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The value of an acute octreotide suppression test in predicting short-term efficacy of somatostatin analogues in acromegaly

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Abstract. Predicting the efficacy of long-acting somatostatin analogues (SSA) remains a challenge. We aim to quantitatively evaluate the predictive value of the octreotide suppression test (OST) in short-term efficacy of SSA in active acromegaly. Sixty-seven newly diagnosed acromegaly patients were assessed with OST. Subsequently, all patients were treated with long-acting SSA for 3 months, followed by reassessment. Nine parameters were tested, including GH_n (the nadir GH during OST), ΔGH1 (= [GH_{0h}-GH_n]/GH_{0h}, GH_{0h} was the baseline GH during OST), ΔGH2 (= [GH_m-GH_n]/GH_m, GH_m was the mean GH on day curve), AUC_(0-6h) (the GH area under the curve during OST), ΔAUC1 (= [GH_{0h}-AUC_(0-6h)]/GH_{0h}), ΔAUC2 (= [GH_m-AUC_(0-6h)]/GH_m), AUC_(m-6h) (the GH AUC during OST where GH_m was used instead of GH_{0h}), ΔAUC1' (= [GH_{0h}-AUC_(m-6h)]/GH_{0h}) and ΔAUC2' (= [GH_m-AUC_(m-6h)]/GH_m). The Youden indices were calculated to determine the optimal cutoffs to predict the short-term efficacy of SSA. ΔGH2 more than 86.83%, ΔAUC2 more than -57.48% and ΔAUC2' more than -57.98% provided the best predictors of a good GH response (sensitivity 93.8%, specificity 85.7%). ΔGH2 more than 90.51% provided the best predictor of a good tumor size response (sensitivity 84.8%, specificity 87.5%). The percentage fall of GH_n (ΔGH) was a better predictive parameter than GH_n. OST showed higher efficiency in predicting the efficacy of octreotide LAR than lanreotide SR. In conclusion, OST is a valid tool to predict both GH and tumor size response to short-term efficacy of SSA in acromegaly, especially for octreotide LAR. GH_m is better to be used as basal GH than GH₀ during OST.

Key words: Acromegaly, Pituitary adenomas, Somatostatin analogues, Pre-surgical medical treatment, Acute octreotide suppression test

ACROMEGALY is an insidious disease associated with a 2 to 2.5 times increased mortality risk

Submitted Apr. 5, 2016; Accepted Jun. 5, 2016 as EJ16-0175
Released online in J-STAGE as advance publication Jul. 12, 2016

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[1]. Control of insulin-like growth factor I (IGF-1), reduction of systemic complications, control of tumor growth, preservation of pituitary function, and extension of life expectancy are the aims of disease management [2-4]. Transsphenoidal surgery has been the first-line therapy for GH-secreting adenomas, but medical therapy is playing an increasingly important role, especially for those patients who are not in remission after surgery or for whom surgery is contraindicated [5]. Somatostatin analogues (SSA) are the most widely used medications for the medical treatment of acromegaly, with biochemical remission (mean GH levels

<2.5µg/L and IGF-1 normalization) rates of approximately 35% [6-8] and tumor size response (tumor volume shrinkage >20%) rates of ~70% [9]. SSA are quite expensive, especially in developing countries in which insurance plans that cover prescriptions are not available, such as China, and importantly, some patients are resistant to SSA. So, it is critical to identify those patients who will benefit from SSA to distinguish them from those who will not.

Lamberts *et al.* initially proposed that acute suppression of GH in response to an octreotide test might predict the long-term efficacy of subcutaneous short-acting octreotide therapy [10, 11]. Since then, the octreotide suppression test (OST) has become routine practice in many endocrine centers [12], but the value of the OST in predicting long-term response to SSA therapy is still controversial [12-15]. Moreover, short-term pre-surgical treatment with SSA in macroadenomas has been supported by a few studies and a recent meta-analysis [16], but it is unclear whether or not the OST can predict the efficacy of SSA after a relatively shorter period of usage, *e.g.* 3 months. In addition, the most frequently used parameter by previous studies was the nadir GH (GH_n) during the OST (shown in Supplementary Table 1), but there might be parameters (such as the percentage fall of the GH_n during the OST, *etc.*) which are more accurate in predicting responses to SSA. Therefore, in addition to nadir GH, we examined additional parameters generated from the OST. The aim of our study was to evaluate the value of the OST in predicting the short-term efficacy of SSA therapy in Chinese patients with active acromegaly.

Subjects and Methods

Patients

This was a retrospective study of prospectively obtained data from patients seen between July 2012 and August 2014 at a tertiary referral center in the East of China. Sixty-seven newly diagnosed and untreated patients with acromegaly (44 women and 23 men, mean age 42.9±12.2 years) were studied. The diagnosis of active disease was based on the clinical features of acromegaly, failure of GH suppression to below 1µg/L in response to a 75g oral glucose load, serum IGF-1 level above the age-appropriate reference range and radiologic evidence of a pituitary tumor. Contrast-enhanced MRI was performed to identify the size and location of pituitary adenomas. Blood sampling was

performed every 30 minutes from 0700 h to 0900 h in order to obtain the mean GH on day curve (GH_m). A standard 6-hour OST was performed in all patients [13, 17]. In brief, after an overnight fast and baseline GH blood sampling the next morning, 100µg of octreotide was administered s.c.. Sampling for GH was continued at hourly intervals for 6 hours. Nine parameters (GH_n, ΔGH1, ΔGH2, AUC_(0-6h), ΔAUC1, ΔAUC2, AUC_(m-6h), ΔAUC1' and ΔAUC2') were generated from the OST and GH_m for further evaluation. After 3 months of long-acting SSA therapy (44 patients with octreotide LAR, 20mg every 4 weeks, and 23 patients with lanreotide SR, 40mg every 2 weeks), patients were evaluated at the end of the period that they were treated with the drug. GH_m, IGF-1 and pituitary MRI were performed to evaluate the response to SSA. A good GH response was defined as a post-treatment GH_m on GH day curve <2.5µg/L or >75% fall compared with the pre-treatment GH_m. This definition was quite consistent with a previous research in which a good GH response was defined as 80% or more reduction in GH [18]. An IGF-1 reduction of ≥50% or IGF-1 below the upper limit of normal range (ULN) was considered as a good IGF-1 response [19]. A good Tumor size response was defined as tumor volume shrinkage >20% [20]. Accordingly, all patients were categorized into GH responders or non-responders, and tumor size responders or non-responders. Parameters generated from the OST were compared between groups. Cutoff values for predicting the efficacy of short-term SSA were obtained after construction of receiver-operating characteristic (ROC) curves.

