

Detection of somatostatin receptor subtypes 2 and 5 by somatostatin receptor scintigraphy and immunohistochemistry: clinical implications in the diagnostic and therapeutic management of gastroenteropancreatic neuroendocrine tumors

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ABSTRACT

Aims and background. Somatostatin receptor scintigraphy (SRS) is the standard method for the detection of somatostatin receptors (SSTRs). It is commonly used in gastroenteropancreatic neuroendocrine tumor (GEP-NET) staging, and represents the criterion of choice for treatment with somatostatin (SST) analogs. Immunohistochemistry (IHC) was reported as a reliable method for the detection of SSTRs with theoretically superior sensitivity over SRS.

Methods and study design. We retrospectively analyzed the sensitivity and specificity of IHC in the detection of SSTRs in a cohort of consecutive patients with GEP-NETs attending our Institute from 1997 to 2007. IHC analysis was restricted to SSTR2 and SSTR5, and the results were interpreted according to two different scoring systems. SRS was used as the gold standard.

Results. Forty-four patients were enrolled; 24 (55%) had foregut carcinoids, 9 (20%) midgut carcinoids, 2 (5%) hindgut carcinoids, and 9 (20%) had GEP-NETs of unknown primary sites. A high concordance rate between IHC and SRS was shown, irrespective of the IHC scoring system applied (73% and 70%). The sensitivity of IHC was 89.3% and 78.6% and the specificity 43.8% and 50%, depending on the scoring system used.

Conclusions. Although SSTR2 was shown to be expressed by IHC in up to 50% of tumors not visualized by SRS, SRS still remains the method of choice in the diagnostic and therapeutic management of GEP-NETs. More pathological and clinical data are needed to properly understand the clinical relevance of immunohistochemical detection of SSTR expression in the absence of tumor uptake at SRS.

Key words: somatostatin receptors, neuroendocrine tumors, somatostatin receptor scintigraphy, immunohistochemistry.

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Introduction

Somatostatin (SST) is a peptide hormone that regulates the endocrine system, affects neurotransmission and cell proliferation, and inhibits the release of numerous secondary hormones. SST acts through interaction with a family of 7 transmembrane domain G-protein-coupled somatostatin receptors (SSTRs) encoded by 5 distinct genes¹⁻³. SSTRs were found to be widely expressed in neuroendocrine tumors (NETs), particularly if they originated from the gastroenteropancreatic (GEP) tract⁴. The use of techniques such as autoradiography, *in situ* hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) has allowed identification of at least 5 subtypes of SSTRs⁵⁻⁸. This finding was the molecular basis

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for the diagnostic and therapeutic use of SST analogs^{9,10}. Currently, identification of the tumor and evaluation of its extent is commonly performed by somatostatin receptor scintigraphy (SRS) and long-acting SST analogs and their radiolabeled compounds represent a valid symptomatic treatment and a promising new tool in the management of metastatic NETs^{11,12}.

It is now clear that the commonly used SST analogs octreotide and lanreotide have a high binding affinity for SSTR2 and SSTR5, low affinity for SSTR3, and no affinity for SSTR1 and SSTR4¹³.

Due to the identification of NET receptor status, SRS has acquired a unique role in clinical practice for disease staging. Moreover, since it provides an *in vivo* demonstration of NET receptor expression (mainly SSTR2 and SSTR5), it has become the main criterion for the selection of patients suitable for SST-analog-based treatment¹⁴. Nevertheless, the diagnostic capability and therapeutic indications of SRS are limited by several factors such as the absence or low density of available receptor binding sites, low affinity subtype receptor expression, small size of tumor lesions, radiopharmaceutical activity, and timing of acquisition^{15,16}. The recent validation of IHC as a low-cost, reproducible, and highly sensitive method for staining SSTRs^{4,7,17,18} is likely to offer new decisional strategies in the management of NETs from the point of view of cost-effectiveness. IHC could in fact help physicians to select patients potentially responsive to treatment with cold or radiolabeled somatostatin analogs, and provide a rationale for the use of SRS in clinical practice. In order to test this hypothesis, we conducted a retrospective study to evaluate the sensitivity and specificity of IHC in the assessment of SSTR expression in GEP-NETs.

Material and methods

From June 1997 to July 2007 247 patients with NETs attended our Institute. We retrospectively enrolled in the study patients with the following characteristics: histopathological diagnosis of GEP-NET, suitable tumor specimens available for IHC analysis, and results of SRS performed as a staging procedure. All tumor specimens were reassessed by a single experienced pathologist (LDT) to confirm the previously formulated diagnosis of GEP-NET. Tumor specimens were obtained from biopsy or surgical sampling of primary or metastatic lesions. The IHC analysis was restricted to SSTR2 and SSTR5 expression. SRS was performed at diagnosis, prior to any therapeutic measure, or later, in the case of disease detectable by conventional endoscopic or radiological procedures.

