Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy

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Typing somatostatin receptor expression in neuroendocrine tumors is of relevance to target somatostatin analogue-based diagnostic approach and treatment. The expanding use of immunohistochemistry to detect somatostatin receptors is to date not paralleled by an accurate methodological setting and standardized interpretation of the results. A multicentric study was designed to compare somatostatin receptor immunohistochemical expression with *in vivo* scintigraphic data and verify its usefulness in the clinical management of neuroendocrine tumors. After methodological setting by testing different somatostatin receptor antibodies, 107 cases of neuroendocrine tumors with available somatostatin receptor scintigraphy data and pathological material were retrospectively analyzed for somatostatin receptor types 2A, 3 and 5 immunohis-tochemical expression, and compared with scintigraphic images and, whenever available, with the clinical response to somatostatin analogue treatment. Restricting 'positive cases' to the presence of a membrane pattern of staining, an overall somatostatin receptor type 2A immunohistochemistry/somatostatin receptor scintigraphy agreement of 77% (χ^2 test $P < 0.0001$) was reached. Lower concordance ratios were detected in preoperative and metastatic tumor samples, possibly as a consequence of somatostatin receptor expression heterogeneity. Pure somatostatin receptor type 2A cytoplasmic staining showed poor correlation with somatostatin receptor scintigraphy (54% concordance rate). The immunohistochemical detection of somatostatin receptor types 3 and 5, which showed almost exclusively a cytoplasmic pattern, did not improve the concordance with scintigraphic data. In a pilot series, somatostatin receptor type 2A immunohistochemistry correlated with clinical response in 75% of cases. In conclusion, we propose a scoring system for somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors correlated with *in vivo* data, based on the evidence that only membrane (rather that cytoplasmic) staining should be considered for a reliable, standardized and clinically relevant report.
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Neuroendocrine tumors belong to a spectrum of lesions with variable degree of differentiation and have various locations and various disease stages: carcinoids (benign and malignant or typical and atypical) lie in the well differentiated extreme of the spectrum, while small and large cell neuroendo-crine carcinomas represent the poorly differentiated variants.[1,2]

There are several therapeutic options for neuroendocrine tumors. Apart from surgery, chemotherapy and biotherapy are generally the first line treatments. The latter includes in both functioning and non-functioning neuroendocrine tumors somatostatin analogue administration, both in medical treatment and as radio-labeled agents, and interferon.[3–5]

Somatostatin is an acidic polypeptide that is widely distributed throughout the central nervous system and different peripheral tissues and organs. Several biological functions related to somatostatin have been described, and include potent inhibition of basal and stimulated secretion from a wide variety of endocrine and exocrine cells, neuromodulatory actions in the central nervous system[6–9] as well as regulatory properties on cell proliferation and differentiation.[10] The physiological actions of somatostatin are initiated by its interaction with a family of specific membrane-bound receptors, the somatostatin receptors family, which encompass to date five different subtypes, named somatostatin receptors types 1–5, displaying a remarkable degree of structural conservation across species (40–60% structural homologies), but mediating different biological actions of somatostatin, via the activation of different intracellular signaling pathways.[11,12]

The rationale for somatostatin analogue (eg octreotide) treatment is the presence of somatostatin receptors on the surface of tumor cells. These bind octreotide with different degrees of affinity, being somatostatin receptors types 2, 3 and 5 those mostly involved in octreotide binding.

In the clinical practice, the presence of functional somatostatin receptors is documented by somatostatin receptor scintigraphy by ^{111}In-pentetreotide: if positive, a binding of the radioligand with one or more of the somatostatin receptors is demonstrated. Unfortunately, this technique does not identify the receptor type and—most importantly—does not determine the cell population containing the binding sites (tumor cells vs other intratumoral cell types). Various methods to detect somatostatin receptors in tumor specimens were reported, including autoradiography, RT-PCR, in situ hybridization, immunoblotting and immunohistochemistry. Several studies have documented that generally these methods correlate each other in somatostatin receptors positive cases, with only minor discrepancies.[13–22]

The currently available somatostatin analogues bind preferentially somatostatin receptors type 2 (which is the most widely expressed subtype in neuroendocrine tumors) and to a lower extent types 3 and 5, and somatostatin receptors profiling in individual patients may be of relevance to better tailor the somatostatin analogue-based treatment. This would hopefully allow to increase the response rate of each patient and reduce costs of biotherapy, which are generally high.

Immunohistochemistry is a cheap, reproducible, and easily accessible procedure that can be performed in most pathology laboratories on fresh and also archival tissue samples. Commercially available antibodies specific for the different receptor subtypes are currently available, but they need to be validated in the clinical practice. No data exist in fact on the quantification and interpretation of somatostatin receptor immunohistochemical findings, although scoring systems have been developed and applied for many years in the analysis of several other receptors (eg estrogen receptor, c-erb-b2, etc).

Aim of the present study is therefore to define the somatostatin receptors immunohistochemical profile in a large series of neuroendocrine tumors of which surgical and/or biopsy material were available, and compare the results with the in vivo data on ^{111}In-pentetreotide scintigraphy and response to somatostatin analogue treatment.