Abbreviated parameters and formulas

GH_{0h} was the baseline GH value during the OST. GH_{1h}, GH_{2h}, GH_{3h}, GH_{4h}, GH_{5h} and GH_{6h} were the GH values from 1h to 6h during the OST. GH_n was the nadir GH during the OST. ΔGH1 = [GH_{0h}-GH_n]/GH_{0h}. ΔGH2 = [GH_m-GH_n]/GH_m. AUC_(0-6h) was the GH area under the curve (AUC) during the OST. AUC_(0-6h) = (GH_{0h}+GH_{1h})/2 + (GH_{1h}+GH_{2h})/2 + (GH_{2h}+GH_{3h})/2 + (GH_{3h}+GH_{4h})/2 + (GH_{4h}+GH_{5h})/2 + (GH_{5h}+GH_{6h})/2. ΔAUC1 = [GH_{0h}-AUC_(0-6h)]/GH_{0h}. ΔAUC2 = [GH_m-AUC_(0-6h)]/GH_m. AUC_(m-6h) was the GH AUC during the OST where GH_m was used instead of GH_{0h}. AUC_(m-6h) = (GH_m+GH_{1h})/2 + (GH_{1h}+GH_{2h})/2 + (GH_{2h}+GH_{3h})/2 + (GH_{3h}+GH_{4h})/2 + (GH_{4h}+GH_{5h})/2 + (GH_{5h}+GH_{6h})/2. ΔAUC1' = [GH_{0h}-AUC_(m-6h)]/GH_{0h} and ΔAUC2' = [GH_m-AUC_(m-6h)]/

GH_m. Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) were calculated as follows: sensitivity = true positives / (true positives + false negatives); specificity = true negatives / (true negatives + false positives); PPV = true positives / (true positives + false positives); NPV = true negatives / (true negatives + false negatives).

Biochemical measurements

GH was measured by a two-site chemiluminescent immunometric assay AutoDELFIA® hGH (PerkinElmer Life and Analytical Sciences, Wallac Oy, Finland), intra-assay CV: 5.3-6.5%, inter-assay CV: 5.7-6.2%, sensitivity: up to 0.01 µg/L (0.026 mU/L).

IGF-1 was measured with the Immulite 2000 solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens Healthcare Diagnostic Products Limited, UK), expected values depending on age (1-6 years: 49-327 µg/L; 7-11 years: 57-551 µg/L; 12-13 years: 143-850 µg/L; 14-16 years: 220-996 µg/L; 17-18 years: 163-731 µg/L; 19-20 years: 127-483 µg/L; 21-35 years: 115-358 µg/L; 36-50 years: 94-284 µg/L; >50 years: 55-238 µg/L; intra-assay CV: 2.3-3.5%; inter-assay CV: 7.0-7.1%; sensitivity: 20 µg/L). IGF-1 index = IGF-1/ ULN [21].

Magnetic resonance imaging (MRI) studies

MRI was performed before and 3 months after SSA pre-surgical treatment. Tumor sizes were calculated by the Di Chiro and Nelson formula: volume = height × length × width × $\pi/6$ [22]. When T2-weighted images were available, the T2 intensity of the solid portion was visually compared with the cerebral grey and white matter in the adjacent temporal lobe. Pituitary adenoma tissue was classified as being hypointense, when the MRI signal was equal to or lower than white matter and as hyperintense when the signal was equal to or higher than grey matter. An isointense signal was defined as a signal intensity between white and grey matter [23]. Patients with T2-hypointense adenomas were compared with T2-iso/hyperintense adenomas [24].

Statistics analysis

Data are presented as mean ± SD (or median with interquartile range) for continuous variables normally (or not normally) distributed, and as frequency for categorical variables. Normality was tested by the Shapiro-Wilk W test. Statistical analyses of categorical vari-

ables were carried out using Chi-square and Fisher's exact tests as appropriate. Means were compared using the Student *t* test when data distribution was normal, or by Wilcoxon rank-sum (Mann-Whitney) test when variables were not normally distributed. Correlations were performed, and Pearson correlation coefficients, or the Spearman Rank correlation coefficients when variables were normally, or not normally distributed, respectively, are reported. After construction of ROC curves, the Youden indices were calculated to determine the optimal cutoffs for the 9 parameters derived from the OST to predict the short-term efficacy of SSA (sensitivity, specificity, PPV, NPV), which included GH_n, ΔGH1, ΔGH2, AUC_(0-6h), ΔAUC1, AUC2, AUC_(m-6h), ΔAUC1' and ΔAUC2'. Linear regression analysis was performed for variables possibly predictive for the outcome of SSA treatment. Statistical evaluation was performed with Statistical software SPSS 16.0. A *p* value <0.05 was considered significant.

Results

Baseline characteristics

Clinical information of the 67 patients pre-treatment was presented in Supplementary Table 2. The median pre-treatment GH_m level was 31.42 (16.69~54.62) µg/L. The median post-treatment GH_m level was 7.00 (2.92~20.48) µg/L. The median percentage fall of GH_m was 72.18 (28.71~89.26) %. The median pre-treatment IGF-1 index was 2.84 (2.35~3.34), which was reduced to 1.98 (1.41~2.47) after treatment. The mean percentage reduction of the IGF-1 index was 30.52±25.24%. Data for volumes were available in 65 cases, which included 62 macroadenomas (95.38%) and 3 microadenomas (4.62%). The median pre-treatment tumor volume was 2.47 (1.62~5.89) cm³. The median post-treatment tumor volume was 1.98 (1.11~4.54) cm³. The mean percentage reduction in tumor volume was 21.39±18.12%. After 3 months of treatment with long-acting SSA, 62 patients received surgery, 4 patients were treated with SSA for another 3-9 months followed by surgery, and 1 patient continued with long-acting SSA as primary therapy.