Somatostatin receptor imaging

SRS was performed using ¹¹¹In-pentetreotide up to December 2005. Octreotide is a peptide that can be

chelated with diethylene-triamine-penta-acetic acid (DTPA) and labeled with indium-111 to obtain ¹¹¹In-pentetreotide. This radiopharmaceutical is used to image SSTR2 and SSTR5, which are usually expressed on GEP-NETs^{19,20}. Whole-body images were acquired 4 and 24 hours after i.v. injection of 185 MBq of ¹¹¹In-pentetreotide using a double-head gamma camera in anterior and posterior views. Single-photon-emission tomography (SPET) studies were acquired. The gamma camera was equipped with low-energy collimators, with a 20% energy window on the ¹¹¹In energy peak (171 keV). Transaxial SPET data were reconstructed and then filtered with a Butterworth filter order 10, cutoff 0.38 cm.

Recently, the clinical efficacy of ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide was compared with that of ¹¹¹In-pentetreotide in a series of patients who were very heterogeneous in terms of underlying pathology²¹. The results showed that the sensitivity of these tracers is similar. Most tumors showed a matching pattern of uptake, indicating that both tracers have a similar binding behavior for SSTR2 and SSTR5⁸.

Since January 2006 we have therefore used ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide for SRS. The radiopharmaceutical was prepared using the recently described formulation³. Whole-body and SPET images were acquired 6 hours after i.v. injection of 370-444 MBq of ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide using a double-head gamma camera equipped with low-energy collimators, with a 20% energy window on ^{99m}Tc energy peaks (140 keV). Transaxial SPET data were reconstructed and then filtered with a Butterworth filter order 10, cutoff 0.66 cm.

SRS uptake was considered positive when it was higher than or equivalent to the normal liver tracer uptake and negative when there was no significant tracer uptake by the corresponding radiological abnormalities that were regarded as a metastasis or as the primary tumor. All data were reviewed by a single experienced nuclear physician (AC; 50 octreotide scintigraphy interpretations per year on average) who was not aware of the clinical presentation or of the tumor's immunostaining but who was aware of the radiological data.

Immunohistochemistry analysis

A representative tumor block was selected from each case. Formalin-fixed, paraffin-embedded tissue sections were consecutively cut and immunostained with antibodies directed against SST antigens SSTR2A [Bio-Trend, clone SS800A, dilution 1:4000, antigen retrieval citrate buffer; detection system Envision (DAKO)] and SSTR5 [Santa Cruz, clone H54, dilution 1:50; retrieval system citrate buffer, detection system Envision (DAKO)]. Analysis for SSTR2A was performed in all cases. Analysis for SSTR5 was performed only in cases with available unstained slides/available material. A strongly immunoreactive endocrine neoplasm was always used as external control. The IHC results were evaluated

semiquantitatively by a single pathologist (LDT) blinded to the scintigraphic results.

When we planned this study, a universally accepted scoring system for SSTR expression evaluation was not available. We hence arbitrarily decided to consider the percentage of immunoreactive tumor cells as the sole criterion for the interpretation of IHC results. We considered as positive cases all tumors showing at least 5% of immunoreactive cells, irrespective of the characteristics of the immunostaining in terms of localization (membrane or cytoplasm) and intensity (1+: faint, i.e., pale staining not easily seen at low magnification; 2+: moderate, i.e., medium staining visible at low magnification, 3+: strong, i.e., dark staining easily seen at low magnification).

More recently, during the final analysis of the data from our series, a study with a similar design conducted in a population of 108 cases of NET was published by Volante *et al.*²², proposing a scoring system in which the subcellular pattern of SSTR expression and the extension of the positive tumor population were criteria for scoring SSTR2 and SSTR3/5 expressing tumors (Table 1). We decided to use in the comparative analysis with SRS both these IHC scoring systems, and the same pathologist (LDT) consequently proceeded with reviewing, according to the criteria proposed by Volante *et al.*, the IHC results initially scored with our system.

Statistical analysis

The sensitivity and specificity with 95% confidence intervals of the 2 scores were calculated. Identification of SSTRs by SRS was considered confirmation of the presence of the receptors and was used as the gold standard. Statistical analysis was carried out using the McNemar chi-square test to compare the 2 scores. By conventional criteria, a *P* value less than 0.05 was considered statistically significant.

Results

Forty-four of 247 patients (18%) met the above inclusion criteria. The primary site of origin inside the gastrointestinal tract and the presence of tumor specimens available for IHC analysis were the main criteria not ful-

Table 1 - Schematic representation of the immunohistochemical criteria proposed by Volante *et al.*²² for assessing SSTR2 and SSTR5 positivity

	Staining	Localization	Extent
SSTR2 – (score 0-1)	–	Cytoplasmic	NA
SSTR2 + (score 2-3)	+	Membranous	Any
SSTR5 –	–	Cytoplasmic	<10% of cells
SSTR5 +	+	Cytoplasmic	≥10% of cells

SSTR2, somatostatin receptor subtype 2; SSTR5, somatostatin receptor subtype 5; NA, not applicable.

filled and determining the limited number of patients included in the study. The patients' characteristics are summarized in Table 2. According to the old embryological classification, 23 patients (53%) had a diagnosis of foregut carcinoid, 9 (20%) of midgut carcinoid, and 3 (7%) of hindgut carcinoid. In the remaining 9 cases