Materials and methods

Case Selection

A series of 107 neuroendocrine tumors (from years 1998 to 2006) treated in three different highly specialized Italian centers of the Universities of Turin, Naples and Varese was selected according to the following criteria: (i) revised histopathological diagnosis; (ii) tissue material available for pathological review and immunohistochemistry; (iii) ^{111}In-pentetreotide scintigraphy data available.

For most cases, medical treatment administered, response to treatment (as progressive disease, partial remission or stable disease) and follow-up data were also accessible.

Somatostatin receptor scintigraphy was performed preoperatively in all but seven patients, who had post-surgical somatostatin receptor scintigraphy in the presence of residual disease. [111]Indium-DTPA[0]-octreotide (135 MBq) was administered immediately after checking the specific radiochemical purity by chromatography, which was always higher than 95%. The scintigraphic procedure included 4- and 24-h planar anterior and posterior images (matrix size 128×128) of the chest, upper and lower abdomen and anterior views of the head and proximal lower legs. At least 500 Kilocounts were recorded over the chest and abdomen and 50–200 Kilocounts were obtained for the head and neck and lower leg views. SPECF of the abdomen was performed 24 h after injection. Sixty-four 40-s views (matrix size: 64×64) were acquired through a 360° arc. Planar and SPECF images were acquired using a large field of view γ camera equipped with a medium-energy, general-purpose collimator. Somatostatin receptor scintigraphy images were scored in four groups according to Kwekkeboom et al.[23]

The tissue material corresponded to 41 preoperative samples (fine-needle aspiration or core biopsies) and 66 surgical samples. All surgical samples were fixed in buffered formalin; preoperative sam-

ples were both fixed in formalin or in alcohol. All cases have been reviewed and diagnosed according to years 2000 and 2004 WHO classifications of Endocrine Tumors.[1,2] They included 70 cases of well-differentiated neuroendocrine tumors (benign carcinoids) or carcinomas (malignant/atypical carcinoids) (63 cases from lung, pancreas and gastrointestinal tract, and 7 cases of liver or lymph node metastases in the absence of evident primary disease), 18 cases of poorly differentiated neuroendocrine carcinomas (mainly from lung), 9 cases of medullary thyroid carcinomas and 7 additional cases of neuroendocrine tumors from skin, paraganglia, thymus and pituitary, as well as 3 cases of adenocarcinomas with neuroendocrine differentiation of the breast and prostate. In 31 cases, tissue material was obtained from metastatic deposits, and in 3 of these cases primary and metastatic lesions from the same patient could be compared. The study was approved by the Local Ethic Committee.

Immunohistochemistry

Serial 5-μm thick paraffin sections were collected onto charged slides and processed by immunohistochemistry in a single Institution (Orbassano, Turin, Italy). In a pilot series of 20 neuroendocrine tumors, as well as in control sections of normal pancreatic tissue, alternative polyclonal somatostatin receptor type 2A antibodies (BioTrend, Cologne, Germany, code SS-800; Santa Cruz Biotechnology, Santa Cruz, CA, USA, code sc-25676; LifeSpan Biosciences, Seattle WA, USA, code LS-A998) were tested. Although all three somatostatin receptor type 2A antibodies were comparable in the majority of normal and neoplastic specimens in terms of staining intensity, in our laboratory protocols BioTrend antibody appeared to be more sensitive as compared to the others, in terms of both presence of positive internal reference controls (ie blood vessels) and demonstration of a membranous staining pattern in normal and neoplastic cell populations. It was therefore selected to be applied to the complete series of cases and for comparison with in vivo data. Pre-absorption experiments, performed in control sections by pre-incubation of the primary antibody with a 10- to 50-fold excess of blocking peptide (BioTrend, code SS-801), could confirm the specificity of such antibody. Negative control experiments by somatostatin receptor type 2A pre-absorption were therefore performed in each immunohistochemical run. In addition, polyclonal antibodies to somatostatin receptor types 3 and 5 from three different sources (BioTrend, codes SS-830 and SS-838, LifeSpan, codes LS-A2622 and LS-A2639, and Abcam, Cambridge, UK, codes ab28680 and ab28618, for subtypes 3 and 5, respectively) were also tested in the pilot series of 20 cases of neuroendocrine tumors. According to the preliminary immunohistochemical findings, Abcam antibo-

dies demonstrated the most intense and reliable results and were therefore employed on the whole case series. Moreover, pre-absorption experiments for BioTrend somatostatin receptor types 3 and 5 antibodies, using the same conditions as for somatostatin receptor type 2A (blocking peptides from BioTrend, codes SS-831 and SS-839-A, respectively) failed to confirm the specificity of these latter primary antibodies, even applying different batches.

For somatostatin receptor immunohistochemistry the following conditions were applied: antigen retrieval was performed in pH 6.0 citrate buffer in a microwave oven (three cycles 5 min each at 750 W) and the primary antibodies were incubated for 4 h at 37°C. Primary antibodies dilutions were 1/3000, 1/1000 and 1/500 for somatostatin receptor types 2A, 3 and 5. Immunoreactions were revealed by a biotin-free dextran-chain detection system (Envision, DakoCytomation, Glostrup, Denmark), and developed using diaminobenzidine as the chromogen. The specificity was also validated in parallel negative control sections by omitting the primary antibodies for each immunohistochemical run.

All somatostatin receptor immunohistochemical findings were analyzed independently by two observers (MV and IR) in one center (Orbassano, Turin, Italy).

