Imaging of Somatostatin Receptors by Indium-111-Pentetreotide Correlates with Quantitative Determination of Somatostatin Receptor Type 2 Gene Expression in Neuroblastoma Tumors

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ABSTRACT

We reported previously that the relative level of gene expression for sst2, a subtype of somatostatin receptors, was positively related to patient outcome in the childhood tumor neuroblastoma (NB). Because sst2 binds with high-affinity octreotide and its scintigraphic derivative, 111In-pentetreotide, we tested the hypothesis of whether NB tumor imaging with 111In-pentetreotide gives similar information to ex vivo measurement of sst2 expression. We, therefore, studied simultaneously nine NB tumors with 111In-pentetreotide single photon emission computed tomography and competitive reverse transcription-PCR for sst2, along with other prognostic markers.

To quantitate the relative abundance of 111In-pentetreotide binding to NB tumors, we developed a simple semi-quantitative method, based on the mathematical analysis of 111In-pentetreotide association to cancer cell receptors at different time points (4 and 24 h). We indeed found that the ratio between the activity in a manually extracted region of interest from pathological (ROI<sub>T</sub>) and background (ROI<sub>nt</sub>) area was increasing between early and late acquisition only in affected tissues. The rate of this pathological increase was quite different among patients and significantly (P < 0.01) related to the abundance of sst2 gene expression, as measured by competitive reverse transcription-PCR on ex vivo tumor samples. Because we demonstrated that in 26 NB patients the density of sst2 is strongly related to survival (P < 0.0005) and apparently independent from N-myc oncogene amplification (P < 0.05), we propose that NB tumor imaging with 111In-pentetreotide may have not only a diagnostic but also a prognostic value.

INTRODUCTION

Since the late eighties it has been recognized that tumor cells of neuroendocrine origin do express SS<sup>3</sup> binding sites (1). By using 123<sup>I</sup>- or 111<sup>In</sup>-labeled SS analogues, the in vivo imaging of SS binding sites was later available (2, 3) and used in several nuclear medicine centers to visualize receptor-positive tumors and their metastases. It was soon evident that SS receptor scanning offers advantages over the other scintigraphic tracers, including the possibility to obtain simultaneous information on tumor localization and tumor responsiveness to SS therapy. Early on, it was also hypothesized that the presence of SS receptor represents a favorable prognostic factor, based on a small series of breast cancer cases (4, 5). Similar results were later on reported for NB tumor (6, 7). In this childhood neuroendocrine cancer, the presence of functioning SS receptors were demonstrated by studies in vitro (7, 8) and confirmed in vivo by tumor imaging with either 123<sup>I</sup>-Tyr-octreotide or 111<sup>In</sup>-pentetreotide (3, 9). Hence, it was generally accepted, yet not demonstrated, that the presence of SS binding sites on cancer cells represents a new marker for a well-differentiated tumor (10).

The possibility to demonstrate a prognostic role of SS receptor in tumors was hampered by the heterogeneity of SS receptors. Up to now five different isoforms of SS receptors have been cloned (sst1–sst5; Refs. 11 and 12) and found to be differentially expressed in human tumors. For example, pancreatic (13) and prostate (14) cancers express sst1 but not sst2 and are, therefore, insensitive to therapy with octreotide (15), which preferentially binds to sst2 (11, 12). Accordingly, 123<sup>I</sup>-Tyr-Octreotide or 111<sup>In</sup>-DTPA-D-Phe-octreotide (111-In-pentetreotide) do not visualize these tumors (3, 14). Conversely, tumors arising from adult and embryonal adrenal medulla, such as pheochromocytoma (16, 17) and NB (18–20), express a relative abundance of sst2 mRNA and are successfully visual-

Received 1/16/97; revised 8/11/97; accepted 9/11/97.

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1 This work was supported by a grant from Associazione Italiana per la Ricerca sul Cancro, Milan, Italy, and also partially supported by Associazione Fiorentina per la Ricerca sulla Crescita Neoplastica, Florence, Italy.