Table 2 - Patient characteristics

	No. of patients	%
Total	44	100
Sex		
Male	25	57
Female	19	43
Median age (range)	59.5 years (17-78)	
MEN-1 syndrome		
Detected	3	7
Undetected	41	93
Hypersecretion syndrome		
Yes	6	14
No	38	86
Site of origin and tumor histology (WHO class.)		
Pancreas	15	34
WDET	3	7
WDEC	9	21
PDEC	2	4
Unknown	9	21
WDEC	9	21
Ileum	7	16
WDET	1	2
WDEC	6	14
Stomach	6	14
WDET	1	2
WDEC	4	9
PDEC	1	2
Mixed	1	2
Colon	5	11
WDEC	3	7
PDEC	2	4
Duodenum	2	4
WDEC	1	2
PDEC	1	2
Extent of disease		
Localized	13	30
Metastatic	31	70
SRS tracer		
¹¹¹ In-pentetreotide	30	68
^{99m} Tc-EDDA/HYNIC-Tyr3-octreotide	14	32
Treatment received		
Surgery	31	70
SSAs	11	25
IFN	6	14
CT	14	32
TACE	2	5
RFA	0	0
PRRT	11	25

WDET, well-differentiated endocrine tumor; WDEC, well-differentiated endocrine carcinoma; PDEC, poorly differentiated endocrine carcinoma; SRS, somatostatin receptor scintigraphy; SSAs, somatostatin analogs; IFN, interferon; CT, chemotherapy; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; PRRT, peptide receptor radiotherapy

(20%) the imaging procedures or explorative surgery did not allow identification of the primary site of the tumor. In Table 2, the tumors are also classified according to the updated WHO classification²³. GEP-NETs were found to be part of the multiple endocrine neoplasia syndrome type 1 (MEN-1) in 3 patients (7%). Thirty-five (100%) of 35 evaluated patients had abnormal levels of serum chromogranin A (CgA); 18 (50%) of 36 evaluated patients had abnormal levels of serum neuron-specific enolase (NSE); 15 (65%) of 23 evaluated patients had abnormal urine levels of 5-hydroxyindoleacetic acid (5-HIAA) (data not shown). Carcinoid syndrome (i.e., flushing and/or diarrhea) was diagnosed in 6 (14%) patients. At the time of the SRS assessment wide variability in terms of extent of disease and tumor bulk was found among the enrolled patients. The most commonly involved sites of disease were the liver, pancreas and lymph nodes. The size of the tumor lesions, calculated on the basis of conventional imaging procedures, varied from a few millimeters to more than 10 centimeters. Wide heterogeneity in terms of treatment received was also reported. Thirty-one patients (70%) underwent radical or palliative surgery. The same number of patients received medical treatment (chemotherapy 32%, somatostatin analogs 25%, interferon 14%). A locoregional procedure (chemoembolization) was attempted in 2 patients (5%) and radiolabeled therapy in 11 patients (25%).

Data regarding the IHC assessment of SSTRs are shown in Table 3. Irrespective of SSTRs detected, the number of immunoreactive cells, the localization of immunoreactivity, and the intensity of staining (Table 4), IHC was positive in 34/44 patients (77%). SSTR2 was expressed in 32 of 44 tumors (73%) and SSTR5 in 17 of 39 evaluated tumors (44%). Both SSTR subtypes were found to be concurrently expressed in 15 tumors (38%). Predictably, SSTR2 appeared to be more frequently expressed than SSTR5 and only 2 (5%) tumors had positive staining for SSTR5 in the absence of positive staining for SSTR2. Pancreatic and colorectal NETs were found to have the highest frequency of SSTR expression (100% in both cases), while only 44% of unknown primary NETs were positive for SSTR2 and/or SSTR5. Most patients presenting with a carcinoid syndrome (83%) had SSTR-expressing tumors.

According to the scoring system proposed by Volante *et al.*²², 30/44 (68%) tumor specimens were positively immunostained for SSTR2 and/or SSTR5. While the percentage of SSTR5-positive tumors was unchanged (44%), fewer SSTR2-expressing tumors were detected (27/44, 61%). Thus, both SSTR subtypes were simultaneously observed in 14 tumors (32%) and SSTR5 was identified as the sole receptor expressed in 3 (7%) cases. Once again, although evaluated with a different scoring system, SSTRs were expressed more frequently in pancreatic and colorectal NETs (93% in both cases) than in unknown-primary NETs (44%), and 5 out of 6 (83%) tu-

mors from patients with a carcinoid syndrome were found to express SSTR2 and/or SSTR5.

Pathological tracer uptake was documented on SRS in 28 patients (64%); as expected, the most commonly involved sites were the liver (45% of patients), lymph nodes (20%), and pancreas (14%).

Comparison between IHC and SRS showed a high concordance rate, irrespective of the method used to evaluate the IHC results. Total agreement in the identification of SSTR expression was revealed in 32 (73%) and 31 (70%) patients when the cutoff of 5% of immunoreactive cells was used as the criterion for an IHC-positive tumor and the scoring system by Volante *et al.*²², respectively. Among the former 32 concordant cases, 25 proved to be positive by both methods and 7 negative by both methods; among the latter 31 concordant cases, 21 were found to be positive by both methods and 10 negative by both methods.

