

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	Analysis of 72 Patients with Colorectal High-Grade Neuroendocrine Neoplasms from Three Chinese Hospitals
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3,4	To delineate the clinicopathologic features and explore the prognostic factors of this rare malignancy  Colorectal HGNENs are rare and aggressive malignancies with poor clinical outcomes. However, patients with younger age, good morphological differentiation and without metastatic disease can have a relatively favorable prognosis.
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6	Colorectal high-grade neuroendocrine neoplasm (HGNEN) is a rare malignancy originating from neuroendocrine cells in the colon and rectum, and it constitutes less than 1% of all colorectal carcinomas.
Objectives	3	State specific objectives, including any prespecified hypotheses	6	Since most previous studies are case reports or small sample reports from single centers and western countries, we conducted a multicenter prospective study and enrolled 72 patients from three different Chinese hospitals, aiming to improve our understanding of the

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clinicopathologic features and oncologic prognosis of patients with colorectal HGNEs.

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## Methods

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Study design	4	Present key elements of study design early in the paper	6,7	We reviewed the electronic medical records from 3 different Chinese institutions and enrolled 72 consecutive colorectal HGNE patients from January 2000 to January 2019, including 47 from the Cancer Hospital Chinese Academy of Medical Sciences, 20 from China-Japan Friendship Hospital and 5 from Beijing Hospital. Information regarding patient demographics, clinicopathologic features, treatment modalities and oncologic outcomes was carefully collected and analyzed.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6	We reviewed the electronic medical records from 3 different Chinese institutions and enrolled 72 consecutive colorectal HGNE patients from January 2000 to January 2019, including 47 from the Cancer Hospital Chinese Academy of Medical Sciences, 20 from China-Japan Friendship Hospital and 5 from Beijing Hospital. Information regarding patient demographics, clinicopathologic features, treatment modalities and oncologic outcomes was carefully collected and analyzed.

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Participants	6	<p>(a) <i>Cohort study</i>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</p> <p><i>Case-control study</i>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls</p> <p><i>Cross-sectional study</i>—Give the eligibility criteria, and the sources and methods of selection of participants</p>	7	All cases were definitively diagnosed with colorectal HGNEN through colonoscopy, abdominal and pelvic enhanced computed tomography scans, tissue biopsy, pathological examination and immunohistochemical evaluation. All patients were confirmed to have a high mitotic rate (over 20/10 high power fields) and/or Ki-67 labeling index (over 20%). Moreover, cases with a component of adenocarcinoma or squamous carcinoma were excluded.
		<p>(b) <i>Cohort study</i>—For matched studies, give matching criteria and number of exposed and unexposed</p> <p><i>Case-control study</i>—For matched studies, give matching criteria and the number of controls per case</p>	/	This is not a matched study.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7	All patients were confirmed to have a high mitotic rate (over 20/10 high power fields) and/or Ki-67 labeling index (over 20%). Moreover, cases with a component of adenocarcinoma or squamous carcinoma were excluded.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6	We reviewed the electronic medical records from 3 different Chinese institutions and enrolled 72 consecutive colorectal HGNEN patients from January 2000 to January 2019, including 47 from the Cancer Hospital Chinese Academy of Medical Sciences, 20 from China-Japan Friendship Hospital and 5 from Beijing Hospital.

Bias	9	Describe any efforts to address potential sources of bias	7	In addition, multivariate analysis was performed using the Cox proportional hazards regression model to identify the independent prognostic factors.
Study size	10	Explain how the study size was arrived at	6	We reviewed the electronic medical records from 3 different Chinese institutions and enrolled 72 consecutive colorectal HGNEN patients from January 2000 to January 2019, including 47 from the Cancer Hospital Chinese Academy of Medical Sciences, 20 from China-Japan Friendship Hospital and 5 from Beijing Hospital.

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7	Quantitative data that followed the normal distribution were expressed as median $\pm$ standard deviation, while quantitative data that did not follow the normal distribution were expressed as median and range. Qualitative data and ordinal data were presented as the number of cases and percentages.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7	Our study received statistical review by one biomedical statistician in our institution. All data were analyzed using the Statistical Package for the Social Sciences (SPSS version 24.0; IBM Corp., Armonk, NY). Quantitative data that followed the normal distribution were expressed as median $\pm$ standard deviation, while quantitative data that did not follow the normal distribution were expressed as median and range. Qualitative data and ordinal data were presented as the number of cases and percentages. Survival time was defined as the time interval between the date of pathological diagnosis and death. Survival rates were calculated by

