in (**Figure 6A**). When divided into the surgical disciplines, the Department of General Surgery, Orthopedics, Gynecology, Cardiac Surgery, Thoracic Surgery were the top 5 disciplines that implemented most ERAS protocols (**Figure 6B**).

## Keywords

Except for the theme words "ERAS" and "fast-track surgery," co-occurrence analysis showed that "colorectal surgery" was the most frequently occurring keyword, with an occurrence of 146 times and total link strength of 211. Followed by "length of stay" (occurrence 109, total link strength 198), "laparoscopy" (occurrence 87, total link strength 156), "perioperative care" (occurrence 69, total link strength 116), "complications" (occurrence 49, total link strength 105). When ranked by year of occurrence, the top five most frequently used keywords occurred around 2016. "bariatric surgery," "bladder cancer," "cystectomy," "Compliance," "elderly" frequently occurred in recent years (**Figure 7A**).

**TABLE 6 |** Top 10 most often published journals.

| Journal | Country | Publications | Citations | IF (2020) |
|---|---|---|---|---|
| Surgical Endoscopy and Other Interventional Techniques | USA | 54 | 816 | 4.584 |
| World Journal of Surgery | USA | 48 | 1,574 | 3.352 |
| Colorectal Disease | England | 42 | 1,234 | 3.788 |
| International Journal of Colorectal Disease | Germany | 42 | 781 | 2.571 |
| Acta Orthopaedica | England | 34 | 1,555 | 3.717 |
| Obesity Surgery | Canada | 32 | 571 | 4.129 |
| British Journal of Surgery | England | 31 | 2,751 | 6.939 |
| Annals of Surgery | USA | 31 | 2,459 | 12.969 |
| Journal of Cardiothoracic and Vascular Anesthesia | USA | 31 | 568 | 2.628 |
| Diseases of the Colon & Rectum | USA | 26 | 949 | 4.785 |

*IF, impact factor.*

In the burst detection, keywords were classified into several clusters, cluster "enhanced recovery after surgery" burst since 2017, "fast-track surgery" burst during 2005–2012, "colorectal surgery" burst during 2016–2018. "Cystectomy," "bladder cancer," "bariatric surgery," "compliance," "elderly" were keywords burst in recent years (**Figure 7B**).

## DISCUSSION

This bibliometric and visualized study identified 20-year original articles in the field of ERAS research and analyzed their characteristics, revealing the global states and hotspots that can help researchers better understand the trends of ERAS research.

The academic impacts of researchers, institutions, and countries in a certain field are usually measured by the publications and citations, however, some limitations must be emphasized. The publication and citation counts can be influenced by the selection of databases, search strategy, and personal bias. A previous bibliometric analysis found that the Web of Science and Scopus exported very similar articles but different citations (18). Besides, the number of citations can be influenced by literature age, some newly published articles had low citations despite their high scientific values. Except for publications and citations, reputations, peer reviews, and impact factors should also be considered (19). Another phenomenon is that articles from inventors tended to be frequently cited, even though the techniques or concepts have been modified or abandoned. Despite these controversies, citations are still the most widely used tool in bibliometric analysis (19). In the present study, the WOS database was retrieved and visualized networks of authors, institutions, and sources based on the publications and citations were presented.

The USA was the largest contributor to original ERAS research, with the highest h-index of 42. Though China published the second large amount of publications, the number of citations was small compared to other countries, indicating a requirement of improved study qualities. When weighed by h-index, Denmark was the second influential country in ERAS research, the h-index was just second to that of the USA. The third country with the greatest number of publications and citations was England, with an h-index of 34. It was worth noting that the Netherlands owned the greatest number

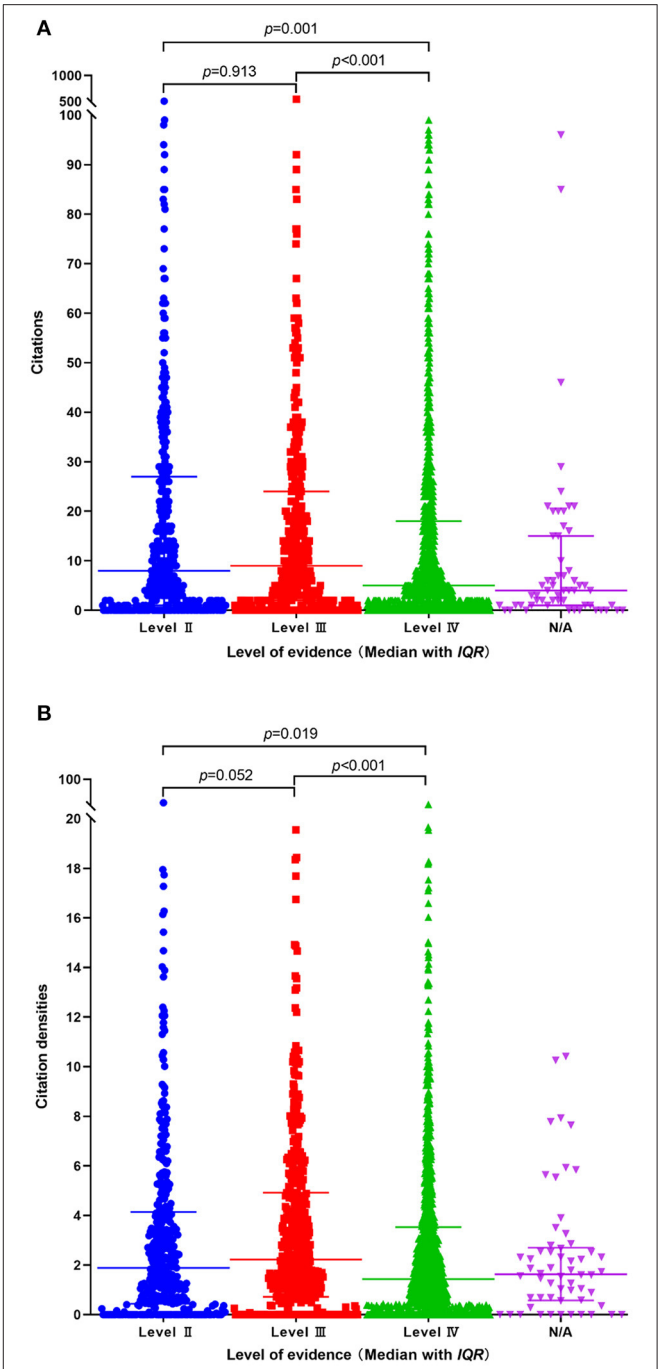



**FIGURE 5 |** Comparison of citations and citation density between different levels of evidence. **(A)** citations. **(B)** citation density.

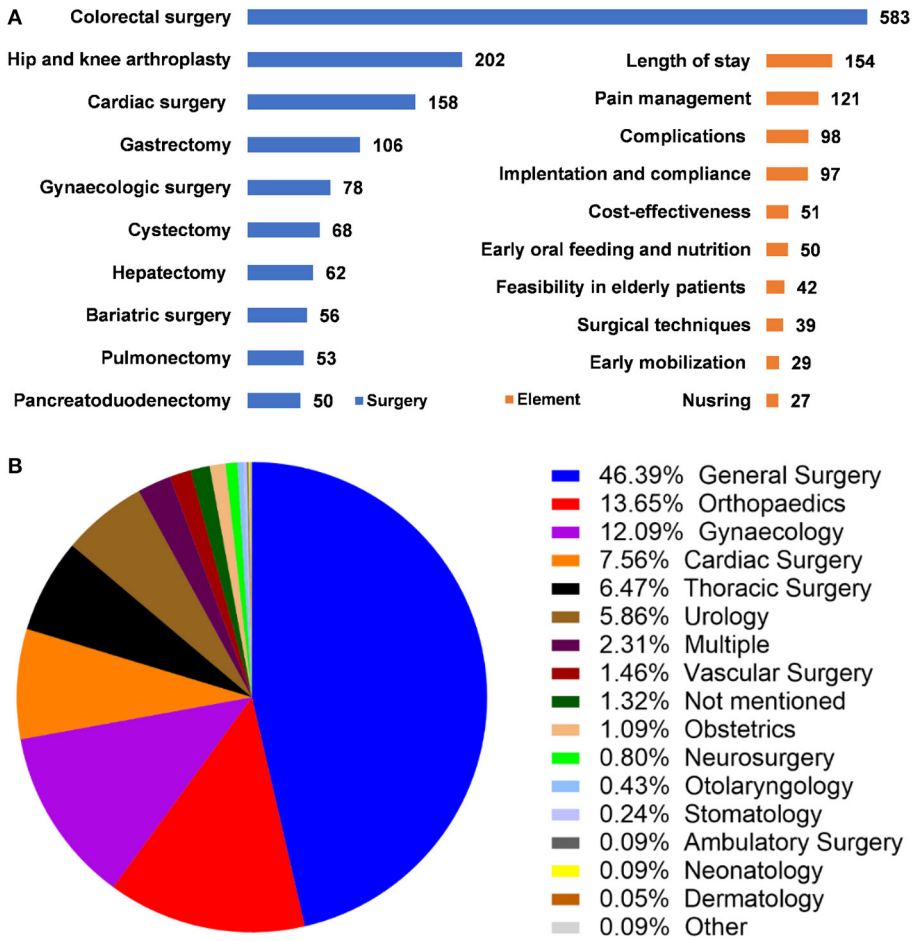


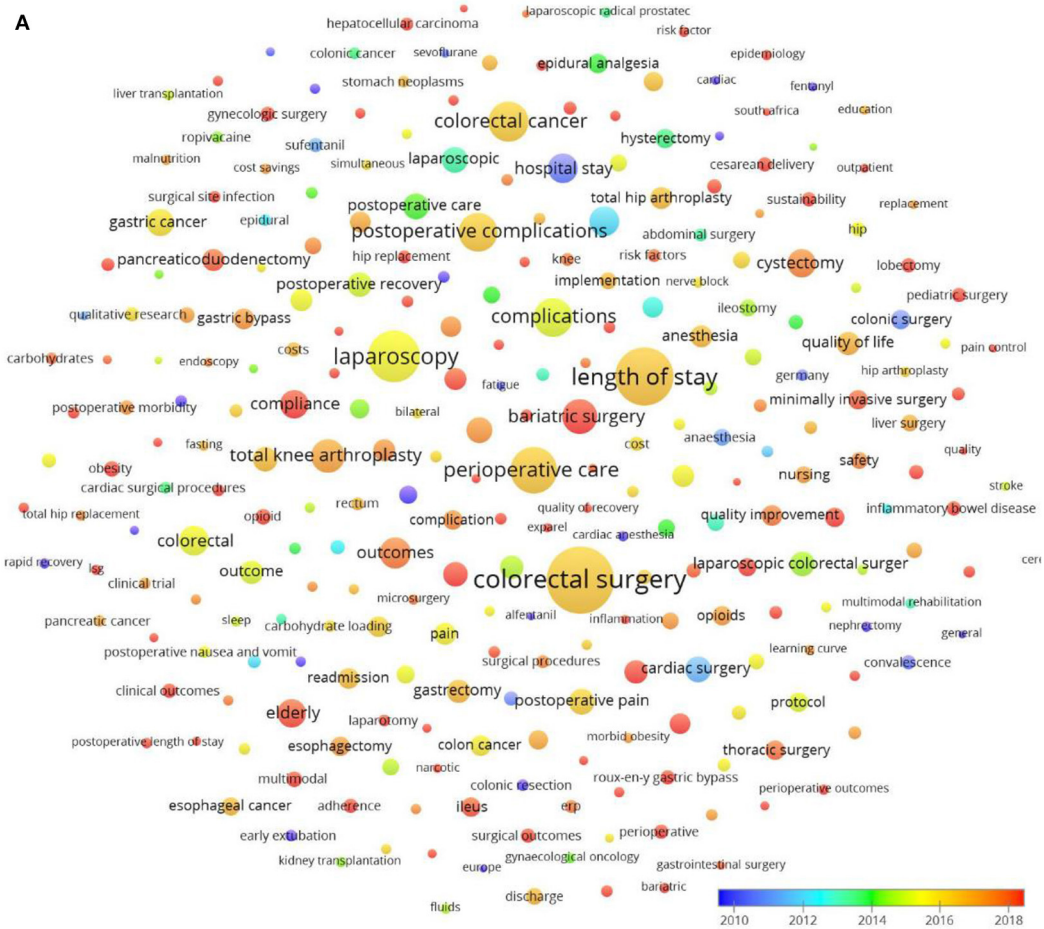

**FIGURE 6 |** Surgeries, most focused elements, and surgical disciplines of ERAS research. **(A)** Top 10 surgeries and most focused ERAS elements. **(B)** Surgical disciplines that implement ERAS protocol.

of citations per item, despite the relatively small number of publications. As was mentioned above, the evaluation of the impact of institutions and countries can be greatly influenced by some productive authors, in this study, most contributions of Denmark were from the University of Copenhagen and its affiliated hospitals.

The high-impact authors and institutions were revealed by the visualized analysis. The bibliographic coupling analysis showed a network of authors and institutions with the greatest number of publications and citations, while the co-citation analysis presented the most frequently cited authors in the reference lists. Keheht H, a surgeon from Rigshospitalet, University of Copenhagen, was the author with the greatest number of publications and citations, as well as the most frequently cited author. He was a member of the first ERAS Study Group and advocated the use of epidural anesthesia for postoperative pain control (4). It should be noticed that the contribution of Rigshospitalet belonged to the University of Copenhagen though it was calculated separately in the visualized network. The second most influential institution was the University of Lausanne, Demartines, Nicolas, and Huebner, Martin were the most productive authors, Gustafsson, Ulf O was the most cited author in this institution.

The top-cited article was from Basse et al. who perfumed a multimodal rehabilitation program of 48-h postoperative stay for patients undergoing colonic resection (8). The article was published in the Annals of Surgery in 2000 and was cited by 547 times in the WOS database. The second most cited article was a multicentre, randomized clinical trial that compared the laparoscopic and open resection of colon cancer combined with fast-track care (9). The article belonged to Vulg, MS et al. and was published in the Annals of Surgery in 2011, cited by 473 times. The third most cited article belonged to Gustafsson et al. (10), who found that improved adherence to ERAS protocol significantly improved the outcomes of patients undergoing colorectal surgery. It was published in the Archives of Surgery in 2011 and cited by 409 times. Among the top 10 most cited articles, all of the ERAS protocols were implemented in colorectal surgery except for one in hip and knee arthroplasty. Most of the highly-cited papers were from

**A**




**B**

| Keywords | Strength | Begin | End | 2000 - 2020 |
|---|---|---|---|---|
| hospital stay | 6.42 | 2002 | 2011 | |
| rehabilitation | 3.26 | 2002 | 2009 | |
| postoperative | 3.43 | 2003 | 2013 | |
| morbidity | 3.96 | 2004 | 2008 | |
| fast-track rehabilitation | 3.35 | 2005 | 2012 | |
| fast-track | 5.66 | 2006 | 2010 | |
| laparoscopic | 3.76 | 2010 | 2014 | |
| rectal cancer | 3.21 | 2012 | 2014 | |
| colorectal cancer | 5.4 | 2016 | 2018 | |
| enhanced recovery after surgery | 47.2 | 2017 | 2020 | |
| era | 19.62 | 2017 | 2020 | |
| enhanced recovery | 12.52 | 2017 | 2020 | |
| colorectal surgery | 7.7 | 2017 | 2018 | |
| outcm | 5.06 | 2017 | 2020 | |
| cystectomy | 4.84 | 2017 | 2018 | |
| c-reactive protein | 4.12 | 2017 | 2018 | |
| compliance | 3.46 | 2017 | 2020 | |
| sleeve gastrectomy | 3.3 | 2017 | 2020 | |
| enhanced recovery after surgery (eras) | 8.24 | 2018 | 2020 | |
| bariatric surgery | 5.64 | 2018 | 2020 | |
| elderly | 4.17 | 2018 | 2020 | |
| bladder cancer | 3.8 | 2018 | 2020 | |

**FIGURE 7 |** Visualized analysis and burst detection of author keywords. **(A)** Co-occurrence analysis of author keywords, weighed by average occurred year. **(B)** The burst detection of keywords.

the British Journal of Surgery and Annals of Surgery, indicating their high reputations within the ERAS research. Revealing the high-impact sources also helps researchers select journals during submission.

In the present study, the OCEM 2011 level of evidence system was used (7). Since the systemic review and meta-analyses were not included, there was no Level I evidence. We found that randomized controlled trials counted only 19.46% among the study designs, though there were more prospective designs, more than half of the studies were case serious or case-control studies and presented level IV evidence. Retrospective analysis of prospectively collected data was considered as retrospective designs, besides, there were a few studies with combined designs. Considering the influence of literature age on citations, we calculated the citation density to weigh the average citations by year. We found that articles with level IV evidence had fewer citations and citation densities compared to that of level II and level III, respectively. While the number of citations and citation densities were comparable between level II and level III studies, suggesting that articles with level IV evidence were less likely to be cited. In short, there was a lack of high-level evidence designs, more prospectively, randomized controlled designs are required in the future.

The ERAS Study Group produced evidence-based protocols to promote perioperative management, which should be implemented by multidisciplinary teams, including surgeons, nurses, anesthesiologists, nutritionists, as well as patients, relatives, and caring members (6, 20). Though the ERAS protocol has been widely recognized around the world, there were still limited changes in most healthcare systems (6). In this study, the majority of the original articles were clinical research, followed by surveys, quality improvement studies, audits, cost-effectiveness analyses, and cross-sectional studies (21–25). We found that only quite a few studies had multidisciplinary interventions, however, most of them focused on elements of ERAS. The top research interests included "length of stay and early discharge after surgery," "perioperative pain management," "postoperative complications," "implementation and compliance with ERAS." In shorts, these most focused research interests may represent the hotspots in the ERAS research.

The ERAS was overwhelmingly implemented in colorectal surgeries (28%), followed by hip and knee arthroplasties, and cardiac surgeries. When classified by surgical disciplines, the department of General Surgery, Orthopedics, Gynecology, Cardiac Surgery, and Thoracic Surgery implemented the greatest number of ERAS protocols. It must be emphasized that the divide of surgical disciplines can differ in institutions. The Department of General Surgery implemented more than 50% ERAS protocols among all disciplines because most abdominal surgeries were included (except for urological, gynecological, and vascular surgeries). The disruption of gastrointestinal function in these surgeries required postoperative rehabilitation urgently. In terms of diseases, most of the ERAS protocols were primarily focused on malignancies, these patients were weak suffered more from surgical trauma. In detail, the ERAS protocols mainly served for colorectal cancers in colorectal surgeries (10, 17) kidney and liver diseases in the transplantation surgeries (26–28) osteoarthritis, degenerative spinal diseases, and hip fractures in orthopedics surgeries (12, 29, 30) coronary artery diseases in cardiac surgeries (31, 32). Fast-track anesthesia mainly focused on postoperative pain control and reduction of opioid use, early extubation, and reduction of postoperative complications (33–35).