In 14 selected cases, somatostatin receptor type 2A immunohistochemistry was repeated in a different laboratory (Varese) following the same working conditions, and the results checked to test inter-laboratory agreement.

Somatostatin Receptor Immunohistochemistry Scoring System

For somatostatin receptor type 2A a semiquantitative scoring system was designed, taking into consideration both the subcellular localization and the extent of the staining, as follows: score 0: absence of immunoreactivity; score 1: pure cytoplasmic immunoreactivity, either focal or diffuse; score 2: membranous reactivity in less than 50% of tumor cells, irrespective of the presence of cytoplasmic staining; score 3: circumferential membranous reactivity in more than 50% of tumor cells, irrespective of the presence of cytoplasmic staining.

For somatostatin receptor types 3 and 5, a cytoplasmic pattern of staining only was observed, and cases were scored as positive in the presence of at least 10% of positive tumor cells.

Response to Treatment

Tumor responses was classified according to the RECIST criteria.[24] Complete response was defined as the absence of radiologically documented lesions. A partial response was defined as at least a 30% decrease in the sum of the longitudinal diameter of target lesions. Progressive disease was defined as at

least a 20% increase in the sum of the longitudinal diameter of target lesions or the appearance of one or more new lesions. Stable disease was defined as neither a sufficient shrinkage to qualify for partial response, nor sufficient increase to qualify for progression disease.

Statistical Analysis

Immunohistochemical results were compared to somatostatin receptor scintigraphy status and available clinico-pathological parameters by χ^2 test using 1 degree of freedom (d.f. = 1), and a level of statistical significance of $P = 0.05$. For statistical comparison, somatostatin receptor type 2A immunohistochemical scores 0 and 1 were considered negative, as opposed to scores 2 and 3 (see also below).

Results

Somatostatin Receptors Distribution in Neuroendocrine Tumors

Immunohistochemistry
Somatostatin receptor types 2A, 3 and 5 antibodies showed the specific signal in internal reference controls (ie blood vessels, pancreatic islets) or in control sections of both formalin- and alcohol-fixed tissue samples. In the case of somatostatin receptor type 2A, pre-absorption experiments abolished the
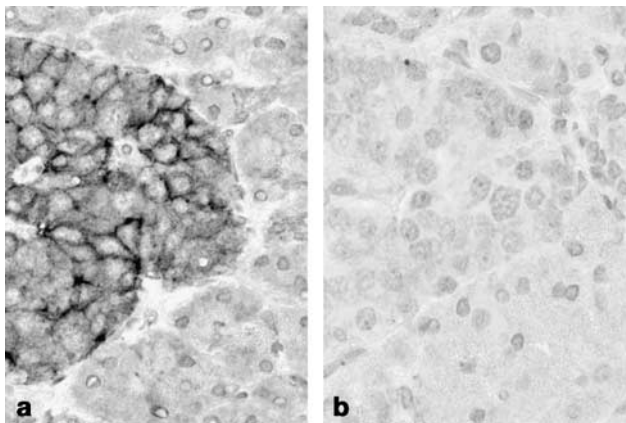

Figure 1 Intense membranous pattern of staining with somatostatin receptor type 2A antibody (BioTrend, code SS-800) in a pancreatic islet (**a**), completely abolished by pre-incubation of the primary antibody with a 50-fold excess of blocking peptide (BioTrend, code SS-801) (**b**).

immunoreactivity (both membranous and cytoplasmic patterns) and confirmed the specificity of the reaction (Figure 1). Grouping somatostatin receptor type 2A immunohistochemical scores 2 and 3 together as positive (see also below), somatostatin receptor type 2A was detected in 79% of well-differentiated neuroendocrine tumors/carcinomas and in 44% of poorly differentiated neuroendocrine carcinomas (Table 1). In somatostatin receptor type 2A score 1 cases, the cytoplasmic reactivity was generally weak to moderate; comparing scores 2 and 3, apart from the percentage of positive tumor cells which determined the inclusion in one or the other group, a slightly weaker and often incomplete membrane staining was observed in score 2 cases, whereas a strong and circumferential membranous pattern was characteristic of score 3 cases (Figures 2 and 3). Inter-observer cross validation between the two independent investigators (MV and IR) was up to 98%; for discrepant cases agreement was reached by reviewing the slides at a multihead microscope. Inter-laboratory cross validation (between the slides stained in Orbassano and in Varese) showed 100% scoring agreement in all 14 cases tested.

Somatostatin receptor types 3 and 5 showed a cytoplasmic pattern only, with very occasional membrane reinforcement. They were positive in 44 and 71% of well-differentiated neuroendocrine tumors/carcinomas and in 17 and 28% of poorly differentiated neuroendocrine carcinomas, respectively for types 3 and 5 (Table 1).

Somatostatin receptor scintigraphy
In vivo somatostatin receptor analysis by ^{111}In-pentetreotide detected positive binding (including scores 2–4 according to Kwekkeboom et al[23] in 78/107 cases (73%), with a decreasing percentage of positive cases comparing well-differentiated lesions (79%), poorly differentiated neuroendocrine carcinomas (44%) and the group miscellaneous neuroendocrine tumors (21%).