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3 The abbreviations used are: SS, somatostatin; NB, neuroblastoma; RT-PCR, reverse transcription-PCR; SPECT, single photon emission computed tomography; mIBG, metaiodobenzylguanidine; ROI, region of interest.
ized with octreotide scintigraphy (3, 9). Therefore, this family of neuroendocrine tumors represents an ideal target to study the relationship between sst2 gene expression and prognosis. By using RT-PCR, we found that 15 of 15 NB tumors investigated expressed sst2 (20), independently from tumor stage and amplification of the N-myc oncogene, a well-known negative prognostic factor (21). However, when we quantitated by competitive RT-PCR the level of expression of sst2 mRNA in the same NB series, we were able to demonstrate that sst2 receptor concentration is related to patient survival (20). Indeed, we found a four-log-unit difference in the level of expression of sst2 among patients. The density of sst2 was elevated in patients with favorable outcome (10^9-4 × 10^9 molecules/µg RNA) and even very low (up to 9 × 10^5 molecules/µg RNA) in patients with poor prognosis (20). Hence, we concluded that a quantitative more than a qualitative evaluation of sst2 gives insights on the biology of NB tumors. Studies in NB cell lines indicate there is a positive correlation between the level of expression of sst2 mRNA, binding of [125I]-SS-14, and biological responsiveness to SS-14 (19, 20). Because we found a sort of biological threshold for sst2 gene expression (7 × 10^6 molecules/µg RNA) separating SS-responsive from unresponsive NB cells (20), we used this threshold as a cutoff to analyze patient survival. In a univariate Kaplan-Meier survival curve, we found that sst2 expression was an important prognostic indicator for NB (20). This finding was in good agreement with previous investigations based on the detection of SS-binding sites in NB tumors (6, 7).

The aim of this study was to extend investigation on the quantitative expression of sst2 in NB tumors and to evaluate whether the quantitative expression of sst2 is related to in vivo imaging of SS receptor, by using 111In-pentetreotide.

**MATERIALS AND METHODS**

**Patients**

Nine children affected by NB were selected at the Department of Pediatrics for the in vivo sst2 SPECT study with 111In-pentetreotide and for in vitro quantitation of sst2 gene expression by competitive RT-PCR. Both sst2 SPECT and RT-PCR were performed on the primary NB tumors, i.e., before any kind of therapy. Clinical characteristics of the children are reported in Table 1. In addition, primary NB tumors were obtained from two other children and were processed only for in vitro quantitation of sst2 gene expression by competitive RT-PCR. These two children were both males, aged 5 and 14 months with a stage I NB located in the adrenal area. They did not show progression of the disease after 22 and 14 months of follow-up, respectively. Informed consent was obtained from parents of all children.

**Somatostatin Receptor Scintigraphy**

**Radiopharmaceutical.** DTPA-d-Phe-1-octreotide (pentetreotide) and 111In-chloride were obtained from Mallinckrodt Medical BV. The radiolabeling was performed according to the instructions of the manufacturer. The chromatography of the radiopharmaceutical was performed using silica gel-imregnated glass fiber strips (ITLC SG; Gelman). The fraction of the peptide bound to 111In was more than 97%. The principal photons useful for detection and imaging studies are 171.3 and 245.4 KeV gamma rays.

**Study Protocol.** 111In-pentetreotide and 111In-mIBG were performed in the same patients a few days apart. For 111In-pentetreotide, they did not receive preliminary preparation and were injected with 5 MBq/kg of 111In-pentetreotide i.v. Three h after tracer injection, a planar whole-body segmentary acquisition was performed to localize the primary tumor and metastasis. The preset count modality was 300,000 cpm for head and neck, 500,000 cpm for thorax and abdomen, anterior and posterior views, with a 128 × 128 matrix, zoom 1.0, and medium energy collimator. Four h after the injection, a tomographic acquisition was performed on the primary tumor, with a 64 × 64 matrix, zoom 1.0, medium energy collimator, 64 frames of 60 s. Twenty-four h after the administration of labeled pentetreotide, SPECT acquisition was repeated with the same parameters on the same body region (22). A diagnostic dose of 131I-mIBG (0.5 MBq/kg) was injected into the patients prior to imaging. The patients were given Lugol’s solution for 2 days before and 3 days after 111In-mIBG administration. A segmentary whole-body posterior and anterior scan was done at 24 and 48 h after the injection. Images were interpreted qualitatively by the observers. A tomographic reconstruction by filtered backprojection was performed, with convolution Butterworth filter (cutoff, 0.25 cycles/pixel; filter order 10) for each tomographic registration.