Hotspots were further revealed by keywords that frequently occurred during a certain period, which were shown in Vosviewer, bursts were detected furtherly by Citespace. Not surprisingly, they exported very similar results. The most frequently occurring keywords "enhanced recovery after surgery" and "fast-track surgery" were not shown in the visualized map to better present other keywords. Though the ERAS concept was formally put forward in 2001, the keywords "ERAS" burst in 2017, before that, "fast-track surgery" was widely used. Research on cardiac surgery burst during 2000–2012, research on colorectal surgery peaked around 2016. In recent years, gastrectomy for bariatric surgery, cystectomy for bladder cancer, compliance with ERAS, and feasibility of ERAS in elderly patients gathered most research interests, which may represent the current trends in ERAS research. We hypothesize that these results were related to the increasing number of obesity, the aged tendency of the population, and the rising morbidity of malignancies. Barriers during the implementation of ERAS were multifactorial, not only from patients but from managers and practitioners (36). Recently, more studies focused on compliance or adherence to ERAS, sustainability of ERAS in community hospitals, and quality improvement programs (36–39).

This study has several limitations. First, the number of included articles was limited because we only retrieved the WOS database, research that followed ERAS protocols but described them as "accelerated recovery," "rapid recovery," "fast recovery" or "multimodal rehabilitation" can be dismissed by using the current search strategy. As was mentioned at the beginning of the article, the ERAS Study Group was established in 2001 and defined the concepts and goals of ERAS, before that, "fast-track recovery" had been used for several years, those studies were not included because of the limited numbers. Second, the contribution of countries and institutions can be influenced greatly by some productive authors, thus contributions from some influential authors and institutions may be underestimated. Furthermore, some influential authors, institutions, and most frequently occurring keywords can not be well-shown in the visualized map. Third, the distribution of countries was determined by corresponding authors, distribution of the authors and institutions was determined by full author lists, while the co-cited authors in the reference list were determined by the first authors, for studies that were performed by cooperation from different institutions and countries, the last corresponding author was regarded as the major contributor, which may lead to bias. Lastly, the level of evidence of the included studies is weak, indicating that most of the ERAS research was observational

studies. This conclusion can be influenced by the included criteria and the grade of evidence level. Despite these facts, this bibliometric analysis presented a clear global distribution and hotspots of original ERAS in the recent 20 years.

## CONCLUSION

This study revealed the global status and trends in the field of ERAS research. Revealing the global states and hotspots can help researchers better understand the trends in ERAS research. The USA was the greatest contributor to ERAS research. Kehlet, H, was the most influential author in the field. Bariatric surgery, compliance with ERAS, and feasibility in the elderly represent the new trend of ERAS research. Most of the ERAS research had low evidence levels, studies with high-level evidence are still required in this field.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

SS designed the research, retrieved the literature, and drafted the manuscript. TW screened the literature, graded the level of evidence, and analyzed the data. RW screened the literature and graded the level of evidence. QL performed the statistical analysis, modified the manuscript, and censored the article. MB designed the work, performed the analysis, performed the interpretation of data, and approved the version to be published. All authors contributed to the article and approved the submitted version.

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# Surgical Correction of Carcinoid Heart Disease Improves Liver Function and 5-Hydroxyindoleacetic Acid Levels

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**Introduction:** Carcinoid heart disease (CHD) is a consequence of neuroendocrine tumors releasing 5-hydroxytryptamine (5-HT) into the systemic circulation, affecting right heart valves, causing fibrosis, and eventually right heart failure. The aim of this study was to determine the effect of valve-replacement on kidney function, liver function, and 5-hydroxyindoleacetic acid (5-HIAA) levels.

**Methods:** A Retrospective study of 17 patients with CHD who had undergone heart-valve replacement surgery between 2010 and 2019, from the Queen Elizabeth Hospital Birmingham. 5-HIAA levels, liver, and kidney function were measured in addition to hepatic inferior vena cava (IVC) diameter and its relationship to carcinoid symptoms.

**Results:** Eleven patients were male and six were female. At time of surgery, average age was 66.6 ± 8.1 years and average BMI was 25.8 ± 5.5 Kg/cm$^2$. Three out of 17 patients had one valve replaced, 13/17 had two replaced (tricuspid and pulmonary), and 1/17 had three replaced (tricuspid, pulmonary and aortic). There was a 31% average decline in 5-HIAA [799.8 (343.6–1078.0) to 555.3 (275.8–817.9), $p = 0.011$], a 35% decline in bilirubin [20 (16–29) to 13 (10–19), $p = <0.001$], and a 15% reduction in the short and long axes of the IVC after valve-replacement surgery [20.0 (18.0–25.0) and 36.5 (29.0–39.8) to 17.0 (14.5–19.3) and 31.0 (26.5–34.3) respectively, $p = <0.001$ and 0.002 respectively].

**Conclusion:** Valve replacement surgery improves 5-HIAA levels alongside improved liver function and hepatic IVC diameter. These findings are consistent with resolution of congestive hepatopathy, and therefore enhanced clearance of 5-HIAA. This suggests that valve-replacement surgery can indirectly have beneficial outcomes on hepatic function and is also associated with a drop in the circulating levels of tumor derived serotonin.

**Keywords: carcinoid heart disease, 5-HIAA, congestive hepatopathy, valve replacement surgery, neuroendocrine tumors (NETs)**

## INTRODUCTION

Carcinoid heart disease (CHD) is a rare condition affecting patients with carcinoid syndrome, which can result in heart failure secondary to the various vasoactive mediators produced by neuroendocrine tumors (1). Serotonin (5-hydroxytryptamine, 5-HT) plays a major role in the development of CHD. 5-HT can bind to 5-HT2B receptors expressed on cardiac valves and myocytes, causing inflammation and fibroblast proliferation, eventually leading to valve fibrosis, retraction of leaflets and valve incompetence (2, 3) typically producing isolated right-sided heart failure (2).

Patients often present late with the signs and symptoms of advanced carcinoid syndrome (cutaneous flushing and diarrhea), CHD and heart failure (4). It is recommended that patients with carcinoid syndrome and/or raised 5-hydroxyindoleacetic acid (5-HIAA, a degradation product of 5-HT) levels have 6–12 monthly N-terminal prohormone of brain natriuretic peptide (NT-proBNP) measurements to screen for CHD (5, 6). Raised NT-proBNP in these patients is a screening tool that, if elevated, mandates the performance of transthoracic echocardiography (TTE) to detect CHD, i.e. heart valve thickening and regurgitation (5).

CHD is treated with a combination of diuretics to reduce fluid overload, and somatostatin analogs to reduce circulating 5-HT levels (4). The definitive treatment for CHD remains heart-valve replacement surgery (see **Figure 1**), which has been shown to improve performance status; however, it has not yet been proven to improve life expectancy – indeed, surgery itself is associated with a 10–20% peri-operative mortality (although the Mayo clinic reports a figure of 5–6% for their latest cohort) (5, 7, 8). Determining when and on whom to operate remains a matter of debate (7). We previously reported in a retrospective study that valve-replacement surgery was associated with a post-operative reduction in 5-HIAA levels (a marker of tumor activity) which suggests that treating CHD may influence tumor activity. The study was limited by the small number of participants (9). A detailed description of peri-operative management is discussed in our previous paper (10).

The purpose of this study therefore is to expand on these findings and to determine whether surgery diminishes circulating tumor hormones. A secondary aim of the study is to investigate whether surgery improves end organ function, specifically the liver.

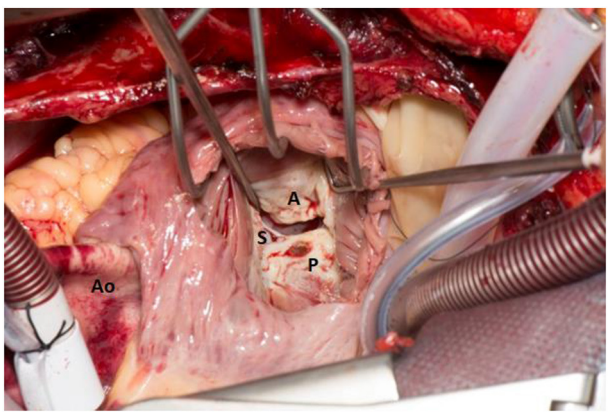


**FIGURE 1 |** The right atrium is opened and retracted exposing the Tricuspid Valve (TV). The cardiopulmonary bypass caval cannulae can be seen. The Aorta (Ao) is on the left hand side. The TV can be seen through the right atrium and the leaflets are labeled respectively (anterior leaflet- A, posterior leaflet- P and septal leaflet- S). The leaflets are thickened and fibrosed and the septal leaflet is plastered onto the ventricular septum inferiorly.

## MATERIALS AND METHODS

### Study Cohort

This is a retrospective, single center study of patients, who underwent valve replacement surgery at the Neuroendocrine Tumor Centre at the Queen Elizabeth Hospital Birmingham. All patients with CHD who underwent heart-valve replacement surgery between 2010 and 2019 (2010 marking the introduction of the "PORTAL" electronic record) were eligible for inclusion (n = 40). Patients were referred for surgery if they met one of the following criteria: stable carcinoid tumor load; severe valvular dysfunction; poor exercise tolerance. Patients were excluded from the study if they died before follow-up tests could be completed (n = 4); did not have complete data, (n = 13); or had concomitant changes to medical management that could confound the 5-HIAA levels, e.g. somatostatin analogs commencement or alteration in dose) or application of interventional radiology treatment (trans-arterial embolization) (n = 6). Conversely, no patients had changes to medical management of heart failure necessitating exclusion.

This left 17 patients within this study.

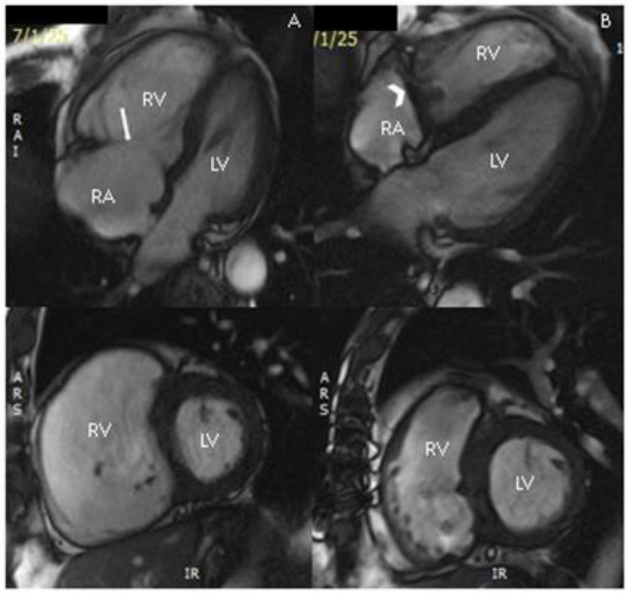


**FIGURE 2 |** Cardiac magnetic resonance images before surgery **(A)** demonstrating severe right ventricular dilatation and flattening of the inter-ventricular septum, and post-tricuspid valve replacement **(B)** demonstrating significant reduction in right ventricular volume; top panel: still from four-chamber cine sequence; lower panel: still from short-axis cine stack at mid-ventricular level. RV, right ventricle; LV, left ventricle; RA, right atrium; arrow, native tricuspid valve; chevron, tricuspid valve replacement.

## Study Measures

All demographic, hematology, biochemical and imaging patient data were drawn from electronic patient records and imaging reports. Within the NET-CHD service, all patients are routinely admitted to hospital for detailed assessment, establishing fitness to proceed with surgery. These admissions were no more than six months from the time of planned surgery.

Renal function was measured using urea and creatinine; liver function: bilirubin, albumin, and PTT; tumor-related hormone activity using 24-h urinary 5-HIAA, with chromogranin-A as a general marker of tumor burden. These data were collected within a range of one to six months either side of surgical intervention.

IVC diameters were measured at the confluence of the hepatic vein with the IVC using routine CT scans that had been performed before and after surgery for the purpose of monitoring cancer progression. Cardiovascular magnetic resonance imaging (CMR) and TTE are routinely performed as part of patient assessment and planning for surgery (see **Figures 2**, **3**).

Carcinoid symptoms were quantified by patient-reported frequency of flushing and diarrhea before and after surgery.

## Data Analysis

Data were tabulated in Microsoft Excel; for each non-parametric measure, the median was calculated. SPSS Statistics (version 23) was used to calculate interquartile ranges, and to apply paired Wilcoxon tests to generate *p*-values. Graphpad Prism 7 was used to generate before and after graphs, which show individual patient data. For parametric data, means were calculated instead; and paired *t*-tests were used to generate *p*-values.

# RESULTS

## Demographics

Of the 17 patients included, 11 were male, six were female. The average age at time of surgery was 66.6 ± 8.1 years. The average BMI at time of surgery was 25.8 ± 5.5 kg/cm$^2$.

## Surgical Perspective

Three patients had one valve replaced (tricuspid); 13 had two valves replaced (tricuspid and pulmonary); and one patient had three valves replaced (tricuspid, pulmonary and aortic). All patients received bioprosthetic valves.

The Medtronic Hancock II stented bioprosthesis was implanted in the tricuspid position in 12 patients (sizes 25–29 mm). The St Jude Epic stented bioprosthesis was implanted in the trucuspid position in five patients, and in the pulmonary position in four patients (sizes 21–29 mm). The Edwards Perimount stented bioprosthesis was implanted in the pulmonary position in 10 patients, and in the aortic position for one patient (sizes 21–23 mm).

In addition, three patients underwent a coronary artery bypass graft (CABG) procedure during valve replacement surgery; and four patients underwent a patent foramen ovale (PFO) closure.

The median length of hospital stay for patients discharged after surgery was 15 days (9–34).

Post-operatively, five patients developed tricuspid regurgitation after a median of 95 weeks (32–349 weeks); and six patients developed pulmonary regurgitation after a median of 28 weeks (21–105). One patient developed stenosis of the tricuspid valve, which was detected on TEE at 93 weeks; another patient developed stenosis of the tricuspid and pulmonary valves at 95 weeks.

## Medical Perspective

Sixteen out of 17 patients had small bowel primary; one patient had bronchial primary. Sites of metastasis is documented in **Table 1**. Sixteen out of 17 patients had metastases to the liver; four to the mesentery; two to bone; and one to mediastinum and retroperitoneum; and one to the pancreas.

Tumor load in the liver was determined at NET MDT; mean tumor load = 52.3% ± 18.2 (range 15–80%). All but one had both lobes involved.

At time of surgery, 2/17 patient were on beta blockers; 10/17 were on diuretics; and 17/17 were on somatostatin analogs.

The frequency and severity of carcinoid symptoms were not collected in a sufficiently systematic fashion before and after surgery. Thus, it was not possible to compare them in a meaningful way, and they have therefore not been included in this paper.

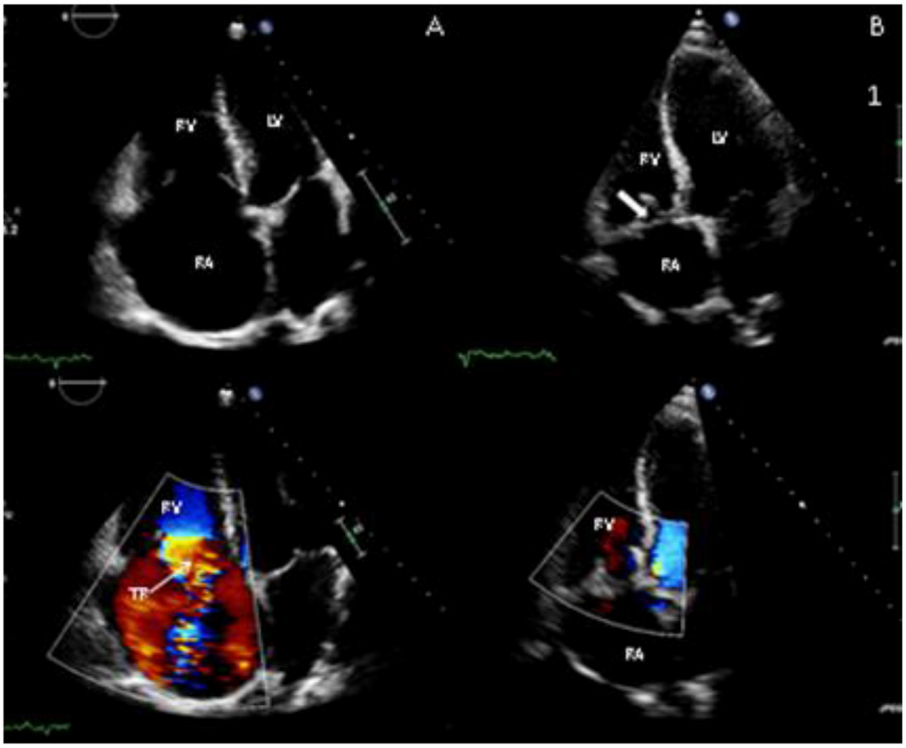


**FIGURE 3 |** Transthoracic echocardiography images before surgery **(A)** and after surgery **(B)** showing apical 4-chamber view of the tricuspid valve (TV). In the pre-surgery images there is failure of leaflet co-aption (note closed position of mitral valve) due to valve degeneration, with severe tricuspid regurgitation (TR) on colour Doppler (lower panels); post-surgery, the right ventricle (RV) and right atrium (RA) have reduced in size and there is a tricuspid valve bioprosthesis (thick arrow) *in situ*, with resolution of TR.

**TABLE 1 |** Sites of metastasis.

| Site of metastasis | Number of patients |
|---|---|
| Liver | 16 |
| Mesentery | 4 |
| Bone | 2 |
| Mediastinum | 1 |
| Pancreas | 1 |
| Retroperitoneum | 1 |

## Survival Data

In our patient cohort, 10/17 were deceased at the time of data capture; of these deceased patients, survival ranged from 9 to 413 weeks, with a median survival of 78 weeks.

## Summary of Results

A summary of results can be found in **Table 2**.

## Tumor Markers

Twenty four hour urinary 5-HIAA (normal range: 0–45 μmol/24 h) improved by 31% following surgery ($p = 0.011$) from a mean of 799.8 (343.6–1078.0) to 555.3 (275.8–817.9) μmol/24 h. Fourteen of 11 patients had an improvement in 24 h urinary 5-HIAA post-surgery (see **Figure 4**). Three patients had increase in 5-HIAA levels of 8, 12, and 42%.

Chromogranin A (normal range: <60 pmol/L) did not significantly change after surgery ($p = 0.839$) (see **Figure 5**).

## Liver Function

The only liver function marker to change significantly ($p < 0.001$) was bilirubin (normal range: <21 μmol/L), which improved from an average of 20 μmol/L (raised) to 13 μmol/L (within normal physiological range), representing a 35% improvement.

The average albumin (normal range: 35–50 g/L) pre-surgery was within normal range (43 *g/L*) and did not significantly improve ($p = 0.134$). Likewise, INR (normal range: 0.8–1.2) did not change on average and remained within normal physiological range (1.2).

Thirteen out of 15 patients had an improvement in bilirubin levels post-surgery (see **Figure 6**). One patient remained the same, one patient worsened slightly.