Most of the patients (93%) with a positive SRS were shown to express SSTRs in at least 5% of tumor cells at IHC. Only 3 (7%) tumors (2 well-differentiated endocrine carcinomas of unknown primary sites and 1 well-differentiated endocrine carcinoma of the ileum) lacking IHC staining for SSTR2 and SSTR5 were visualized by SRS (2 visualized by SRS with ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide, 1 visualized by SRS with ¹¹¹In-pentetreotide). On the other hand, 9 tumors (20%) with positive IHC staining were not identified by SRS (SRS was performed in 7 cases using ¹¹¹In-pentetreotide and in the remaining 2 cases using ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide), resulting in 89.3% (CI 95%: 77.8; 100) and 43.8% (CI 95%: 19.4; 68.1) sensitivity and specificity, respectively (Figure 1).

When positive and negative IHC results were defined according to the scoring system by Volante *et al.*²², the percentage of IHC-positive tumors not visualized by SRS remained consistently high (18%, 8 of the 9 cases described above), and in 14% of the entire series (6/44, 3 new cases other than the 3 described above) IHC failed to detect SSTR2 and SSTR5 in the presence of a positive SRS, resulting in 78.6% (CI 95%: 63.4; 93.8) and 50.0% (CI 95%: 25.5; 74.5) sensitivity and specificity, respectively.

In all cases where – regardless of the scoring criteria used – the lack of concordance between IHC and SRS was the result of the absence of tumor SSTR expression (SRS-positive/SSTR-negative tumors), a repeat IHC analysis of the available tumor specimens was performed that further confirmed the original findings.

Consistently with what was observed for the concordance rate, the reliability of IHC in detecting the expression of SSTRs did not differ significantly in either sensitivity ($P = 0.249$) or specificity ($P = 1.0$), irrespective of the criteria to evaluate the results of immunostaining. Moreover, considering sensitivity and specificity simultaneously, no significant differences were found between the 2 scoring systems ($P = 0.135$).