OST

Values of the 9 parameters derived from the OST were shown in Table 1. Among the 67 patients assessed by the standard 6-hour OST, the median time to reach the GH_n was 3 (2~5) h. The GH_n occurred

Table 1 Baseline values of parameters derived from the OST (median with interquartile range)

Parameter	Median with interquartile range
GH _n	2.19 (0.77 ~ 7.77) µg/L
ΔGH1	89.80 (71.37 ~ 95.90) %
ΔGH2	89.81 (72.82 ~ 96.83) %
AUC _(0-6h)	39.17 (19.43 ~ 84.77)
ΔAUC1	-48.70 (-165.75 ~ 7.31) %
ΔAUC2	-35.27 (-148.67 ~ 16.89) %
AUC _(m-6h)	41.40 (19.51 ~ 95.94)
ΔAUC1'	-59.51 (-190.63 ~ -2.93) %
ΔAUC2'	-32.27 (-151.88 ~ 12.03) %

at 1h in 16.4%, at 2h in 23.9%, at 3h in 17.9%, at 4h in 16.4%, at 5h in 14.9%, and at 6h in 10.4% of the patients. There was no correlation between the time to reach GH_n and the pre-treatment GH_m level, pre-treatment IGF-1 index or pre-treatment tumor volume ($p=0.565, 0.348, 0.774$, respectively). GH_n, AUC_(0-6h) and AUC_(m-6h) was negatively correlated with the reduction of GH_m and tumor volume. ΔGH1, ΔGH2, ΔAUC1, ΔAUC2, ΔAUC1' and ΔAUC2' was positively correlated with the reduction of GH_m, IGF-1 index and tumor volume. There was no correlation between GH_n, AUC_(0-6h), AUC_(m-6h) and the reduction of IGF-1 index (Supplementary Table 3).

Linear regression analysis

GH_n, ΔGH1, ΔGH2, ΔAUC1 and ΔAUC2 were also tested in linear regression analysis. We found that GH_m reduction was strongly associated with GH_n, ΔGH1, ΔGH2, ΔAUC1 and ΔAUC2 when applied individually [GH_n (Beta=-0.517, $R^2=0.311$, $p=0.000$), ΔGH1 (Beta=0.721, $R^2=0.538$, $p=0.000$), ΔGH (Beta=0.711, $R^2=0.538$, $p=0.000$), ΔAUC1 (Beta=0.706, $R^2=0.501$, $p=0.000$), ΔAUC2 (Beta=0.682, $R^2=0.492$, $p=0.000$)], respectively (Supplementary Table 4).

Comparison between GH responders / non-responders and between tumor size responders / non-responders

There were 32 patients assessed as GH responders, 35 as GH non-responders, 33 as tumor size responders and 32 as tumor size non-responders. The GH_n, AUC_(0-6h) and AUC_(m-6h) levels were significantly lower in GH responders than in GH non-responders (0.81µg/L vs 7.27µg/L, $p<0.0001$; 25.68 vs 74.58, $p=0.0002$; 25.5 vs 74.13, $p=0.0003$; Fig. 1A and Supplementary Fig. 1). The ΔGH1, ΔGH2, ΔAUC1, ΔAUC2, ΔAUC1', ΔAUC2' levels were significantly higher in GH responders than in GH non-responders (95.91% vs 71.78%,

96.69% vs 76.32%, 6.44% vs -164.90%, 16.14% vs -137.36%, -0.87% vs -169.19%, 11.62% vs -141.23%, all $p<0.0001$, Fig. 1A and Supplementary Fig. 1). Similarly, the GH_n, AUC_(0-6h) and AUC_(m-6h) levels were significantly lower in tumor size responders than in tumor size non-responders (1.23µg/L vs 6.41µg/L, $p<0.0001$; 28.71 vs 65.06, $p=0.0108$; 32.1 vs 64.14, $p=0.0191$; Fig. 1B and Supplementary Fig. 2). The ΔGH1, ΔGH2, ΔAUC1, ΔAUC2, ΔAUC1' and ΔAUC2' levels were significantly higher in tumor size responders than in tumor size non-responders (95.16% vs 73.86%, 95.31% vs 77.77%, 3.04% vs -138.11%, 13.76% vs -127.61%, -5.83% vs -146.05%, 5.47% vs -131.19%, all $p<0.0001$, Fig. 1B and Supplementary Fig. 2).

The predictive value of various parameters derived from the OST for the short-term efficacy of SSA treatment

The optimal cutoff values of the 9 parameters derived from the OST and GH_m in predicting a good GH response were shown in Table 2. The cutoff values of ΔGH2, ΔAUC2 and ΔAUC2' were 86.83%, -57.48% and -57.98%, respectively, which showed the best power in predicting a good GH response to treatment with SSA, with a PPV of 85.7% and a NPV of 93.8% (sensitivity 93.8% and specificity 85.7%, Fig. 2A). The cutoff value of GH_n was 3.37µg/L, with a PPV of 71.8% and a NPV of 85.7% (sensitivity 87.5% and specificity 68.6%, Fig. 2A).

The optimal cutoff values of the 9 parameters in predicting a good tumor size response were shown in Table 3. The cutoff value of ΔGH2 was 90.51%, which showed the best power in predicting a good tumor size response to treatment with SSA, with a PPV of 87.5% and a NPV of 84.8% (sensitivity 84.8% and specificity 87.5%, Fig. 2B). The cutoff value of GH_n was 3.37 µg/L, with a PPV of 71.1% and a NPV of 77.8% (sensitivity 81.8% and specificity 65.6%, Fig. 2B). The cutoff values of ΔGH1, ΔAUC1, ΔAUC2, ΔAUC1' and ΔAUC2' were 90.32%, -38.9%, -42.81%, -57.57% and -24.57%, respectively, with PPV of 87.1%, 84.4%, 84.8%, 83.9% and 87.1%, NPV of 82.4%, 81.8%, 84.4%, 79.4%, and 82.4%, respectively (Fig. 2B).

A good IGF-1 response, defined as $\geq 50\%$ IGF-1 reduction or below the ULN, was achieved in 15 of the 63 patients whose IGF-1 were available before and after SSA treatment. Parameters from the OST did not efficiently separate responders from non-responders with regards to IGF-1 reduction (Supplementary Table 5).

A

B

Fig. 1 Comparison of GHn, Δ GH1 and Δ AUC1 between GH (A) and tumor size (B) responders / non-responders ($p < 0.0001$) [Wilcoxon rank-sum (Mann-Whitney) test].