Correlation of Immunohistochemistry with In Vivo Data

When checking the correlation of somatostatin receptor type 2A antibody reactivity with in vivo data among the different immunohistochemical groups, scores 2 and 3 showed a high concordance with somatostatin receptor scintigraphy, being correlated to scintigraphic positivity in 87 and 94% of

Table 1 Somatostatin receptors expression in 107 neuroendocrine tumors

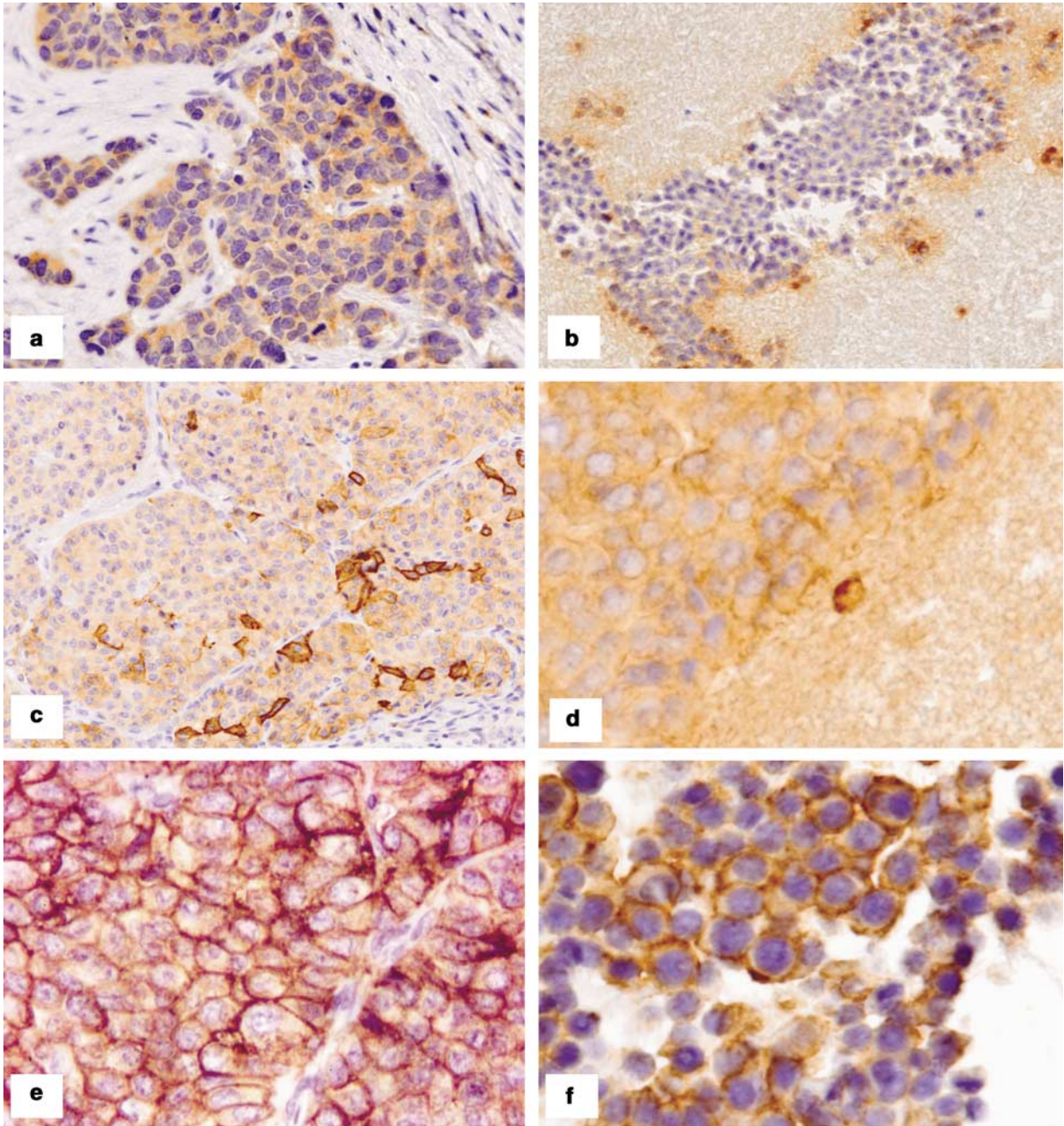|	Somatostatin receptor type 2A (scores 2 and 3)	Somatostatin receptor type 3	Somatostatin receptor type 5
WD NET/NEC (70 cases)	79%	44%	71%
PD NEC (18 cases)	44%	17%	28%
Others (19 cases)	21%	53%	74%

Figure 2 Representative illustration of somatostatin receptor type 2A immunohistochemical scores in surgical (left-sided pictures; (**a**) lymph node metastasis of medullary thyroid carcinoma; (**c**) atypical carcinoid of the lung, (**e**) well-differentiated neuroendocrine carcinoma of the pancreas, not functioning) and preoperative cytological (right-sided pictures; (**b**) liver metastasis of well-differentiated neuroendocrine carcinoma of the pancreas; (**d**) lymph node metastasis of well-differentiated neuroendocrine carcinoma, primary unknown; (**f**) liver metastasis of well-differentiated neuroendocrine carcinoma of the small ileum) samples. The lesions represented included: (**a**, **b**) Score 1 (**a** and **b**) included pure cytoplasmic reactivity, either moderate/strong (**a**) or weak (**b**). Score 2 (**c** and **d**) was considered in the presence of a membranous pattern of staining in less than 50% of tumors cells, either in scattered cells with complete membrane outlining (**c**) or with partial membrane staining in most tumor cells (**d**). Score 3 was clearly recognizable by a membranous, usually intense, staining in more than 50% of tumors cells (**e** and **f**).

cases, respectively, and were grouped together for further statistical comparison. By contrast, immunohistochemical scores 0 and 1 showed a poor correlation with somatostatin receptor scintigraphy results, and therefore were grouped together as negative for further statistical analysis (Figure 3).