## Hepatic IVC Diameter

Both the short and long axes of the hepatic portion of the IVC were significantly reduced by an average of 15%

**TABLE 2 |** Summary of markers, before and after surgery.

| Marker | Normal range | Pre-Treatment | Post-Treatment | *P*-value |
|---|---|---|---|---|
| Urea, *mmol/L* ($N = 16$) | 2.5-7.8 | 6 (5–8) | 7 (5–8) | 0.463 |
| Creatinine, μ*mol/L* ($N = 16$) | 64-104 | 94 (74–120) | 94 (68–108) | 0.349 |
| Bilirubin, μ*mol/L* ($N = 15$) | <21 | 20 (16–29) | 13 (10–19) | <0.001 |
| Albumin, g/L ($N = 15$) | 35–50 | 43 ± 5 | 41 ± 5 | 0.134 |
| INR ($N = 9$) | 0.8–1.2 | 1.2 (1.1–1.3) | 1.2 (1.1–1.2) | 0.984 |
| 24 hr urinary 5-HIAA, μ*mol/L/24 hours* ($N = 14$) | 0–45 | 799.8 (343.6–1078.0) | 555.3 (275.8–817.9) | 0.011 |
| Chromogranin A, *pmol/L* ($N = 13$) | <60 | 468.0 (246.5–1586.0) | 1366.0 (360.5–2340.0) | 0.839 |
| Maximum short axis of the hepatic portion of the IVC, *mm* ($n = 17$) | n/a | 20.0 (18.0–25.0) | 17.0 (14.5–19.3) | <0.001 |
| Maximum axial diameter of the hepatic portion of the IVC, *mm* ($n = 17$) | n/a | 36.5 (29.0–39.8) | 31.0 (26.5–34.3) | 0.002 |

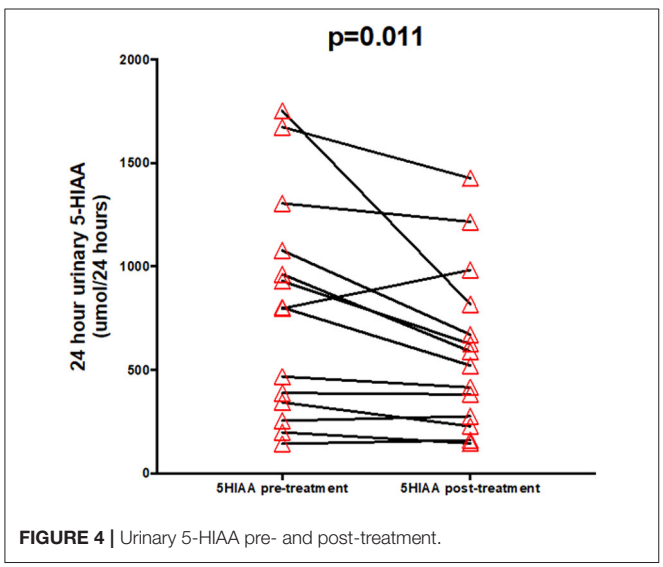


**FIGURE 4 |** Urinary 5-HIAA pre- and post-treatment.

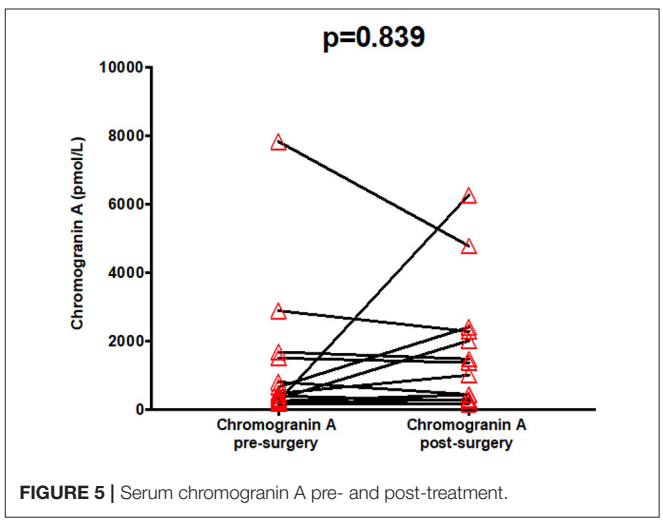


**FIGURE 5 |** Serum chromogranin A pre- and post-treatment.

following surgery ($p = <0.001$, 0.002 respectively). When examined individually, 15/17 patients showed an improvement in both axes of measurement (see **Figures 7**, **8**). In both instances, two patients showed an increase in the axes of measurement.

## Renal Function

There was no significant change in serum urea (normal range: 2.5–7.8 mmol/L) and creatinine (normal range: 64–104 μmol/L) post-surgery ($p = 0.463$, 0.349 respectively) after surgery, and remained within physiological range (6 to 7, 94 to 94 μmol/L respectively).

# DISCUSSION

## Primary Outcomes

For patients with CHD, heart-valve replacement surgery is associated with a reduction in 5-HIAA levels and bilirubin levels. These findings reflect improvement in liver function. These results complement our previous observations showing a reduction in 5-HIAA levels with cardiac surgery (9).

Of the 14/17 patients with complete sets of 5-HIAA measurements, three experienced a rise after surgery; with increases of 8, 12, and 42%, despite a reduction in serum bilirubin levels. However, two out of three of these patients had cancer progression on cross-sectional imaging and rising

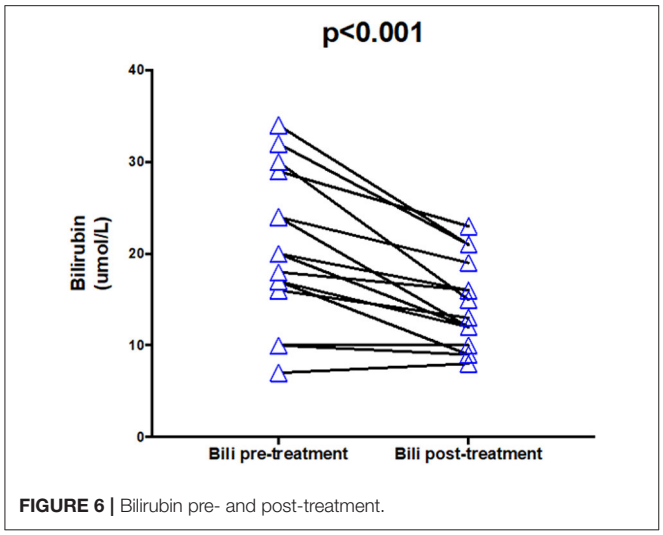


**FIGURE 6 |** Bilirubin pre- and post-treatment.

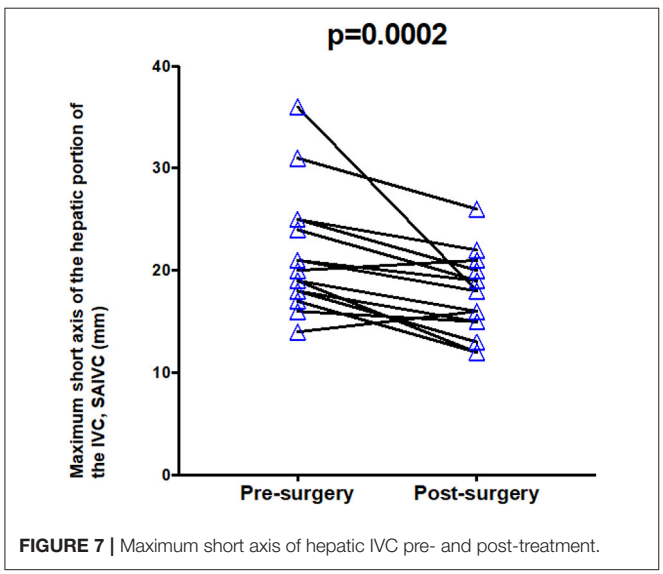


**FIGURE 7 |** Maximum short axis of hepatic IVC pre- and post-treatment.

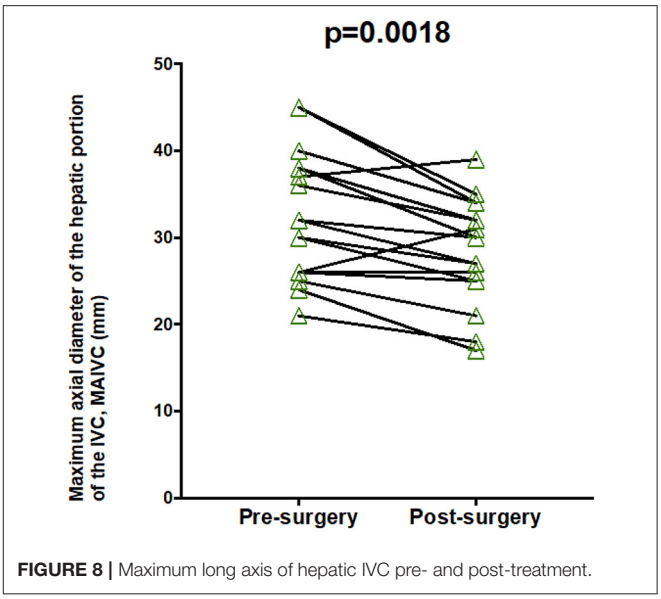


**FIGURE 8 |** Maximum long axis of hepatic IVC pre- and post-treatment.

serum chromogranin-A levels, indicating that a rising 5-HIAA level post cardiac surgery warrants investigation for tumor progression.

Increased liver enzymes (particularly ALP and GGT), bilirubin, and INR with a reduced albumin has been described in right heart failure (11). In our case series with CHD, we noted that other markers of liver function (ALP, GGT, albumin and INR) were within normal physiological ranges before surgery and did not change with surgery. Bilirubin was the only marker that was elevated and showed significant decrease following surgery in line with previous reports in tricuspid regurgitation (11). Bilirubin could therefore serve as a sensitive marker of liver dysfunction in CHD.

Renal function (urea and creatinine) did not significantly change following surgery. Based on these findings, improvement in renal function is unlikely to play a role in the reduction of 5-HIAA levels.

Overall, these findings are consistent with our hypothesis that reduction in 5-HIAA can be attributed to improvement in congestive hepatopathy and hence liver function and enhanced clearance of 5-HIAA.

Both the long and short axes of the hepatic IVC were reduced after surgery, which reflects improvements in the pressure within this vessel.

Right heart overload and regurgitation of blood in to the IVC is well-known to cause what is termed "congestive hepatopathy" as seen in children and young adults with univentricular hearts (12). This creates a constant back pressure on the liver leading to an increase in liver enzymes and bilirubin, liver fibrosis and eventually cirrhosis (12).

A similar mechanism seems to be occurring in patients with tricuspid regurgitation due to CHD. The regurgitation of blood from the right ventricle into the IVC and then hepatic veins can often be seen on CT scan by analyzing the flow of contrast in the blood vessels.

Replacement of the tricuspid valve resolves the regurgitation (see **Figures 7**–**9**.) and is proposed to be the mechanism for the improvement in serum bilirubin levels (13). This suggests that an approach to managing CHD that prioritizes improving liver function may help to improve 5-HIAA levels and likely patient.

## Limitations

The retrospective study design is inherently less powerful than a prospective study design.

After application of exclusion criteria, 17 out of 40 patient were included in the study, which means that the results may be subject to some selection bias.

It was not possible to accurately quantify the severity of carcinoid syndrome with the existing quality of life questionnaires i.e. QLQ C30 and QLQ GINET21 as they

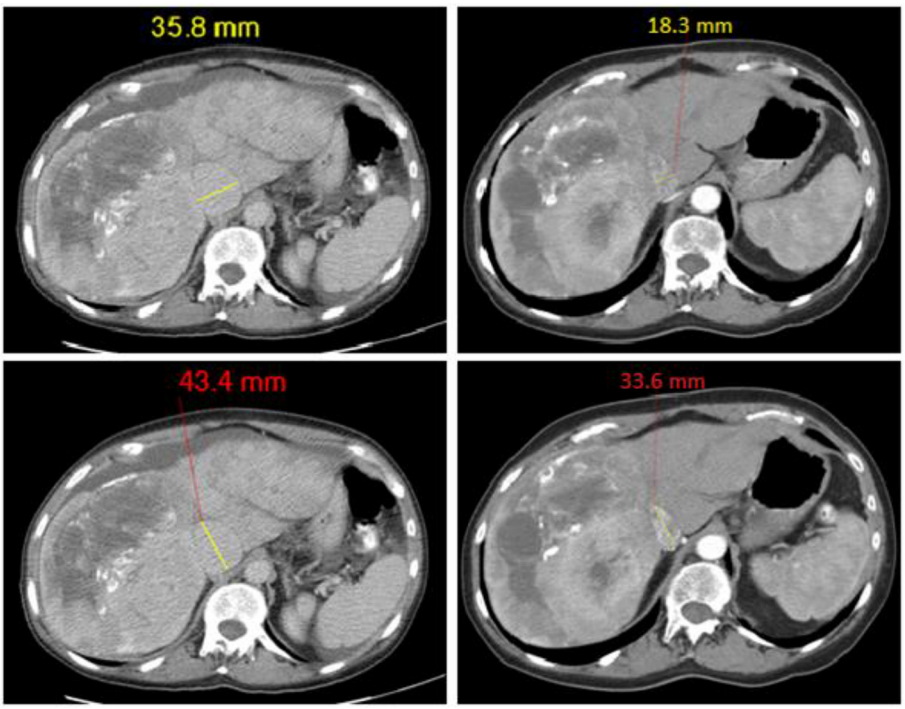


**FIGURE 9 |** Representative imaging for data in **Figures 5**, **6** showing reduction in IVC diameters following tricuspid valve replacement; before surgery images on left hand side, after surgery images on right hand side.

do not capture carcinoid syndrome in terms of frequency or severity.

## CONCLUSION

Right-sided heart-valve replacement in CHD leads to major physiological changes due to changes in hemodynamics and IVC pressures brought about by right heart failure and regurgitation into the inferior vena cava.

Here we present novel data showing for the first time an improvement in serum 5-HIAA levels, a marker strongly related to carcinoid syndrome symptoms and CHD, in a cohort of patients where confounding variables have been excluded.

Furthermore, we present evidence for the first time that improvement in 5-HIAA levels occur in parallel with an improvement in liver function, namely a significant reduction in bilirubin (which appears to be a more sensitive marker of liver dysfunction). We demonstrate that after valvular surgery the raised IVC pressure from right heart failure improves as evidenced by a significant reduction in IVC diameters. Reduction in IVC diameter and bilirubin levels suggest resolution of congestive hepatopathy and hence a reduction in 5-HIAA levels. In addition to the improvement in cardiac function following valvular repair in CHD, our results demonstrate that there are additional benefits leading to improved liver function and reduction in 5-HT levels and thus may improve the options for additional tumor directed therapies in these patients.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Audits and Registries Management Service (CARMS). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

Conception and design: HS and TS. Collection and assembly of data: HS. Data analysis and interpretation: HS, VS, and TS. All authors contributed in manuscript writing and final approval of manuscript.

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# Impact of Surgery on Non-Functional Pancreatic Neuroendocrine Tumors ≤2 cm: Analyses With Propensity Score–Based Inverse Probability of Treatment Weighting

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**Purpose:** The impact of surgery on non-functional pancreatic neuroendocrine tumors (NF-PNETs) ≤2 cm is controversial. This study sought to demonstrate the impact of surgery on the prognosis of NF-PNETs ≤2 cm with different biological behaviors.

**Methods:** Patients with NF-PNETs ≤2 cm from 2004 to 2015 in the Surveillance, Epidemiology, and End Results database were included in this study. An inverse probability of treatment weighting (IPTW) method was used to reduce the selection bias. Kaplan–Meier survival analysis and Cox proportional hazards regression were used to evaluate the effect of surgery on the prognosis.

**Results:** In the IPTW-adjusted Cox proportional hazards regression analysis, surgery improved the cancer-specific survival (CSS) in the overall cohort (hazard ratio [HR], 0.187; 95% confidence interval [CI], 0.102–0.343; $p < 0.001$), patients with poorly differentiated or undifferentiated tumor grades (HR, 0.238; 95% CI, 0.105–0.64; $p < 0.001$), patients with distant metastasis (HR, 0.102; 95% CI, 0.021–0.496; $p = 0.005$), and patients with local invasion (HR, 0.059; 95% CI, 0.005–0.683; $p = 0.002$). Surgery did not improve the CSS in patients with lymph node metastasis only (HR, 0.26; 95% CI, 0.0462–1.461; $p = 0.126$) or patients with well or moderate differentiation while without distant and lymph node metastasis (HR, 0.387; 95% CI, 0.146–1.028; $p = 0.057$).

**Conclusions:** Among patients with NF-PNETs ≤2 cm, different biological behaviors correlate with different prognostic impacts of surgery. As long as distant metastasis does not occur and the grade is well–moderately differentiated, these patients will not benefit from surgery no matter whether lymph node metastasis occurs or not. However, when local invasion appears in this group of patients, surgery should be performed. Moreover, patients with a tumor grade of poorly differentiated or undifferentiated or those with distant metastases may benefit from surgery.

**Keywords: non-founctional pancreatic neuroendocrine tumors, 2 centimeters, surgery, SEER, survival analysis**

## INTRODUCTION

With a continuously increasing occurrence rate over the last 20 years (1), pancreatic neuroendocrine tumors (PNETs) now account for 1% of all pancreatic neoplasms (2). This trend may be attributed to increased awareness, diagnostic techniques advancement, or other unidentified environmental factors or genetic factors (3). PNETs may be divided into functional and non-functional (NF-PNET) tumors. In total, 60%–90% of PNETs are clinically non-functional (4, 5). Traditionally considered as less biologically aggressive than pancreatic cancer, PNETs are increasingly recognized for their highly variable pathological potential (6, 7). Many PNETs are indolent with a low metastasis trend and favorable long-term prognosis. In contrast, other high-grade tumors show relentless early metastasis, making their biology more aggressive than ductal adenocarcinoma. Through the generic term "PNET," a very heterogeneous disease has unfolded before us, which can be defined either as a pancreatic neoplasm or a carcinoma (8).

Surgical resection is the only radical way to treat PNETs. In general, functional PNETs and non-functional tumors >2 cm should be resected according to the recommendations of the National Comprehensive Cancer Network (V2.2021) (9). However, the management of NF-PNETs ≤2 cm remains controversial. Today, many centers (10, 11), as well as the European Neuroendocrine Tumor Society (ENETS) (12) recommend a surveillance strategy for such lesions given their low malignant potential, slow growth, and high incidence of postoperative mortality and morbidity. On the contrary, some studies have reported that surgical resection of NF-PNETs ≤2 is associated with a better survival rate (13). There is increasing recognition that the presence of small, high-grade tumors may result in aggressive behavior (14). In addition, there is increasing evidence that nodules, distant metastases, and recurrence may present in tumor cases that meet the preoperative criteria for benign disease (i.e., intrapancreatic tumors ≤2 cm) (15–18). Therefore, there is no consensus on the optimal management of NF-PNETs ≤2 cm nor regarding the impact of surgery on these patients.

The aim of this population-based study was to determine whether surgical treatment exerted a beneficial effect on overall survival (OS) and cancer-specific survival (CSS) of patients or not. In this study, we enrolled patients with NF-PNETs ≤2 cm with different oncological characteristics, including grade (poorly differentiated, undifferentiated or well–moderately differentiated), lymph node status (lymph node metastasis or not), distant metastasis status (distant metastasis metastasis or not), and regional extension (local invasion or not). We present the following article in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting checklist.