Table 2 Optimal cutoff values of parameters derived from the OST in predicting a good GH response (sensitivity, specificity, PPV, NPV)

	AUC **	Youden index	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	<i>p</i>
GH _n	0.877	0.561	3.37	87.5	68.6	71.8	85.7	0.000 *
ΔGH1	0.946	0.769	82.57%	96.9	80.0	81.6	96.6	0.000 *
ΔGH2	0.935	0.795	86.83%	93.8	85.7	85.7	93.8	0.000 *
AUC _(0-6h)	0.763	0.420	72.31	90.6	51.4	63.0	85.7	0.000 *
ΔAUC1	0.947	0.763	-52.75%	90.6	85.7	85.3	90.9	0.000 *
ΔAUC2	0.921	0.795	-57.48%	93.8	85.7	85.7	93.8	0.000 *
AUC _(m-6h)	0.754	0.413	57.29	81.3	60.0	65.0	77.8	0.000 *
ΔAUC1'	0.921	0.721	-25.41%	75.0	97.1	96.0	81.0	0.000 *
ΔAUC2'	0.943	0.795	-57.98%	93.8	85.7	85.7	93.8	0.000 *

* *p*<0.05. AUC **, the area under the ROC curve. **Fig. 2** ROC curve analysis of GH_n, ΔGH2 and ΔAUC2 during the OST in predicting a good GH response (A) and a good tumor size response (B) after treatment with SSA for 3 months.**Table 3** Optimal cutoff values of parameters derived from the OST in predicting a good tumor size response (sensitivity, specificity, PPV, NPV)

	AUC **	Youden index	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	<i>p</i>
GH _n	0.794	0.474	3.37	81.8	65.6	71.1	77.8	0.000 *
ΔGH1	0.869	0.693	90.32%	81.8	87.5	87.1	82.4	0.000 *
ΔGH2	0.872	0.723	90.51%	84.8	87.5	87.5	84.8	0.000 *
AUC _(0-6h)	0.683	0.349	62.12	81.8	53.1	64.3	73.9	0.000 *
ΔAUC1	0.848	0.662	-38.90%	81.8	84.4	84.4	81.8	0.000 *
ΔAUC2	0.848	0.692	-42.81%	84.8	84.4	84.8	84.4	0.000 *
AUC _(m-6h)	0.669	0.321	57.29	75.8	56.3	64.1	69.2	0.000 *
ΔAUC1'	0.829	0.632	-57.57%	78.8	84.4	83.9	79.4	0.000 *
ΔAUC2'	0.858	0.693	-24.57%	81.8	87.5	87.1	82.4	0.000 *

* *p*<0.05. AUC **, the area under the ROC curve.

The differential predictive value of Δ GH2 for the short-term response to octreotide LAR versus lanreotide SR

The prognostic profiles of Δ GH2 in predicting a good GH and tumor size response to treatment with octreotide LAR versus lanreotide SR were shown in Fig. 3. The cutoff value of Δ GH2 <82.21% during the OST had a PPV of 92.3% and a NPV of 94.4% in predicting a good GH response in patients treated with octreotide LAR. The PPV and NPV for predicting a good tumor size response was 91.7% and 84.2% with the cutoff value of Δ GH2 <90.53%. A less optimal prognostic profile was obtained for subjects treated with Lanreotide SR with the cutoff value of Δ GH2 <86.83% during the OST providing a PPV of 70% and a NPV of 100%, in predicting a good GH response. The PPV and NPV for predicting a good tumor size response was 75% and 85.7% with the cutoff value of Δ GH2 <89.63%.

The predictive value of age, gender and T2-weighted MRI for the short-term efficacy of SSA treatment

The cutoff value of age was 37.0y in predicting a good GH response to treatment with SSA, with a PPV of 60.5% and a NPV of 75.0% (sensitivity 81.3% and specificity 51.4%, AUC 0.641, $p=0.048$). 45.45% (20/44) female vs 47.83% (11/23) male were GH responders ($p=0.853$). 23.81% (10/42) female vs 23.81% (5/21) male were IGF-1 responders ($p=1.000$). 51.16% (22/43) female vs 50.0% (11/22) male were tumor size responders ($p=0.929$). T2-weighted MRI was available in 13 subjects. 7 (53.85%) were hypointense, 2 (15.38%) were isointense, 4 (30.77%) were hyperintense. 71.43% (5/7) hypointense adenoma vs 33.33% (2/6) iso/hyperintense adenoma were GH responders ($p=0.286$). 42.86% (3/7) hypointense adenoma vs 0% (0/6) iso/hyperintense adenoma were IGF-1 responders ($p=0.192$). 71.43% (5/7) hypointense adenoma vs 50.0% (3/6) iso/hyperintense adenoma were tumor size responders ($p=0.592$).

Fig. 3 ROC curve analysis of Δ GH2 in predicting a good GH response (A) and a good tumor size response (B) after treatment with octreotide LAR (OCT) versus lanreotide SR (LAN).

Discussion

SSA is a cornerstone of acromegaly management. The value of primary therapy (pre-surgical administration) is controversial, but a recent meta-analysis by Fougner *et al.* suggested a trend favoring pre-operative SSA treatment [16]. However, one third of patients responded poorly or was resistant to SSA [25]. Those patients who are identified as not responding to SSA would receive surgery directly and not use SSA as pre-surgical administration, which will save medical expenses. Factors that have been shown to predict response to SSA include the followings: age, gender, GH level, tumor size, MRI T2 signal, OST, somatostatin receptor (SSTR) phenotype, granular pattern, *gsp* mutation, Ki-67 expression, SST5TMD4 truncation, arylhydrocarbon-interacting protein (AIP) mutation, zinc finger regulator of apoptosis and cell cycle arrest 1 (ZAC1) expression, E-cadherin expression, and raf kinase inhibitory protein (RKIP) expression [26]. However, most of these factors can be only evaluated by pathology, and the clinical factors are not strong enough predictors to be useful in clinical practice. The OST is one of the most promising methods to predict efficacy of SSA therapy in *de novo* patients with acromegaly. The OST was first proposed by Lamberts *et al.* in 1988 to evaluate the suppression extent of short-acting SSA and to help determine the number of injections per day. After the long-acting formulations became available, several studies evaluated the efficacy of OST as a predictor of long-term response (shown in Supplementary Table 1). However, the results of different studies were conflicting, but with more publications in favor of using the OST [13-15, 27-31], than discarding it [7, 12, 25, 32]. The conflicting results might be caused by different OST protocols, octreotide dosages, outcome criteria and various parameters selected for evaluation. The purpose of our study was to investigate the predictive value of the OST using rigorous and homogenous methods.

Various OST protocols have been used in different centers. For example, the doses of octreotide used were 50 to 300 µg, while the sampling time was from 3 to 10 hours. Originally, the OST was performed with 50 µg octreotide s.c. and lasted for 6 to 10 hours. Later, Biermasz *et al.* performed a shorter test (hourly sampling $\times$ 3 hours after i.v. administration of 50 µg of octreotide) [14] and concluded that 2 hours were sufficient. In our study, the median time to reach the GH_n

was 3 (2~5) h. 39 cases (58.2%) needed 3 hours to reach the GH_n and 28 cases (41.8%) needed 4-6 hours to reach the GH_n. From the results of our study, we conclude that 6 hours are required to determine the nadir GH in most cases.