**ROI Extraction.** A ROI was manually extracted on the tumor area (ROI_T) in a transaxial slice (Fig. 1). The same ROI was also repositioned in the same slice on a different area out of the tumor and with local (vascular) and surrounding (scatter) conditions similar to ROI_T. Because liver and kidney are directly involved in the metabolic clearance of the tracer, they were not considered for ROI_N. In our series, adrenal (n = 7) and lung (n = 2) were considered for calculation of ROI_N. Both tissues contain somatostatin receptors that bind 111In-pentetreotide (23). This procedure was applied to 4- and 24-h acquisition images.

**Data Processing.** A ratio between the ROI_T and the ROI_N was calculated and expressed as global count activity (cpm) and average counts/pixel (cpm/pixel) for each transaxial SPECT slice. The same processing was repeated at least three times, and results are expressed as mean ± SD. Results from two independent investigators were not different. Results were evaluated as described previously (22) under three assumptions: (a) the activity in ROI_N is due to tracer binding to the sst2 in normal tissue; (b) the density of sst2 in normal tissue is a constant by time; (c) the activity retained in ROI_T and ROI_N at 4 and 24 h is due uniquely to receptor bound tracer. According to the SPECT time, ROI_T/ROI_N is defined, respectively, ROI_T/ROI_N at 4 h and ROI_T/ROI_N at 24 h. Under the above assumptions, if the activity in ROI_T is due to overexpression of sst2, the ratio ROI_T/ROI_N should increase between the SPECT at 4 and 24 h. Conversely, if the activity in ROI_T at 4 h is due to “nonspecific” uptake, the ratio ROI_T/ROI_N should be stable or decreased between 4 and 24 h.

The increase (INC) of the ROI_T/ROI_N ratios is expressed as mean ± SD by:

\[
\text{INC} = \frac{(\text{ROI}_{T}\text{ROI}_{N}24h - \text{ROI}_{T}\text{ROI}_{N}4h)}{(\text{ROI}_{T}\text{ROI}_{N}4h)}
\]
Table 1  Clinical and biological characteristics of the nine NB patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (mo)/Sex</th>
<th>Stage</th>
<th>Serum markers</th>
<th>Site</th>
<th>DNA ploidy</th>
<th>Chromo(^{2}) 1p36</th>
<th>Degree of maturation(^{2})</th>
<th>BM(^{3}) disease</th>
<th>Treatment(^{6})</th>
<th>Follow-up(^{8})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7/M</td>
<td>I</td>
<td>46/13.4/768</td>
<td>Ad</td>
<td>A</td>
<td>nl</td>
<td>Diff.</td>
<td>No</td>
<td>Total Res</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>3/F</td>
<td>IVS</td>
<td>40/121/1260</td>
<td>T + Ad + L</td>
<td>A</td>
<td>nl</td>
<td>Diff.</td>
<td>Yes</td>
<td>Biopsy</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>4/F</td>
<td>IVS</td>
<td>46/114/601</td>
<td>Ad + L</td>
<td>A</td>
<td>nl</td>
<td>Diff.</td>
<td>Yes</td>
<td>Biopsy</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>3/M</td>
<td>II</td>
<td>25/12.2/525</td>
<td>T</td>
<td>A</td>
<td>nl</td>
<td>Diff.</td>
<td>No</td>
<td>Total Res</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>42/M</td>
<td>IV</td>
<td>2537/-1893</td>
<td>Ad + L + Bo</td>
<td>D</td>
<td>nl</td>
<td>Undiff.</td>
<td>Yes</td>
<td>Subtotal Res</td>
<td>D-CECat (\times) 4, EX + ADR, EX + VP16, BUS-TT-LPAM + PSCC</td>
</tr>
<tr>
<td>6</td>
<td>168/M</td>
<td>IV</td>
<td>2557/62.4/</td>
<td>Ad + T + Bo</td>
<td>D</td>
<td>nl</td>
<td>Undiff.</td>
<td>Yes</td>
<td>Subtotal Res</td>
<td>D-CECat (\times) 4, EX + ADR, EX + TT (\times) 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2/M</td>
<td>II</td>
<td>283/40/933</td>
<td>T</td>
<td>A</td>
<td>nl</td>
<td>Diff.</td>
<td>No</td>
<td>Total Res</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>42/M</td>
<td>IV</td>
<td>104/-735</td>
<td>Ad + Bo + Br</td>
<td>D</td>
<td>nl</td>
<td>Diff.</td>
<td>Yes</td>
<td>Subtotal Res</td>
<td>D-CECat (\times) 4, EX + ADR, EX + VP16, BUS-TT-LPAM + PSCC</td>
</tr>
<tr>
<td>9</td>
<td>11/M</td>
<td>IV</td>
<td>12/-1260</td>
<td>Ad + Bo + Br</td>
<td>D</td>
<td>nl</td>
<td>Undiff.</td>
<td>Yes</td>
<td>Subtotal Res</td>
<td>None</td>
</tr>
</tbody>
</table>