Table 3 - Comparison of SRS results and IHC staining for SSTR2 and SSTR5

Pts	Sex	Age	Primary site	Histology	MEN	CS	Sample	Procedure	Site/measure of detectable disease	SRS tracer	SRS	SSTR2	SSTR5	sstr2	sstr5
1	m	59	Unknown	WDEC	No	Yes	Brain	Surgery	Liver/140 mm	^{99m} Tc	+	+	NA	+	NA
2	m	67	Ileum	WDEC	No	No	Ileum	Surgery	Liver/16 mm	^{99m} Tc	+	-	-	-	-
3	f	66	Unknown	WDEC	No	No	Liver	Biopsy	Liver/25 mm	^{99m} Tc	-	-	-	-	-
4	m	34	Rectum	PDEC	No	No	Rectum	Surgery	Rectum/25 mm	¹¹¹ In	-	+	-	-	-
5	m	53	Ileum	WDEC	No	No	Ileum	Surgery	Liver/20 mm	¹¹¹ In	+	+	-	+	-
6	m	48	Ileum	WDET	No	No	Ileum	Surgery	Ileum/20 mm	¹¹¹ In	+	+	+	+	+
7	f	55	Stomach	WDEC	No	No	Liver	Biopsy	Liver/20 mm	^{99m} Tc	+	+	-	-	-
8	f	64	Ileum	WDEC	No	No	Ileum	Surgery	Ileum/60 mm	^{99m} Tc	+	+	-	+	-
9	m	51	Pancreas	WDEC	No	No	Liver	Surgery	Liver/40 mm	¹¹¹ In	+	+	-	+	-
10	f	68	Pancreas	WDEC	No	No	Pancreas	Surgery	Lymph node/30 mm	¹¹¹ In	+	+	-	-	-
11	m	73	Pancreas	WDET	No	No	Pancreas	Surgery	Pancreas/14 mm	^{99m} Tc	+	+	NA	+	NA
12	m	49	Stomach	WDEC	No	No	Stomach	Surgery	Stomach/10 mm	¹¹¹ In	-	-	NA	-	NA
13	m	49	Pancreas	WDEC	No	No	Pancreas	Surgery	Pancreas/25 mm	¹¹¹ In	-	+	+	+	+
14	f	17	Pancreas	WDET	Yes	NA	Pancreas	Surgery	Pancreas/12 mm	¹¹¹ In	-	+	+	+	+
15	m	71	Unknown	WDEC	No	No	Liver	Surgery	Liver/45 mm	¹¹¹ In	+	-	-	-	-
16	f	75	Unknown	WDEC	No	No	Liver	Biopsy	Liver/30 mm	^{99m} Tc	+	-	-	-	-
17	m	29	Pancreas	WDEC	Yes	No	Pancreas	Surgery	Pancreas/45 mm	¹¹¹ In	-	+	-	+	-
18	f	47	Pancreas	PDEC	No	No	Liver	Biopsy	Liver/80 mm	^{99m} Tc	+	+	-	+	-
19	f	46	Pancreas	PDEC	No	No	Pancreas	Biopsy	Pancreas/60 mm	¹¹¹ In	+	+	-	+	-
20	f	60	Stomach	Mixed	No	No	Liver	Surgery	Lymph node/100 mm	¹¹¹ In	-	-	-	-	-
21	m	55	Pancreas	WDEC	No	No	Liver	Biopsy	Liver/48 mm	¹¹¹ In	+	+	+	+	+
22	f	57	Colon	PDEC	No	No	Colon	Surgery	Liver/25 mm	¹¹¹ In	+	+	-	+	-
23	f	76	Ileum	WDEC	No	No	Ileum	Surgery	Ileum/50 mm	^{99m} Tc	+	+	+	+	+
24	m	35	Duodenum	WDEC	No	No	Duodenum	Surgery	Duodenum/19 mm	¹¹¹ In	-	-	-	-	NA
25	m	49	Unknown	WDEC	No	No	Liver	Biopsy	Liver/70 mm	¹¹¹ In	+	+	+	+	+
26	f	64	Pancreas	WDEC	No	No	Pancreas	Surgery	Pancreas/10 mm	¹¹¹ In	-	+	+	-	+
27	f	69	Stomach	WDEC	No	No	Stomach	Biopsy	Stomach/10 mm	¹¹¹ In	-	+	-	+	-
28	m	64	Pancreas	WDEC	No	No	Pancreas	Surgery	Pancreas/65 mm	^{99m} Tc	+	+	+	+	+
29	m	71	Colon	WDEC	No	Yes	Liver	Biopsy	Liver/85 mm	¹¹¹ In	+	+	+	+	+
30	m	69	Ileum	WDEC	No	No	Ileum	Surgery	Ileum/40 mm	¹¹¹ In	+	+	-	-	-
31	m	65	Colon	WDEC	No	Yes	Colon	Surgery	Liver/35 mm	¹¹¹ In	+	-	+	-	+
32	m	62	Stomach	WDET	No	No	Stomach	Surgery	Stomach/30 mm	^{99m} Tc	-	+	+	+	+
33	f	66	Unknown	WDEC	No	No	Liver	Biopsy	Liver/70 mm	¹¹¹ In	-	-	-	-	-
34	f	63	Unknown	WDEC	No	No	Liver	Surgery	Liver/36 mm	¹¹¹ In	+	+	NA	+	NA
35	m	55	Stomach	PDEC	No	No	Stomach	Biopsy	Lymph node/65 mm	¹¹¹ In	+	+	+	+	+
36	f	25	Pancreas	WDEC	Yes	Yes	Pancreas	Surgery	Liver/60 mm	¹¹¹ In	+	+	+	+	+
37	f	50	Unknown	WDEC	No	No	Liver	Biopsy	Liver/70 mm	^{99m} Tc	+	+	+	+	+
38	m	78	Duodenum	PDEC	No	No	Duodenum	Surgery	Duodenum/20 mm	^{99m} Tc	-	+	-	+	-
39	f	67	Pancreas	WDET	No	No	Pancreas	Surgery	Pancreas/14 mm	¹¹¹ In	+	+	+	+	+
40	m	67	Unknown	WDEC	No	No	Liver	Biopsy	Lymph node/25 mm	^{99m} Tc	-	-	-	-	-
41	f	62	Rectum	WDEC	No	No	Liver	Surgery	Liver/40 mm	¹¹¹ In	+	+	+	+	+
42	m	50	Ileum	WDEC	No	Yes	Ileum	Surgery	Liver/30 mm	¹¹¹ In	-	-	-	-	-
43	m	59	Pancreas	WDEC	No	No	Pancreas	Biopsy	Pancreas/120 mm	¹¹¹ In	-	-	+	-	+
44	m	40	Pancreas	WDEC	NA	Yes	Pancreas	Surgery	Pancreas/33 mm	¹¹¹ In	+	+	-	+	-

f, female; m, male; WDET, well-differentiated endocrine tumor; WDEC, well-differentiated endocrine carcinoma; PDEC, poorly differentiated endocrine carcinoma; MEN, multiple endocrine neoplasia; CS, carcinoid syndrome; SRS, somatostatin receptor scintigraphy; ¹¹¹In, ¹¹¹In-pentetreotide; ^{99m}Tc, ^{99m}Tc-EDDA/HYNIC-Tyr3-octreotide; SSTR2 and SSTR5, somatostatin receptor subtype 2 and 5, evaluated according to our immunohistochemical scoring system; sstr2 and sstr5, somatostatin receptor subtype 2 and 5, evaluated according to the immunohistochemical scoring system proposed by Volante *et al.*²²; NA, not applicable.

Discussion

The identification of SSTR expression in GEP-NETs has drastically changed the diagnostic and therapeutic management of these tumors, representing the rationale for the clinical application of SST analogs^{5,9,11}. These drugs are the standard treatment of the hypersecreting syndrome commonly observed in GEP-NETs¹¹ and can be used effectively also as antitumor agents, ensuring tumor growth control and better patient outcome.

Moreover, the availability of new active radiolabeled analogs has opened new perspectives for the treatment of these neoplasms¹².