Most criteria for long-term efficacy of SSA reported in the literature were set with mean GH <2.5 µg/L with or without IGF-1 normalization. Based on our previous finding [33], long-term administration of SSA made the tumor texture harder, facilitating both the formation of pseudocapsules and subsequent surgical removal. However, it would be a surgical obstacle if the tumor texture were too fibrotic. Therefore, we have adopted the relatively short-term (3 months) administration of pre-surgical SSA for acromegaly management. The aim of pre-surgical SSA treatment is the achievement of satisfactory GH reduction and tumor shrinkage, the latter making surgical removal easier and safer, rather than the complete remission of the disease. In our study, a good GH response was defined as a post-treatment GH_m on GH day curve <2.5 µg/L or >75% reduction compared with the pre-treatment GH_m, a good IGF-1 was considered as $\geq$ 50% IGF-1 reduction or below the ULN [19] and a good tumor size response was defined as the tumor volume shrinkage >20% [20]. The definition of a good GH response in our study was quite consistent with a previous research in which a good GH response was defined as 80% or more reduction in GH [18].

As indicated in Supplementary Table 1, the widely used parameters in the literature were either nadir GH (1 µg/L, 2 µg/L, 2.5 µg/L or 5 µg/L as cutoff value) or percentage reduction of GH (50% or 75% as cutoff) during the OST, with PPVs ranging from 33%~100%, and NPVs ranging from 46%~100%, which were not well enough to predict the efficacy of SSA. Cutoff values for nadir GH were inconsistent in different studies [13, 30], and the predictive value of the percent reduction of GH during the OST was scarcely evaluated. In addition, most studies of OST aimed to predict the biochemical response to SSA, instead of tumor size response. There has been only one study which examined the value of OST in predicting tumor size response. The study found that neither acute nor long-term octreotide effects on GH levels were associated with degree of tumor size reduction [7]. As delineated in Supplementary Table 1, among the 13 papers published from 1988 to 2011, there was a great degree of inconsistency, which has led to conflicting conclusions.

In addition, distinct from previous studies, our study focused on predicting the short-term efficacy, instead of long-term efficacy, which differentiated it from other studies. Furthermore, we have tested the second largest cohort to date, and we used more accurate and rigorous parameters than in previous studies, as shown in Tables 2 and 3.

In our study, the cutoffs were determined for maximal predictive value of both the GH_n and the percentage reduction in GH_n (ΔGH1) during the OST. ΔGH2 was generated when GH_m was used as the baseline GH, instead of using GH_{0h} . In order to utilize as much information as possible from the OST curve, we generated AUC values [$\text{AUC}_{(0-6h)}$ and $\text{AUC}_{(m-6h)}$] and their relative transformations (ΔAUC1 , ΔAUC2 , $\Delta\text{AUC1}'$ and $\Delta\text{AUC2}'$). The predictive values of the various parameters generated from the OST were separately evaluated for both GH and tumor size response to the short-term administration of pre-surgical SSA therapy. As indicated in Table 2 and Table 3, the delta values were significantly more efficient than GH_n and AUC in predicting the efficacy of pre-surgical SSA therapy. Compared with other parameters, ΔGH2 , ΔAUC2 and $\Delta\text{AUC2}'$ showed higher PPV and NPV (85.7% and 93.8%, respectively) in predicting a good GH response. In addition, ΔGH2 had higher positive and negative predictive values than the other 8 parameters in predicting a good tumor size response (PPV 87.5% and NPV 84.8%). According to our study we recommend ΔGH2 as the best parameter to predict both GH and tumor size response to short-term efficacy of SSA in acromegaly, which is very useful for clinicians who are faced with decisions about whether to use an SSA in patients with acromegaly preoperatively. Not surprisingly, the 9 parameters derived from the OST did not reach a high enough efficacy in predicting IGF-1 response, possibly due to the long half-life of IGF-1 and short observation (3 months) of our study. Gadelha *et al.* summarized that there were at least 3 factors (age, gender, MRI T2W signal), which would be assessed before operation, could predict the efficacy of SSA. We also tested these factors in our cohort, and found that only the age cutoff of 37y could predict a good GH response to some extent (sensitivity 81.3%, specificity 51.4%, PPV 60.5%, NPV 75%, AUC 0.641, $p=0.048$), but the efficiency was obviously lower than the OST. Neither Gender

nor MRI T2W signal could distinguish SSA responders from non-responders. This result conflicts with Heck *et al.*'s [34], possibly due to the small size of the present study (MRI T2W signal was only available in 13 patients). Whether MRI T2W signal could predict the efficacy of SSA needs further evaluation. Interestingly, our data revealed that OST is more useful in predicting the efficacy of octreotide LAR than lanreotide SR, which is supported by the result of a previous study [13].

Our study is the first to use AUC and delta AUC to predict the efficacy of SSA pre-surgical treatment to aid in decision making with regard to acromegaly management. Although the sample size of the cohort in our study is one of the largest (only inferior to Colao *et al.* [17]) in the literature, more patients are still needed to evaluate if ΔAUC values are superior to the traditional parameters (GH_n and ΔGH) or not.

In conclusion, the OST is a clinically useful test to predict short-term efficacy of SSA treatment with predictive values greater than 85% (even >90% to predict the efficacy of octreotide LAR alone). Compared with GH_0 , Mean GH on day curve (GH_m) is better to be used as basal GH during the OST. The percentage reduction of GH_n (ΔGH) and ΔAUC are better predictive parameters than the GH_n and AUC and capture information from the entire OST time curve. Whether or not the ΔAUC values are superior to the traditional parameters (GH_n and ΔGH) needs further evaluation.

Acknowledgments

The authors thank Dr. Karen Klahr Miller for participating in modification the manuscript. This study was supported by the following grants: from National Natural Science Foundation of China (No. 81370938, No.81172391, No.81370884, No.81471041), Sheng Kang Hospital Development Center (SHDC12012220), Shanghai Rising-Star Tracking Program (12QH1400400), Shanghai Municipal Commission of Health and Family Planning (XYQ2011002, XYQ2013120).

Conflict of Interest

None of the authors have any potential conflicts of interest associated with this research.

Supplementary Table 1 Prediction of long-term response to SSA by the OST. Review of the literature.

