Immunoreactivity Score Using an Anti-sst2A Receptor Monoclonal Antibody Strongly Predicts the Biochemical Response to Adjuvant Treatment with Somatostatin Analogs in Acromegaly

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Immunoreactivity Score Using an Anti-sst2A Receptor Monoclonal Antibody Strongly Predicts the Biochemical Response to Adjuvant Treatment with Somatostatin Analogs in Acromegaly

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Context: Somatostatin receptor subtype 2 (sst2A) protein expression has been demonstrated to positively correlate with somatostatin analog treatment outcome in GH-secreting adenomas. Recently, a new rabbit monoclonal anti-sst2A antibody (clone UMB-1) has been validated as a reliable method to selectively detect sst2A protein levels in formalin-fixed tissues.

Objective: The aim of the study was to establish whether the evaluation of sst2A protein levels, assessed with a routine reproducible immunohistochemistry protocol using UMB-1 antibody, may predict the successful adjuvant therapy with somatostatin analogs in acromegalic patients.

Design, Setting, and Patients: Thirty-six acromegalic patients from our referral hospital were evaluated retrospectively. Sst2A expression analysis was performed by immunohistochemistry in 25 patients and by quantitative RT-PCR in 26 patients. Sst2A immunoreactivity was assessed with an immunoreactivity score (IRS), which takes into account both the percentage of positive cells and staining intensity.

Interventions: Patients with persistent disease after surgery (n = 26) were treated with somatostatin analogs for a median duration of 6 months.

Main Outcome Measure: GH and IGF-I levels were measured before and after postoperative treatment.

Results: Sst2A IRS showed a significant positive correlation with both GH (P = 0.039) and IGF-I (P = 0.001) suppression by octreotide. Sst2A IRS was negatively associated with IGF-I levels reached after treatment (P = 0.001), and patients that achieved IGF-I normalization showed significantly higher sst2A IRS compared to the group that was not normalized (P = 0.002). A sst2A IRS of at least 5 showed a sensitivity of 86% and a specificity of 91% in predicting IGF-I normalization during adjuvant octreotide treatment.

Conclusion: Sst2A IRS with the anti-sst2A antibody UMB-1 represents a valid tool in the clinical practice to identify acromegalic patients likely to be responders to adjuvant therapy with the currently available somatostatin analogs. (J Clin Endocrinol Metab 98: E0000–E0000, 2013)
T
he clinically available somatostatin analogs (SSAs), octreotide and lanreotide, preferentially bind the somatostatin receptor subtype 2A (sst2A), and the GH-lowering effect of these drugs has been positively correlated with both the level of mRNA and protein receptor expression (1–3). Sst2A protein has been mainly evaluated by immunohistochemistry (IHC) using different polyclonal antibodies (4–6). However, more recently, an IHC protocol performed with an sst2A rabbit monoclonal antibody (UMB-1 clone) has been demonstrated to be as effective as the "gold standard" in vitro method to quantify sst2 levels in tumor tissues, namely autoradiography (7). In fact, using the UMB-1 monoclonal antibody, sst2A protein has been mainly evaluated by immunohistochemistry (IHC) using different polyclonal antibodies (4–6). However, more recently, an IHC protocol performed with an sst2A rabbit monoclonal antibody (UMB-1 clone) has been demonstrated to be as effective as the "gold standard" in vitro method to quantify sst2 levels in tumor tissues, namely autoradiography (7).

Patients and Methods

Patients, tumors, and assays

Thirty-six acromegalic patients (18 men, 18 women; age range, 19–70 yr) that underwent transsphenoidal neurosurgery were evaluated. Twenty-seven patients (75%) had a macroadenoma, and nine had a microadenoma. Eleven patients were treated before surgery with octreotide long-acting repeatable (LAR) (20–30 mg/4 wk). No patient had received radiotherapy before or during the study period.

Patients with persistent disease after surgery (n = 26) started adjuvant octreotide LAR treatment (median duration, 6 months; range, 3–78 months) with a starting dose of 20 mg/4 wk. After 3 months, in patients not adequately controlled (IGF-I above reference range), octreotide LAR was increased up to 30 mg/4 wk.