## MATERIALS AND METHODS

### Database and Patient Identification

We selected potential patients who were eligible for inclusion in a retrospective cohort study from 2004 to 2015 from the Surveillance, Epidemiology, and End Results (SEER) database. The inclusion criteria for this study were as follows: (a) NF-PNETs were included on the basis of International Classification of Diseases for Oncology, third edition, (b) the tumor was ≤2 cm in size, and (c) patients had a pathological diagnosis. Patients with (a) secondary or multiple primary cancers; (b) an age of <20 years at the time of diagnosis; or (c) missing or incomplete information about survival or months of follow-up, cause of mortality, or other necessary characteristics were excluded. The patient-selected pathway is shown in **Figure 1**. Ethics approval and informed consent were not required for this study because the SEER study data are publicly available.

### Study Covariables and Outcomes

The variables included in this analysis were divided into 3 categories by type of information, as follows: patient-related demographics, tumor-related information, and treatment-related variables. Patient-related information included race (White, Black, or another race), diagnosed age, sex, insurance status (uninsured, insurance, or Medicaid), diagnosed year (2004–2010 or 2010–2015), and marital status (married, unmarried, divorced, separated, or widowed). Tumor-related information included tumor site (head, body, tail, and other sites), tumor size, lymph node invasion, regional extension, distant metastasis, and tumor grade (well–moderately or poorly undifferentiated). Treatment-related variables included surgery and chemotherapy.

The primary outcomes of interest were overall survival (OS) and cancer-specific survival (CSS). OS was defined as the time from the NF-PNET diagnosis date to the date of death (event occurred) or last contact (censor). CSS was defined as the time from the NF-PNET diagnosis date to the date of death due to NF-PNET (event occurred) or last contact (censor).

### Statistical Analysis

Based on the missing at random assumption approach, we used the multiple imputation method to impute missing data for race (1.69% missing), insurance (8.65% missing), marital status (5.86% missing), tumor site (9.04% missing), lymph node status (3.44% missing), and grade (20.47% missing), distant metastasis (6.26% missing), regional extension (2.68% missing), and tumor size (6.6% missing). Then, we compared the baseline characteristics before and after multiple imputation and found no significant difference (**Table 1**).

Continuous variables were described using median with interquartile range (IQR) or mean ± standard error values. Categorical variables were presented as numbers and percentages. We compared baseline characteristics between the surgery group and non-surgery group. The Wilcoxon rank-

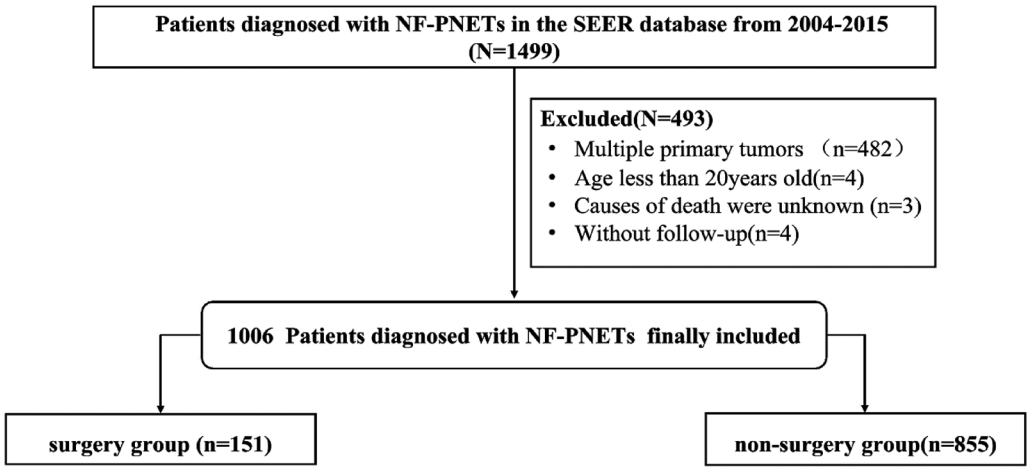


**FIGURE 1** | Flowchart of patient selection from the Surveillance, Epidemiology, and End Results database.

sum test was used to compare continuous variables, and categorical variables were compared using the chi-squared test. The balance in covariates was assessed using the standardized mean difference (SMD) approach. An imbalance in factors between the 2 groups was defined by an SMD of >0.1.

In non-randomized studies, the effect of treatment on outcomes can be impacted by treatment-selection bias wherein the treated cohort systematically differs from the control cohort. To account for section bias and confounding factors between the surgery group and the non-surgery group when comparing outcomes, inverse probability of treatment weighting (IPTW) was performed to balance differences in baseline demographical and clinical variables between patients who received surgery and those who did not. The Kaplan–Meier method using log-rank statistics was used to compare OS and CSS between the surgery and non-surgery groups for the IPTW-adjusted population. An IPTW-adjusted Cox proportional hazards regression analysis was performed for estimating the independent effect of surgery on the prognosis of NF-PNETs ≤2 cm.

In addition, we further conducted subgroup analyses according to grade, distant metastasis, regional extension, and lymph invasion. In each group, we also compared the OS and CSS by Kaplan–Meier analysis in the IPTW-adjusted population. An IPTW-adjusted Cox proportional hazards regression analysis was completed for each subgroup. Finally, we also conducted a sensitivity analysis for the population with missing values.

The present study conformed to the STROBE guideline (19). Statistical significance was defined by a 2-tailed $p$ value of <0.05. SPSS version 24 (IBM Corporation, Armonk, NY, USA) and R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org) were used for the statistical analyses.

## Subgroup Definition

To explore the impact of surgery on the prognosis of NF-PNETs ≤2 cm with different biological behaviors, we performed subgroup analyses, comparing the OS and CSS of the surgery and non-surgery groups according to grade (G), lymph node status (N), distant metastasis status (M), and regional extension status like invasion of surrounding tissues beyond the pancreatic capsule, including blood vessels, nerves, and fat. (E). Therefore, we divided the total population into the following 5 groups: G1, M0N0G0, M0N0G0E1, M0N1G0, and M1N0G0. The G1 group included patients whose tumors appeared poorly differentiated or undifferentiated with any lymph node status, distant metastasis status, and regional extension status. The M0N0G0 group included patients whose tumors did not appear to have distant metastasis or lymph node metastasis and appeared well–moderately differentiated with any regional extension status. The M0N0G0E1 group included patients whose tumor did not appear to have distant metastasis or lymph node metastasis and appeared well–moderately differentiated with local invasion. The M0N1G0 group included patients whose tumors appeared to have lymph node metastasis, a well–moderately differentiated tumor grade, and any regional extension status without distant metastasis. Finally, the M1N0G0 group included patients whose tumors appeared to have distant metastasis (to tissue or organs, except the pancreas and lymph nodes) and appeared well–moderately differentiated without lymph node invasion.

# RESULTS

## Baseline Characteristics in the Unadjusted and Adjusted Populations

Using the inclusion and exclusion criteria, we identified 1,006 patients in the SEER database diagnosed with NF-PNETs ≤2 cm between 2004 and 2015. Of these, there were 855 (85.0%) patients who were treated with surgery. In the overall cohort, patients without distant metastasis accounted for 89.2%, patients with tumors of a well–moderate grade

**TABLE 1** | Patient characteristics.

| Characteristic | Category | Before imputation | After imputation | *p*-value |
|---|---|---|---|---|
| *N* | | 1006 | 1006 | |
| Age (%) | | | | 1.000 |
| | 20–40 | 93 (9.24%) | 93 (9.24%) | |
| | 40–60 | 412 (40.95%) | 412 (40.95%) | |
| | ≥60 | 501 (49.80%) | 501 (49.80%) | |
| Race (%) | | | | 0.979 |
| | White | 758 (75.35%) | 768 (76.34%) | |
| | Black | 115 (11.43%) | 117 (11.63%) | |
| | Other | 116 (11.53%) | 121 (12.03%) | |
| | NA | 17 (1.69%) | – | |
| Sex (%) | | | | 1.000 |
| | Female | 541 (53.78%) | 541 (53.78%) | |
| | Male | 465 (46.22%) | 465 (46.22%) | |
| Year of diagnosis (%) | | | | 1.000 |
| | 2004–2010 | 233 (23.16%) | 233 (23.16%) | |
| | 2011–2015 | 773 (76.84%) | 773 (76.84%) | |
| Insurance (%) | | | | 0.956 |
| | Insured | 816 (81.11%) | 889 (88.37%) | |
| | Uninsured | 18 (1.79%) | 20 (1.99%) | |
| | Medicaid | 85 (8.45%) | 97 (9.64%) | |
| | NA | 87 (8.65%) | – | |
| Marital status (%) | | | | 1.000 |
| | Married | 632 (62.82%) | 674 (67.00%) | |
| | Unmarried | 158 (15.72%) | 168 (16.70%) | |
| | Divorced | 88 (8.75%) | 93 (9.24%) | |
| | Separated | 10 (0.99%) | 10 (0.99%) | |
| | Widowed | 59 (5.86%) | 61 (6.06%) | |
| | NA | 59 (5.86%) | – | |
| Tumor site (%) | | | | 0.997 |
| | Head | 256 (25.45%) | 286 (28.43%) | |
| | Body | 219 (21.77%) | 239 (23.76%) | |
| | Tail | 338 (33.60%) | 369 (36.68%) | |
| | Other | 102 (10.14%) | 112 (11.13%) | |
| | NA | 91 (9.04%) | – | |
| Tumor size (median [IQR]) | | 15.00 (10.00–18.00) | 15.00 (10.00–18.00) | 1.000 |
| Grade (%) | | | | 0.094 |
| | Well–moderate | 777 (77.24%) | 962 (95.63%) | |
| | Poorly undifferentiated | 23 (2.29%) | 44 (4.37%) | |
| | NA | 206 (20.47%) | – | |
| Distant metastasis (%) | | | | 0.805 |
| | Negative | 845 (84.00%) | 898 (89.26%) | |
| | Positive | 98 (9.74%) | 108 (10.74%) | |
| | NA | 63 (6.26%) | – | |
| Regional extension (%) | | | | 0.776 |
| | Local[a] | 872 (86.68%) | 892 (88.67%) | |
| | Extended[b] | 107 (10.64%) | 114 (11.33%) | |
| | NA | 27 (2.68%) | – | |
| Lymph invasion (%) | | | | 0.659 |
| | Negative | 867 (86.18%) | 892 (88.67%) | |
| | Positive | 104 (10.38%) | 114 (11.33%) | |
| | NA | 35 (3.44%) | – | |
| Surgery (%) | | | | 1.000 |
| | No | 151 (15.01%) | 151 (15.01%) | |
| | Yes | 855 (84.99%) | 855 (84.99%) | |
| Chemotherapy (%) | | | | 1.000 |
| | No | 954 (94.83%) | 954 (94.83%) | |
| | Yes | 52 (5.17%) | 52 (5.17%) | |
| Cancer-specific death (%) | | | | 1.000 |
| | No | 909 (90.36%) | 909 (90.36%) | |
| | Yes | 97 (9.64%) | 97 (9.64%) | |
| Overall survival (%) | | | | 1.000 |
| | No | 876 (87.08%) | 876 (87.08%) | |

(*continued*)

**TABLE 1** | Continued

| Characteristic | Category | Before imputation | After imputation | *p*-value |
|---|---|---|---|---|
| | Yes | 130 (12.92%) | 130 (12.92%) | |
| Survival months (median [IQR]) | | 36.00 (21.00–56.00) | 36.00 (21.00–56.00) | 1.000 |

*Abbreviation: IQR, interquartile range.*
[a]*Lesions confined to the pancreas.*
[b]*Lesions invaded the surrounding tissues of the pancreas.*

accounted for 95.6%, patients with local invasion accounted for 11.3%, and patients with lymph invasion accounted for 11.3% of the population, respectively. The baseline patient, tumor, and treatment characteristics of the cohort before and after IPTW are shown in **Table 2**. Before IPTW adjustment, there were significant differences between the 2 groups. Most notably, compared to the non-surgery group, the surgery group contained more patients with well–moderately differentiated tumors (97.5% vs. 84.8%, $p < 0.001$), fewer patients with distant metastasis (3.9% vs. 49.7%, $p < 0.001$), more patients without chemotherapy (98.2% vs. 75.5%, $p < 0.001$), and more patients without local invasion (89.8% vs. 82.1%, $p = 0.009$). Additionally, in the surgery group, there were more insured patients ($p = 0.036$) and married patients ($p < 0.001$), which indicated a degree of selection bias in the retrospective cohort. Age, race, sex, diagnosis year, and lymph invasion were not significantly different between the 2 groups ($p = 0.131$ for age, $p = 0.189$ for race, $p = 0.142$ for sex, and $p = 0.699$ for lymph node invasion). Following IPTW adjustment, there was no significant difference between the 2 groups with SMD < 0.1 for all covariables, which indicated a favorable balance of baseline patient, tumor, and treatment characteristics between the surgery group and non-surgery group.

## Surgery Group vs. Non-Surgery Group in the Overall Population

The OS rates at 1, 3, and 5 years were 95.1%, 94.4%, and 91.8% in the surgery group and 71.5%, 53.8%, and 44.0% in the non-surgery group, respectively. Meanwhile, the CSS rates at 1, 3, and 5 years were 97.8%, 96.3%, and 91.8% in the surgery group and 74.3%, 57.2%, and 49.1% in the non-surgery group. After IPTW adjustment, surgery was associated with improved OS and CSS (**Figure 2**). During the IPTW-adjusted Cox proportional hazards regression analysis, surgery was associated with significant OS (hazard ratio [HR], 0.205; 95% confidence interval [CI], 0.116–0.361; $p < 0.001$; **Table 3**) and CSS (HR, 0.187; 95% CI, 0.102–0.343; $p < 0.001$; **Table 3**) benefits.

## Subgroup Analysis

In the G1 and M1N0G0 groups, patients who had undergone surgery had better OS ($p = 0.0001$ for adjusted OS of the G1 group, **Figure 3A**; $p = 0.0008$ for adjusted OS of the M1N0G0 group, **Figure 3E**) and CSS ($p = 0.0016$ for adjusted CSS of the G1 group, **Figure 4A**; $p = 0.0009$ for adjusted CSS of the M1N0G0 group, **Figure 4E**) compared to those who had not undergone surgery after IPTW adjustment.

In the M0N0G0 group, the OS of the surgery group was higher than that of the non-surgery group ($p = 0.0029$ for adjusted OS, **Figure 3C**), but there was no difference in CSS ($p = 0.1082$, **Figure 4C**). In the M0N0G0E1 group, surgery was associated with improved OS and CSS ($p = 0.0401$ for adjusted OS, **Figure 3D**; $p = 0.0018$ for adjusted CSS, **Figure 4D**). In the M0N1G0 group, there were no significant differences between adjusted CSS and OS of the 2 groups ($p = 0.2506$ for adjusted OS, **Figure 3B**; $p = 0.1913$ for adjusted CSS, **Figure 4B**).

## Independent Role of Surgery for Survival Outcomes

During the IPTW-adjusted Cox proportional hazards regression analysis, we obtained almost the same results as those retrieved from the Kaplan–Meier survival analysis. Surgery was associated with significant OS and CSS benefits in the G1 group (HR of all-cause mortality, 0.289; 95% CI, 0.136–0.613; $p = 0.001$; **Figure 5** and HR of cancer-specific mortality, 0.238; 95% CI, 0.105–0.64, $p < 0.001$; **Figure 6**) and the M1N0G0 group (HR of all-cause mortality, 0.133; 95% CI, 0.039–0.455; $p = 0.001$; **Figure 5** and HR of cancer-specific mortality, 0.102; 95% CI, 0.021–0.496; $p = 0.005$; **Figure 6**). The OS and CSS in the M0N1G0 group were not improved by surgery (HR of all-cause mortality, 0.296; 95% CI, 0.536 −1.636; $p = 0.163$; **Figure 5** and HR of cancer-specific mortality, 0.26; 95% CI, 0.0462–1.461; $p = 0.126$; **Figure 6**). Surgery was associated with superior CSS in the M0N0G0E1 group (HR, 0.059; 95% CI, 0.005–0.683; $p = 0.002$; **Figure 6**), while there was no significant OS benefit related to surgery in the M0N0G0 group (HR, 0.387; 95% CI, 0.146–1.028; $p = 0.057$; **Figure 6**).

## Sensitivity Analysis

Since we performed multiple imputation on the total population, we conducted a sensitivity analysis of the population with missing values, and the results showed that surgery was associated with improved OS and CSS after IPTW adjustment ($p = 0.0295$ for adjusted OS, **Supplementary Figure S1A**; $p = 0.0475$ for adjusted CSS, **Supplementary Figure S1B**). The IPTW-adjusted Cox proportional hazards regression analysis showed that surgery had significant benefits on OS (HR, 0.223; 95% CI, 0.115–0.434; $p < 0.001$; **Table 3**) and CSS (HR, 0.198; 95% CI, 0.097–0.404; $p < 0.001$; **Table 3**).

# DISCUSSION

With the widespread use of high-quality, cross-sectional imaging, NF-PNETs ≤2 cm are now increasingly identifiable (20). However, the optimal management strategy for patients with such tumors remains controversial. In this study, it was proved that surgery benefits the CSS of the overall population

**TABLE 2** | Comparison of baseline characteristics before and after IPTW.

| Group | Unmatched | | | | IPTW | | | |
|---|---|---|---|---|---|---|---|---|
| | Level | Non-surgery | Surgery | *p*-value | Level | Non-surgery | Surgery | *p*-value |
| Age (%) | 20–40 | 14 (9.3) | 79 (9.2) | 0.131 | 20–40 | 14.5 | 9.5 | 0.485 |
| | 40–60 | 51 (33.8) | 361 (42.2) | | 40–60 | 37.8 | 41.2 | |
| | ≥60 | 86 (57.0) | 415 (48.5) | | ≥60 | 47.7 | 49.3 | |
| Race (%) | White | 118 (78.1) | 650 (76.0) | 0.189 | White | 79 | 76.4 | 0.582 |
| | Black | 21 (13.9) | 96 (11.2) | | Black | 12.5 | 11.3 | |
| | Other | 12 (7.9) | 109 (12.7) | | Other | 8.5 | 12.2 | |
| Sex (%) | Female | 90 (59.6) | 451 (52.7) | 0.142 | Female | 51.7 | 53.1 | 0.82 |
| | Male | 61 (40.4) | 404 (47.3) | | Male | 48.3 | 46.9 | |
| Year of diagnosis (%) | 2004–2010 | 44 (29.1) | 189 (22.1) | 0.074 | 2004–2010 | 21.7 | 23.1 | 0.777 |
| | 2011–2015 | 107 (70.9) | 666 (77.9) | | 2011–2015 | 78.3 | 76.9 | |
| Site (%) | Head | 56 (37.1) | 230 (26.9) | 0.036* | Head | 29.1 | 27.6 | 0.788 |
| | Body | 33 (21.9) | 206 (24.1) | | Body | 20.7 | 23.5 | |
| | Tail | 43 (28.5) | 326 (38.1) | | Tail | 35.5 | 37.7 | |
| | Other | 19 (12.6) | 93 (10.9) | | Other | 14.7 | 11.2 | |
| Grade (%) | Well–moderate | 128 (84.8) | 834 (97.5) | <0.001* | Well–moderate | 95.8 | 96.4 | 0.675 |
| | Poorly undifferentiated | 23 (15.2) | 21 (2.5) | | Poorly undifferentiated | 4.2 | 3.6 | |
| Distant metastasis (%) | Negative | 76 (50.3) | 822 (96.1) | <0.001* | Negative | 89.9 | 91.7 | 0.426 |
| | Positive | 75 (49.7) | 33 (3.9) | | Positive | 10.1 | 8.3 | |
| Chemotherapy (%) | No | 114 (75.5) | 840 (98.2) | <0.001* | No | 94.9 | 96.9 | 0.188 |
| | Yes | 37 (24.5) | 15 (1.8) | | Yes | 5.1 | 3.1 | |
| Regional extension (%) | Local | 124 (82.1) | 768 (89.8) | 0.009* | Local | 88.3 | 89.1 | 0.855 |
| | Extended | 27 (17.9) | 87 (10.2) | | Extended | 11.7 | 10.9 | |
| Lymph invasion (%) | Negative | 132 (87.4) | 760 (88.9) | 0.699 | Negative | 88.1 | 88.8 | 0.88 |
| | Positive | 19 (12.6) | 95 (11.1) | | Positive | 11.9 | 11.2 | |
| Insurance (%) | Insured | 126 (83.4) | 763 (89.2) | 0.036* | Insured | 88.3 | 88.9 | 0.284 |
| | Uninsured | 2 (1.3) | 18 (2.1) | | Uninsured | 0.5 | 1.9 | |
| | Medicaid | 23 (15.2) | 74 (8.7) | | Medicaid | 11.2 | 9.2 | |
| Marital status (%) | Married | 78 (51.7) | 596 (69.7) | <0.001* | Married | 64.2 | 67.7 | 0.942 |
| | Unmarried | 27 (17.9) | 141 (16.5) | | Unmarried | 19 | 17.2 | |
| | Divorced | 25 (16.6) | 68 (8.0) | | Divorced | 8.7 | 8.5 | |
| | Seperated | 3 (2.0) | 7 (0.8) | | Seperated | 1.2 | 1.2 | |
| | Widowed | 18 (11.9) | 43 (5.0) | | Widowed | 6.9 | 5.4 | |
| Tumor size (mm, mean ± SD) | | 14.54 ± 5.02 | 13.56 ± 4.59 | 0.017* | | 13.78 (4.89) | 13.61 (4.59) | 0.778 |

*Abbreviations: SD, standard deviation; IPTW, inverse probability of treatment weighting.*
*$p < 0.05$.

and G1, M1N0G0, and M0N0G0E1 groups, while patients in the M0N0G0E0 and M0N1G0 groups do not gain such a benefit. In a further step, we used multivariate Cox models to find that surgical treatment is an independent prognostic factor of CSS in the overall population and G1, M0N0G0E1, and M1N0G0 groups. Because of the heterogeneity of oncologic behavior and prognosis of PENTs, we should be prudent in discerning the impact of surgery on the overall population, and we believe it is reasonable and necessary to explore the prognostic impact of surgery in PNETs with different biological behaviors.