\(^{a}\) Ferr, ferritin (ng/mL); LDH, lactate dehydrogenase (U/L); NSE, neuron-specific enolase (ng/mL).

\(^{b}\) Ad, adrenal; T, thorax; L, liver; Bo, bone; Br, brain.

\(^{c}\) A, aneuploid; D, diploid.

\(^{2}\) Chrom, chromosome; nl, normal.

\(^{3}\) Diff., differentiating histology; Undiff., undifferentiated histology.

\(^{4}\) BM, bone marrow.

\(^{5}\) S, surgery; CT, chemotherapy; Res, resection; D-CECat, deferoxamine/cyclophosphamide/etoposide/carboplatinum; EX, cyclophosphamide; ADR, adriamycin; VP16, etoposide; TT, thiopeta; BUS, busulfan; LPAM, melphalan; PSCC, peripheral blood staminal cells.

\(^{6}\) DOD, dead of disease; NED, no evident disease; AWD, alive with disease.
In Vivo CATCGACCGA-3') using a 480 thermal cycler from Perkin-
Elmer programmed with an initial incubation at 95°C for 1
20 s, followed by 40 cycles of 60 s at 95°C and 120 s at 62°C, with
a final extension step for 7 min at 62°C. Each sample was also
followed by 15 mm at 70°C with GeneAmp Thermostable
60°C.

The degree of N-myc amplification was determined by a
two-step competitive PCR as described previously using a DNA
multiple competitor (pONC: Ref. 27). By the first competitive
PCR, we determined the exact concentration of sample DNA by
referring to the single-copy reference gene, β-globin, whereas in
the second one, the copies of N-myc were evaluated. Each PCR
cycle for both β-globin and N-myc included 1 min at 94°C, 1
min at 60°C, and 1 min at 72°C. After a 40-cycle PCR and an
electrophoresis on a 12% polyacrylamide gel, the results were
calculated on the basis of the densitometric ratios of competitor
and genomic bands as described for mRNA evaluation.

Statistical Analysis
Progression-free survival was estimated from the date of
diagnosis to the date of last follow-up or until disease progression
was noted or death occurred. Estimates of progression-free survival
of various subgroups of children with NB were calculated using the
Kaplan and Meier method (28). Student’s t test and linear regres-
sion were estimated after logarithmic transformation of the data to
obtain a normal distribution. Results are expressed as mean ± SD.