Author & year	Test dose & time	Study types	No.	Long-term treatment		OST positive criteria	Predictive value of the OST	Conclusion
				Duration	Goal			
Lamberts [10] 1988	50µg s.c., 10h	Retrospective	15	96 weeks	Decrease in mean GH & normal IGF-1	Not mentioned	Not mentioned	Useful for s.c. octreotide
Colao [17] 1996	100µg s.c., 6h	Retrospective	68	1-3 months	GH < 5µg/L	mean GH 50% fall	PPV 43% NPV 78%	Not recommended
Jenkins [27] 2004	50, 100 and 200µg s.c., 10h	Prospective	6	6 months	< 5mU/L (2.5µg/L)	Nadir GH < 5mU/L (2.5µg/L)	Not mentioned	Maybe useful
Halach [28] 2004	100µg s.c., 6h	Not mentioned	10	6 months	Mean GH < 2.5µg/L	Nadir GH < 2.5µg/L	PPV 70%	Maybe useful
Lindsay [15] 2004	50 or 100µg s.c., 8h	Not mentioned	23	3 years	GH < 5mU/L (2.5µg/L) & normal IGF-1	nadir GH < 5mU/L (2.5µg/L) nadir GH < 10mU/L (5µg/L)	PPV 57% (GH < 5mU/L) 14% (normal IGF1) PPV 50% (GH < 5 mU/L) 33% (normal IGF1)	Useful
De Herder [32] 2005	50µg s.c., 465min	Not mentioned	24	At least 9 months	Normal IGF-1	mean GH 50% fall mean GH 75% fall nadir GH < 1.1µg/L nadir GH < 2µg/L	PPV 58% NPV 100% PPV 73% NPV 77% PPV 65% NPV 69% PPV 82% NPV 46%	Not recommended
Karavitaki [13] 2005	100µg s.c., 6h	Not mentioned	30	6 months	GH < 5mU/L (2.5µg/L)	Nadir GH < 5.25mU/L (2.125µg/L) (octreotide) or < 6.05mU/L (3µg/L) (lanreotide)	PPV 94% NPV 100% (octreotide) PPV 92% NPV 67% (lanreotide)	Reliable for octreotide
Gilbert [29] 2005	50µg s.c., 6h	Retrospective	33	15 months	GH < 5mU/L (2.5µg/L)	nadir GH < 5mU/L (2.5µg/L)	sensitivity 80% specificity 83%	Useful
Biermasz [14] 2005	50µg i.v., 3h	Not mentioned	18	4.1±0.4 years	GH < 5mU/L (2.5µg/L) & normal IGF-1	nadir GH < 5mU/L (2.5µg/L) nadir GH < 2.5mU/L (1.25µg/L)	PPV 73% NPV 57% PPV 80% NPV 63%	Useful
Pokrajac [12] 2006	50 or 100µg s.c., 9h	Not mentioned	42	2 years	GH < 5mU/L (2.5µg/L) & normal IGF-1	nadir GH < 5mU/L (2.5µg/L) mean GH 50% fall mean GH 75% fall	PPV 82 & 83% NPV 50 & 61% PPV 69 & 68% NPV 38 & 50% PPV 72 & 72% NPV 40 & 47%	Not recommended
Halperin [30] 2008	100µg s.c., 6h / 2h	Retrospective	18	12 months	IGF-1 < 2 SD IGF-1 < 3 SD	nadir GH < 3.6µg/L nadir GH < 9.2µg/L	PPV 33% NPV 89% PPV 75% NPV 67%	Useful
Bandgar [31] 2010	50µg s.c., 6h	Prospective	10	12 months	Nadir GH < 1µg/L & normal IGF-1	nadir GH < 1µg/L	PPV 100% NPV 100% PPV 100% NPV 83.3%	Useful
Carlsen [7] 2011	50µg s.c., 4h	Prospective	31	6 months	GH < 2mU/L & normal IGF-1	mean GH 50% fall	Predict GH reduction, but not tumor shrinkage	Not recommended

Supplementary Table 2 Patients' data and GH and tumor size parameters before and after short-term pre-surgical therapy with SSA