To the best of our knowledge, the present study is the first to provide compelling evidence to support different impacts of surgical treatment on patients with NF-PNETs ≤2 cm in different subgroups, including those divided according to M status, N status, regional extension, and grade, at the same time. A striking conclusion was obtained in this study by applying multiple imputation to missing data, which reduced the estimation and improved the validity. Moreover, in order to reduce the selection bias or another bias caused by the limitations of real-world research, IPTW was used to weigh the population in the adjusted analysis, which is potentially a more beneficial approach than using common matching techniques, such as retaining all the samples. After learning from prior proposals about conducting survival analysis, the influence of treatment effects on survival was analyzed in the present study, and CSS and OS were used to mitigate the unmeasured selection bias of the treatment effect.

Characterized by their indolent course and lacking early symptoms, the management of NF-PNETs ≤2 cm is still considered to be controversial. The guidelines or expert

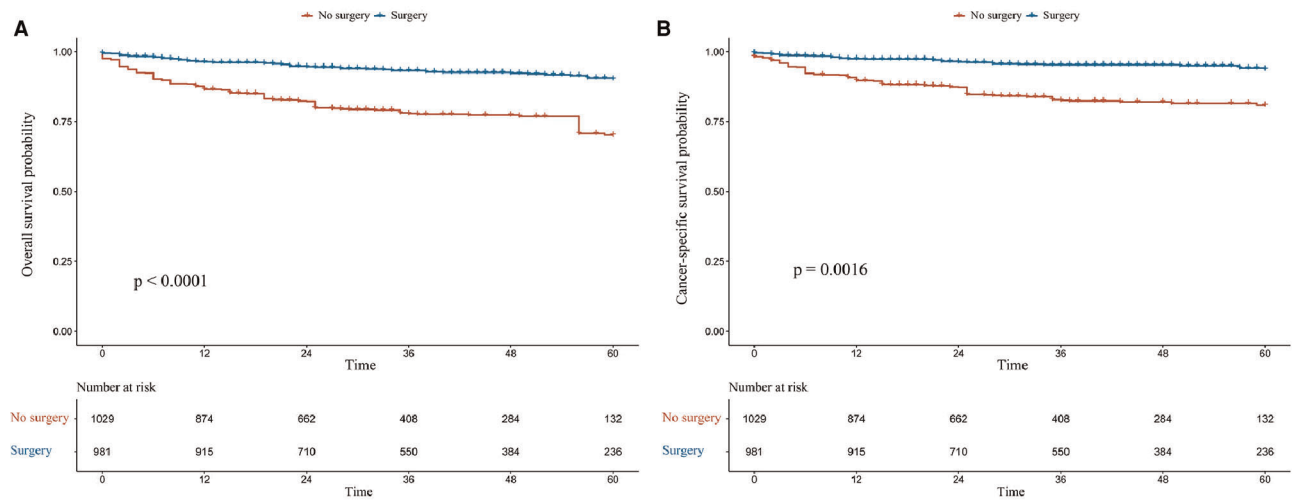


**FIGURE 2** | Kaplan–Meier survival estimates of overall (**A**) and cancer-specific (**B**) survival in the overall population after inverse probability of treatment weighting adjustment.

**TABLE 3** | Multivariable Cox proportional hazards models in the weighted population.

| | | | Overall survival[a] | | | Cancer-specific survival[b] | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | *p*–value | HR | 95% CI | *p*–value |
| Overall population | Surgery | No | 1.00 | – | – | 1.00 | – | – |
| | | Yes | 0.205 | 0.116–0.361 | <0.001* | 0.187 | 0.102–0.343 | <0.001* |
| Patients with missing data | Surgery | No | 1.00 | – | – | 1.00 | – | – |
| | | Yes | 0.223 | 0.115–0.434 | <0.001* | 0.198 | 0.0969–0.404 | <0.001* |

*Abbreviation: HR, hazard ratio.*

[a]*The OS multivariable model was constructed with surgery, age, sex, race, tumor site, tumor size, grade, distant metastasis, reginal extension, and lymph node invasion.*

[b]*The CSS multivariable model was constructed with pre-specified variables (surgery, sex, age, grade, reginal extension and distant metastasis) to avoid overfitting considering the limited number of outcomes.*

**p < 0.05.*

consensuses that have been published in different regions are not consistent at present. For example, the ENETS guidelines (12) suggest that observation is a reasonable option for patients with NF-PNETs ≤2 cm. However, other studies have pointed out that, even when the tumors are small (≤2 cm), they showed signs of malignant behavior, such as extra-pancreatic extension, lymph nodal metastasis, distant-organ metastasis, and recurrence, which may lead to disease-related death (21). A consensus statement announced by the Chinese Study Group for Neuroendocrine Tumors (CSNET) (22) suggested that a more aggressive approach be undertaken, except in some selected cases of NF-PNETs <1 cm or patients with incidentally discovered and unacceptable surgical risks. Patients with NF-PNETs ≤2 cm should be treated with tumor resection and careful postoperative surveillance. Meanwhile, it was suggested in the North American Neuroendocrine Tumor Society (NANETS) guidelines (23) that observation is an optimal choice for NF-PNETs ≤1 cm, while the management of NF-PNETs 1–2 cm should be considered on an individual basis according to some factors, including patient age, comorbidities, endoscopic ultrasonography–fine-needle aspiration or endoscopic ultrasonography–biopsy findings (grade, Ki-67), tumor growth status, anatomical location, extent of procedure required for complete resection, patient preferences, and access to long-term follow-up. In a meta-analysis of 714 patients with NF-PNETs <2 cm, it was discovered that PNET excision was linked to better 1-, 3-, and 5-year OS rates (13). The conclusion that surgery improves patient survival was then pushed through the literature as proof of its superiority. Therefore, whether patients with NF-PENTs ≤2 cm ought to be treated with surgery should be further explored by more accurate and specific grouping of this population. Our research has solved some of the differences in this area.

Consistent with another study (24) that evaluated 709 patients who had stage I disease according to the eighth edition of the American Joint Committee on Cancer criteria, our subgroup analysis of the M0N0G0 group found that, while surgical resection was associated with improved OS, there was no benefit offered to CSS. Compared to this earlier

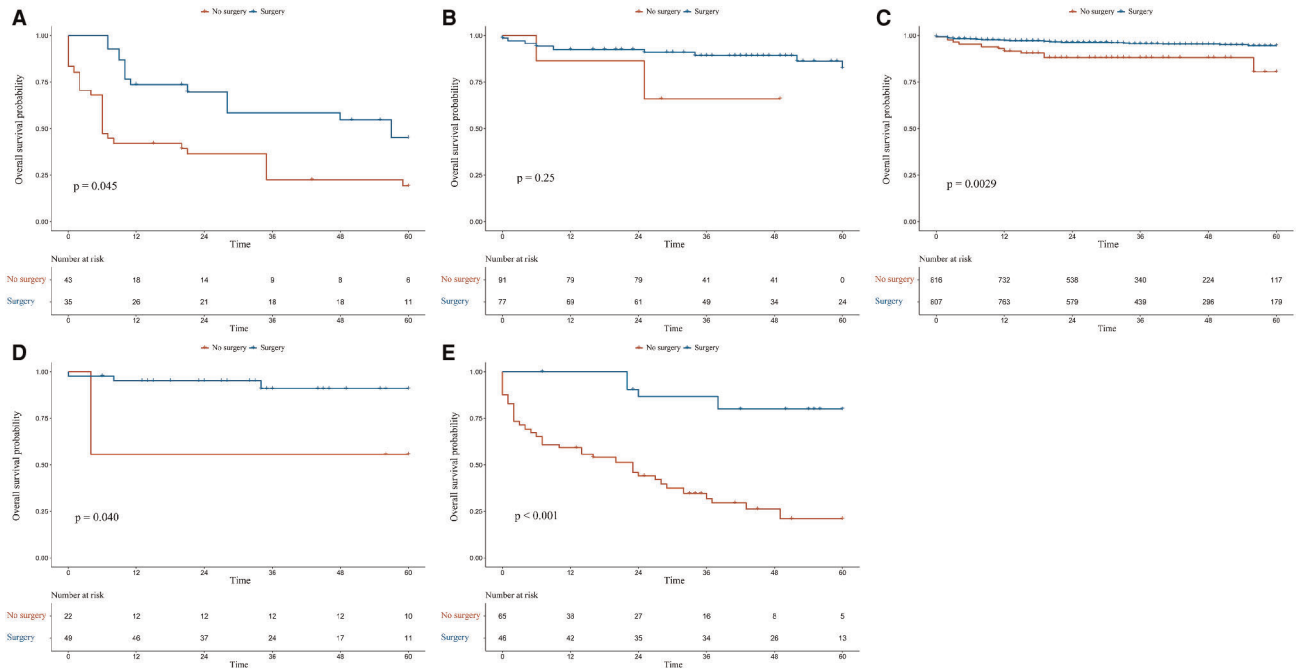


**FIGURE 3** | Kaplan–Meier survival estimates of overall survival in different subgroups after inverse probability of treatment weighting adjustment: (**A**) G1, (**B**) M0N1G0, (**C**) M0N0G0, (**D**) M0N0G0E1, and (**E**) M1N0G0.

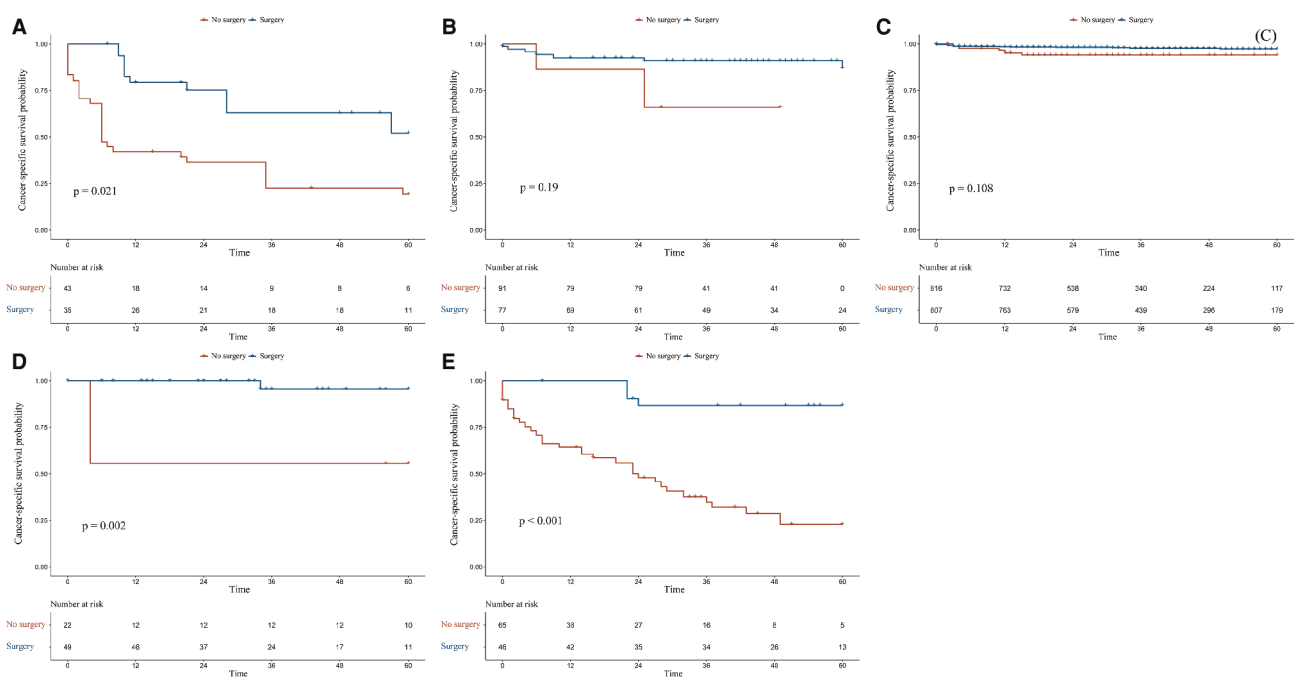


**FIGURE 4** | Kaplan–Meier survival estimates of cancer-specific survival in different subgroups after inverse probability of treatment weighting adjustment: (**A**) G1, (**B**) M0N1G0, (**C**) M0N0G0, (**D**) M0N0G0E1, and (**E**) M1N0G0.

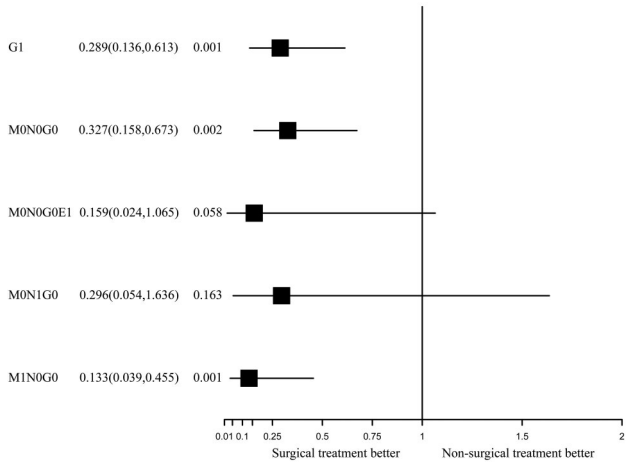


**FIGURE 5** | Forest plot depicting inverse probability of treatment weighting–adjusted hazard ratios of all-cause mortality in different subgroups of surgery versus non-surgery. The multivariable model was constructed with pre-specified variables (surgery, sex, age) to avoid overfitting, considering the limited number of outcomes.

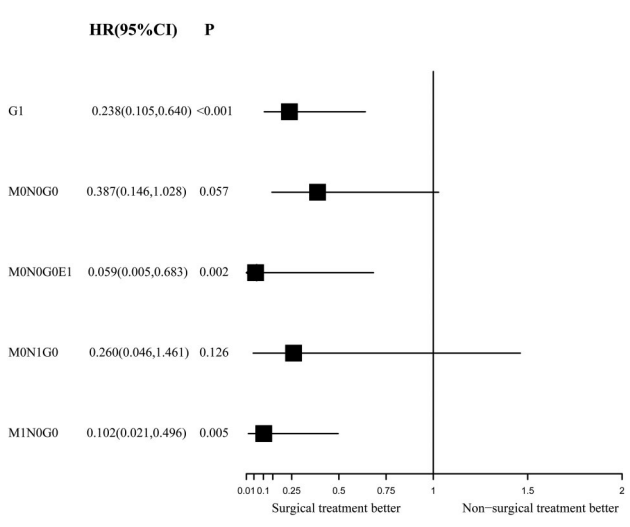


**FIGURE 6** | Forest plot depicting inverse probability of treatment weighting–adjusted hazard ratios of cancer-specific mortality in different subgroups of surgery versus non-surgery. The multivariable model was constructed with pre-specified variables (surgery, sex, age) to avoid overfitting, considering the limited number of outcomes.

study, our investigation further confirmed that surgical treatment did not improve the OS or CSS of M0N1G0 patients, and this result may be related to the unclear risk-stratification of lymph node metastasis and the inconsistent impact of lymph node metastasis on survival among patients with NF-PENTs ≤2 cm (25–28). Another study using the SEER database analyzed 2,158 patients with a median tumor size of 5 cm and found that lymph node status was not a predictor of overall survival (29). These findings reinforced the observation that close follow-up is a reasonable strategy for M0N0G0 and M0N1G0 patients with NF-PNETs ≤2 cm. In addition, our study conducted a subgroup analysis of the population with local invasion (M0G0N0E1 group), where CSS was significantly improved by surgical treatment. This result may be related to the higher degree of malignancy of NF-PENTs invading the surrounding tissues of the pancreas. In addition, regional extension is related to the tumor growth rate and its anatomical location, which can be monitored by imaging methods like magnetic resonance imaging/computed tomography (30). A retrospective multi-institutional analysis that included 119 cases with confirmed NF-PNETs and resection suggested that vascular/perineural invasion is a significant prognostic factor of recurrence (31).

In order to explore the role of surgical treatment in a population with different tumor grades, a study including 380 patients (32) reported an interesting conclusion that surgical resection provides a survival benefit for patients with NF-PNETs ≤2 cm, even those with favorable well-differentiated and moderately differentiated histologies, and tumor size and margin status were not predictors of survival. Meanwhile, other studies have pointed out that poor pathological grades are signs of malignant behavior and commonly considered to be risk factors affecting the prognosis of NF-PNETs ≤2 cm (15, 33, 34). We support the conclusion that patients with poorly differentiated or undifferentiated tumors could benefit from surgical treatment, regardless of distant metastasis, lymph node metastasis, or regional extension. However, we believe that the conclusion that patients with favorable well-differentiated tumors could benefit from surgical treatment should be considered on a case-by-case basis. As mentioned earlier, we found that surgical treatment could not provide survival benefits to patients with well-differentiated tumors (those in the M0G0N0E0 and M0N1G0 groups) so long as their tumors did not appear to have regional extension or distant metastasis.