RESULTS
Fig. 1 shows a typical SPECT study for the evaluation of the
ROI4/ROI3T ratio at different times (4 and 24 h) in the
affected or unaffected adrenal of a patient with NB (patient 3).
As shown in the lower part of Fig. 1, we found a positive
ROI4/ROI3T at both the time points investigated. However, we
detected a specific increase by time in ROI4/ROI3T ratio only in
the affected tissue. According to a previous study (22), this
finding is suggestive for the presence of octreotide binding sites
in the tumor. Of the nine NB patients studied by SPECT, we
found a substantial increase by time in ROI4/ROI3T ratio in
five of nine (range, 1.45–0.33), a borderline value in two of nine
patients (0.23–0.16), and a lack of increase in two of nine patients
(0.04–0.01). Results from SPECT studies with 111In-pentet-
reotide are shown in Table 2, along with those obtained with
111In-miBG, the classical diagnostic tool for NB. By using com-
petitive RT-PCR on the primary tumors of the same patients, we
found specific transcripts for sst2 in all of the NBs investigated
with a rather different degree of expression among tumors
(Table 2). Although the number of patients in this study is very
limited, results obtained by competitive RT-PCR and semiquan-
titative SPECT are quite consistent and overall in agreement
with those of a previous report (20). Indeed, they indicate that
quantitation of sst2 gives significant insights into the biology of
NB tumors. A low expression of sst2 was found in patients with
unfavorable stage of disease [stage IV, log10 (sst2) = 7.87 ± 0.5
molecules/μg RNA, n = 4] when compared to stages with a

Competitive RT-PCR for sst2 mRNA and N-myc
Amplification Measurement
Total RNA and DNA were extracted by conventional phe-
nol-chloroform procedure (24, 25) from surgical or biop
tical specimens of the primary NB tumors. Competitor for sst2
mRNA measurement was prepared by a modification of the
overlap extension method and cloned in the plasmid pSSR-I
downstream to the T7 RNA polymerase promoter, as detailed
previously (20). The RNA competitor was obtained by in vitro
run-off transcription of pSSR-I with T7 RNA polymerase and
quantified by spectrophotometric reading. Competitor concen-
tration was expressed in terms of molecules/μl.

For the assay, 1 μg of total RNA was reverse-transcribed
by antisense primer (sst2-R, 5'-GCAAAGACAGATGATGAG-
GTGA-3'), along with an increasing number of RNA competitor
molecules (from 7 × 102 to 7 × 105) by a 5-min incubation at
60°C, followed by 15 min at 70°C with GeneAmp Thermostable
rTth reverse transcriptase RNA PCR kit (Perkin-Elmer, Nor-
walk, CT). The resulting cDNA was subsequently amplified in
the presence of sst2-L primer (sst2-L, 5'-CAGTCATGAG-
CATCGACCGA-3') using a 480 thermal cycler from Perkin-
Elmer programmed with an initial incubation at 95°C for 120 s,
followed by 40 cycles of 60 s at 95°C and 120 s at 62°C, with
a final extension step for 7 min at 62°C. Each sample was also
submitted to conventional PCR with the same primers and cycling
but without reverse transcription to exclude the presence of residual
genomic DNA in extracted specimens. Ten μl of each reaction was
run on a 12% polyacrylamide gel, stained with ethidium bromide,
and quantified by CCD video camera and image analysis as re-
ported previously (26). The ratios between the two amplification
products were then plotted against the amount of competitor mol-
ecules initially added to the sample, and the equivalence (i.e., the
concentration of competitor giving a 1:1 competitor:target ratio)
corresponded exactly to the number of mRNA molecules present in
the initial sample.

Although the number of patients in this study is very
limited, results obtained by competitive RT-PCR and semiquan-
titative SPECT are quite consistent and overall in agreement
with those of a previous report (20). Indeed, they indicate that
quantitation of sst2 gives significant insights into the biology of
NB tumors. A low expression of sst2 was found in patients with
unfavorable stage of disease [stage IV, log10 (sst2) = 7.87 ± 0.5
molecules/μg RNA, n = 4] when compared to stages with a