Treated patients were investigated for GH and IGF-I levels immediately before (basal values) and after (posttreatment values) postoperative treatment. Moreover, an acute octreotide test


| TABLE 1. General characteristics, tumor size, and clinical data of the 25 patients investigated for sst2A IRS |
|---|---|---|---|---|---|---|---|---|
| 1 | F, 19 | Macro | Yes | — | — | 6.63 | 88 | 90 | 6 | — |
| 2 | F, 38 | Macro | — | — | — | 95 | 9 | 0.40 |
| 3 | M, 60 | Macro | — | — | — | 87 | 6 | 0.54 |
| 4 | F, 38 | Micro | — | — | — | 83 | 6 | 0.21 |
| 5 | F, 42 | Macro | — | — | 26 | 2.6 | 82 | 4 | 0.22 |
| 6 | M, 36 | Macro | Yes | — | 17 | 4.7 | 8 | 1 | 0.07 |
| 7 | M, 58 | Micro | Yes | — | 56 | 0.94 | 40 | 8 | 0.21 |
| 8 | M, 34 | Micro | Yes | — | 19 | 1.96 | 8 | 3 | 0.43 |
| 9 | M, 44 | Micro | — | — | — | 6 | 0.10 |
| 10 | F, 40 | Macro | Yes | — | 35 | 5.0 | 8 | 1 | 0.03 |
| 11 | F, 44 | Macro | Yes | — | 84 | 1.2 | — | 55 | 2 | 0.05 |
| 12 | M, 24 | Macro | Yes | — | 67 | 1.8 | 88 | 72 | 4 | 0.17 |
| 13 | M, 37 | Macro | Yes | — | 72 | 0.5 | 62 | — | 4 | 0.17 |
| 14 | M, 49 | Macro | Yes | — | 57 | 1.5 | 76 | — | 4 | 0.11 |
| 15 | M, 49 | Macro | Yes | — | 66 | 1.0 | — | — | 6 | 0.20 |
| 16 | M, 27 | Macro | Yes | — | — | 2.6 | — | 205 | 66 | 1 | 0.14 |
| 17 | F, 35 | Macro | Yes | — | — | 15 | 0.4 | 86 | 47 | 1 | 0.21 |
| 18 | F, 40 | Macro | Yes | — | 35 | 0.8 | 67 | — | 9 | 0.10 |
| 19 | F, 48 | Macro | — | — | — | 88 | 1 | 0.17 |
| 20 | M, 58 | Macro | — | — | — | 6 | 0.20 |
| 21 | F, 70 | Macro | Yes | — | 37 | 1.2 | — | — | 6 | 0.25 |
| 22 | M, 39 | Macro | Yes | — | 8 | 2.5 | — | 21 | 2 | 0 |
| 23 | M, 51 | Micro | — | — | — | 88 | 12 | 6 | 0 |
| 24 | M, 44 | Macro | Yes | — | 58 | 0.83 | 87 | 95 | 6 | 0.10 |
| 25 | F, 32 | Macro | Yes | — | 50 | 0.7 | — | — | 6 | 0 |

F. Female; M, male; OCT, octreotide; ULN, upper limit of normal; —, not available.

a Patients treated with SSA (also) before neurosurgery.

b Data in parentheses indicate percentage of IGF-I decrease categorized. IGF-I was scored 2 when normalized during therapy, 1 when reduced more than 50% but not normalized, and 0 when reduced less than 50%.

Data in boldface indicate patients that normalized IGF-I during adjuvant SSAs treatment.
was performed in 21 patients, as reported before (10). Approval from the Medical Ethical Committee of the Erasmus MC and informed consent to use the tumor tissues for research purposes were obtained.

Both GH and IGF-I concentrations were determined by use of a nonisotopic, automatic chemiluminescence immunoassay system (Immune; Diagnostic Products Corp., Los Angeles, CA). Not all parameters were available for each patient.

Quantitative RT-PCR

Sst2A mRNA expression analysis was performed in 26 tumor samples by use of quantitative RT-PCR, as previously described (1). The sequences, final concentrations, and PCR efficiencies of the hypoxanthine phosphoribosyltransferase (hprt) and sst2 primer-probe pairs have been described previously (1). Samples were measured on an ABI Prism 7900 Sequence Detection System (Perkin-Elmer, Foster City, CA) and normalized against the expression of the housekeeping gene hprt.

Immunohistochemistry

Before immunostaining, formalin-fixed paraffin-embedded tissues from 25 GH-secreting pituitary adenomas (see Table 1 for general and clinical characteristics of patients) were cut (5 μm), deparaffinized, and rehydrated. Tissue slides were heated in Tris-EDTA buffer (pH 9.0) for 20 min (microwave) for antigen retrieval and bathed in a 3% H2O2/PBS solution for 15 min at room temperature in the dark to quench endogenous peroxidase. After washing with Tris/HCl/Tween 0.5%, sections were incubated with the anti-sst2 primary antibody [rabbit monoclonal (SS-8000-RM, clone UMB-1), dilution 1:50; Biotrend, Köln, Germany] overnight at 4 C. After several washes, two drops of horseradish peroxidase rabbit/mouse (Dako Detection System; Dako Netherlands, Heverlee, Belgium) were added to tissues and incubated for 30 min. Bound antibody was visualized with freshly prepared 100 μl of Dako Detection System twice for 5 min at room temperature in the dark. Staining was then stopped by rinsing with water. Slides were counterstained with hematoxylin and coverslipped. For negative controls, the primary antibody was omitted.