In addition, many studies have pointed out that distant metastasis is associated with the poor prognosis of NF-PNETs ≤2 cm (35–37), and we suggested that M1N0G0 patients should undergo surgery to obtain survival benefits unlike patients with pancreatic cancer, even if the grade is well-differentiated. Ye et al. (38) evaluated 758 NF-PNET patients with distant metastasis and reported that the median OS of patients treated with surgery was noticeably higher than that of those who were not treated with surgery (79 vs. 24 months). The median CSS of patients who underwent surgical resection of the primary tumor was 81 months, while that of patients who did not undergo surgical resection of the primary tumor was 26 months. However, this study included 627 NF-PNETs > 2 cm, and the NF-PNET ≤2 cm cohort was not analyzed separately from this group, so the study conclusions may be driven by these larger tumors. Therefore, our research is more convincing in terms of providing accurate findings for patients with NF-PNETs ≤2 cm with distant metastasis.

The current study has several limitations. First, given its retrospective nature, the present study has some inherent selection bias. Although the measured confounders that may influence the treatment choice and final treatment effect of patients were well balanced, any remaining unbalanced and unmeasured confounders would still bring about some bias. Second, the variables used for the survival analysis and multivariate Cox regression analysis were only a subset of the clinical and pathological features. Some important tumor markers, such as the Ki-67 index, and some positive prognostic variables, such as surgical margin status, were not available in the SEER datasets. Third, subgroup analysis was not performed for tumors ≤1 cm or 1–2 cm, and some studies have suggested these values are more rational tumor size cutoffs to identify malignancy (39–41). However, this discrepancy does not completely limit the unique effect of surgical resection of NF-PNETs ≤2 cm that we report here.

In conclusion, we have demonstrated that, first, as long as there is no distant metastasis and the tumor is well–moderately differentiated, whether lymph node metastasis occurs or not, patients may not benefit from surgery. However, if this population shows local invasion, surgery should be performed. Second, surgery should be performed if the grade is poorly undifferentiated, regardless of distant metastasis, lymph node metastasis, or regional extension. At last, patients with distant metastasis, regardless of their tumor grade, can benefit from surgery. These findings could help clinicians make better decisions about whether to choose surgery for patients with NF-PNETs ≤2 cm.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov.

## AUTHOR CONTRIBUTIONS

JY and HW had contributed equally to this work and share first authorship. JY and HW conceived the study, wrote the manuscript, searched the database, reviewed the studies, collected the data and performed the statistical analyses. LJ and LC are the guarantors for this study. LJ and LC performed revision of the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2022.890564/full#supplementary-material.

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# Diagnostic work-up and advancement in the diagnosis of gastroenteropancreatic neuroendocrine neoplasms

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Neuroendocrine neoplasms (NENs) are a heterogeneous group of neoplasms ranging from well-differentiated, slowly growing tumors to poorly differentiated carcinomas. These tumors are generally characterized by indolent course and quite often absence of specific symptoms, thus eluding diagnosis until at an advanced stage. This underscores the importance of establishing a prompt and accurate diagnosis. The gold-standard remains histopathology. This should contain neuroendocrine-specific markers, such as chromogranin A; and also, an estimate of the proliferation by Ki-67 (or MIB-1), which is pivotal for treatment selection and prognostication. Initial work-up involves assessment of serum Chromogranin A and in selected patients gut peptide hormones. More recently, the measurement of multiple NEN-related transcripts, or the detection of circulating tumor cells enhanced our current diagnostic armamentarium and appears to supersede historical serum markers, such as Chromogranin A. Standard imaging procedures include cross-sectional imaging, either computed tomography or magnetic resonance, and are combined with somatostatin receptor scintigraphy. In particular, the advent of $^{111}$In-DTPA-octreotide and more recently PET/CT and $^{68}$Ga-DOTA-Octreotate scans revolutionized the diagnostic landscape of NENs. Likewise, FDG PET represents an invaluable asset in the management of high-grade neuroendocrine carcinomas. Lastly, endoscopy, either conventional, or more advanced modalities such as endoscopic ultrasound, capsule endoscopy and enteroscopy, are essential for the diagnosis and staging of gastroenteropancreatic neuroendocrine neoplasms and are routinely integrated in clinical practice. The complexity and variability of NENs necessitate the deep understanding of the current diagnostic strategies, which in turn assists in offering optimal patient-tailored treatment. The current review article presents the diagnostic work-up of GEP-NENs and all the recent advances in the field.

KEYWORDS

neuroendocrine tumor, ki67 proliferation index, chromogranin A, gut peptide hormones, NETest, somatostatin receptor scintigraphy, 68-gallium-DOTA-Octreotate

# 1. Introduction

Neuroendocrine neoplasms (NEN) are rare and heterogeneous tumors that are phenotypically similar and derive from the diffuse neuroendocrine cell system. These neoplasms demonstrate a rising prevalence and incidence. This is likely a result of the deeper and better understanding of these tumors, but also of the advent and integration of more advanced diagnostic means (1–4). In general, NENs exhibit slow growth and often absence of specific symptoms, which may in turn delay the diagnosis until at an advanced stage, when overt symptoms may develop. Among the several distinct sites of origin, gastroenteropancreatic NENs (GEP NENs) represent the commonest subtype, accounting for nearly 60% of all NENs. Among these, small bowel- (SBNEN) and pancreatic- NENs (pNEN) are the most frequent (5–8).

In addition to the variable primary sites, tumor heterogeneity is also evident by their variable biologic behavior. Often these tumors run a "benign" course with no ostensible disease progression and excellent prognosis. However, non-uncommonly, they may also be truly malignant, associated with an aggressive course, poor prognosis and a very limited life expectancy, mimicking other cancers (9).

This complexity and variability of NENs necessitate the deeper and better understanding of the current diagnostic armamentarium and strategic approach, and integration in clinical practice of all novel diagnostic tools. This in turn is pivotal to determine the optimal (tailored) treatment, including accurate selection of surgical candidates. The diagnostic cascade should be initiated once there is clinical suspicion. Initial work-up involves assessment of serum Chromogranin A and, in selected patients, measurement of gut peptide hormones. Recently, the measurement of multiple NEN-related transcripts or the detection of circulating tumor cells has been introduced and will play a key role, and seems to be superior to historical serum markers, such as Chromogranin A. Cross-sectional imaging, combined with somatostatin receptor scintigraphy and PET scan will complement the diagnostic approach and assist in disease stratification. Ultimately, the gold-standard of the diagnosis remains histopathology. The present review discusses the diagnostic work-up of GEP-NENs and presents all the novel diagnostic means that emerged over the last years. **Table 1** summarizes current diagnostic modalities and their clinical utility.

## 1.1. Clinical presentation

Not uncommonly, GEP-NENs are incidentally discovered. The rate of such presentation varies; for instance, in pNENs not producing hormones incidental diagnosis can exceed 50% (10), whereas it can be as high as 80% in the case of appendiceal NENs (11). Often NENs cause non-specific symptoms, such as abdominal pain or discomfort, weight loss, change in bowel habits or diarrhea. These symptoms are often attributed to other causes, such as gastritis, irritable bowel syndromes, or other relevant disorders, before a diagnosis is established. In contrast to such presentation, however, NENs may also overproduce hormones, such as 5-hydroxytryptamine (5-HT, serotonin), that results is associated symptoms. Such tumors are termed functional NENs, in contrast to non-functional NENs. For instance, the "carcinoid syndrome" is a syndrome that is mostly present in SBNENs, in the presence of hepatic or retroperitoneal metastases. This is caused due to 5-HT reaching the systemic circulation. As a result, patients present with a variety of symptoms, often precipated by a variety of foods, alcohol, stress, ot other triggers. Most commnonly subjects present with paroxysmal flushing, chronic diarrhea, wheezing and less frequently carcinoid heart disease (CHD), among others. Analogous to this, the secretion of other hormones by this subgroup of NENs, termed functionally active, such as insulin, gastrin, vasoactive intestinal peptide, glucagon, somatostatin, and others, can lead to specific syndromes, which are discussed later in more detail (6, 8). **Table 2** summarizes functional pNENs and their respective presentation.

# 2. Pathology

Histopathological confirmation represents the gold standard for the diagnosis of NENs and is recommended by the European Neuroendocrine Tumor Society (ENETS) (12). Tissue diagnosis should be pursued when clinically feasible. It should be noted that a biopsy is deemed superior to a fine needle aspirate (FNA) when, this is feasible (12, 13). This is of particular interest in the context of Endoscopic Ultrasound (EUS), where if not enough material is available, there is a risk of under-grading the tumor (6, 14, 15). In a recent study by our study group, data of patients who underwent EUS-guided tissue sampling of suspicious pancreatic lesions over a 13-year period were analyzed. Lesions underwent EUS-FNA or FNB sampling, or a combination of the two, and the accuracy and safety of different EUS-guided sampling methods for confirmed pNENs were investigated. Diagnostic yield of EUS-FNA and EUS-FNB alone, including the inadequate specimens, was 77.5% (95% CI: 68.9%–86.2%) and 85.4% (95% CI: 74.6%–96.2%), respectively, whereas the combination of both sampling modalities established the diagnosis in over 95% of cases. Diagnostic sensitivity among the adequate samples for EUS-FNA, EUS-FNB and for the combination of the two methods was 88.4% (95% CI: 80.9%–96.0%), 94.3% (95% CI: 86.6%–100%) and 100% (95% CI: 100%–100%). These findings clearly illustrated that EUS-FNB improves diagnostic sensitivity and provides further information than cytological assessment alone, in patients with pNENs (16).

When a NEN is considered or clinically suspected, in addition to the conventional histopathological analysis, immunohistochemistry should be performed, to assess the tumor phenotype and Ki-67. Immunohistochemical staining with synaptophysin, and Chromogranin A (CgA) is also required. Ki-67 is a cell proliferation–associated nuclear marker, that is critical in assessing the differentiation of NENs, and as a result their respective course. CgA is a protein commonly secreted by neuroendocrine tumor cells (17).

TABLE 1 Current diagnostic tools in neuroendocrine neoplasms (NENs).

| Modality | Indication | Strengths | Limitations |
|---|---|---|---|
| **Histopathology (tissue diagnosis)** | – To be pursued in all NENs, when feasible | – Gold standard for diagnosis | – Expert pathologist input recommended |
| **Biomarkers** | | | |
| Serum Chromogranin A (CgA) | – At diagnosis and during follow-up | – Well-studied biomarker<br>– Can be used in functional and non-functional NENs | – Moderately sensitive, variable specificity<br>– Not useful prognosticator<br>– False positive results due to several factors<br>– international standard for CgA assay lacks |
| Urinary 5-hydroxyindoleacetic acid (5-HIAA) | – At diagnosis and during follow-up<br>– Particularly useful in patients with carcinoid syndrome | – Well-studied biomarker<br>– Significant sensitivity and specificity especially in carcinoid syndrome | – Not useful prognosticator<br>– Dietary restrictions prior to urine collection<br>– Falsely elevated or low due to various factors |
| Gut Peptide Hormones (insulin, gastrin, VIP, glucagon, somatostatin) | – Used for functional NENs, especially pancreatic and duodenal | – Inappropriate elevation of the appropriate, specific serum hormonal marker required for diagnosis | – Should be interpreted with caution, and within a relevant clinical context<br>– Various factors affect levels |
| **Cross-sectional imaging** | | | |
| Contrast-enhanced computed tomography (CT) | – Backbone of diagnosis, staging, follow-up and assessment of treatment response | – Broadly available<br>– Well established modality<br>– Best modality to assess vascular infiltration<br>– Useful in the pre-operative setting | – Radiation exposure<br>– Variable sensitivity<br>– Less accurate in the diagnosis of gastric, duodenal, rectal and colonic NENs (still important for staging) |
| Contrast-enhanced magnetic resonance imaging (MRI) | – Similar to CT<br>– Modality of choice, or complementary to CT | – Not contraindicated in patients allergic to iodine contrast<br>– No radiation exposure<br>– Superior to CT in assessing bone, brain, or abdominal disease<br>– Superior to CT when hepatocyte-specific contrast is used | – Less available than CT<br>– Contraindicated in patients with metallic implants |
| **Nuclear Medicine and Hybrid Imaging** | | | |
| $^{68}$Gallium-DOTA-peptides | – Investigation of choice for well-differentiated NENs | – Mean sensitivity and specificity: 88%–93% and 88%–95%, respectively<br>– Superior to cross-sectional imaging in bone metastases<br>– Sensitive in detecting even subtle lymph node or small peritoneal metastases<br>– Unaffected by the use of somatostatin analogs before examination<br>– Lower exposure to radiation than classical scintigraphy | – Still not broadly available<br>– More expensive than other modalities |
| $^{18}$FDG PET/CT | – More useful in high-grade poorly differentiated neuroendocrine carcinomas (NEC) | – More sensitive than other modalities in detecting even subtle high-grade NECs | – Still not broadly available<br>– More expensive than other modalities<br>– Falsely positive results in active inflammation or infection |
| **Endoscopy** | | | |
| Gastroscopy/Colonoscopy | – Investigation of choice for gastric, duodenal, rectal and colonic NENs | – Allows biopsy of neoplasm<br>– Primary NEN may be resected when indicated | – Invasive procedure<br>– Associated with adverse events, in particular in frail patients<br>– Biopsies may be misleading as NENs are subepithelial lesions |
| Endoscopic Ultrasound (EUS) | – Indicated in gastric, duodenal, and pancreatic NENs<br>– Useful for diagnosis and staging<br>– FNB should be preferred over FNA | – Increased sensitivity and specificity<br>– Enables detection of previously unidentified tumors<br>– Permits tissue diagnosis and histological evaluation (superior to conventional endoscopy) | – Invasive procedure<br>– Associated with adverse events, in particular in frail patients<br>– Depends on endoscopists skills |
| | | | – Risk of capsule retention |

(*continued*)

TABLE 1 Continued

| Modality | Indication | Strengths | Limitations |
|---|---|---|---|
| Small Bowel Capsule Endoscopy (SBCE) | – May have a role in detecting multifocal SBNENs pre-operatively or metastatic disease of unknown primary | – Enables detection of primary NEN or multiple NENs in small bowel (variable sensitivity)<br>– -Could determine extent of resection | – Relevant expertise required<br>– Further research required |
| Balloon Enteroscopy (BE) | – May have a role in detecting multifocal SBNENs pre-operatively or metastatic disease of unknown primary<br>– Complementary to SBCE | – Enables detection of primary NEN or multiple NENs in small bowel (variable sensitivity)<br>– Could determine extent of resection | – Invasive procedure<br>– Associated with adverse events, in particular in frail patients<br>– Relevant expertise required<br>– Further research required |

TABLE 2 Functional pancreatic neuroendocrine tumor syndromes.

| | Biologically active peptide secreted | Tumor location | Associated with MEN-1, % | Main symptoms/signs |
|---|---|---|---|---|
| **Insulinoma** | Insulin | Pancreas (>99%) | 4–5 | Hypoglycaemic symptoms |
| **Zollinger-Ellison syndrome** | Gastrin | Duodenum (70%); Pancreas (25%); Other sites (5%) | 20–25 | Abdominal pain; peptic ulcer disease; diarrhoea; oesophageal symptoms (reflux) |
| **VIPoma (Verner-Morrison syndrome)** | Vasoactive intestinal peptide | Pancreas (90%, adult) | 6 | Profuse watery diarrhoea; hypokalaemia; dehydration |
| **Glucagonoma** | Glucagon | Pancreas (100%) | 1–20 | Dermatitis (necrolytic migratory erythema); glucose intolerance; weight loss; deep vein thrombosis |
| **Somatostatinoma** | Somatostatin | Pancreas (55%); duodenum/ jejunum (44%) | 45 | diabetes mellitus; cholelithiasis; diarrhoea (steatorrhea) |

The AJCC (American Joint Committee on Cancer), ENETS, UICC (International Union for Cancer Control), WHO (World Health Organization) developed a series of systems to classify NENs. Similar to other neoplasms, these classification systems are used to stage the disease, and are essential for treatment selection and prognostication (18–25). In particular, the WHO proposes a universal definition system for neuroendocrine neoplasia based on differentiation and proliferative grading. Table 3 summarises the novel WHO NEN classification.It integrates the mitotic count, and most importantly the nuclear antigen Ki-67, as markers of the proliferation activity of these neoplasms. Ki67 is more accurate and reproducible than the mitotic index (24, 25). Historically, a Ki-67 > 20% was believed to define poorly differentiated neoplasms, indicating an overall unfavorable prognosis. However, emerging evidence indicated that there is a distinct category of well-differentiated grade 3 neuroendocrine tumors (NETs), that is clearly different from the very aggressive poorly differentiated grade 3 neuroendocrine carcinomas (NECs), the latter associated with an unfavorable prognosis (19–21). More recently, the 5th edition of the WHO Classification of Endocrine Tumors was published, termed Classification of Endocrine and Neuroendocrine Tumors. This up-to-date classification system integrates this emerging evidence. In particular, the novel WHO 2022 system describes NECs, epithelial poorly differentiated neoplasms, composed of cells with severe cellular atypia and severely deranged molecular/genetic profiles, that broadly retain neuroendocrine markers. These NECs are further subclassified in small or large cell types, and Ki-67 is >20%, often >70%. This is in contrast to well differentiated grade 3 NETs. This is shown to have clinical implications on prognosis: Grade 3 NECs for instance were shown to have a 4 months' shorter median survival than G3 NETs and responded better to platinum-based chemotherapy (26, 27).

# 3. Biomarkers used in the diagnosis of neuroendocrine tumors

## 3.1. Classical blood and urine biomarkers

### 3.1.1. Chromogranin A

Over the years, a considerable number of biomarkers have been integrated in clinical practice, and are used for diagnostic purposes, but also to follow-up patients with established disease. The most important among them is the general serum biomarker CgA. This is an acid glycoprotein present in the secretory dense core granules of most neuroendocrine cells. It is also secreted from neuroendocrine-derived tumors, including GEP-NENs, pheochromocytomas, and others. Of note, both functional and non-functional NENs may result in elevated CgA levels (28). CgA is a moderately sensitive marker, whereas specificity largely relies upon the type and tumor burden (for instance, specificity of approximately 100% has been reported in metastatic tumors). In particular, specificity of assays ranges from 68 to 100% and sensitivity ranges from 42%–93%, depending upon tumor primary site, grade, or disease burden (29). Significantly elevated CgA levels are unlikely to be encountered in other disease than NENs, with the exception maybe of patients receiving protein pump inhibitors (PPIs) (30–34). Although this is a marker that has been well validated for diagnostic and follow-up purposes, CgA cannot steadily be used for prognostication (35, 36).