An overall somatostatin receptor type 2A immunohistochemistry/somatostatin receptor scintigraphy agreement of 77% (82/107 cases, χ^2 $P < 0.0001$) was reached (Table 2). Among the 82 concordant cases, 61 were both methods positive (Figure 4a–c) and 21 were both methods negative. Consistent
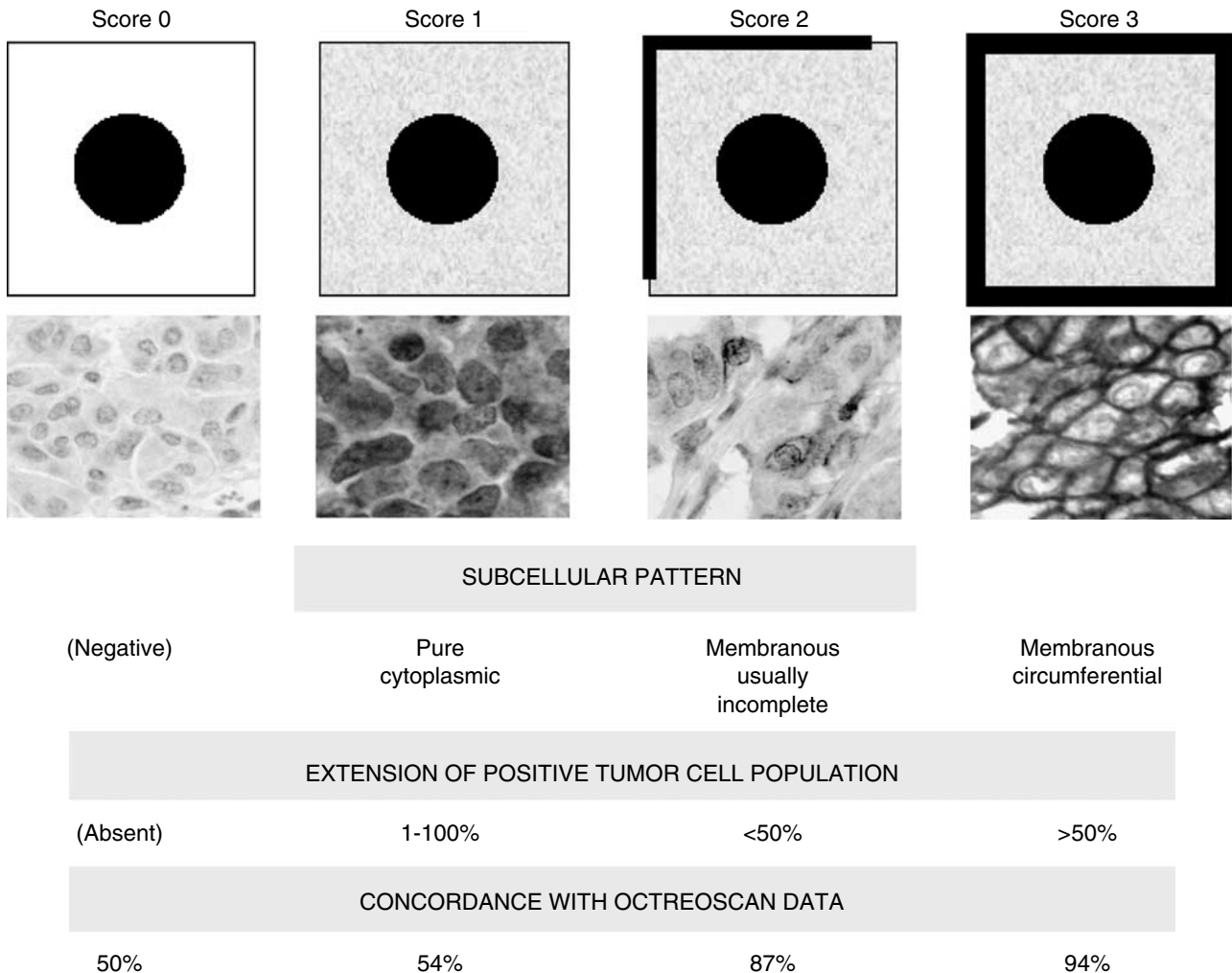
Figure 3 Schematic illustration of somatostatin receptor type 2A immunohistochemical scoring system and its concordance rate with ^{111}In-pentetreotide *in vivo* imaging.

concordance rates were observed in the group of well-differentiated (79%, χ^2 $P = 0.05$) and poorly differentiated (94%, χ^2 $P < 0.0001$) neoplasms. Conversely, considering individual tumor locations, a low concordance rate was observed in the case of medullary thyroid carcinomas and occult primary neuroendocrine carcinomas (67 and 54% immunohistochemistry/somatostatin receptor scintigraphy agreement, respectively). The latter group showed mainly an immunohistochemistry negative/somatostatin receptor scintigraphy positive profile (Figure 4d–f).