Patient: (no.)	Sex	Age (y)	SSA *	GH _{mean} pre-SSA (μg/L)	GH0 (μg/L)	GH1 (μg/L)	GH2 (μg/L)	GH3 (μg/L)	GH4 (μg/L)	GH5 (μg/L)	GH6 (μg/L)	GH _{mean} post-SSA (μg/L)	IGF-1 index pre-SSA	IGF-1 index post-SSA	Tumor volume pre-SSA (cm ³)	Tumor volume post-SSA (cm ³)
1	M	27	LAN	9.12	9.85	2.31	1.73	1.92	2.35	2.69	3.65	8.88	2.41	2.50	1.99	1.66
2	F	51	OCT	17.73	37.23	2.19	0.77	0.58	0.46	0.50	0.50	2.18	4.98	1.12	1.84	0.90
3	M	51	OCT	31.42	29.62	12.50	8.54	8.65	12.04	11.31	13.46	8.74	2.71	2.07	7.19	7.17
4	F	38	LAN	123.24	31.85	4.38	3.00	4.58	8.08	13.31	18.92	4.33	—	2.19	2.08	1.53
5	F	28	OCT	86.15	96.92	38.35	29.00	28.31	33.81	27.35	42.31	91.38	2.35	2.09	11.35	11.62
6	M	34	OCT	31.58	13.42	1.62	0.92	0.73	0.73	0.65	0.96	7.00	3.17	2.44	3.35	2.01
7	F	47	OCT	20.78	20.04	1.85	0.88	0.92	1.35	2.35	4.12	3.59	2.37	1.60	0.99	0.68
8	M	45	LAN	10.87	18.38	1.12	0.69	0.77	0.69	1.08	1.27	1.53	3.02	1.58	0.60	0.40
9	F	33	LAN	39.24	36.04	8.42	7.77	9.54	13.00	11.19	15.38	48.45	2.84	2.25	2.46	2.25
10	F	39	OCT	43.15	22.42	1.42	0.92	1.00	1.65	1.96	3.46	11.74	2.83	1.67	1.90	1.72
11	M	54	LAN	86.08	71.92	22.85	17.65	22.42	24.19	16.58	25.81	59.77	2.26	2.25	15.55	14.06
12	F	31	OCT	33.75	30.88	13.88	12.96	16.08	16.58	18.46	24.31	33.27	2.55	2.34	6.80	6.53
13	F	41	OCT	53.38	55.77	25.81	20.23	30.23	37.73	46.15	45.00	28.56	3.48	2.30	9.01	8.49
14	F	28	OCT	132.69	96.54	8.96	2.58	2.12	2.38	3.27	4.92	1.11	3.07	0.61	5.54	3.30
15	M	17	OCT	49.69	54.62	26.69	35.35	35.96	53.08	46.54	40.38	69.46	2.15	1.04	51.30	48.46
16	F	44	OCT	71.23	65.38	13.08	2.77	1.73	1.15	0.77	1.04	5.60	2.07	1.57	2.39	1.20
17	F	25	LAN	31.21	20.85	4.35	2.58	1.88	2.08	3.35	4.50	9.49	2.59	1.96	5.90	3.53
18	M	57	LAN	16.62	13.12	2.73	1.50	1.19	0.88	1.27	1.31	14.24	3.77	2.66	1.71	1.41
19	F	65	LAN	3.32	2.50	0.65	0.88	1.19	2.31	2.35	2.88	3.10	3.07	2.34	0.78	0.52
20	M	31	LAN	26.12	20.23	8.92	5.50	6.00	4.00	5.42	6.08	23.16	3.65	2.96	5.34	5.41
21	F	60	LAN	13.88	11.12	2.62	3.35	3.42	4.62	5.35	8.81	7.38	2.27	1.96	1.02	0.84
22	M	36	LAN	27.98	31.00	5.08	2.96	3.35	3.65	2.46	2.85	14.54	3.83	3.67	0.81	0.48
23	F	68	OCT	89.62	95.00	10.62	18.50	8.85	6.92	7.88	41.92	12.91	2.44	1.63	4.91	3.31
24	F	65	OCT	32.17	28.50	5.69	2.08	1.92	1.73	1.96	2.15	3.21	2.47	—	2.23	1.74
25	F	59	OCT	77.31	106.15	74.62	56.92	90.00	96.15	91.92	84.23	76.92	3.25	2.89	1.99	1.88
26	F	42	OCT	33.79	32.27	17.88	6.00	6.08	5.58	6.88	9.88	7.92	3.70	2.52	5.09	4.56
27	F	45	OCT	40.72	10.42	1.15	0.88	0.85	0.81	0.77	0.92	3.59	2.66	1.62	1.81	1.19
28	F	44	OCT	16.70	22.42	11.27	13.00	9.81	11.12	14.15	12.85	17.18	2.70	2.49	7.16	5.28
29	M	60	LAN	12.65	11.19	1.12	0.50	0.42	0.35	0.31	0.38	1.16	3.19	1.13	—	—
30	M	43	OCT	43.50	57.31	13.00	4.73	3.00	2.04	2.42	2.19	10.61	—	2.22	4.86	3.84
31	M	61	LAN	35.71	43.46	18.00	18.12	20.85	19.31	20.77	25.04	26.37	—	2.92	12.18	12.42
32	M	38	LAN	110.38	127.31	50.38	57.31	55.77	63.08	55.77	65.00	78.69	5.63	4.29	9.21	8.06
33	F	30	OCT	22.61	14.42	6.96	5.35	5.69	6.27	7.65	9.85	7.59	3.06	2.46	2.26	1.24
34	F	34	LAN	77.15	76.92	23.04	18.27	19.85	19.50	21.50	26.38	43.43	2.21	1.43	4.42	4.42
35	F	55	OCT	3.79	3.38	1.65	2.04	2.88	2.62	3.08	3.65	3.42	3.35	2.28	6.27	6.26
36	M	47	OCT	23.27	23.27	1.42	0.54	0.88	0.73	0.62	0.73	2.56	4.63	3.14	0.49	0.38
37	F	34	LAN	60.89	43.46	4.04	2.00	0.85	1.08	1.46	3.04	4.25	2.04	0.64	1.71	1.18
38	F	34	LAN	25.88	24.73	6.85	6.23	5.69	11.65	13.12	4.92	15.54	1.68	1.73	4.61	4.51
39	F	61	OCT	46.92	53.46	5.88	1.85	1.23	1.73	1.42	0.65	1.85	3.53	0.64	1.03	0.57
40	F	57	OCT	11.43	12.88	1.19	0.73	0.27	0.88	0.77	0.54	2.06	3.28	1.34	1.44	0.80
41	F	56	OCT	37.92	36.88	3.69	1.27	0.62	0.88	0.88	1.81	2.92	4.37	1.76	2.04	1.15
42	F	17	OCT	10.50	11.77	15.04	16.46	23.04	12.54	8.19	7.23	6.13	1.18	1.35	15.49	14.57
43	F	19	OCT	114.08	65.00	34.58	49.23	64.23	51.92	50.77	66.54	79.69	1.70	1.33	28.59	28.17
44	F	32	OCT	8.80	6.54	0.81	0.54	0.50	0.54	0.73	0.69	1.10	1.66	0.68	0.42	0.33
45	M	39	OCT	94.77	86.15	17.88	6.19	4.15	3.46	3.46	3.27	1.44	4.99	0.65	3.10	1.98
46	M	35	OCT	54.62	52.69	17.19	10.42	12.50	11.27	10.54	13.42	47.46	3.13	3.21	20.21	13.01
47	F	35	OCT	11.42	9.62	1.15	0.73	0.69	0.88	1.00	1.27	1.18	2.09	1.50	2.47	1.60
48	M	56	OCT	18.85	19.58	1.81	2.19	1.92	1.65	1.81	3.31	3.62	2.74	2.00	7.40	2.83
49	F	38	OCT	21.81	27.08	3.81	1.54	1.35	1.23	2.04	2.31	2.57	3.11	1.31	1.41	0.79
50	M	41	LAN	70.23	74.62	7.92	2.12	1.08	0.81	0.58	0.58	6.10	3.40	1.96	2.59	1.42
51	F	33	OCT	126.00	107.69	22.92	8.00	5.46	4.31	4.00	6.31	5.33	3.73	1.28	5.88	2.88
52	F	31	LAN	24.42	22.85	3.54	3.92	5.73	9.46	14.96	23.38	23.05	2.02	1.88	1.85	1.36
53	F	54	OCT	6.54	6.62	2.42	2.08	2.23	2.23	2.04	2.15	2.60	3.18	2.24	2.24	2.51
54	M	41	OCT	15.69	7.15	1.12	0.65	0.58	0.50	0.50	0.42	0.65	2.19	0.76	0.89	0.89
55	F	42	OCT	6.12	7.65	2.19	2.50	2.81	3.54	3.46	3.88	3.02	2.33	1.74	2.88	2.51
56	F	36	OCT	16.65	12.81	3.73	3.46	4.92	6.27	7.77	11.38	7.92	2.79	1.84	5.22	4.46
57	F	33	LAN	56.15	79.23	25.00	18.73	16.19	12.35	8.31	8.08	15.04	1.95	2.68	4.17	3.52

Supplementary Table 2 Cont.