TABLE 3 WHO 2022 classification system for neuroendocrine tumors.

| Neuroendocrine Neoplasm | Classification | Diagnostic Criteria |
|---|---|---|
| Well-differentiated neuroendocrine tumor (NET) | Grade 1 | < 2 mitoses/2 mm$^2$ and/or Ki67 < 3% |
| | Grade 2 | 2–20 mitoses/2 mm$^2$ and/or Ki67 3%–20% |
| | Grade 3 | > 20 mitoses/2 mm$^2$ and/or Ki67 > 20% |
| Poorly differentiated neuroendocrine carcinoma (NEC) | Small cell NEC | > 20 mitoses/2 mm$^2$ and/or Ki67 > 20% (often > 70%), and small cell cytomorphology |
| | Large cell NEC | > 20 mitoses/2 mm$^2$ and/or Ki67 > 20% (often > 70%), and large cell cytomorphology |

In addition, CgA should be interpreted with caution as several conditions may lead to falsely positive elevation. Reasons for false positive CgA elevation include renal disease, Parkinson disease, uncontrolled hypertension, pregnancy, hypergastrinemia, chronic atrophic gastritis, among others. In addition, treatment with antisecretory medications, especially PPIs have been associated with falsely elevated CgA levels, and PPIs should be interrupted, leaving a clearance of at least 3 half-lives, prior to testing, where this is possible and safe for the patient. Analogous to this, steroid treatment or glucocorticoid excess can lead to upregulation of CgA. A limitation of CgA is also the fact that a recognized international standard for CgA assay is not available and variations in assay types may influence results. It is thus recommended that reference laboratories should be preferred when available, and that serial measurements should be performed using the same assay (28).

### 3.1.2. Urinary 5-hydroxyindoleacetic acid

As already discussed, NENs may secrete 5-hydroxytryptamine (5-HT, serotonin) and other hormones, and in some subjects particularly with SBNENs this may mediate the carcinoid syndrome. Urinary 5-hydroxyindoleacetic acid (5-HIAA) is the urinary metabolite, following breakdown of 5-HT. Urinary 5-HIAA has proven of great value, and has been integrated in the diagnosis and follow-up of patients with carcinoid syndrome (28). The sensitivity and specificity of this marker has been reported to be 70% and 90%, respectively. Midgut NENs, such as SBNENs, produce more serotonin than the rest of the NENs, and it is when 5-HIAA is most useful. It should also be noted that urinary 5-HIAA levels depend upon the respective volume of the neoplasm and thus could be normal in individuals with no metastases (37, 38). Like CgA, there is no sound scientific evidence supporting the role of urinary 5-HIAA for prognosis. To increase accuracy, specific dietary restrictions should be followed prior to urine collection. Falsely low urinary 5-HIAA levels may be encountered in cases of impaired kidney function or on haemodialysis. Lastly, one needs to consider that urinary 5-HIAA levels may be falsely elevated in cases of malabsorptive disease, not treated. Examples include untreated coeliac disease, tropical sprue, Whipple disease, etc (28).

A less common manifestation of carcinoid syndrome is CHD, which affects nearly 20% of patients with "carcinoid syndrome". In CHD, plaque-like, fibrous endocardial thickening of the cardiac valves develops. Patients with CHD have a poor prognosis. This is due to the gradual development of heart valve dysfunction and finally progressive heart failure. CHD is believed to be caused by this same tumor secretion of vasoactive hormonal products. Bhattacharyya et al. prospectively followed-up more than two hundred fifty patients with carcinoid syndrome for a median follow-up of 29 months. 44 of the included individuals either developed *de novo* CHD or exhibited deterioration in the pre-existing valvular dysfunction. This was associated with a synchronous elevation in the median levels of urinary 5-HIAA 5-HIAA levels of more than 300 μmol/24 h were reported to independently predict CHD development or progression, among other factors (39).

### 3.1.3. N-terminal pro-brain natriuretic peptide

N-terminal pro-brain natriuretic peptide (NT-proBNP) is a natriuretic peptide, and it is an established diagnostic test for heart failure and its management. In the context of NENs, it should be used to screen for CHD, with proven and well-established value (40). Bhattacharyya et al. reported that in patients with carcinoid syndrome and valvular involvement, NT–proBNP was significantly higher than in patients without CHD. A cutoff level of 260 pg/ml was reported to be 92% sensitive and 91% specific to diagnose CHD in subjects with carcinoid syndrome (41). It is recommended that this biochemical marker should be routinely included in the diagnostic assessment and subsequent follow-up of patients with NENs and carcinoid syndrome.

### 3.1.4. Gut peptide hormones

In functional tumors, measurement of specific hormones is appropriate [glucagon in glucagonoma, vasoactive intestinal peptide (VIP) in VIPoma, gastrin in gastrinoma, insulin in insulinoma, etc.]. There are two distinct clinical syndromes that need to be exceptionally presented: gastrinoma and Zollinger Ellison Syndrome (ZES); and insulinoma.

Gastrinomas are usually located in the duodenum or pancreas, secrete gastrin, and cause a clinical syndrome known as ZES. This results from hyperproduction of gastric acid by the parietal cells of the stomach, triggered by gastrin hypersecretion from the NEN. This in turn causes peptic ulcer disease, abdominal pain and chronic diarrhoea and malabsorption. The most common presentation of ZES is with duodenal ulcers, peptic ulcer symptoms, GERD symptoms or ulcer complications and diarrhea. Conversely, multiple ulcers or ulcers in unusual locations are a less frequent presenting feature than in the past. ZES should be suspected in cases of recurrent, severe or familial peptic ulcer disease, in particular when H. pylori is not detected or other risk factors are absent. In addition, peptic ulcer disease that is resistant to treatment, or associated with severe GORD or severe complications, should also prompt the physician to consider ZES (42). It should be noted that hypergastrinemia can occur much more frequently outside the context of a ZES cause, such as hypo- or achlorhydria secondary to chronic atrophic gastritis, pernicious anaemia, helicobacter pylori, or due to the use of proton-pump inhibitors (8). For ZES to be confidently diagnosed, inappropriately elevated fasting serum gastrin (FSG)

level should be shown in the presence of gastric acid secretion. ZES can be diagnosed when FSG is more that 10-fold elevated and the gastric pH <2. However, in about 60% these requirements are not present (43), and additional tests are required, i.e., secretin test (44). It is acknowledged that the diagnosis of ZES is becoming more challenging, primarily due to increasing unreliability of commercial gastrin assays; the lack of availability of secretin to perform secretin provocative tests, where indicated; and the widespread use of PPIs (8).

Insulinoma is a rare pancreatic NEN (pNEN) that secretes insulin. This secretion is not properly regulated by glucose, and as a result Insulinomas continuously and inappropriately secrete insulin causing hypoglycaemia. Typically, patients with such tumors develop hypoglycaemia while fasting or during exercise, which improve by eating (8). In a consensus report from the US Endocrine Society, the proposed criteria for the diagnosis of Insulinoma were as follows: endogenous hyperinsulinism documented by the finding of symptoms, signs, or both; with plasma concentrations of glucose <55 mg/dl (3.0 mmol/litre), insulin ≥ 3.0 $\mu$U/ml (18 pmol/litre), C-peptide ≥0.6 ng/ml (0.2 nmol/litre), and proinsulin ≥5.0 pmol/litre (45).

Most pNENs occur sporadically in a non-inherited fashion. Nevertheless, a variable proportion of them can emerge as part of an inherited syndrome. Multiple Endocrine Neoplasia type 1 (MEN1) is the most important inherited syndrome that accounts for up to 30% of gastrinomas and <5% of Insulinomas. This is an autosomal-dominant genetic condition involving the development of multiple tumors, arising from neuroendocrine cells. These tumors more frequently occur in endocrine glands, mainly the parathyroids, GEP system and pituitary gland (8). The diagnosis of MEN1 in patients with a functional pNEN is frequently markedly delayed (5–9.5 years) (46, 47). Patient diagnosed with ZES should be routinely screened for MEN1, due to the association between the two conditions. Relevant guidelines recommend that parathormone level in the serum, ionized calcium levels and prolactin are routinely performed at diagnosis, and then annually in these patients. Additionally, if a patient is diagnosed with insulinoma before the age of 20 or with multiple insulinomas at any age, MEN1 should also be suspected, and patient screened (8, 42). Overall, we would recommend that all patients with functional pNENs are screened, as above, despite some paucity of relevant data.

# 4. The role of contrast-enhanced imaging and nuclear imaging in the diagnosis of neuroendocrine neoplasms

Computed Tomography (CT) with contrast of the neck-chest-abdomen and pelvis, including three-phase CT of the liver, represents the cornerstone for the diagnosis, staging and follow-up of NENs. Magnetic resonance imaging (MRI) with contrast should be the examination of choice to assess the liver, pancreas, brain and bone, when possible. It is recommended that somatostatin receptor imaging is used for staging, follow-up, and on a pre-operative basis; $^{68}$Ga-DOTATATE is recommended when available. Lastly, $^{18}$FDG-PET/CT is of greater value in cases of higher glucose metabolism and less somatostatin receptor expression. Therefore, we recommend that $^{18}$FDG-PET/CT is considered for high grade NENs, mainly G3 tumors (48–52).

## 4.1. Cross-sectional imaging

### 4.1.1. Contrast-enhanced computed tomography

CT scan represents the backbone of the NEN diagnosis; and is also broadly used for staging, surveillance and monitoring treatment response. Its' use is well-established as a result of its diagnostic accuracy and broad and steady availability. CT is the best modality to assess vascular infiltration and is very helpful in the pre-operative setting. The sensitivity and specificity for CT to diagnose individuals with NEN ranges from 61 to 93% and 71 to 100%, respectively (48).

In particular, the diagnostic accuracy of CT for the diagnosis of pNEN ranges from 69%–94% (53–56). For the diagnosis of small bowel NENs, CT enteroclysis exhibits sensitivity of up to 85% and 97%, respectively (57–59). CT enterography is similar to CT enteroclysis, primarily differing in the method of contrast administration. The sensitivity of both methods is comparable (60). Gastric (gNENs), duodenal (dNENs), rectal (rNENs) and NENs of the colon are often diagnosed by endoscopy, either conventional or EUS. Therefore, CT has a limited role for the diagnosis of these tumors, but should be used for staging (48). CT sensitivity and specificity for diagnosing liver metastases ranges from 75%–100% and 83%–100%, respectively (61–64). Careful consideration is required, however, as CT scan cannot steadily differentiate NEN liver metastases from liver metastases originating from other malignancies (48). Additionally, CT is less accurate in detecting smaller lesions (< 1cm) and bone metastases (sensitivity <60%) and this limitation needs to be considered (65–67).

### 4.1.2. Contrast-enhanced magnetic resonance imaging

MRI is increasingly available and has the advantage of no exposure to radiation. Additionally, as is the case of other types of cancer, MRI appears superior to CT in assessing bone, brain, or abdominal disease, and may be preferred as the imaging modality of choice, or complementary to CT. In particular, MRI has higher tissue resolution than CT and should be preferred for assessing bone metastases. In addition, on patients who are allergic to iodine contrast agents, MRI should also be the preferred modality (68). Furthermore, diffusion weighted imaging (DW imaging) allows detection of subtle neoplastic tissue changes and is highly sensitive in detecting NEN-related liver metastases (48).

In the assessment of pNENs, tumor volume affects the accuracy of MRI. In particular, it is 70% sensitive for primary NENs larger than 2.5 cm, and this sensitivity decreases for lesions < 1.5 cm (69, 70). The accuracy of DW-MRI for the detection of primary NENs and metastatic disease is comparable to PET/CT (71–73). MRI is helpful in surgical planning and assessment of the relationship of the tumor to the main pancreatic duct if enucleation is planned (74). Several features of the neoplasms, such as its size and

shape; enhancement pattern, vascular invasion, and involvement of lymph nodes can assist in determining tumor grade (74). MRI also enables additional sequences for pancreatic neoplasms; and in particular MRCP should be used to determine the anatomical relationship between the tumor and the pancreatic and common bile ducts (48).

The use of hepatocyte-specific contrast media renders MRI scan superior to CT in characterizing liver lesions (48, 74). In a prospective study that compared MRI, CT, and somatostatin receptor scintigraphy (SRS), MRI was reported to detect more metastasic sites compared to the other modalities. In particular, the respective sensitivity for detecting liver metastases was 95.2%, 78%, and 49.3% (75). If hepatic surgery is considered, MRI of the liver should be considered for better assessment of the hepatic tumor load prior to the surgical intervention.

Lastly, in the assessment of SBNENs, a recent study by Dohan et al., reported that the overall sensitivity of MR-enterography for small bowel NENs detection was 74% (95% CI: 54%–89%) on a per-lesion basis and 95% (95% CI: 74%–100%) on a per-patient basis, providing direct evidence of the diagnostic value of MRI in this setting too (76).

#### 4.1.3. The role of imaging in assessing carcinoid heart disease

Echocardiography (ECHO) is of paramount importance in the diagnosis of CHD and is the gold standard in determining severity of the condition. It is a prerequisite that only physicians experienced in its use are involved. Diffuse thickening of the valve leaflets; isolated thickening of a single valve leaflet without significant reduction in leaflet mobility; or the development of valvular regurgitation, may all be seen in CHD. 3-dimensional trans-thoracic-ECHO or trans-oesophageal ECHO may be preferred to examine the pulmonary and tricuspid valves (41). ENETS recommend echocardiography to be performed at baseline and then six monthly to annually in relevant patients (77).

Cardiac magnetic resonance imaging (CMRI) and cardiac computed tomography (CCT) are useful additional modalities, and can complement ECHO. The former is an excellent tool when echocardiographic windows are poor or when structures such as the pulmonary valve cannot be visualised. It also allows measurement of heart metastases and provides information on invasion of extra-cardiac structures (41). CCT can assist in examining the heart valves and right ventricular size and function; the coronary arteries before heart surgery; and depict myocardial metastases and their relationship with the affected valve(s) (78, 79).

### 4.2. Nuclear medicine and hybrid imaging

Somatostatin is a cyclic peptide that exerts strong regulatory effects in the body. The action of this protein is mediated through membrane-bound receptors. These receptors are expressed in high volumes in neuroendocrine cells, and currently five subclasses 1–5 have been cloned (sst1–sst5). These somatostatin receptors are also expressed in high volumes in NENs (80, 81). The landscape was revolutionised following the advent of PET/CT; and novel tracers have been developed, including somatostatin analogs (SSAs), such as $^{68}$Ga-DOTA, and metabolic markers, such as $^{18}$F-FDG (82, 83). DOTATOC OTANOC, and DOTATATE are the main DOTA-peptides that bind to somatostatin receptors. As a result, they are currently broadly used both in the diagnostic/staging cascade, but also for therapeutic purposes, i.e. Peptide receptor radionuclide therapy (PRRT) (84, 85). PET/CT with $^{68}$Ga-DOTA-peptides is more sensitivity than cross-sectional imaging with CT or classical scintigraphy for detecting well-differentiated tumors (52, 86). Conversely, $^{18}$F-FDG PET/CT is used for less differentiated NETs, due to the presence of increased glucose in these tumors. This also illustrates the increased propensity for more aggressive course and poor prognosis (87).

#### 4.2.1. The advent of $^{68}$Ga-DOTA-peptides

Historically, $^{111}$In-pentetreotide (Octreoscan$^{TM}$) represented the mainstay of SRS. $^{68}$Ga-DOTA-SSAs has superseded and replaced classical scintigraphy in an increasing number of healthcare settings, owing to the greater accuracy and lower exposure to radiation, and it is now considered the investigation of choice for well-differentiated NENs. Nevertheless, primarily due to financial limitations, Octreoscan$^{TM}$ still represents the backbone of scintigraphy in many centers, especially in healthcare settings with restricted resources. At present, we recommend that SRS should only be used only when PET/CT imaging is unavailable. Overall, the sensitivity of $^{111}$In-pentetreotide scintigraphy for the detection of these neoplasms ranges from 60%–80% and the specificity from 92%–100% (88–92). Conversely, $^{68}$Ga-DOTA-SSAs exhibits greater diagnostic accuracy, albeit small variations reported in the literature. In recent systematic reviews and meta-analyses comprising overlapping studies, mean sensitivities and specificities for NEN detection varied from 88%–93% and 88%–95%, respectively. $^{68}$Ga-DOTA-SSAs is superior to cross-sectional imaging in the detection of bone metastases, that are often subtle. Likewise, lymph node metastases may be characterised, and the detection of small peritoneal metastases is facilitated by $^{68}$Ga-DOTA-SSAs (93–96).

Yang et al. in their metanalysis included ten studies comprising 416 patients with NENs. The pooled sensitivity and sensitivity of $^{68}$Ga-DOTATOC in the diagnosis of NENs was 93% (95% confidence interval [CI] 89%–96%) and 85% (95% CI 74%–93%), respectively. The pooled sensitivity and specificity of $^{68}$Ga-DOTATATE PET in diagnosing NENs was 96% (95% CI 91%–99%) and 100% (95% CI 82%–100%), respectively (93). In a recent retrospective study of patients with pNENs across three tertiary UK NET referral centers $^{68}$Ga-DOTA PET/CT was assessed. It was reported that the findings of $^{68}$Ga-DOTA PET/CT imaging provided extra information in more than 50% of the studied subjects and had an impact on management decisions in nearly 40% (97). These studies clearly illustrate that $^{68}$Ga-DOTA PET/CT significantly upgraded and enhanced our diagnostic armamentarium. The quality of this diagnostic modality was also found to be unaffected by the use of SSAs before the

examination, a key advantage. Thus, it is recommended against discontinuing short-acting SSAs before the examination (98).

#### 4.2.2. The role of PET/CT in high-grade disease

In contrast to $^{68}$Ga-DOTA PET/CT imaging, $^{18}$FDG PET/CT is generally better in the context of high-grade disease, indicating likely a more aggressive course. With increasing tumor proliferation, somatostatin receptor expression declines and so does uptake on SRS or $^{68}$Gallium-DOTA-SSAs (99, 100). Conversely, these lesions generally become more avid on $^{18}$FDG PET/CT with increasing proliferation. Therefore, $^{18}$FDG PET/CT is more appropriate for high-grade poorly differentiated G3 tumors, which generally have higher glucose metabolism. $^{18}$FDG PET/CT has been reported to be 37%–72% sensitive for the detection of these high-grade tumors. In general, findings of $^{18}$FDG-positive tumors at PET/CT are indicative of unfavorable prognosis (87, 101–104).

#### 4.2.3. Other applications of nuclear medicine in the diagnosis of neuroendocrine neoplasms

MEN1 syndrome may be associated with tumors developing in several sites, more frequently in the parathyroids, GEP tract and pituitary gland (8). In fact, 90% of patients with MEN1 develop primary hyperparathyroidism before the age of 50. Parathyroid imaging is of paramount importance in the management of parathyroid disease and its aim is to localise all sites of excess hormone secretion before surgery. The spectrum of parathyroid imaging comprises single-photon scintigraphy with Tc-99m-Sestamibi, or dual tracer Tc-99m-pertechnetate and Tc-99m-sestamibi with or without SPECT or SPECT/CT. Combination of cross-sectional imaging and molecular imaging enables optimisation of our diagnostic potential, and grants the ability to have concrete structural and functional information in a single investigation (105).

## 5. The use of endoscopy in the diagnostic cascade

Conventional endoscopy is pivotal in detecting and treating NENs in the upper or lower GI tract (106). More recently, the introduction of EUS in clinical practice enhanced out diagnostic potential. Lastly, small bowel capsule endoscopy (SBCE) and balloon enteroscopy have also emerged as novel and helpful techniques.

