Correlation and Comparison of Somatostatin Receptor Type 2 Immunohistochemical Scoring Systems with ^{68}Ga -DOTATATE Positron Emission Tomography/Computed Tomography Imaging in Gastroenteropancreatic Neuroendocrine Neoplasms

Jiangyuan Yu^a Fang Cao^b Xinya Zhao^b Qing Xie^a Ming Lu^c Jie Li^c
Zhi Yang^a Yu Sun^b

^aKey Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing, China; ^bKey Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Pathology, Peking University Cancer Hospital & Institute, Beijing, China; ^cKey Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Oncology, Peking University Cancer Hospital & Institute, Beijing, China

Keywords

Somatostatin receptor type 2 · Immunohistochemical scoring systems · ^{68}Ga -DOTATATE PET/CT imaging · Gastroenteropancreatic neuroendocrine neoplasms

Abstract

Introduction: The overexpression of somatostatin receptor type 2 (SSTR2) is a unique characteristic of gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs), which establishes the basis for both diagnosis and therapy. The SSTR status can be evaluated by immunohistochemical staining (IHC) and ^{68}Ga -DOTATATE positron emission tomography/computed tomography (PET/CT) imaging. This study attempted to determine the relationship between IHC and ^{68}Ga -DOTATATE PET/CT imaging and to explore the optimal

cutoff value for SSTR IHC reading. **Patients and Methods:** A total of 100 GEP-NENs with SSTR PET/CT and pathological data were retrospectively analyzed, which consisted of neuroendocrine tumor (NET) G1 ($n = 9$), NET G2 ($n = 64$), NET G3 ($n = 13$), neuroendocrine carcinoma ($n = 10$), and mixed neuroendocrine-non-NENs ($n = 4$). SSTR2-IHC results were interpreted by 4 well-established semiquantitative scoring systems, including human epidermal growth factor receptor 2 (HER2) score, Volante score, H score, and immunoreactive score. **Results:** In the homogeneous SSTR2 expression group (accounting for 57% of all cases), the 4 scoring systems were highly concordant with each other (Kendall's Tau-b coeffi-

Jiangyuan Yu and Fang Cao contributed equally to this work. Prof. Yu Sun and Prof. Zhi Yang are co-corresponding authors.

cient range: 0.80–0.96, $p < 0.001$) and also highly correlated with the ^{68}Ga -DOTATATE PET/CT imaging results (Spearman's rank correlation coefficients: 0.71, 0.86, 0.80, and 0.71, $p < 0.001$). In the heterogeneous group (43%), the 4 scoring systems revealed a lower level of concordance (the Kendall Tau-b coefficient range: 0.40–0.75, $p < 0.01$), and the correlation with ^{68}Ga -DOTATATE PET/CT imaging was also lower, albeit statistically significant (Spearman's rank correlation coefficients: 0.53, 0.38, 0.36, and 0.33, $p < 0.05$). Heterogeneous SSTR2 expression was mainly observed in the HER2 2+ cases, for which the combination with H score could help identify positive cases with increased sensitivity and specificity. The highest sensitivity and specificity of H scores in predicting the imaging results were achieved at 86.10 and 89.30% when defining the cutoff value as 160, indicating that 80% of the tumor cells were moderately positive or 55% were strongly positive. **Conclusions:** SSTR2 IHC was found to predict ^{68}Ga -DOTATATE PET/CT imaging accurately, especially in the homogeneous expression group. According to the positive ^{68}Ga -DOTATATE PET/CT outcomes, 80% of the tumor cells moderately positive or 55% strongly positive was the cutoff values for SSTR2-IHC reading.

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Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) arise from diffuse neuroendocrine cells of the digestive system and exhibit heterogeneous biological characteristics with respect to differentiation and prognosis. The overexpression of somatostatin receptors (SSTRs) on the tumor cell surface is a distinctive feature of GEP-NENs, especially SSTR2. These characteristics form the basis of pathological diagnosis, molecular imaging, somatostatin analogs, and peptide receptor radionuclide therapy (PRRT).

Positron emission tomography/computed tomography (PET/CT) with ^{68}Ga -labeled somatostatin analogs play a crucial role in the diagnosis, staging, and management of GEP-NENs. In June 2016, the US Food and Drug Administration approved a kit for the synthesis of ^{68}Ga -DOTATATE. As an in vivo imaging modality, ^{68}Ga -DOTATATE PET/CT can be used to indicate the distribution and density of SSTR expression levels throughout the whole body and is considered to be a gold standard for SSTR evaluation. However, SSTR PET/CT imaging has some limitations. For example, the primary or metastatic lesions are too small to be identified or already resected after surgery. In addition, this tech-

nique has limited usage because of its high cost and relatively complex operating technology.

In terms of pathology, the SSTR protein levels are detected routinely by immunohistochemistry (IHC), which is a reproducible method performed in laboratories all across the world. This method is preferred due to its cost effectiveness and clinical values. There are several semi-quantitative scoring systems for SSTR2 evaluation, including human epidermal growth factor receptor 2 (HER2) score, Volante score, H score, and immunoreactive score (IRS), some of which exhibit high inter-laboratory comparability [1–3]. However, each scoring system is based on different criteria and none of them have been reported to be widely adopted in the routine clinical practice. No conclusive evidence is available with respect to the systematic comparison between the existing IHC scoring systems and the outcomes of in vivo image analysis.