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Two transaxial 111In-pentetreotide SPECT slices through the
left and right adrenals from patient 3 (stage IVs neuroblastoma). A, 
imaging of transaxial SPECT sections with manual extracted ROIs (1 = 
ROI1 and 2 = ROI2). Region 2 (ROI2) was symmetrically positioned 
with respect to region 1 (ROI1) to have the same vascular content and 
scatter contribution from the underlying renal superior pole. The left 
section shows a slice at 4 h, whereas the right one shows a slice at 24 h. 
B, size and counts activity (as counts/pixel and total counts) of transaxial 
SPECT sections. The ratio ROI4/ROI3T is also shown for each section. 
Note the positive ROI4/ROI3T ratio for 111In-pentetreotide uptake. At the 
relatively increase in ROI4/ROI3T (0.33) corresponds a high level of 
expression of sst2, as measured *ex vivo* in the tumor by competitive 
RT-PCR (sst2 = 1 × 109 molecules/μg RNA).
amplification by PCR also has a highly significant poor prognosis. \(N_{-nvc}\) expression by the NB tumors of sst2 receptors. expression of sst2 is positively related to a favorable outcome dramatically affected by previous study (20). We found that progression-free survival is \(sst2\) reotide SPECT studies and with those derived from a previous study (20). The univariate hazard ratio was calculated for \(sst2\) expression, we used methodologies to describe the same biological phenomenon, \(r\), and more powerful than amplification of the \(N_{-nvc}\) oncogene. a well-known negative prognostic factor that \(sst2\) expression with an undifferentiated histology \(\log \text{INC} = -1.4 \pm 0.6, \(n = 3; P < 0.05\). Similar results were obtained in \(111\text{-In}-\text{pentetreotide} \text{SPECT}\) studies. In fact, by using the aforementioned semiquantitative approach, we found a higher \(\text{ROI}_{\text{NT}}/\text{ROI}_{\text{INT}}\) ratio increase in patients with a favorable stage \(\log \text{INC} = -0.18 \pm 0.23, \(n = 5\) and differentiating tumors showed a higher expression of \(sst2\) \(\log \text{INC} = -0.26 \pm 0.28, \(n = 6\) than in stage IV \(\log \text{INC} = -1.21 \pm 0.62, \(n = 4, P < 0.05\) or with an undifferentiated histology \(\log \text{INC} = -1.4 \pm 0.6, \(n = 3; P < 0.005\). In addition, the lowest level of \(sst2\) expression and \(111\text{-In}-\text{pentetreotide} \text{SPECT}\) ratio increase was found in the two patients that did not survive, whereas the highest levels of receptors were detected with both methodologies in two patients at stage IVs. In Fig. 2 is depicted the relationship between detection of \(sst2\) expression by competitive RT-PCR and \(111\text{-In}-\text{pentetreotide} \text{SPECT}\) ratio increase. Because results are correlated \(r = 0.76, P < 0.01\), it is possible that the two methodologies describe the same biological phenomenon, \(i.e.,\) expression by the NB tumors of \(sst2\) receptors.

Although an eye-ball inspection of the present data obtained either with RT-PCR or SPECT indicates that an elevated expression of \(sst2\) is positively related to a favorable outcome (Fig. 2), our study population is too small to calculate univariate hazard ratios. We, therefore, analyzed data from patients as in Table 1 together with those from two other NB patients from the Departments of Pediatrics that did not perform \(111\text{-In}-\text{pentetreotide} \text{SPECT}\) studies and with those derived from a previous study (20). The univariate hazard ratio was calculated for \(sst2\) gene expression and \(N_{-nvc}\) amplification, a well-known negative prognostic factor for NB. For \(sst2\) gene expression, we used a cutoff of 7 \(\times 10^3\) molecules/\(\mu\)g RNA, a value derived from a previous study (20). We found that progression-free survival is dramatically affected by \(sst2\) expression \(P = 0.00034, n = 26;\) Fig. 3A). In the same study population, evaluation of \(N_{-nvc}\) amplification by PCR also has a highly significant poor prognostic value \(P < 0.00007, n = 25;\) Fig. 3B). To identify the most powerful prognostic factor, we performed a bivariate analysis with the Cox proportional-hazards model containing \(sst2\) and \(N_{-nvc}\). In this model, we found that \(sst2\) gene expression is still a significant prognostic factor \(P = 0.022\), whereas \(N_{-nvc}\) lost its predictive power \(P = 0.41\).