A rather poor correlation was observed in the group of preoperative biopsy samples (73% immunohistochemistry/somatostatin receptor scintigraphy agreement, χ^2 $P = 0.09$) and in patients from whom metastatic tumor samples were available (65% immunohistochemistry/somatostatin receptor scintigraphy agreement, χ^2 $P = 0.3$).

When correlating scintigraphic data with all three somatostatin receptor antibodies, the overall agreement in the presence of at least one positive somatostatin receptor type did not change (82/107 cases, 77%), whereas an increase of immunohistochemistry positive/scintigraphy negative cases was observed (with a loss of 'specificity' if considering ^{111}In-pentetreotide scintigraphy the reference method; Table 3). In 28 patients, all bearing well-differentiated neuroendocrine carcinomas from pancreas and gastrointestinal tract or unknown origin, homogeneous somatostatin analogue treatment was administered until progression, consisting in intramuscular injection of the long-acting release octreotide (octreotide L-A-R) at a dose of 20 mg every 28 days. Immunohistochemical somatostatin receptor type 2A status correlated to response to treatment in 75% of cases (21/28); among these 21 concordant cases, 16 patients had a disease stabilization and 2 cases had a partial response to treatment in the presence of somatostatin receptor type 2A immunoreactivity, whereas the 3 remaining cases, negative to somatostatin receptor type 2A immuno-

Table 2 Concordance between immunohistochemistry and ^{111}In-pentetreotide scintigraphy in detecting the presence of somatostatin receptor type 2A in neuroendocrine tumors

Total (107)	Location	Concordant group			Discordant group		
		No. (%) 82 (77)	IHC/srs+ 61	IHC/srs− 21	No. (%) 25 (23)	IHC+/srs− 6	IHC−/srs+ 19
WD	Lung (17)	**13 (*76*)**	11	2	**4 (*24*)**	1	3
NET/NEC	Pancreas (28)	**24 (*86*)**	23	1	**4 (*14*)**	2	2
(70)	GI tract[a] (18)	**16 (*89*)**	15	1	**2 (*11*)**	2	—
	Primary unknown (7)	**2 (*29*)**	1	1	**5 (*71*)**	—	5
PD NEC	Lung (13)	**12 (*92*)**	5	7	**1 (*8*)**	1	—
(18)	GI tract (1)	**1**	—	1	—	—	—
	Primary unknown (4)	**4 (*100*)**	2	2	—	—	—
Others	Thyroid MTC (9)	**6 (*67*)**	1	5	**3 (*33*)**	—	3
(19)	Merkel cell ca. (3)	**2**	2	—	**1**	—	1
	Paraganglioma/Pheo (2)	**1**	1	—	**1**	—	1
	Thymic carcinoid (1)	—	—	—	**1**	—	1
	ADCA with NE diff (3)	**1**	—	1	**2**	—	2
	Pituitary (1)	—	—	—	**1**	—	1
Preoperative samples (41)		**30 (*73*)**	25	5	**11 (*27*)**	3	8
Metastatic samples (31)		**20 (*65*)**	17	3	**11 (*35*)**	1	10

ADCA, adenocarcinoma; GI, gastrointestinal; IHC, immunohistochemistry; MTC, medullary thyroid carcinoma; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; PD, poorly differentiated; srs, somatostatin receptor scintigraphy; WD, well differentiated.
Bold values represent the total.
Italic values represent the percentage.
[a]Including one case of primary WD NEC of the liver and one of the gallbladder.

histochemistry, had disease progression. By contrast, all seven discordant cases had disease progression in spite of somatostatin receptor type 2 protein immunohistochemical expression.

Discussion

The wide expression of somatostatin receptors in neuroendocrine tumors has been largely investigated and led nowadays to the development of clinically relevant diagnostic and therapeutic strategies.[25] However, which methodology could better determine the somatostatin receptor status of a given tumor and could predict its possible visualization by somatostatin receptor scintigraphy and response to somatostatin-analogue therapy is far from being settled in the clinical practice. At present for the clinicians, somatostatin receptor scintigraphy remains the golden standard method to detect somatostatin receptors. In fact, octreotide scintigraphy provides information on the whole tumor mass including metastases and its signal intensity is used for radiotherapeutic decisions.[23] Among alternative methods, immunohistochemistry seems to be a reliable tool to detect the somatostatin receptor profile in neuroendocrine tumors, due to the following advantages: detection of the receptor protein (instead of RNA, for example in PCR-based methods), possibility of detecting the cellular type expressing the receptor (neoplastic cells vs blood vessels or reactive lymphocytes, etc), availability of subtype specific antibodies, applicability in archival material, low cost/benefit ratio which renders this

method applicable in most laboratories.[13,15–17,19,21] On the contrary, major disadvantages are related to the lack of standardization of the method (from both technical and interpretation viewpoints) which is a great limitation in diagnostic applications, the failure of demonstrating 'functional' receptors (as opposed to autoradiography), and undetermined sensitivity of the technique, since limited evidence has been reported on the correlation between immunohistochemistry and other in vivo techniques. In a recent paper, Korner et al[15] tested different somatostatin receptor type 2A antibodies in different human tumors, and correlated the immunohistochemical pattern with previous autoradiographic data on 37 cases, demonstrating a good correlation between the two methods applying the same antibody selected in our study. Previous reports testing somatostatin receptor immunohistochemistry in lung[18] and gastrointestinal[16] neuroendocrine tumors and in pheochromocytomas[17] in correlation to somatostatin receptor scintigraphy are limited by the relative low number of cases compared and by a general lack of standardization of the immunohistochemical interpretation (ie membranous vs cytoplasmic pattern). Therefore, we designed the present study on a large multicentric series of neuroendocrine tumors to validate the reproducibility of the immunohistochemical method and to compare the results with somatostatin receptor scintigraphy imaging.