Hence, as methods for investigating the SSTR expression levels, ^{68}Ga -SSTR PET/CT is an in vivo evaluation method, while IHC is an in vitro reflection of the protein expression status. The correlation between the 2 modalities indicates great value in optimizing SSTR PET/CT application, selecting suitable candidates for SSTR-related therapy, and evaluating differentiation or other biological characteristics of GEP-NENs. In this study, 4 established IHC scoring systems were compared to evaluate the SSTR2 expression, and the data obtained were correlated with the ^{68}Ga -DOTATATE PET/CT imaging results. To the best of our knowledge, this is the first comparative analysis of the SSTR2 expression both from pathology and imaging in a relatively large cohort of GEP-NEN patients. The aims of this study were to determine the concordance and correlation between IHC and ^{68}Ga -DOTATATE PET/CT and explore the optimal cutoff value for the SSTR IHC reading, which can be universally applied for routine pathological reporting.

Materials and Methods

Case Selection and Samples

A total of 148 formalin-fixed paraffin-embedded non-lung neuroendocrine tumor (NET) samples from 141 patients were first included. These patients were diagnosed at the Department of Pathology, Peking University Cancer Hospital (Beijing, China) between January 2016 and July 2019. All tumors were subjected to histological verification by 2 independent pathologists according to the 2019 WHO Classification of Digestive System Tumors. All patients were staged by ^{68}Ga -DOTATATE PET/CT, and imaging data for 114 tumors from 108 patients were obtained from the Department of Nuclear Medicine, Peking University Cancer Hospital

Table 1. Overview of the 4 different scoring systems applied for SSTR2 immunohistochemistry evaluation

A. Volante score and HER2 score			
Classification	Volante score	HER2 score	
0	Absence of staining	Absence or incomplete membrane staining in <10% tumors cells	
1+	Pure cytoplasmic staining	Faint/barely perceptible or weak incomplete membrane staining in ≥10% tumor cells	
2+	Membranous staining in <50% of tumor cells ^a	Weak to moderate complete membranous or cytoplasmic staining in ≥10% of tumor cells	
3+	Complete membranous staining in >50% of tumor cells ^a	Strong complete membranous or cytoplasmic staining in ≥10% of tumor cells	
B. IRS score			
classification	IRS score	intensity of staining X	percentage of positive cells ^b
0	0–1	0	0 = negative
1	2–3	1+ = weak	1 = <10% positive cells
2	4–8	2+ = moderate	2 = 10–50% positive cells
3	9–12	3+ = strong	3 = 51–80% positive cells 4 = >80% positive cells
C. H score			
classification	H score	intensity of staining	calculation ^b
0	0–49	0	H score = [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]
1	50–99	1+ = weak	
2	100–199	2+ = moderate	
3	200–300	3+ = strong	

SSTR2, somatostatin receptor type 2; IRS, immunoreactive score; HER2, human epidermal growth factor receptor 2. ^a Cytoplasmic staining was excluded. ^b Incomplete membranous staining or cytoplasmic staining was included.

(Beijing, China). The remaining 33 patients showed no tumors at the time of imaging. Finally, 100 GEP-NENs from 95 patients were included in the study after exclusion of carcinoid tumors from the mediastinum, the ovary, and the thymus. Out of the total cases, 9 were of NET G1, 64 were of NET G2, 13 were of NET G3, 10 were of neuroendocrine carcinoma (NEC), and 4 were of mixed neuroendocrine-non-NENs (MiNENs). The MiNEN cases were mixed adenoneuroendocrine carcinoma with NEC ranging from 40 to 90%. Approval for this retrospective analysis was sought from the Ethics Committee of Peking University Cancer Hospital.

In this cohort, samples for SSTR2 evaluation included surgical and biopsy specimens ($n = 39$ and 61 , respectively) of primary and metastatic tumors ($n = 57$ and 43 , respectively). More than half of the primary tumors ($33/57$ and 57.9%) were surgically resected, while biopsy was mainly performed on metastatic tumors ($37/43$, 86.0%). Majority of the metastatic tumors were extracted from the liver ($40/43$, 93.0%) and a few from the lymph nodes ($3/40$, 7.0%). Five of them were primary tumors paired with liver metastasis.

Immunohistochemistry and the Scoring Systems

For each of the formalin-fixed paraffin-embedded tumor samples, sections ($4\text{-}\mu\text{m}$ thickness) were prepared and mounted on positively charged slides. SSTR2-IHC analysis was performed by staining with a rabbit anti-SSTR2 monoclonal antibody (working solution; clone EP149; ZSGB-BIO, Beijing, China) for 15 min at 37°C on an auto-stainer (BOND-MAX; LEICA, Leica Biosystems Newcastle Ltd., Newcastle, UK) with specified second antibody and visualization system. Positive SSTR2 immunoreactivity was defined as the presence of membrane immunoreactivity and the absence of stromal immunoreactivity. In some cases, the positive SSTR2 expression was recorded in the cytoplasm after preoperative treatment with SSTR analogs ($11/100$, 11%). Four different scoring systems, namely HER2 score, Volante score [2], H score, and IRS [1, 4] were applied during the evaluation. The HER2 scoring system is a 4-point classification system based on the subcellular localization, the amount of positive cells, and the intensity of staining. The Volante scoring system is based on the subcellular localization and extent of staining, with a rating system from 0, 1+,