**DISCUSSION**

NB is a relatively common embryonal tumor characterized by a remarkable biological heterogeneity (29) and, therefore, by an enigmatic and often unpredictable clinical outcome. Hence, it represents a therapeutic challenge. If NBs are categorized according to clinical (tumor site, age at diagnosis) and biological features (lactate dehydrogenase, histopathology, DNA ploidy, chromosome 1p deletion, and \(N_{-nvc}\) amplification), different risk groups can be identified (30, 31) and treated accordingly. High-risk group NB patients are treated with intensive multiple-agent chemotherapy, autologous bone marrow or peripheral stem cell transplantation, immunotherapy, and radiotherapy (32). Conversely, low-risk group NB patients, characterized by even spontaneous regression, are treated with minimal therapy (32). Although a careful evaluation of the known clinical and laboratory variables helps in establishing a correct guideline for therapy, the overall cure rates for NB is not improved much. Therefore, there is the need for new prognostic factor(s). The ideal one would be a sensitive, repeatable, nontoxic, and cheap indicator of the biology of the disease. We now provide evidence that measurement of the neuroendocrine marker \(sst2\) with competitive RT-PCR and especially with \(111\text{-In}-\text{pentetreotide} \text{SPECT}\) might fulfill such criteria.

In a previous study, we found that measuring \(sst2\) gene expression in NB primary tumors gives insights on patient outcome (20). We now confirm in a larger study population that an elevated concentration of \(sst2\) correlates with a good prognosis, indicating that expression of this neuroendocrine marker is related to a more favorable biology of the tumor. In addition, we found that the prognostic value of \(sst2\) is independent from and more powerful than amplification of the \(N_{-nvc}\) oncogene, a well-recognized, strongly unfavorable outcome predictor (21, 30, 31). Hence, it is worthwhile to test the clinical value of \(sst2\) measurement in even larger series to verify whether \(sst2\) expres-

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**Table 2** Expression of \(sst2\) and amplification of \(N_{-nvc}\) (competitive PCR) as compared with scintigraphic \(111\text{-In}-\text{pentetreotide}\) and \(131\text{-mIBG}\) uptake in nine patients affected by NB

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>(sst2) (mol/(\mu)g RNA)</th>
<th>(\text{ROI}<em>{\text{NT}}/\text{ROI}</em>{\text{INT}}) 4h Mean ± SD</th>
<th>(\text{ROI}<em>{\text{NT}}/\text{ROI}</em>{\text{INT}}) 24h Mean ± SD</th>
<th>(\text{INC}) (\text{ROI}<em>{\text{NT}}/\text{ROI}</em>{\text{INT}}) Mean ± SD</th>
<th>(131\text{-mIBG}) N\text{-}mIBG copies</th>
<th>Follow-up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(5 \times 10^6)</td>
<td>8.15 ± 0.06</td>
<td>20.05 ± 0.04</td>
<td>1.45 ± 0.02</td>
<td>PT</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>(6 \times 10^6)</td>
<td>1.51 ± 0.06</td>
<td>2.60 ± 0.03</td>
<td>0.71 ± 0.06</td>
<td>PT; M</td>
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<tr>
<td>3</td>
<td>(1 \times 10^6)</td>
<td>1.45 ± 0.05</td>
<td>1.94 ± 0.04</td>
<td>0.33 ± 0.07</td>
<td>PT; M</td>
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<td>4</td>
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<td>2.14 ± 0.05</td>
<td>3.20 ± 0.03</td>
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<td>PT</td>
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<td>(2 \times 10^6)</td>
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<td>0.16 ± 0.005</td>
<td>PT</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>(4 \times 10^7)</td>
<td>1.03 ± 0.02</td>
<td>1.07 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>PT; M</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>(8 \times 10^7)</td>
<td>2.02 ± 0.03</td>
<td>3.50 ± 0.03</td>
<td>0.72 ± 0.04</td>
<td>PT</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
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<td>2.16 ± 0.05</td>
<td>2.67 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>PT; M</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>(2 \times 10^9)</td>
<td>1.00 ± 0.01</td>
<td>1.01 ± 0.01</td>
<td>0.01 ± 0.001</td>
<td>M</td>
<td>1</td>
</tr>
</tbody>
</table>

\(\text{INC}\) [\(1TN4 24h - \text{TNT}\) 4h(\(\text{TNT}\) 4 h)].