The adenomas were scored semiquantitatively on the basis of a well-established immunoreactivity scoring system (IRS) (11). The IRS is calculated by the product of the percentage of positive cells (4, >80%; 3, 51–80%; 2, 10–50%; 1, <10%; 0, 0%) and the intensity of the staining (3, strong; 2, moderate; 1, mild; and 0, no staining), which results in IRS scores between 0 (no staining) and 12 (maximum staining).

Statistical analysis

The data were statistically analyzed using SPSS software version 15.0 for Windows (SPSS, Chicago, IL). When data distribution was normal, means ± SE were used; otherwise, median values (median, range: minimum-maximum) were calculated. Between-group comparisons were analyzed by the Mann-Whitney U test, and correlation coefficients were calculated by the Spearman rank order R. Assessment of the predictive discrimination of sst2A IRS was made using the receiver-operating characteristic (ROC) curve. Differences were taken to be statistically significant at P < 0.05.

Results

Short- and long-term response to octreotide treatment

Basal (morning, overnight fasting) mean GH levels (n = 31) were 16.1 ± 3.6 (1.2 to 76.4 μg/liter), and basal mean IGF-I levels [expressed as upper limit of normality range (ULNR), n = 28] were 3.02 ± 0.34 (1.2 to 7.7 ULNR). Basal GH and IGF-I values were directly correlated (r = 0.57; P = 0.004; n = 24).

After postoperative therapy, the mean IGF-I decrease was 36.9 ± 7.1%. IGF-I normalized in 12 patients (responders), reduced more than 50% without reaching normalization in three patients (partial responders), and reduced less than 50% in the remaining 11 patients (poor responders). GH levels were reduced by more than 50% in 10 patients and less than 50% in the other 10, with a mean percentage suppression of 42.2 ± 10.7. The percentages of GH and
IGF-I decrease during treatment were significantly and directly correlated ($r = 0.55; P = 0.011; n = 20$).

The percentage of GH suppression during the acute octreotide test ranged from 31.3 to 95.0% (basal vs. nadir) and was significantly higher ($P = 0.012; n = 14$) in the group of patients achieving IGF-I normalization after adjuvant treatment, compared with patients who were not normalized.

**Correlation between sst2A mRNA and protein expression and GH lowering during octreotide test**

A heterogeneous expression of sst2A mRNA content was recorded in our adenoma samples, with an 18-fold difference between the lowest and highest levels measured. Similarly, sst2A protein expression evaluated by IRS was variable in the different samples. Seven tumors showed low IRS (IRS 1–2), five showed intermediate (IRS 3–4), 11 showed high-intermediate (IRS 6–9), and two adenomas received the maximum score (IRS 12) (Fig. 1). Neither the patients’ general characteristics (age, sex) nor tumor size (micro- or macroadenoma) and SSA treatment before surgery were significantly related to sst2A expression (both mRNA and protein), although a trend for lower sst2A IRS in samples from SSA-pretreated patients was observed ($P = 0.086$). Both sst2A mRNA and protein levels were inversely correlated with basal IGF-I values ($r = -0.54, P = 0.018$; and $r = -0.50, P = 0.026$, respectively). Sst2A IRS showed a trend for direct correlation with mRNA expression ($r^2 = 0.39; P = 0.097; n = 19$).

The sst2A protein expression (IRS) was strongly and directly correlated with the percentage of GH suppression after sc 100-μg octreotide administration ($r = 0.73; P = 0.003; n = 14$; Fig. 2A). A similar correlation was observed for mRNA level as well ($r = 0.64; P = 0.010; n = 15$; Fig. 2B).

**Sst2A IRS and adjuvant treatment with long-acting SSAs**

No correlation between sst2A mRNA levels and GH and/or IGF-I lowering after adjuvant treatment with octreotide was found. Conversely, a significant positive association was found between sst2A IRS and both GH ($r = 0.55; P = 0.039; n = 14$) and IGF-I ($r = 0.70; P = 0.001; n = 18$) suppression by octreotide LAR administered as adjuvant treatment. In line with this, sst2A IRS was negatively associated ($r = -0.82; P = 0.001; n = 18$) with the IGF-I levels (as ULNR) after treatment (Fig. 2C). It is noteworthy that the group of patients that achieved IGF-I normalization showed significantly higher sst2A IRS compared with the non-normalized group ($P = 0.002; n = 18$) (Fig. 2D).