Table 2. Clinicopathologic characteristics and SSTR2 expression in 100 GEP-NENs

Variable	All tumors 100	NET G1 9 (100.0)	NET G2 63 (100.0)	NET G3 14 (100.0)	NEC 10 (100.0)	MiNENs 4 (100.0)	<i>p</i> value
Gender, <i>n</i> (%)							
Male	57	4 (44.4)	38 (60.3)	6 (42.9)	5 (50.0)	4 (100.0)	0.527
Female	43	5 (55.6)	25 (39.7)	8 (57.1)	5 (50.0)	0 (0.0)	
Age, yr, <i>n</i> (%)							
<60	70	6 (66.7)	46 (73.0)	10 (71.4)	6 (60.0)	2 (50.0)	0.697
≥60	30	3 (33.3)	17 (27.0)	4 (28.6)	4 (40.0)	2 (50.0)	
Localization, <i>n</i> (%)							
Stomach	13	2 (22.2)	6 (9.5)	1 (7.1)	1 (10.0)	3 (75.0)	0.040
Small intestine	13	2 (22.2)	10 (15.9)	0 (0.0)	1 (10.0)	0 (0.0)	
Colorectal	17	2 (22.2)	10 (15.9)	2 (14.3)	2 (20.0)	1 (25.0)	
Pancreas	49	1 (11.2)	34 (54.0)	10 (71.5)	4 (40.0)	0 (0.0)	
Others*	8	2 (22.2)	3 (4.7)	1 (7.1)	2 (20.0)	0 (0.0)	
Ki67							
Median (range)	10 (1–80)	1 (1–2)	5 (3–20)	30 (24–60)	75 (50–80)	80 (75–80)	0.000
TNM stage, <i>n</i> (%)							
M0	26	4 (44.4)	13 (20.6)	2 (14.3)	4 (40.0)	3 (75.0)	0.070
M1	74	5 (55.6)	50 (79.4)	12 (85.7)	6 (60.0)	1 (25.0)	
SSTR2 patterns, <i>n</i> (%)							
Membrane	89	7 (77.8)	57 (90.5)	13 (92.9)	8 (80.0)	4 (100.0)	0.645
Cytoplasm + membrane	11	2 (22.2)	6 (9.5)	1 (7.1)	2 (20.0)	0 (0.0)	
SSTR2 heterogeneity, <i>n</i> (%)							
No	57	5 (55.6)	39 (61.9)	5 (35.7)	6 (60.0)	2 (50.0)	0.586
Yes	43	4 (44.4)	24 (38.1)	9 (64.3)	4 (40.0)	2 (50.0)	
IHC scoring							
HER2 Pos, <i>n</i> (%)	85 (85)	8 (88.9)	56 (88.9)	11 (78.6)	7 (70.0)	3 (75.0)	0.495
Volante Pos, <i>n</i> (%)	90 (90)	8 (88.9)	57 (90.5)	13 (92.9)	9 (90.0)	3 (75.0)	0.885
H mean (±SD)	189.5±99.9	235.0±95.2	204.7±92.7	169.7±111.62	115.5±92.2	92.5±77.2	0.000
IRS mean (±SD)	7.4±3.9	8.9±3.9	7.8±3.7	6.9±4.2	5.3±3.4	4.0±2.8	0.025
SSTR2 imaging							
Pos, <i>n</i> (%)	72 (72)	8 (88.9)	51 (81.0)	8 (57.1)	3 (30.0)	2 (50.0)	0.007

MiNENs were all mixed adenoneuroendocrine carcinoma with neuroendocrine carcinoma ranging from 40 to 90%. * Others included 7 cases from abdominal or liver metastasis in the absence of evident primary tumors and 1 case from the spleen diagnosed as NET G2. IHC, immunohistochemical staining; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; SSTR2, somatostatin receptor type 2; GEP-NENs, gastroenteropancreatic neuroendocrine neoplasms; MiNENs, mixed neuroendocrine-non-neuroendocrine neoplasms; HER2, human epidermal growth factor receptor 2; SD, standard deviation.

2+, and to 3+. Tumors with a Volante score or a HER2 score of 2+ or 3+ were considered positive. The H and IRS scores are semi-quantitative scoring systems based on the staining intensity and the percent of positive cells. The H score was calculated by adding the product of different staining intensities (0, 1+, 2+, and 3+) with the percent of positive cells, thereby resulting in a score ranging from 0 to 300. The IRS score was determined by multiplying the staining intensity in 4 gradations (0, 1+, 2+, and 3+) with the percent of positive cells in 5 gradations (0, <10, 10–50, 51–80, and >80%). As suggested by Remmele and Stegner [5], the grade of intensity, which was the most predominant, was used and the IRS score of 0–12 points was finally calculated [6]. For the purpose of statistical analysis, the H and IRS scores were subclassified into 4 groups. The details of these 4 scoring systems are illustrated in Table 1.

⁶⁸Ga-DOTATATE PET/CT Imaging

Patients fasted for at least 6 h before undertaking the PET/CT scan. Images were acquired 1 h after the administration of 100–200-MBq ⁶⁸Ga-DOTATATE injection. A whole-body scan was performed from the mid-skull to the upper thigh level in 2-dimensional Flow Motion mode (Biograph64; Siemens, Erlangen, Germany). The correction of attenuation and anatomical localization was performed by using a low-dose, noncontrast CT. After taking the CT scan, a PET image was obtained with an acquisition speed of 1 mm/s. Reconstructed attenuation-corrected PET images, CT images, and fused images of matched pairs of PET and CT sections were evaluated by 2 experienced nuclear medicine physicians. A PET-positive lesion is defined as any area with uptake greater than that of the normal liver background that cannot be identified as a physiological uptake. The Krenning scoring system was used as a