Many techniques have been developed and used to identify SSTR expression, and have led to a reasonable prediction of the utility of diagnostic procedures and the efficacy of the above-mentioned therapeutic agents. At present, SRS is the standard diagnostic procedure utilized for *in vivo* evaluation of SSTR expression. In particular, in patients with GEP-NETs, this procedure

Table 4 - Characteristics of immunohistochemical staining for SSTR2 and SSTR5

Pts	Primary site	Histology	SSTR2			SSTR5		
			% of cells	Intensity	Localization	% of cells	Intensity	Localization
1	Unknown	WDEC	40	3+	m/c	NA	NA	NA
2	Ileum	WDEC	-	-	-	-	-	-
3	Unknown	WDEC	-	-	-	-	-	-
4	Rectum	PDEC	70	1+	c	-	-	-
5	Ileum	WDEC	50	1+	m/c	-	-	-
6	Ileum	WDET	40	3+	m/c	10	3+	m/c
7	Stomach	WDEC	80	2+	c	-	-	-
8	Ileum	WDEC	80	1+	m/c	-	-	-
9	Pancreas	WDEC	100	3+	m/c	-	-	-
10	Pancreas	WDEC	90	1+	c	-	-	-
11	Pancreas	WDET	40	3+	m/c	NA	NA	NA
12	Stomach	WDEC	-	-	-	NA	NA	NA
13	Pancreas	WDEC	100	3+	m/c	10	2+	m/c
14	Pancreas	WDET	100	1+	m/c	100	2+	m
15	Unknown	WDEC	-	-	-	-	-	-
16	Unknown	WDEC	-	-	-	-	-	-
17	Pancreas	WDEC	100	3+	m/c	-	-	-
18	Pancreas	PDEC	60	2+	m	-	-	-
19	Pancreas	PDEC	100	3+	m/c	-	-	-
20	Stomach	Mixed	-	-	-	-	-	-
21	Pancreas	WDEC	100	3+	m	100	3+	m/c
22	Colon	PDEC	10	3+	m/c	-	-	-
23	Ileum	WDEC	10	1+	m	30	3+	m/c
24	Duodenum	WDEC	-	-	-	NA	NA	NA
25	Unknown	WDEC	100	3+	m	100	3+	m/c
26	Pancreas	WDEC	30	1+	c	30	1+	m/c
27	Stomach	WDEC	80	3+	m/c	-	-	-
28	Pancreas	WDEC	100	3+	m	100	3+	m/c
29	Colon	WDEC	100	3+	m/c	100	3+	m/c
30	Ileum	WDEC	100	1+	c	-	-	-
31	Colon	WDEC	-	-	-	100	3+	m
32	Stomach	WDET	100	3+	m/c	10	3+	m/c
33	Unknown	WDEC	-	-	-	-	-	-
34	Unknown	WDEC	100	3+	m/c	NA	NA	NA
35	Stomach	PDEC	100	3+	m	100	2+	m/c
36	Pancreas	WDEC	100	3+	m/c	10	3+	m
37	Unknown	WDEC	100	3+	m	100	3+	c
38	Duodenum	PDEC	30	2+	m/c	-	-	-
39	Pancreas	WDET	100	3+	m/c	100	3+	m/c
40	Unknown	WDEC	-	-	-	-	-	-
41	Rectum	WDEC	100	2+	m/c	50	2+	m
42	Ileum	WDEC	-	-	-	-	-	-
43	Pancreas	WDEC	-	-	-	100	3+	m/c
44	Pancreas	WDEC	70	3+	m/c	-	-	-

WDET, well-differentiated endocrine tumor; WDEC, well-differentiated endocrine carcinoma; PDEC, poorly differentiated endocrine carcinoma; m, membrane; c, cytoplasm; NA, not applicable.

has the double aim of disease staging and selection of patients suitable for treatment with SST analogs^{9,16}.

Unfortunately, several factors can invalidate the diagnostic accuracy of SRS^{15,16}. Moreover, SRS does not allow the identification of the specific tumor SSTR profile and can give confounding results through the visualization of cellular binding sites not properly belonging to the tumor population.

IHC was demonstrated to be a reliable, highly effective and affordable method of assessing the specific SSTR expression pattern^{4,7,17,18,24-27}. However, concerns remain about the routine use of this procedure in clinical practice, in association with or possibly in substitu-

tion for SRS. These concerns are mostly related to the methods used, with variable reliability of the polyclonal antibodies employed, and to difficulties in interpreting the information acquired, partly because of the lack of a standard evaluating system and the scarcity of available clinical data.