\(\text{PT}\), primary tumor; \(M\), metastasis; \(\text{DOD}\), dead of disease; \(\text{NED}\), no evident disease; \(\text{AWD}\), alive with disease.
sion detects patients at high risk among those who do not have N-myc amplification. In these children, more intensive therapy may be considered. However, one of the main limitations in the quantification of sst2 gene expression by RT-PCR is the availability of tumoral tissue for RNA extraction. For example, sst2 measurement by RT-PCR is not an ideal tool for longitudinal studies in the same patient.

In this report, we described a simple, semiquantitative method for evaluation of in vivo sst2 expression in NB by using $^{111}$In-pentetreotide SPECT. Three lines of evidence indicated that a semiquantitative $^{111}$In-pentetreotide SPECT is the method of choice to visualize in vivo sst2 receptors in NB patients: (a) $^{111}$In-pentetreotide is derived from the cyclic octapeptide octetride that binds the sst2 subtype of SS receptors with the highest affinity (11, 12); (b) there is a strict correlation between the concentration of binding sites for SS and the sst2 gene expression in a wide panel of NB cell lines (19, 20); and (c) $^{111}$In-pentetreotide imaging in gastroenteropancreatic tumors correlates with sst2 gene expression, as measured by semiquantitative RT-PCR (33), and tumor markers (34). In a preliminary study, we noted that the association kinetic of $^{111}$In-pentetreotide to tumoral binding sites was still increasing after 24 h from the administration of the tracer, whereas in nontumoral targets, this increase was not present (22). Because there was concordance between the $^{111}$In-pentetreotide association study and a parallel cold-displacement study, we inferred that the percentage of the increase of $^{111}$In-pentetreotide binding between an early (4 h) and a late (24 h) time point represents an in vivo index of sst2 receptor density (22). In this study, we analyzed the value of this scintigraphic index in nine NB patients and compared results with those derived from competitive RT-PCR studies in the same subjects. Results we obtained with the two methodologies were in close agreement ($P < 0.01$). This is striking indeed, taking into account that RT-PCR measures sst2 gene expression in tumoral tissue obtained ex vivo, whereas $^{111}$In-pentetreotide SPECT visualizes sst2 binding sites in vivo. Although the number of patients investigated with both methods is limited, it is noteworthy that children with a low density of sst2 by RT-PCR and sst2 by scintigraphy have a poor outcome, whereas the highest concentration of receptors was found in two patients at stage IVs, characterized by a relative good prognosis (35). Assuming that further studies confirm the concordance between semiquantitative $^{111}$In-pentetreotide SPECT and competitive RT-PCR in evaluating sst2 in NB, we believe that the former method provides more information than the latter one. Indeed, NB imaging with $^{111}$In-pentetreotide not only has a potential prognostic value but also retains a definitive diagnostic value, allowing localization of primary tumors and/or metastasis (3, 9). In addition, NB imaging with $^{111}$In-pentetreotide offers the possibility of serial measurements over the course of the disease, monitoring spontaneous and therapy-induced regression or differentiation along a more mature chromaffin phenotype.

In conclusion, in this study, we provided evidence that measuring sst2 gene expression by competitive RT-PCR provides useful insights for NB patient outcome, apparently independently from amplification of N-myc. In addition, we showed, although in a limited number of patients, that similar information was acquired with competitive RT-PCR and a with a semiquantitative imaging of sst2, based on a time-related increase of $^{111}$In-pentetreotide binding to NB cells.

**Fig. 2** Linear relationship between the log of the concentration of sst2 mRNA molecules, as determined by competitive RT-PCR, and the log of ROI$_f$/ROI$_{ve}$ increase between 4 and 24 h for $^{111}$In-pentetreotide SPECT in nine NB tumors. The relative follow-up is reported.

**Fig. 3** Kaplan-Meier survival curves for 26 patients with neuroblastoma classified for sst2 mRNA expression ($\geq 7 \times 10^7$ molecules/mg RNA; A) and for N-myc amplification (B). Differences in survival times between the two groups were significant on the basis of the Cox-Mantel test.
ACKNOWLEDGMENTS

We thank Dr. V. Giachè for his contribution to the statistical analysis of the data. We thank Professor A. Pupi for helpful comments on the manuscript.

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Imaging of somatostatin receptors by indium-111-pentetreotide correlates with quantitative determination of somatostatin receptor type 2 gene expression in neuroblastoma tumors.

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