Fig. 1. Representative images of the SSTR2-IHC scores and ^{68}Ga -DOTATATE imaging are shown as colonic NEC (**a, b**), rectum NET G2 (**c, d**), and liver metastasis from gastric NET G1 (**e, f**). The lower right images are corresponding images at higher magnification. Scale bars are at the bottom left of the images. The lesions represented included HER2 score 1+ or Volante score 2+ with discontinuous moderate membranous IHC staining (**a**) with a corresponding SUVmax of colon lesion (yellow arrow) as only 3.7, which is interpreted as negative in ^{68}Ga -DOTATATE PET/CT imaging (**b**). The HER2 and Volante scores of 2+ with heterogeneous mod-

erate membranous IHC staining in <50% of tumor cells (**c**) and thickened rectum wall showing heterogeneous uptake from high to low (**d**). The HER2 and Volante scores of 3+ with strong complete membranous IHC staining (**e**) and a corresponding SUVmax of liver metastasis as 22.5, which is interpreted as positive in ^{68}Ga -DOTATATE PET/CT imaging (**f**). IHC, immunohistochemical staining; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; SUVmax, standardized uptake value; PET/CT, positron emission tomography/computed tomography; SSTR2, somatostatin receptor type 2; HER2, human epidermal growth factor receptor 2.

semiquantitative method for assessing the degree of tracer uptake (0: none; 1: much lower than that of the liver; 2: slightly less than or equal to that of the liver; 3: greater than that of the liver; and 4: greater than that of the spleen) [7]. The maximum standardized uptake value (SUVmax) was measured for metastatic and primary

lesions, and the highest SUVmax value obtained from each of the patients was applied in the final analyses.

SSTR molecular imaging was performed preoperatively in 70 patients, and postoperatively in 25 patients who had some amount of residual disease. The sites of pathological sampling and imaging

Table 3. The distribution of the heterogeneity of SSTR2 expression in the 4 IHC scoring systems

Classifications	Total cases (cases with heterogeneous SSTR2 expression, %), N (n, %)				p value
	0	1+	2+	3+	
HER2 score	9 (0, 0)	6 (1, 16.7)	42 (26, 61.9)	43 (16, 37.2)	0.000
Volante score	8 (0, 0)	2 (0, 0)	31 (21, 67.7)	59 (22, 37.3)	0.000
H score	16 (1, 6.3)	6 (4, 66.7)	24 (17, 70.8)	54 (21, 38.9)	0.000
IRS score	10 (0, 0)	6 (1, 16.7)	49 (33, 67.3)	35 (9, 25.7)	0.000

IHC, immunohistochemical staining; SSTR2, somatostatin receptor type 2; IRS, immunoreactive score; HER2, human epidermal growth factor receptor 2.

evaluation showed consistency in 75 tumors and inconsistency in 25 tumors. Out of these, 21 pathological samples were drawn at the primary tumors, while evaluation of the imaging was performed in the metastatic tumors, and vice versa, in 4 cases. The median interval time between pathological examination and imaging evaluation was 40 days (range: 1–1,868 days), with 84% of the cases occurring within 1 year and 10% in >2 years.

Statistical Analyses

The SPSS 17.0 version (IBM Corporation, Armonk, NY, USA) was used for statistical analysis of the data. Continuous variables were summarized as means $\pm$ standard deviation. Categorical variables were expressed as counts and percent. Kendall's Tau-b and Spearman's rank correlation were used for comparing the immunohistochemical scoring systems with each other and with the SSTR imaging status. The association between SSTR2 expression and SUV values with the clinical-pathological variables was analyzed by employing Pearson's χ^2 and Fisher's exact test. Receiver operating characteristic curve analysis was performed to determine the diagnostic accuracy of the H score and IRS score for the purpose of selecting positive SSTR imaging. The sensitivity and specificity were calculated using a cutoff value as determined by maximization of sensitivity and specificity. $p < 0.05$ was considered to be statistically significant, and $p < 0.01$ was considered to be highly significant.

Results

Clinicopathological Characteristics of Patients and SSTR2 Expression

Immunohistochemistry

The clinicopathological features and the SSTR2 expression of 100 GEP-NENs from 95 patients are illustrated in Table 2. A vast majority of SSTR2-positive immunoactivity was observed in the membrane ($n = 89$, 89%), while lesser immunoactivity was recorded in the cytoplasm and the membrane ($n = 11$, 11%). No significant difference was observed between the different histological grades and the SSTR2 expression patterns ($p = 0.645$). Previous studies have reported heterogeneity

of the SSTR2 expression. The heterogeneous SSTR2 expression was detected in 43% of the cases. Neither the different histological grades nor the primary tumor sites or pathological sampling methods (surgical or biopsy) demonstrated any significant influence on the heterogeneity of the SSTR2 expression (Pearson's χ^2 test, $p > 0.05$).

The SSTR2 expression was found to be positive in 85% of the cases by using the HER2 scoring system and in 90% of the tumors using the Volante scoring system. The positive rates for NET G1/G2, NET G3, or NEC were 88.9, 78.6, and 71.4% (HER2 score) and 90.3, 92.9, and 85.7% (Volante score), respectively. Some representative images are shown in Figure 1a, c, and e. No significant difference existed between the positive rates in tumors, with variable histological grades and different primary sites and with/without liver metastasis by using the HER2 and Volante scoring systems. The SSTR2 scores of NET G1/G2 were significantly higher than those of NEC involving the H and IRS scoring systems ($p = 0.004$ and 0.043 , respectively), although no significant differences were noted between NETs or between NET G3 and NEC ($p > 0.05$). No significant association was observed between the SSTR2-positive rates and gender, age, sampling methods for pathological specimens (surgical or biopsy), or the source of tumors (primary or metastatic) involving the 4 scoring systems ($p > 0.05$).