As a first aim, a practical immunohistochemical scoring system for the most common somatostatin receptor subtype—namely type 2A—based on the percentage of positive tumor cells and the pattern of
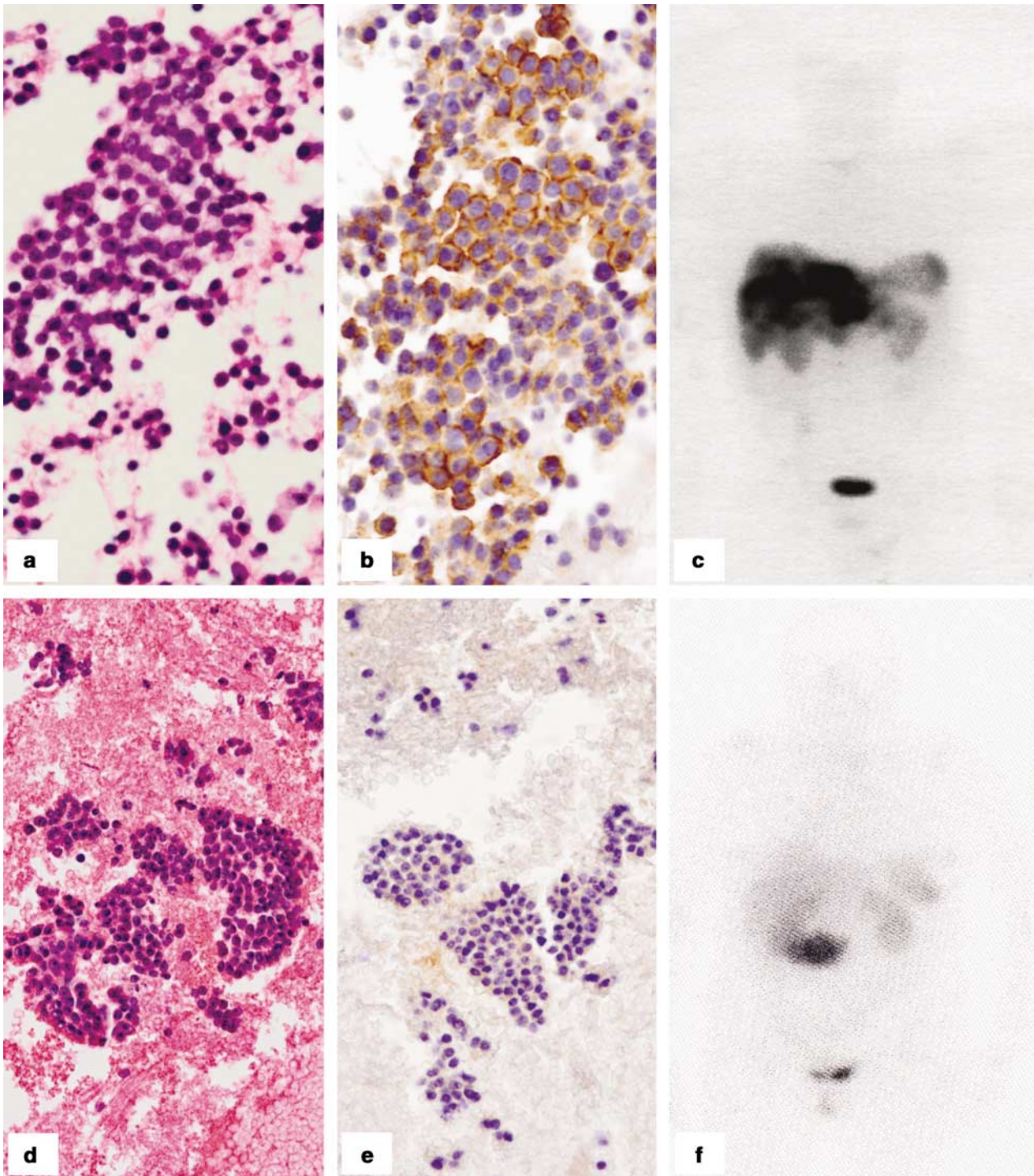
Figure 4 Fine-needle aspiration biopsy of a liver metastasis from a well-differentiated pancreatic neuroendocrine carcinoma (**a**) strongly expressing somatostatin receptor type 2A by immunohistochemistry (**b**, immunohistochemical score 3) and clearly visible by ^{111}In-pentetreotide scintigraphy (**c**). By contrast, a case of fine-needle aspiration biopsy of a para-caval lymph node metastasis from a well-differentiated neuroendocrine carcinoma of primary unknown origin (**d**) showing discrepant results between negative immuno-histochemistry (**e**, immunohistochemical score 1) and positive scintigraphy (**f**).