### 5.1. Conventional endoscopy in neuroendocrine tumors

Upper gastrointestinal endoscopy with careful appraisal of tumors and background gastric mucosa is still the gold standard in diagnosing gastric and duodenal NENs. In the case of gNENs, gastroscopy establishes the diagnosis. It is critical that multiple biopsies are taken from the antrum and gastric body and fundus, in addition to the largest lesions/polyps (107). dNENs are commonly incidentally found during endoscopy for other indications. As the duodenum is within the reach of conventional endoscopy, histological evaluation and staging, and even curative endoscopic treatment are enabled. Gastroscopy with biopsies can accurately diagnose dNEN, whereas EUS can solidify the diagnosis and complete (local) staging, as discussed later. Some dNENs, such as gastrinomas causing ZES, may be missed on both conventional endoscopy and EUS, and these are diagnosed by hormone assays as described in detail in a previous section (108). Although beyond the scope of this review article, it should be noted that endoscopic management also represents the first line treatment for localized type 1 gNENs, followed by active surveillance. This approach ascertains acceptable oncologic outcomes combined with peri-procedural safety. Classic polypectomy, endoscopic mucosal resection (EMR), or endoscopic submucosal dissection (ESD) modalities are used in clinical practice. Any gNEN > 10mm should be considered for endoscopic treatment, unless suspicion or confirmation exists of muscularis propria invasion or lymph node metastasis, when surgical resection should be considered (106).

In analogy to this, most rNENs are diagnosed incidentally during colonoscopy performed for other indications. rNENs are small, usually 10mm lesions, that resemble benign hyperplastic rectal polyps. Commonly, the endoscopist resects the "hyperplastic polyp", which proves to be a rNEN on histopathology. This emphatically illustrates why endoscopists should be familiarised with the identification of such lesions. It is also important to be able to distinct NENs from other subepithelial lesions, such as lipomas, which usually do not require treatment. Endoscopic biopsies could be misleading as rNENs are submucosal lesions frequently escaping the diagnosis when biopsies with conventional endoscopy are taken. In addition, random biopsies can cause tissue fibrosis, which may challenge subsequent endoscopic resection (109). This underscores why biopsies should not be taken routinely if a rNEN is strongly suspected, and the critical role of EUS is evident.

### 5.2. Advances in endoscopy in the assessment of the small bowel

#### 5.2.1. The role of small bowel capsule endoscopy in the management of neuroendocrine tumours

The role of SBCE in the diagnosis of SBNENs is not yet well established, in contrast to other endoscopic modalities, and consensus guidelines recommend its use upon local expertise (7). At present, it seems that SBCE may be of value in detecting multifocal SBNENs, in particular in the pre-operative setting to determine the extent of resection. Additionally, the use of SBCE could be considered in cases of metastatic disease of unknown origin before laparotomy. Occasionally, the primary site may remain unclear despite thorough investigations (110). On surgical exploration, most such tumors are detected in the small bowel (111). Nevertheless, one should consider that in NENs of unknown primary, SBCE is 75% sensitive, and only 38% specific compared to laparotomy (112). Additionally, in SBCE

contractions of the small bowel or extrinsic compression may give the (false) impression of lesions. Likewise, when a true mass is detected, localization may be inaccurate and additional procedures, such as balloon enteroscopy, may be required for confirmations and pathological evaluation.

#### 5.2.2. The role of balloon enteroscopy in the management of neuroendocrine tumors

The diagnostic yield of bowel enteroscopy (BE) for all small bowel masses varies in different studies (113, 114). In individuals with suspected NENs but inconclusive initial investigations, the diagnostic yield is estimated approximately 33% (115). In a recent study, BE was reported to have 88% sensitivity for the detection of the primary SBNEN, compared to approximately 60% for CT, 54% for MRI, and 56% for somatostatin receptor imaging. In this study, 21.2% of the patients had their primary tumors missed on imaging. Notably, 92.3% of those who had BE, had their primary tumor ultimately identified (116). Similar to SBCE, this modality can identify multifocal NENs pre-operatively. In a retrospective study of subjects who had small bowel resection, pre-operative BE was shown to detect additional lesions in over 50% of patients, compared to 18% with capsule endoscopy (117). The mail limitation of BE would be the fact that it is only or primarily available in referral centres. Considering this, the North American (NANETS) guidelines recommend that multifocal tumors may be most accurately identified at the time of surgery, by examining the entire bowel (118). Overall, the use of BE should be reserved only for centers where it is available and relevant expertise exists.

Overall, BE has the advantage of being an invasive modality, enabling the performance of biopsies, among others. In cases of suspected small bowel lesions, SBCE is usually performed first-line, followed by BE in the case of positive findings (119). Sound relevant evidence or guidance lack in the diagnostic cascade of neuroendocrine neoplasms. Where local expertise does exist, and in an appropriate clinical context as outlined above, these two modalities should have a role, and we believe this is complementary, similar to other small bowel lesions.

### 5.3. The role of endoscopic ultrasound

The advent of EUS revolutionised the field. In particular, it enables detection of previously unidentified tumors; contributes to staging of GEP-NETS, and finally permits tissue diagnosis and histological evaluation.

#### 5.3.1. Endoscopic ultrasound and pancreatic neuroendocrine neoplasms

In particular, it seems that EUS is the most sensitive method for pNENs, being 82%–93% sensitive and 86%–95% specific in this context (120–122). In a series of studies involving more than 200 patients, the detection rate of EUS was ranging from 75 to 97% (55, 99, 100, 123–128). Notably, in a recent review by Ishi et al., tumor grading between EUS-FNA and surgical samples showed a concordance rate of 77.5% (95% CI = 0.59–0.71, $p < 0.01$). (129). Likewise, intraoperative US (IOUS) was also shown to sensitive for pNEN, having a detection rate of 74 to 96% (99, 130–132).

#### 5.3.2. Endoscopic ultrasound and gastroduodenal neuroendocrine neoplasms

For dNENs and lymph node metastases, the detection rate of EUS was 63% in 2 studies comprising 59 patients (99, 126). Forceps biopsy during standard endoscopic examination can diagnose most dNENs, and not uncommonly, endoscopy can offer a curative option for small sporadic dNENs. Nevertheless, for larger tumors over 1 cm, local staging by EUS is recommended before resection. Duodenal NENs are typically submucosal lesions, but they can rarely extend beyond this layer. EUS can accurately establish the degree of submucosal involvement. EUS can accurately assess locoregional lymph node metastases, and this is of particular importance as dNENs can be associated with such metastases in up to 40%–60%, especially gastrinomas. All the above are critical to determine optimal treatment, and candidacy for endoscopic or surgical treatment. Lastly, the pancreas can also be fully interrogated for small tumors, that can be linked to MEN-1 (133–135).

Lastly, gNENs can be classified into three subtypes: type 1 g-NETs which are the most frequent and develop due to hypergastrinaemia in the context of autoimmune atrophic gastritis; type 2 that are linked to increased gastric secretion, in the context of gastrin-secreting tumors, often in patients with MEN-1 and as part of a ZES; lastly, type 3 tumors are sporadic and usually poorly differentiated, mimicking malignant neoplasms of the stomach (136). EUS is recommended for type 1 gNENs > 1 cm prior to endoscopic resection. Similarly, for patients suspected to have type 2 neoplasms, this modality assesses for the presence of dNENs or pNENs. Lastly, for the assessment of type 3 gNENs, EUS evaluates the depth of invasion into the mucosal layers or beyond, and the presence of lymph nodes in the gastro-hepatic and peri-gastric areas (137).

#### 5.3.3. Other application of endoscopic ultrasound

Regarding the role of EUS in the management of rNENs, according to the current ENETS consensus, EUS should follow endoscopic evaluation of a suspected rNEN (138). EUS can accurately assess tumor size, depth of invasion and locoregional lymph node metastases. This can be of paramount importance when determining surgical candidates, and can assist in determining appropriate treatment (109, 139).

## 6. Future perspectives in neuroendocrine neoplasms diagnostics

### 6.1. Novel biomarkers for the diagnosis of neuroendocrine neoplasms

Most of the aforementioned biomarkers, widely used to date, fail to capture the biologic complexity of a NEN; and even the

historical Ki67 appears to have some limitations. There is an emerging need to embrace advances in the field and integrate molecular genomic tools into clinical practice. The argument is that contrary to the current biomarkers, multianalyte analysis assess the tumor molecular genomic mechanism. Multianalyte biomarkers include tumor-derived components such as ctDNA, circulating tumor cells (CTCs), miRNA, extracellular vesicles, and "tumor-educated" platelets (140). Key novel biomarkers are summarised in **Table 4**.

### 6.1.1. NETest

The NETest is the most successful multianalyte biomarker assessed to date in the management of NENs. This mRNA genomic biomarker measures a series of relevant transcripts in the blood, which is considered the biological signature of the neoplasm. It is considered a liquid biopsy procedure, assessing the circulating expression level of genes involved in oncogenesis, cell proliferation, signalling and metastasis formation through a peripheral blood real-time polymerase chain reaction (RT-PCR). The respective results are expressed as an activity index (NETest score), ranging between 0 and 100. A score ranging from 21 to 40% represents "stable" disease, whereas a score > 40% reflects "progressive" disease (140). Oberg et al. in their recent meta-analysis reported that the diagnostic accuracy of this tests is approximately 96%. The diagnostic accuracy of this test to differentiate between stable and progressive disease was reported to be between 84.5% and 85.5%. The NETest was 91.5%–97.8% accurate as a marker of natural history and 93.7%–97.4% accurate as an interventional/response biomarker (141).

TABLE 4 Novel biomarkers for the diagnosis of neuroendocrine neoplasms (NENs).

| Biomarker | Function | Role | Strengths |
|---|---|---|---|
| **NETest** | – mRNA genomic biomarker<br>– Measures a series of transcripts in the blood<br>– Results are expressed as an activity index (NETest score) | – May have a role in the diagnosis, assessment of the effectiveness of surgery, monitoring therapeutic efficacy [including Peptide Receptor Radionuclide Therapy (PRRT)] | – Significant diagnostic accuracy |
| **Circulating tumor cells (CTCs)** | – Tumor cells shed from the primary tumor or metastasis loci and intravasate into the peripheral blood circulation system | – May have a role as accurate prognostic markers | – Optimal CTC threshold to predict PFS and OS in metastatic pNENs and SB-NENs studied<br>– These thresholds can stratify patients in clinical practice and clinical trials |

PRRT is a very effective treatment modality for patients with metastatic and/or inoperable NENs. A radionuclide linked to a SSA is used, and this allows to accurately deliver radiotherapy to somatostatin receptor–expressing neoplasms, such as the great majority of NENs (142). Bodei et al. prospectively evaluated NETest as a surrogate biomarker for Response Evaluation Criteria in Solid Tumors (RECIST). Notably, in over a hundred subjects assessed, NETest significantly decreased in patients who responded to treatment, as per RECIST criteria, and remained elevated in those who did not "respond" to PRRT. Notably, the reported accuracy of treatment response was 98% (143). Similar to the NETest, NEN transcript expression in blood integrated with tumor grade provides a PRRT predictive quotient (PPQ) which also stratifies patients who "respond" to PRRT from those who do not. PPQ response prediction was accurate in 97% with a 99% accurate positive and 93% accurate negative prediction. NETest significantly decreased in PPQ-predicted "responders" and remained elevated or even further increased in PPQ-predicted patients who did not respond to treatment. Interestingly, CgA did not correlate that well with the outcome of PRRT, decreasing in only 38% of treatment "responders" (143).

### 6.1.2. Circulating tumor cells

CTC measurement has also been assessed in several malignancies. In particular, the CellSearch platform was approved by the US Food and Drug Administration for use in several malignancies, after trials reported the prognostic value of CTCs at defined thresholds (144–146). Although CTCs are also detectable in patients with NENs, studies on the measurement of CTCs in the diagnosis of NENs are not equally enthusiastic (140). On the contrary, CTCs appear more accurate as prognostic markers (147, 148). Interestingly, Mandair et al. defined optimal prognostic CTC thresholds in pNENs and midgut NENs. They used CellSearch to enumerate CTCs in almost 200 subjects with metastatic NENs, as above. These subjects were then followed-up for at least 3 years or until death. CTCs were detected in 33% of patients with pNEN and 51% of midgut NENs. In the multivariate Cox hazard regression analysis for progression free survival (PFS) in subjects with pNEN, 1 or greater CTC had a hazard ratio (HR) of 2.6, whereas 2 or greater CTCs had an HR of 2.25 in midgut NENs. In the multivariate Cox hazard regression analysis for overall survival (OS) in pNEN, 1 or greater CTCs had an HR of 3.16 and in midgut NENs, 2 or greater CTCs had an HR of 1.73 (149).

## 6.2. The impact of radiomics in diagnosis and staging neuroendocrine tumors

The quantitative analysis of medical images data and the extraction of imaging features, also called "radiomics", represent an emerging approach in personalized medicine and advanced diagnostics, especially for disease characterization or outcome prediction. Similar to other neoplasia, this appears to be a promising tool in the context of NENs. A recent study by Mori et al. evaluated preoperative CT radiomic features as a predictor

of tumor grade, the presence of lymph nodes metastases or distant metastases, and microvascular invasion of pNENs. This retrospective study included over 100 patients who underwent surgery for pNEN. They showed that combining few radiomic and clinic-radiological features resulted in presurgical prediction of histological characteristics of pNENs (150). In a different study by Chiti et al., the authors assessed whether CT scan radiomics analysis could predict GEP-NEN grade according to the recent WHO classification; they also concluded that CT-radiomics analysis may contribute to differentiating the histological grade for these tumors (151). Although these studies, among others, illustrate that radiomics may be an invaluable tool in the future, further studies will be required to validate the results.

## 6.3. Recent advents in nuclear medicine in the diagnosis of neuroendocrine tumors

### 6.3.1. The role of the novel glucagon-like peptide-1 receptors scintigraphy

Insulinomas are rare functional pNENs that secrete insulin arbitrarily, as already discussed. Surgery remains the preferred treatment modality, whenever possible, being linked to cure rate exceeding 98% (152–157). Surgical treatment of insulinomas may be challenged due to difficulties in localizing it using conventional diagnostic modalities, however. In <5%–10% of such individuals, investigations can be negative and non-conclusive (8). Even highly sophisticated and advanced $^{68}$Gallium-DOTA-SSAs are positive in less than 30% (158). Conversely, insulinomas exhibit a very high density of glucagon-like peptide-1 receptors (GLP-1R). As a result, receptor scintigraphy with radiolabelled GLP-1 receptor analogues is a very promising modality, albeit hampered by its limited availability so far (159–162). In one of the first relevant studies, Christ et al. tested the $^{111}$In-labeled GLP-1R agonist $^{111}$In-DOTA-exendin-4 in localizing insulinomas. They found that the GLP-1R scans successfully detected the insulinomas and contributed to the successful surgical resection of insulinomas in all subjects (162).

### 6.3.2. Dual $^{68}$Gallium DOTATATE and $^{18}$F-FDG PET/CT in patients with metastatic gastroenteropancreatic neuroendocrine neoplasms

Recent studies explored whether combining $^{68}$Gallium-DOTA-SSAs and $^{18}$F-FDG PET/CT would enhance our ability to determine prognosis. A recent study by Hayes et al. investigated the prognostic utility of a classification system combining the findings of $^{68}$Ga-DOTATATE and $^{18}$F-FDG PET/CT and the researchers reported that such a classification tool could indeed correlate with prognosis (163). In addition, Panagiotidis et al. investigated whether $^{68}$Ga-DOTATATE and $^{18}$F-FDG PET/CT could influence treatment decisions. The results changed the therapeutic plan in 80.8% of patients. In approximately 21%, $^{18}$F-FDG PET/CT affected the decision-making, prompting mostly the initiation of chemotherapy. In nearly 50% the treatment cascade was influenced by $^{68}$Ga-DOTATATE, resulting in consideration of PRRT (164). More recently, a multicentre study assessed and aimed to validate the NETPET score as a prognostic biomarker in metastatic GEP-NENs. The combination of $^{68}$Gallium-DOTA-SSAs and $^{18}$F-FDG PET/CT, i.e., "dual PET imaging", provides a comprehensive overview of the status of the disease. The NETPET score, a 5-point scoring system for dual PET reporting in subjects with metastatic NENs, summarises the information provided by the two modalities into a single parameter. The NETPET score correlated with histological grade ($p < 0.001$), and importantly it was significantly associated with overall survival and time to progression on univariate and multivariate analysis ($p < 0.01$) (165).

## 6.4. Decoding the genetic and molecular profiles of neuroendocrine neoplasms

The advent of pre-clinical models appears to be promising for the design, assessment and evolution of genuinely tailored personalised treatment. In particular, primary culture cells originating from solid neoplasms, have gained significant importance in individualised anti-cancer treatment. In analogy to this, patient-derived xenografts (PDXs) in mice represent an *in vivo* model for the development of individualised precision medicine. To produce PDXs, tumors collected following surgical resection or biopsy, are inoculated as pieces or single-cell suspensions subcutaneously usually into the flank of an animal model (166). More recently, zebrafish PDX (zPDX) emerged as a promising option (167). In the former case, the main advantage is the potential to assess the efficacy of different anti-tumor treatment options in a short time, and also to perform preliminary pre-clinical studies for the identification of novel molecular targets (168, 169). In the latter case, it is anticipated that the effects of antitumor compounds on tumor-induced angiogenesis, invasiveness, metastatic dissemination and tumor cell proliferation can be assessed within very few days after implantation.

Although some of the novel revolutionary techniques presented in the current review are currently in the developmental pipeline, it is surely an insight into the future and illustrate the major paradigm shift currently taking place in medical oncology. Accurate and early diagnosis is critical, whereas there has been some progress, and we definitely believe that the research focus should be in establishing robust prognosticators and prognostic scores.

# 7. Conclusions

It is evident that NENs are complex and heterogeneous tumors, and as such require a sophisticated diagnostic approach. This is critical, as early and accurate diagnosis and staging largely

influence prognosis and patient outcomes. Pathology still represents and will likely remain the gold standard in the foreseeable future. Likewise, cross-sectional imaging is still the backbone in the diagnosis and staging of these tumors, and in addition, the advances in CT and MRI have also improved the diagnostic yield. Nevertheless, the genuine revolution in the field follows the advances in nuclear medicine, and the emergence of novel biomarkers assessing the tumor molecular genomic mechanisms. The former, comprising $^{68}$Ga-DOTA PET/CT exhibits unprecedented diagnostic accuracy, and has been shown to influence and update management in a significant number of patients (97, 164), limited primarily by its high-cost and consequent limited availability. Likewise, multianalyte biomarkers appear promising tools also leading to new horizons. The NETest in particular provides accurate information about the diagnosis, completeness of surgical resection and the presence of residual disease in patients with NENs; it can also predict the therapeutic efficacy of SSAs and PRRT; and lastly it is standardized, reproducible and not influenced by age, gender, ethnicity, fasting or other medications (141, 170, 171). It is evident that we do live in exciting times. Our deeper understanding of these rare neoplasms, the progress already made in the diagnosis and treatment of NENs, and finally these new promising developments, all bring us one step closer to tailored treatment and improved outcomes.

## Author contributions

AK, AG, AP, PB, DG, VNP, CT: conception and design; AK, AK, AP: article draft; AK, AG, AP, PB, DG, VNP, CT: critical revision for important intellectual content, final approval of the article. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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