⁶⁸Ga-DOTATATE PET/CT Imaging

Positive SSTR imaging was detected in 72% of the tumors. Some representative images are shown in Figure 1b, d, and f. The positive rate in NET was significantly higher than that in NEC (77.9 and 35.7%, respectively; $p = 0.001$), which decreased gradually from NET G1 (88.9%) to NET G3 (57.1%). Positive SSTR imaging was more common in pancreatic NET G2 than in colorectal NET G2 (94.1 and 63.6%, respectively; $p = 0.025$). The ef-

Table 4. The absolute agreement and Kendall's Tau-b correlation between the 4 IHC scoring systems

	Heterogeneity					
	no (<i>n</i> = 57)			yes (<i>n</i> = 43)		
	agreement, <i>n</i> (%)	Kendall's Tau-b (s)	<i>p</i> value	agreement, <i>n</i> (%)	Kendall's Tau-b (s)	<i>p</i> value
HER2 score versus volante score	42 (73.7)	0.816	<0.001	34 (79.1)	0.652	<0.001
HER2 score versus H classification	43 (75.4)	0.851	<0.001	33 (76.7)	0.752	<0.001
HER2 score versus IRS classification	54 (94.7)	0.966	<0.001	34 (79.1)	0.594	<0.001
Volante score versus H classification	44 (77.2)	0.867	<0.001	31 (72.1)	0.658	0.008
Volante score versus IRS classification	39 (68.4)	0.810	<0.001	27 (62.8)	0.404	<0.001
H Classification versus IRS classification	43 (75.4)	0.851	<0.001	26 (60.4)	0.534	<0.001

IHC, immunohistochemical staining; IRS, immunoreactive score; HER2, human epidermal growth factor receptor 2.

Table 5. Spearman's rank correlation between the 4 IHC scoring systems and the ⁶⁸Ga-DOTATATE PET/CT imaging

	Heterogeneity			
	no (<i>n</i> = 57)		yes (<i>n</i> = 43)	
	rho	<i>p</i> value	rho	<i>p</i> value
HER2 score versus ⁶⁸ Ga-DOTATATE PET/CT imaging	0.715	<0.001	0.529	<0.001
Volante score versus ⁶⁸ Ga-DOTATATE PET/CT imaging	0.862	<0.001	0.380	0.012
H score versus ⁶⁸ Ga-DOTATATE PET/CT imaging	0.795	<0.001	0.337	0.027
IRS score versus ⁶⁸ Ga-DOTATATE PET/CT imaging	0.711	<0.001	0.365	0.016

⁶⁸Ga-DOTATATE PET/CT imaging was classified into 5 groups as described. IHC, immunohistochemical staining; PET/CT; positron emission tomography/computed tomography; IRS, immunoreactive score; HER2, human epidermal growth factor receptor 2.

fect of primary tumor diameters on the imaging status was found to be statistically insignificant (positive: 2.99 ± 2.32 cm vs. negative: 4.47 ± 3.11 cm, $p = 0.128$).

Comparison of the 4 Different Scoring Systems

A comparative description of the distribution of the 4 scoring systems and the heterogeneity of SSTR2 expression is presented in Table 3. In general, after reclassification into 4 separate groups, the 4 scoring systems showed significant positive correlation with one another (Kendall's Tau-b range: 0.836–0.932; $p < 0.001$). The heterogeneous expression of SSTR2 was most common in the 2+ groups (range: 61.9–70.8%). In cases without heterogeneous SSTR2 expression (accounting for 57% of all cases), these 4 scoring systems were highly consistent (Kendall's Tau-b range: 0.81–0.97). However, in cases with heterogeneous expression, HER2 score and H score were more

consistent (Kendall's Tau-b = 0.752), while the Volante score and IRS score showed poor consistency (Kendall's Tau-b coefficient <0.5), as shown in Table 4.

Correlation of SSTR2 Immunohistochemistry with ⁶⁸Ga-DOTATATE PET/CT Imaging

The correlation between IHC scores and the ⁶⁸Ga-DOTATATE PET/CT imaging results are presented in Table 5. In cases without heterogeneous SSTR2 expression, the 4 scoring systems all correlated well with ⁶⁸Ga-DOTATATE PET/CT imaging (Spearman's rank correlation range: 0.711–0.862; $p < 0.001$). In cases with heterogeneous SSTR2 expression, the correlation between IHC and ⁶⁸Ga-DOTATATE PET/CT was found to be relatively low with the Spearman's rank correlation range of 0.337–0.529 ($p < 0.05$). The area under a ROC curve value of the H and IRS scores in predicting the ⁶⁸Ga-DOT-

ATATE PET/CT imaging outcomes was 0.951 and 0.934, respectively, with the highest sensitivity and specificity achieved when defining the cutoff value as 160 (H score) and 6 (IRS score) (Fig. 2).

The IHC diagnostic values for ^{68}Ga -DOTATATE PET/CT imaging are illustrated in Table 6. The data of imaging were further reclassified into 5 groups: 0, 1, 2, 3, and 4, as mentioned earlier, and groups 3 and 4 were considered as positive. The IHC scores of 2+ and 3+ were considered as positive in the HER2 score and Volante score. For the H score and IRS score, cases with a score higher than the cutoff value in receiver operator characteristic analysis were considered as positive. The diagnostic concordance rate was observed in 87, 87, 82, and 79% of the tumors using the HER2 score, H score, Volante score and IRS score, respectively. The combination of the HER2 score and H score is considered as an effective approach to improve the predictive accuracy for SSTR imaging assessment.