In the present study we performed a retrospective comparative evaluation of SSTR expression, detected by both IHC and SRS, in patients with GEP-NETs. Our study showed a high concordance rate between IHC and SRS, irrespective of the IHC scoring system applied (73% and 70%). Even if our analysis was restricted to SSTR2 and SSTR5 and did not include SSTR3 detection, the results

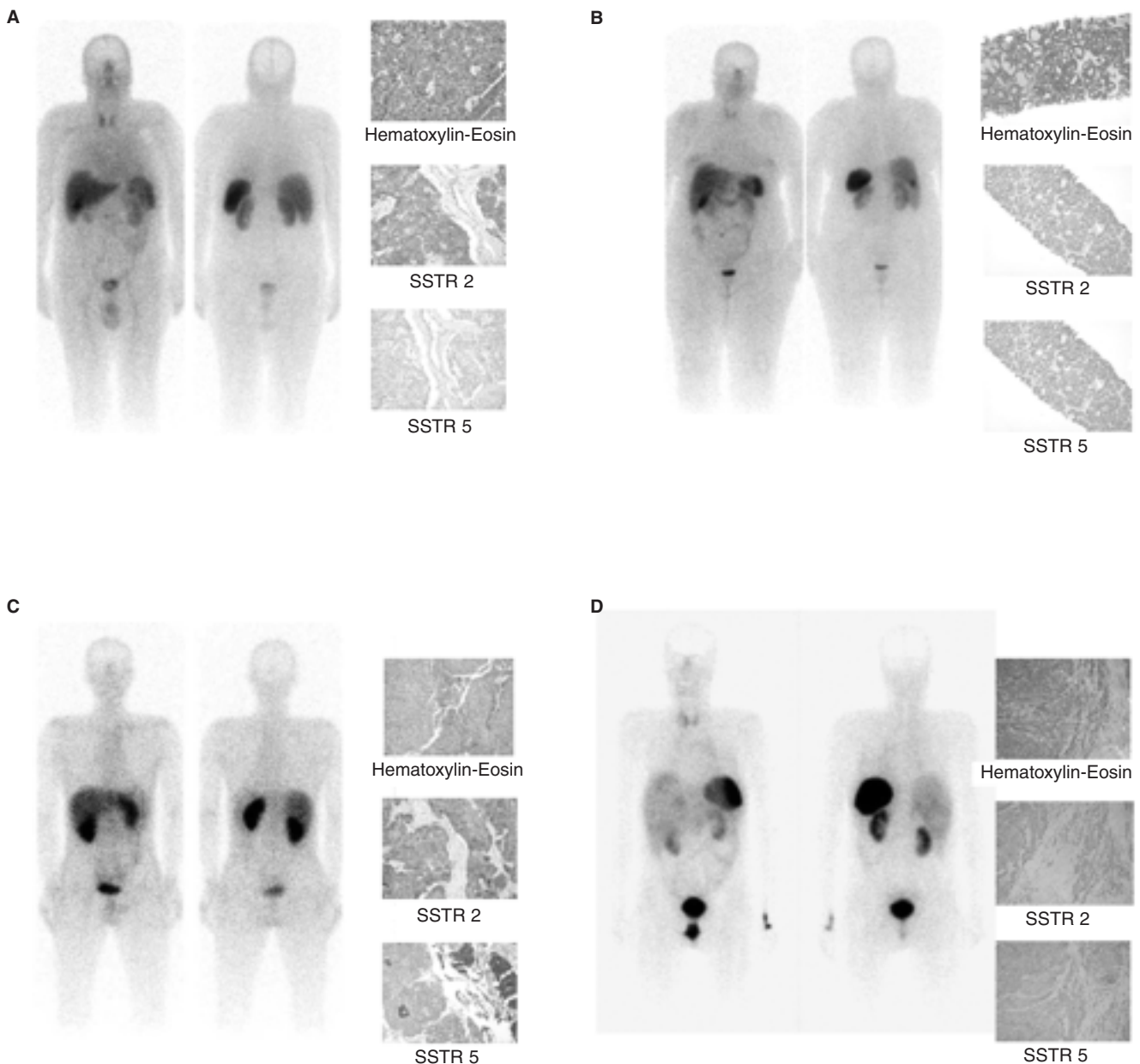


Figure 1 - Correlation between SRS and IHC for SSTR2 and SSTR5. A) (patient No. 11): SSTR2 expression at IHC and positive SRS in a well-differentiated endocrine carcinoma of the pancreas. B) (patient No. 16): Absence of SSTR expression and positive SRS in a well-differentiated endocrine carcinoma from an unknown primary site. C) (patient No. 17): SSTR2 expression at IHC and negative SRS in a well-differentiated endocrine carcinoma of the pancreas. D) (patient No. 40): Absence of SSTR expression and negative SRS in a well-differentiated endocrine carcinoma from an unknown primary site.

were consistent with the concordance rate reported in the study by Volante *et al.*²² On the other hand, when the 2 IHC scoring systems were compared directly, the application in our series of the criteria proposed by Volante *et al.* resulted in a reduced number of positively scored cases (30 *vs* 34), consistent with the more restrictive criteria observed. This circumstance, however, did not significantly change the specificity of IHC and generated only a slight trend towards a statistically significant difference in sensitivity in favor of our scoring system.