staining was proposed and assessed. The correlation with ^{111}In-pentetreotide scintigraphy data indicate that the higher concordance rates were achieved only when a membranous pattern of staining was observed (corresponding to scores 2 and 3), whereas pure cytoplasmic staining (score 1) presented a rather poor and random concordance rate and was therefore considered as negative for further analysis. Although 'functional' mechanisms for somatostatin receptor type 2A cytoplasmic positivity (ie receptor

Table 3 Concordance between immunohistochemistry and ^{111}In-pentetreotide scintigraphy, according to somatostatin receptor type considered

	Somatostatin receptor type 2A	Somatostatin receptor type 2A and/or 3	Somatostatin receptor type 2A and/or 5	At least one somatostatin receptor type
Overall agreement	77%	80%	77%	77%
Sensitivity[a]	80%	91%	89%	93%
Specificity[a]	67%	60%	41%	35%

[a]Sensitivity and specificity were calculated considering arbitrarily ^{111}In-pentetreotide scintigraphy the reference method.

internalization) have been discussed in the literature,[26] it might be speculated that in the diagnostic practice careful search for membranous pattern (associated or not with a faint cytoplasmic pattern) should be pursued to define a positive staining. The same consideration should be deserved for somatostatin receptor types 3 and 5 antibodies, which showed almost exclusively a cytoplasmic pattern of staining, and which did not improve significantly the capability of somatostatin receptor type 2A alone to correlate with octreotide scintigraphy.

Immunohistochemical somatostatin receptor type 2A scores 2 and 3 were higher, as expected, in well-differentiated neuroendocrine tumor/carcinoma group as compared to poorly differentiated carcinomas (Table 1), without significant differences considering the organs of origin, except for the cases of metastatic samples of unknown primary origin which showed a lower rate of somatostatin receptor type 2A expression. When comparing the complete somatostatin receptor type 2A immunohistochemical data with somatostatin receptor scintigraphy, an overall high agreement (77%, χ^2 test $P < 0.0001$) was observed, irrespective of the degree of differentiation. However, when going through the major causes of discrepancy, some considerations might be drawn. First of all, in most instances the source of discrepancy was related to a negative somatostatin receptor type 2A immunohistochemical result in the presence of positive somatostatin receptor scintigraphy. In this respect it is difficult, if not useless, to determine which method is more sensitive or specific. It is well known, in fact, that false somatostatin receptor scintigraphy positivity may be related to necrotic areas or inflammation which may be frequent within an otherwise somatostatin receptor negative tumor,[18] and therefore immunohistochemistry represents, rather than an alternative, a useful adjunct to somatostatin receptor scintigraphy. Moreover, the possible role of other somatostatin receptor subtypes (namely types 3 and 5), which possess high affinity for the currently available somatostatin analogues, was investigated, although their analysis did not improve the overall concordance between scintigraphy and immunohistochemistry, and their application in the clinical practice still needs the availability of satisfactory commercial reagents, in our opinion.

By contrast, it should be speculated that immunohistochemistry may present a lower sensitivity than ^{111}In-pentetreotide scintigraphy and the tissue material investigated may not represent the somatostatin receptor type 2A expression pattern of the whole tumor. The frequent heterogeneity of somatostatin receptor distribution in tumor tissues may cause discrepant (either positive or negative) immunohistochemical results as compared to somatostatin receptor scintigraphy, and this is more evident in our study when analyzing separately preoperative (ie small tissue fragments) and metastatic samples, which showed lower concordance rates (73 and 65%, χ^2 $P = 0.09$ and $P = 0.3$, respectively). Technical artifacts related to tissue fixation and preservation are instead unlikely to explain discrepancies between the two methods considered, since comparable results were observed in both formalin and alcohol-fixed tissue specimens, and irrespective of the length of tissue storage.

It is important to note that the correlation with somatostatin receptor scintigraphy represents only one side of the matter, and the predictive value of somatostatin receptor immunohistochemistry in determining the clinical response to somatostatin analogues treatment would have comparable if not greater clinical impact. Such a correlation was limited in our study because of heterogeneous medical treatments of the patients and different intrinsic biological behavior of the diseases (ie from well- to poorly differentiated forms). However, in a group of 28 cases with comparable disease and therapeutic schedules, positive somatostatin receptor type 2A immunohistochemistry correlated with somatostatin analogues response in up to 75% of cases. Large clinical trials should be designed to validate the role of somatostatin receptor immunohistochemical profile in the prediction of clinical response.

In conclusion, our data indicate that (i) immunohistochemistry represents a reliable and useful method to characterize somatostatin receptor expression in neuroendocrine tumors, with special reference to type 2A, provided that accurate methodological conditions and standardized interpretation of the immunohistochemical results (membranous vs cytoplasmic pattern) are obtained; (ii) a standardized scoring system for immunohis-

tochemistry is proposed, with good clinical correlates; which support its role as a useful adjunct to somatostatin receptor scintigraphy in the clinical management of these patients, providing additional information on the tissue distribution of the receptors, heterogeneity among primary and metastatic lesions, and picking up false-positive scintigraphic images; (iii) somatostatin receptor type 2A immunohistochemistry may predict clinical response to somatostatin analogue therapy, although further validation on large series is needed.

Acknowledgement

Disclosure/conflict of interest

The authors have no conflict of interest to declare.

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