Comparative analysis of the clinicopathological factors in consistent and inconsistent cases is depicted in Table 7. The histological grades and Ki67 index of GEP-NENs have a significant impact on the consistency of SSTR imaging and pathological assessment involving the HER2 score and Volante score. The consistency in NET G1 and G2 was significantly higher than that in NET G3 and NEC ([67/70], 95.7% and [20/27], 74.1%, respectively) for HER2 score and ([66/70], 94.3% and [16/27], 59.3%, respectively) for Volante score. However, with respect to the H score and IRS score, the heterogeneity of SSTR2 expression had a significant impact on the consistency. The consistency was significantly higher in cases lacking heterogeneity when compared to those with heterogeneity, ([56/57], 98.2% and [31/40], 77.5%, respectively) for H score and ([56/57], 98.2% and [22/40], 55%, respectively) for IRS score. Other factors including the primary tumor sites, the SSTR2 expression patterns, biopsy or surgery, and the interval time between pathological and imaging assessment showed no significant effect on the consistency.

Discussion

In this study, 4 different immunohistochemical scoring systems for the SSTR2 expression in 100 GEP-NENs were compared and subjected to correlational analyses with the results of ^{68}Ga -DOTATATE PET/CT imaging. It was found that SSTR2-IHC staining indicated high predictive accuracy with regard to the imaging outcomes. The HER2 score is a common clinical method suitable for

Fig. 2. The AUC of the H and IRS scores in predicting ^{68}Ga -DOTATATE PET/CT imaging outcomes are shown. The data of ^{68}Ga -DOTATATE PET/CT imaging was classified as negative or positive. **a** The AUC value of the H score in predicting the ^{68}Ga -DOTATATE PET/CT imaging outcomes was 0.951. The highest sensitivity and specificity were 86.1 and 89.3%, respectively, with a cutoff value defined as 160. **b** The AUC value of the IRS score in predicting the ^{68}Ga -DOTATATE PET/CT imaging outcomes was 0.934. The highest sensitivity and specificity were 73.6 and 92.9%, respectively, with a cutoff value defined as 6. ROC, receiver operator characteristic; PET/CT; positron emission tomography/computed tomography; IRS, immunoreactive score; AUC, area under a ROC curve.

SSTR2-IHC evaluation. In HER2 2+ cases, the combination of HER2 and H score is an effective approach to improve the predictive accuracy for ^{68}Ga -DOTATATE PET/CT imaging. For the H score and IRS score, the optimal cutoff values were 160 and 6, respectively, reflecting the highest sensitivity and specificity in the prediction of imaging results. This can be of great value for the clinical

Table 6. The diagnostic values of the 4 IHC scoring systems in predicting ^{68}Ga -DOTATATE PET/CT imaging

⁶⁸ Ga-DOTATATE PET/CT imaging					
	positive (<i>n</i> = 72)	negative (<i>n</i> = 28)	<i>p</i> value	sensitivity, %	specificity, %
HER2 score					
Positive	72	13	<0.001	100	53.6
Negative	0	15			
Volante score					
Positive	72	18	<0.001	100	35.7
Negative	0	10			
H score					
>160	62	3*	<0.001	86.10	89.30
≤160	10	25			
IRS score					
>6	53	2	<0.001	73.60	92.90
≤6	19	26			
HER2 score 2+ (<i>n</i> = 42)					
H score					
>160	19	3*	<0.001	65.5	76.9
≤160	10	10			
Volante score 2+ (<i>n</i> = 31)					
H score					
>160	7	1*	<0.001	46.7	93.8
≤160	8	15			

* Indicates that these 3 tumors were discordant between SSTR2 IHC and ⁶⁸Ga-DOTATATE PET/CT imaging in 3 scoring systems, including the H score, HER2 score, and Volante score. In 2 of the tumors, the residual tumors were too small, which may cause false negatives on imaging assessments. The interval time between IHC evaluation and imaging assessment in the third tumor was 4.78 years, which may be responsible for the inconsistency. IHC, immunohistochemical staining; PET/CT; positron emission tomography/computed tomography; SSTR2, somatostatin receptor type 2; HER2, human epidermal growth factor receptor 2.

management of patients with GEP-NENs. SSTR2 IHC may be the best substitute for preoperative SSTR imaging. First, a positive IHC result contributes to the selection of ^{68}Ga -SSTR PET/CT imaging for restaging and follow-up. Second, the correlations also guide clinicians for the identification of patients for further synthetic somatostatin analog or PRRT. The SSTR expression status also reflects the biological characteristics of NENs, such as differentiation [8] and heterogeneity [9], which is proved to be helpful in the pathological diagnosis.

Based on the ^{68}Ga -DOTATATE PET/CT imaging results, the 4 different IHC scoring systems in both homogeneous and heterogeneous SSTR2 expression groups were compared. The latter group indicated lower correlation relative to the former, while the ^{68}Ga -DOTATATE PET/CT indicated intratumoral and/or intertumoral heterogeneity in 22% of all cases. Heterogeneity is a unique biological characteristic of GEP-NENs, which accounts

for the increase in the difficulty of clinical diagnosis and therapy. A recent analysis [9] of the heterogeneity of the SSTR2 expression in 156 liver metastases from small intestinal NENs indicated intertumoral heterogeneity in nearly 33% of the patients and intratumoral heterogeneity in 69% of the tumors. Our previous study [10] also indicated that 29.6% of GEP-NENs with liver metastases exhibited variable SSTR expression performance. The heterogeneous SSTR2 expression complicates the accurate computation of percent with respect to the staining intensity [11]. Therefore, the correlation between IHC and SSTR molecular imaging was analyzed separately in homogeneous and heterogeneous expression groups. The heterogeneity also induced differences in response to SSTR-based therapy among different grades of tumors. *Graf* reported that patients with heterogeneous SSTR expression treated with PRRT had significantly lower overall survive time and time to progression [12].