Despite the above-reported high concordance rate, a non-negligible number of discrepancies were observed. It is remarkable that, using our IHC interpreting system, up to 8 of 16 tumors (50%) not visualized by SRS were found to express SSTR2, the receptor subtype which mediates the biological effects of SST analogs and for whose detection most reliable polyclonal antibodies are actually available. In order to identify the causes of the apparent failure of SRS (false positivity of IHC according to the statistical design of the study), we reviewed the

data of these patients. None of the above-mentioned limiting factors that modify SRS sensitivity were clearly found to justify all the observed discrepancies between SRS and IHC. Rather, a wide variability in terms of tumor sites, histological types, and lesion diameters was demonstrated. In only 3 of 9 cases (one of these also characterized by a low grade of IHC SSTR expression), the potentially detectable tumor was near the spatial resolution limit of SRS. Two more cases of SRS failure could be caused by the weak intensity of the IHC staining both in terms of staining intensity and number of immunoreactive cells. We do not have any explanation for the remaining 4 patients, in whom the tumor bulk ranged from 20 to 120 mm and strong and homogeneous immunoreactivity for SSTR2 and/or SSTR5 was documented. Besides, given the comparable accuracies reported in the literature for ^{111}In -pentetretotide and $^{99\text{m}}\text{Tc}$ -EDDA/HYNIC-Tyr3-octreotide, we assumed the results of our study were not biased by the use of 2 similar radiopharmaceuticals. In fact, we were only looking at the presence or absence of radiopharmaceutical uptake in a determined lesion, without considering the overall lesion detection rate. Since we studied a limited number of patients, we cannot draw any conclusions from the fact that 7 out of 9 tumors were missed by ^{111}In -pentetretotide.

As previously reported, the specificity of IHC did not appear to be influenced by the scoring system applied. Only 1 of these 8 IHC+/SRS- tumors was considered SSTR negative when the analysis was performed according to the scoring system proposed by Volante *et al.* (specifically, case 4 was scored as negative due to exclusive cytoplasmic localization of SSTR2 expression), even if in 2 tumors the IHC positivity was attributable to SSTR5 expression only.

IHC also showed some limits in our study. Three of 10 tumors not expressing SSTRs with IHC according to our IHC evaluation method were positive on SRS. We hypothesized that this unexpected result could be related to the intrinsic heterogeneity of the tumors' SSTR expression²⁸ and to the expression of SSTR3, a receptor subtype with a moderate binding affinity for radiolabeled octreotide¹³, which was not assessed by IHC in the present study. In any case, adding detection of SSTR3 expression to the IHC analysis did not result in a higher concordance rate between SRS and IHC.

The number of false negative IHC results (6 IHC-/SRS+ cases) was even larger when SSTR expression was reassessed according to the more restrictive criteria by Volante *et al.*²² This was not particularly surprising if we consider the interesting – and not consistent with previous reports – unusually high proportion of tumors showing cytoplasmic immunostaining (25/32 cases for SSTR2 and 13/17 cases for SSTR5) correlated with the presence of an internalized receptor.

The possibility of taking IHC analysis as a surrogate for SSTR expression, traditionally detected by SRS,

could optimize the use of SRS in the clinical management of GEP-NETs by limiting its diagnostic and therapeutic applications. In a hypothetical diagnostic algorithm we could reserve the use of SRS to patients positive for SSTRs on IHC, who were suitable for surgery or locoregional treatment, in whom the identification of distant metastasis or the primary tumor could reasonably modify the therapeutic management. Besides, the assessment of SSTRs by IHC at diagnosis could offer an immediate answer about the suitability of SST-analog-based treatment.

This is the first study evaluating the reliability of IHC, in comparison with SRS, in the detection of SSTR expression in a series of GEP-NETs, and comparing 2 different IHC scoring systems.

Although our data showed that SRS failed to detect SSTRs in up to 20% of GEP-NET patients (up to 50% of SRS-negative patients), which would suggest that these patients, on the basis of the SRS result, might be excluded from useful treatment with SST analogs²⁹, more exhaustive clinical data are needed to confirm the clinical suitability of SSTR detection by IHC. IHC can in fact only identify the presence of the receptor, without offering any information about its functional status and ability to mediate the biological effects of SST analogs. Moreover, the variable identification of subcellular SSTR distribution raises concerns about the clinical usefulness of cytoplasmic immunostaining. Whether this aspect reflects tumor differentiation or correlates with actual binding of SST analogs and subsequently with any beneficial clinical response to such agents is an interesting question to be addressed according to complete pathological and clinical data. For the same reasons it is not possible to support the use of a particular IHC scoring system until this is legitimated and validated by the strength of the clinical effectiveness or ineffectiveness of a SST-analog-based treatment.

Finally, any proposal for the routine application of IHC analysis instead of SRS cannot be supported given the non-negligible number of false negative cases reported, irrespective of the IHC scoring system used and probably due to heterogeneous intratumoral SSTR expression. Until the clinical reliability and reproducibility of the IHC results is clearly demonstrated in a prospective trial, we think that SRS should remain the gold standard in the diagnostic workup and therapeutic decisional process of GEP-NETs. This is particularly true if we consider the recent demonstration of the superior sensitivity of gallium-68-labeled peptides used with PET-CT in comparison with SRS. Nevertheless, whenever SRS is not available or potentially weakened by technical and/or biological variables, IHC analysis could represent a reasonable alternative to guide clinicians in the decision of the appropriate treatment strategy.

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