The prognostic profile of sst2A IRS in predicting normalization of IGF-I on treatment with octreotide LAR is graphically represented in Fig. 2E. A sst2A IRS of at least 5 (computed cutoff by ROC curve analysis) showed a sensitivity of 86% and a specificity of 91% (positive predictive value, 86%; and negative predictive value, 91%) in predicting normalization of IGF-I on treatment with octreotide LAR.
predicting IGF-I normalization during adjuvant octreotide LAR treatment.

Discussion

Surgery still represents the first-line therapy in the majority of patients with acromegaly (12). However, in case of persistent disease, SSAs are the first choice for adjuvant medical treatment (10, 13–16).

A large number of in vitro and in vivo studies already demonstrated that sst2A receptor expression correlates with the effectiveness of SSAs, both in terms of biochemical and tumor growth control, in different subsets of GH-secreting adenomas (2, 17, 18). In particular, in recent years, a number of studies focused on the correlation between the response to SSA treatment and sst2A protein expression (evaluated by IHC), highlighting sst2A expression as a crucial factor for successful treatment (4–6, 8). However, most of these studies investigated the correlation between sst2A immunoreactivity and therapy outcome in patients treated with SSA before surgery. In two of these studies, it is noteworthy that the authors already speculated about a possible role of long-term SSA treatment in affecting sst2A expression, reporting a significantly lower sst2A immunoreactivity in tumor samples of patients pretreated with SSAs, compared with untreated (6, 8).

In our study, we selected mainly patients naive to SSA treatment to minimize the influence of pretreatment on sst2 expression, and we focused on the response to post-surgery adjuvant therapy to achieve clearer indications from a homogenous cohort of patients.

Since its validation, the new rabbit monoclonal anti-sst2A antibody UMB-1 appeared to be a very reliable method to selectively detect sst2A expression in paraffin-embedded formalin-fixed tissues, facilitating the establishment of routine performance of sst2A IHC in human tumor samples, with a high quality of specific membranous staining (7, 9). Moreover, the evaluation of sst2A expression with a semiquantitative IRS system, which takes into account the intensity of the staining as well as the percentage of positive cells, resulted in a strong and convincing direct correlation between sst2A expression and IGF-I normalization, percentage IGF-I decrease, and lower IGF-I absolute values (as ULNR) after adjuvant treatment with SSAs. It is noteworthy that the predictive value of sst2A IRS was also confirmed when correlating sst2A protein levels with the amount of GH decrease after an acute octreotide test with a correlation coefficient and a significance comparable to that obtained by evaluation of sst2A mRNA in the same population, as already described (1, 18). However, despite the strong concordance observed for sst2A mRNA and protein data in predicting acute GH lowering, the correlation between sst2A mRNA and protein level (as IRS) in the same subjects was not statistically significant, as already observed by other authors in nonpituitary tumors (19).

This is the first study showing that a sst2A IRS of at least 5, comparable to that greater than 50% of moderately stained cells, which is easily visible with a low-power objective, results in an 86% positive (and 91% negative) predictive value to IGF-I normalization after a mean of 6 months adjuvant treatment with octreotide. This approach can be combined and may strengthen the already described positive predictive value of biochemical features explored by other authors (octreotide test and basal hormones assessment). In fact, in our study group, the amount of GH decrease after an octreotide test and the basal IGF-I values were also valuable predictors for IGF-I normalization after adjuvant treatment and, when combined with sst2A IRS (best predictor), could result in an improvement of the general predictive power. Interestingly, our finding of an inverse correlation between basal IGF-I values and sst2A IRS of the tumor could provide a reasonable pathophysiological explanation for the clinical observation of a lower rate of IGF-I normalization after SSA treatment in those patients with higher basal IGF-I values (20).

In conclusion, sst2A IRS using UMB-1 may represent a valid tool in clinical practice, due to the feasibility and reproducibility of a relatively low-cost method, as well as the general availability of formalin-fixed samples, to identify those patients likely to be good responders, in terms of IGF-I normalization, to adjuvant therapy with the currently available SSAs. Moreover, in the light of the recent availability of other medical treatment-effective modalities for patients with acromegaly, such as GH-receptor antagonists and SSTR panligands, a trustable standardized evaluation of sst2A expression could be crucial to lead the best individualized medical approach, avoiding a delay in the establishment of an effective medical treatment, also in terms of health systems costs.

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