Table 7. Comparative analysis of the clinicopathological factors in consistent and inconsistent cases involving the 4 IHC scoring systems and ^{68}Ga -DOTATATE PET/CT imaging

	Consistent cases versus inconsistent cases (<i>p</i> value)			
	HER2 score	Volante score	H score	IRS score
Tumor locations	–	–	–	–
SSTR2-IHC patterns	–	–	–	–
SSTR2 heterogeneity	–	–	0.003	0.000
Tumor histological types	0.004	0.000	–	–
Ki67 index	0.000	0.000	–	–
TNM stage	–	0.04	–	–
Surgical or biopsy materials	–	–	–	–
Interval time between IHC and imaging	–	–	–	–

Three tumors were excluded. They showed inconsistency between SSTR2 IHC and ^{68}Ga -DOTATATE PET/CT imaging in 3 scoring systems, including the H score, HER2 score, and Volante score. “–” means $p > 0.05$. IHC, immunohistochemical staining; PET/CT, positron emission tomography/computed tomography; SSTR2, somatostatin receptor type 2; IRS, immunoreactive score; HER2, human epidermal growth factor receptor 2.

Although several studies [13–15] have verified the concordance and correlation between ^{68}Ga -SSTR PET/CT and SSTR IHC, none discussed the role of heterogeneity and the cutoff values in SSTR IHC evaluation. As methods for investigating the SSTR expression levels, ^{68}Ga -SSTR PET/CT is an in vivo evaluation, while IHC is an in vitro reflection of the protein expression status. The IRS and HER2 scoring systems were developed for the IHC detection of estrogen and HER2 receptors in mammary carcinoma, respectively [5, 16]. The H scoring system, despite being quite complicated, is more precise and hence has a wider application and provides a more detailed analysis [17]. The Volante scoring system was the first method developed specifically for SSTR2 evaluation [2]. According to the criteria of the Volante scoring system, any occurrence of membranous staining is interpreted as positive (score 2+ or 3+), and the difference between the scores reflects >50% of circumferential membranous reactivity. Highest sensitivity (100%) and lowest specificity (35.7%) was exhibited by the Volante scoring system. Clearly, the positive threshold of this system is not optimal for clinical application. All 4 scoring systems exhibited good concordance, especially in the case of the homogeneous groups. Because of its simplicity and its potential for use on a large scale, the HER2 scoring system has been recommended as a standard method for SSTR2 evaluation [18]. Moreover, 10 out of 14 (71.4%) of NECs or MiNENs with NEC components were interpreted as positive by employing the HER2 score, which was much higher than that obtained through ^{68}Ga -DOTATATE

PET/CT imaging ([5/14], 35.7%). It was found that the 10% cutoff value applied to the HER2 scoring system was not accurate for SSTR2 staining.

The cutoff value for H score was defined as 160 for predicting the ^{68}Ga -DOTATATE PET/CT imaging results, which indicated the highest sensitivity and specificity at 86.1 and 89.3%, respectively. Moreover, 65% of all cases had an H score of >160 points (65/100). In addition, 95.4% (62/65) of the cases were positive in the imaging assessments and only 4.6% (3/65) were negative. The mismatch observed in the 3 tumors was further analyzed. It was concluded that the excessively small size of residual lesions for imaging and the long interval time between pathology and imaging (4.78 years) may be the probable reason for the inconsistency. On the other hand, in 35 of the 100 cases with H scores ≤ 160 , 71.4% (25/35) of cases were observed to be negative during imaging, which included 15 cases with a HER2 score of 0 or 1+ and 10 cases with a HER2 score of 2+. In addition, 28.6% (10/35) of the cases, with H scores ranging between 120 and 160 points and all with a HER2 score of 2+, showed positive outcomes in ^{68}Ga -DOTATATE PET/CT imaging. All these cases had heterogeneous SSTR2 expression, which probably affected the pathological evaluation, resulting in inconsistency.

With respect to the HER2 scores, the outcomes of ^{68}Ga -DOTATATE PET/CT imaging were observed to be positive in 100% of the cases (43 cases) with the HER2 scores of 3+ and all negative in cases (15 cases) with the HER2 scores of 0 or 1+. In cases with the HER2 scores of 2+, the positive rate in ^{68}Ga -DOTATATE PET/CT imaging was

69% (29/42), and 65.5% (19/29) of tumors had H scores of >160. For cases with 2+ HER2 scores, the sensitivity and specificity of H scores for predicting the outcomes of ⁶⁸Ga-DOTATATE PET/CT imaging was 65.5 and 76.9%, respectively. In addition, the heterogeneous SSTR2 expression was mainly found in cases with 2+ HER2 scores. Therefore, in such cases, the combination of H score and HER2 score is expected to help identify positive cases with increased sensitivity and specificity. It indicated that 80% of tumor cells were moderately positive or 55% strongly positive were the cutoff value for SSTR2-IHC reading according to the positive PET/CT results.

The present study has several limitations. This is a retrospective single-center research study. In order to optimize the cutoff value and explore the predictive value for SSTR-related therapy, we plan to design a multicenter prospective study in the near future.