				the Kaplan-Meier method and further compared through the log-rank test. In addition, multivariate analysis was performed using the Cox proportional hazards regression model to identify the independent prognostic factors. A P-value <0.05 was considered statistically significant.
		(b) Describe any methods used to examine subgroups and interactions	7	Survival rates were calculated by the Kaplan-Meier method and further compared through the log-rank test.
		(c) Explain how missing data were addressed	/	/
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	/	/
		(e) Describe any sensitivity analyses	/	No sensitivity analyses was done.
<b>Results</b>				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7	A total of 72 patients with a median age of 59.5 years old (range, 18-82 years old), including 52 (72.2%) males and 20 (27.8%) females, were enrolled in our study.
		(b) Give reasons for non-participation at each stage	/	No non-participation was observed in this study.
		(c) Consider use of a flow diagram	/	/
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8	The average BMI was $23.8 \pm 3.4$

kg/m2. The common symptoms were as follows: hematochezia 37 (51.4%), abdominal pain 23 (31.9%), changes in bowel habits 23 (31.9%), abdominal distention 5 (9.6%), weight loss 3 (4.2%), and anemia 2 (2.8%). Two patients were asymptomatic, and cancer was detected through routine health examinations. No patients had functional tumors or presented with carcinoid syndrome. The rectum (N=46, 63.9%), especially low rectum, was the most common primary site. Among the 46 patients with rectum HGNEs, 28 (60.9%) were located in the low rectum. More than half of the patients (51.4%) presented metastatic diseases at the date of diagnosis, and liver and distant lymph nodes were the two most common metastatic sites.

		(b) Indicate number of participants with missing data for each variable of interest	21,22,23	Table 1, Table 2, Table 3
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	/	This is a observational study.
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	/	This is a observational study.
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	/	This is a observational study.
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	/	/

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	23	Table 4
		(b) Report category boundaries when continuous variables were categorized	23	Table 4 age<70, age≥70
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	/	This is a observational study.

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	/	No subgroup in this study.
<b>Discussion</b>				
Key results	18	Summarise key results with reference to study objectives	9	<p>All patients were followed up for a median duration of 15.5 months (range 1-190 months). A median survival of 31 months was achieved in the whole cohort, and the 3-year and 5-year survival rates were 44.3% and 36.3%, respectively. A significantly decreased median survival of 13 months was observed for the patients with metastatic disease. Since more than half of the patients without distant metastasis (67%) survived through the end of follow-up, the median survival of these patients could not be calculated. Univariate analysis demonstrated that age (<math>P&lt;0.001</math>), pathologic type (<math>P=0.033</math>), neoplasm macroscopic type (<math>P=0.037</math>), distant metastasis (<math>P&lt;0.001</math>), positive EMVI (<math>P=0.047</math>), elevation of pretreatment serum lactate dehydrogenase (<math>P=0.015</math>), and resection of the primary site (<math>P&lt;</math></p>

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0.001) were associated with the overall survival of patients with colorectal HGNEC (Figure 1). For unclear reasons, no significant survival advantage was found in patients with a low Ki-67 index (Ki-67<55%), as reported in previous studies. To identify the independent prognostic factors, multivariate analysis was subsequently performed. Based on previous studies and knowledge, we enrolled 6 variables: gender, age, tumor location, pathological type, distant metastasis, and resection of the primary site. Given the missing data for the pretreatment level of serum lactate dehydrogenase, tumor macroscopic type, EMVI and Ki-67 index, these variables were not included in the multivariate analysis. Consequently, age  $\geq 70$  (HR=3.926; 95% confidence interval, 1.740-8.858; P= 0.001), pathologic type of NEC (HR=6.647; 95% confidence interval, 1.759-25.119; P= 0.005)

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				and distant metastasis (HR=6.356; 95% confidence interval, 2.543-15.889; P<0.001) were confirmed to be independent risk factors for poor prognosis (Table 4).
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16	Our study had several limitations as follows: 1) It is a retrospective study, and the bias from patient selection and information collection is unavoidable. 2) The period of our study is within a span of nearly 20 years, the nomenclature and classification of colorectal NETs has been changing, and the early pathological reports are not as normative as they are now. This leads to the lack of vital information, such as the Ki-67 index and pathological type (small cell or large cell), in some patients and makes it difficult to evaluate their value in predicting prognosis.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16	In conclusion, colorectal HGNETs are rare and

				heterogeneous groups of malignancies. They present distinct clinicopathologic characteristics with colorectal adenocarcinoma and show dismal prognosis. Patients with pathologic type NETs G3, younger age and without distant metastasis might have relatively good clinical outcomes.
Generalisability	21	Discuss the generalisability (external validity) of the study results	16	colorectal HGNETs are rare and heterogeneous groups of malignancies. They present distinct clinicopathologic characteristics with colorectal adenocarcinoma and show dismal prognosis.
<b>Other information</b>				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1	Supported by Medicine and Health Technology Innovation Project of Chinese Academy of Medical Sciences, No. 2017-12M-1-006 and China Cancer Research Foundation, Beijing Terry Fox Run Special Fund, No. LC2017L03, No. LC2017A19

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).