Patient: (no.)	Sex	Age (y)	SSA *	GH _{mean} pre-SSA (μg/L)	GH0 (μg/L)	GH1 (μg/L)	GH2 (μg/L)	GH3 (μg/L)	GH4 (μg/L)	GH5 (μg/L)	GH6 (μg/L)	GH _{mean} post-SSA (μg/L)	IGF-1 index pre-SSA	IGF-1 index post-SSA	Tumor volume pre-SSA (cm ³)	Tumor volume post-SSA (cm ³)
58	M	44	OCT	3.73	3.08	0.65	0.42	0.38	0.46	0.69	0.88	1.08	3.30	1.23	0.35	0.35
59	F	60	LAN	16.69	21.92	8.85	8.73	7.27	9.23	12.31	18.54	9.52	3.49	3.59	0.53	0.49
60	M	44	LAN	21.25	29.27	3.23	1.46	1.27	1.08	1.00	1.69	4.19	3.25	2.19	1.17	1.06
61	M	52	OCT	37.35	25.73	4.81	2.85	2.81	2.88	3.00	3.65	4.01	4.95	3.23	2.04	1.44
62	M	50	OCT	32.67	22.92	4.12	2.50	2.65	1.81	2.73	3.50	20.48	2.95	2.25	3.21	2.12
63	F	50	OCT	40.27	33.19	21.31	27.96	30.08	32.46	35.12	36.15	34.05	2.35	2.11	1.52	1.88
64	F	32	OCT	67.69	56.54	29.85	43.08	47.31	60.00	52.69	51.54	50.00	2.37	1.90	—	—
65	F	50	OCT	17.65	12.27	1.46	0.54	0.46	0.58	0.62	1.00	0.41	2.41	1.13	1.12	0.75
66	F	54	OCT	21.08	16.58	5.12	4.19	4.73	5.88	6.77	9.69	3.15	2.50	2.50	4.00	3.48
67	F	41	LAN	18.32	22.00	5.58	2.81	2.35	2.19	2.23	2.77	1.72	3.04	2.74	6.77	5.54

* LAN: lanreotide SR, 40 mg/2 weeks ×12 weeks; OCT: octreotide LAR, 20 mg/4 weeks ×12 weeks.

Supplementary Table 3 The correlation analysis between the 9 parameters and the reduction in GH_m, IGF-1 and tumor size (*r* and *p*)

	GH _m reduction		IGF-1 reduction		tumor size reduction	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
GH _n	-0.538	0.000 *	-0.250	0.410	-0.434	0.000 *
ΔGH1	0.705	0.000 *	0.440	0.000 *	0.649	0.000 *
ΔGH2	0.704	0.000 *	0.445	0.000 *	0.605	0.000 *
AUC _(0-6h)	-0.479	0.000 *	-0.218	0.077	-0.368	0.003 *
ΔAUC1	0.670	0.000 *	0.437	0.000 *	0.586	0.000 *
ΔAUC2	0.665	0.000 *	0.438	0.000 *	0.535	0.000 *
AUC _(m-6h)	-0.465	0.000 *	-0.213	0.084	-0.364	0.003 *
ΔAUC1'	0.626	0.000 *	0.428	0.000 *	0.572	0.000 *
ΔAUC2'	0.672	0.000 *	0.445	0.000 *	0.554	0.000 *

* *p*<0.05

Supplementary Table 4 Results from linear regression analysis with the percentage of GH_m reduction after 3 months treatment with SSA as dependent variable

	Unadjusted estimates						Adjusted estimates					
	Beta	R ²	B	95%CI	<i>P</i>		Beta	R ²	B	95%CI	<i>P</i>	
GH _n	-0.538	0.289	-1.714	-2.379	-1.048	0.000 *	-0.517	0.311	-1.648	-2.323	-0.973	0.000 *
ΔGH1	0.705	0.497	1.482	1.112	1.851	0.000 *	0.721	0.538	1.517	1.144	1.889	0.000 *
ΔGH2	0.704	0.496	1.394	1.046	1.743	0.000 *	0.711	0.539	1.408	1.064	1.753	0.000 *
ΔAUC1	0.670	0.449	0.170	0.123	0.217	0.000 *	0.706	0.501	0.179	0.132	0.227	0.000 *
ΔAUC2	0.665	0.442	0.157	0.113	0.201	0.000 *	0.682	0.492	0.161	0.118	0.205	0.000 *

* *p*<0.05

Supplementary Table 5 Optimal cutoff values of the 9 parameters in predicting a good IGF-1 response (Sensitivity, Specificity, PPV, NPV)

	AUC **	Youden index	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	<i>p</i>
GH _n	0.813	0.517	0.67	91.7	60.0	69.2	88.0	0.000 *
ΔGH1	0.837	0.633	93.71%	80.0	83.3	60.0	93.0	0.000 *
ΔGH2	0.817	0.659	94.15%	86.7	79.2	56.5	95.0	0.000 *
AUC _(0-6h)	0.678	0.338	11.20%	93.8	40.0	66.7	83.3	0.047 *
ΔAUC1	0.833	0.659	-4.67%	86.7	79.2	56.5	95.0	0.000 *
ΔAUC2	0.796	0.600	-33.53%	93.3	66.7	46.7	97.0	0.000 *
AUC _(m-6h)	0.681	0.405	14.49	93.8	46.7	70.0	84.9	0.036 *
ΔAUC1'	0.813	0.592	-22.52%	80.0	79.2	54.5	92.7	0.000 *
ΔAUC2'	0.815	0.675	9.41%	80.0	87.5	66.7	93.3	0.000 *

* *p*<0.05. AUC **, the area under the ROC curve.

Supplementary Fig. 1 Comparison of $\Delta GH2$, $AUC_{(0-6h)}$, $\Delta AUC2$, $AUC_{(m-6h)}$, $\Delta AUC1'$ and $\Delta AUC2'$ between GH responders and non-responders [Wilcoxon rank-sum (Mann-Whitney) test].

Supplementary Fig. 2 Comparison of $\Delta GH2$, $AUC_{(0-6h)}$, $\Delta AUC2$, $AUC_{(m-6h)}$, $\Delta AUC1'$ and $\Delta AUC2'$ between tumor size responders and non-responders [Wilcoxon rank-sum (Mann-Whitney) test].

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