Conclusions

Owing to the strong correlation, SSTR2 immunohistochemistry staining can predict the outcomes of ⁶⁸Ga-DOTATATE PET/CT imaging accurately, especially in the homogeneous expression group. According to the ⁶⁸Ga-DOTATATE PET/CT imaging, 80% of tumor cells were moderately positive or 55% strongly positive were the cutoff value for SSTR2 immunohistochemistry reading. These results are useful for optimizing ⁶⁸Ga-DOTATATE PET/CT application and guiding clinicians to identify suitable patients for further SSTR-related therapy.

References

- 1 Kasajima A, Papotti M, Ito W, Brizzi MP, La Salvia A, Rapa I, et al. High interlaboratory and interobserver agreement of somatostatin receptor immunohistochemical determination and correlation with response to somatostatin analogs. *Hum Pathol*. 2018;72:144–52.
- 2 Volante M, Brizzi MP, Faggiano A, La Rosa S, Rapa I, Ferrero A, et al. Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. *Mod Pathol*. 2007;20(11):1172–82.
- 3 Kaemmerer D, Peter L, Lupp A, Schulz S, Sanger J, Baum RP, et al. Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastroenteropancreatic neuroendocrine tumors. *Int J Clin Exp Pathol*. 2012;5(3):187–94.
- 4 Specht E, Kaemmerer D, Sanger J, Wirtz RM, Schulz S, Lupp A. Comparison of immunoreactive score, HER2/neu score and H score for the immunohistochemical evaluation of somatostatin receptors in bronchopulmonary neuroendocrine neoplasms. *Histopathology*. 2015;67(3):368–77.
- 5 Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. *Pathology*. 1987;8(3):138–40.
- 6 McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KSSr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal anti-receptor antibodies. *Arch Pathol Lab Med*. 1985;109(8):716–21.
- 7 Krenning EP, Bakker WH, Breeman WA, Koper JW, Kooij PP, Aulsema L, et al. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. *Lancet*. 1989;1(8632):242–4.
- 8 Yu J, Li N, Li J, Lu M, Leal JP, Tan H, et al. The correlation between [(68)Ga]DOTATATE PET/CT and cell proliferation in patients with GEP-NENs. *Mol Imaging Biol*. 2019;21(5):984–90.
- 9 Charoenpitakchai M, Liu E, Zhao Z, Koyama T, Huh WJ, Berlin J, et al. In liver metastases from small intestinal neuroendocrine tumors, SSTR2A expression is heterogeneous. *Virchows Arch*. 2017;470(5):545–52.
- 10 Panpan Z, Jiangyuan Y, Jie L, Lin S, Nan L, Hua Z, et al. Clinical and prognostic value of PET/CT imaging with combination of (68)Ga-DOTATATE and (18)F-FDG in gastroenteropancreatic neuroendocrine neoplasms. *Contrast Media Mol Imaging*. 2018.

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Statement of Ethics

This retrospective analysis was approved by the Ethics Committee of Peking University Cancer Hospital in July 20, 2019.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Jiangyuan Yu, Fang Cao, Zhi Yang, and Yu Sun carried out conception and design. Jiangyuan Yu, Fang Cao, and Yu Sun developed methodology. Jiangyuan Yu, Fang Cao, Xinya Zhao, and Yu Sun acquired the data. Fang Cao, Qing Xie, Ming Lu, and Jie Li carried out analyses. All the authors were involved in writing the manuscript and had final approval of the final versions.

- 11 Specht E, Kaemmerer D, Sanger J, Wirtz RM, Schulz S, Lupp A. Comparison of immunoreactive score, HER2/neu score and H score for the immunohistochemical evaluation of somatostatin receptors in bronchopulmonary neuroendocrine neoplasms. [Histopathology](#). 2015;67(3):368–77.
- 12 Graf J, Pape UF, Jann H, Denecke T, Arsenic R, Brenner W, et al. Prognostic significance of somatostatin receptor heterogeneity in progressive neuroendocrine tumor treated with Lu-177 DOTATOC or Lu-177 DOTATATE. [Eur J Nucl Med Mol Imaging](#). 2020;47(4):881–94.
- 13 Diakatou E, Alexandraki KI, Tsolakis AV, Kontogeorgos G, Chatzellis E, Leonti A, et al. Somatostatin and dopamine receptor expression in neuroendocrine neoplasms: correlation of immunohistochemical findings with somatostatin receptor scintigraphy visual scores. [Clin Endocrinol](#). 2015;83(3):420–8.
- 14 Korner M, Waser B, Schonbrunn A, Perren A, Reubi JC. Somatostatin receptor subtype 2A immunohistochemistry using a new monoclonal antibody selects tumors suitable for in vivo somatostatin receptor targeting. [Am J Surg Pathol](#). 2012;36(2):242–52.
- 15 Kaemmerer D, Peter L, Lupp A, Schulz S, Sanger J, Prasad V, et al. Molecular imaging with ⁶⁸Ga-SSTR PET/CT and correlation to immunohistochemistry of somatostatin receptors in neuroendocrine tumours. [Eur J Nucl Med Mol Imaging](#). 2011;38(10):1659–68.
- 16 Koeppen HK, Wright BD, Burt AD, Quirke P, McNicol AM, Dybdal NO, et al. Overexpression of HER2/neu in solid tumours: an immunohistochemical survey. [Histopathology](#). 2001;38(2):96–104.
- 17 Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. [J Clin Oncol](#). 2003; 21(20):3798–807.
- 18 Miederer M, Seidl S, Buck A, Scheidhauer K, Wester HJ, Schwaiger M, et al. Correlation of immunohistopathological expression of somatostatin receptor 2 with standardised uptake values in ⁶⁸Ga-DOTATOC PET/CT. [Eur J Nucl Med Mol Imaging](#). 2009;36(1):48–52.