Monitoring of Treatment Success in Patients With Acromegaly: The Value of Serum Insulin-Like Growth Factor Binding Protein-3 and Serum Leptin Measurements in Comparison to Plasma Insulin-Like Growth Factor I Determination

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The aim of the present study was to determine the value of serum insulin-like growth factor binding protein-3 (IGFBP-3) and serum leptin measurements in comparison with plasma insulin-like growth factor I (IGF-I) measurements as indicators of treatment success in patients with acromegaly. Thirty-five acromegaly patients, 25 female and 10 male, divided into groups of patients with postadenomectomy "active" acromegaly (n = 20) and patients with postadenomectomy "controlled" acromegaly (n = 15), and 44 healthy volunteers sex- and age-matched with the acromegaly patients were included in the present study. We comparatively analyzed plasma IGF-I, serum IGFBP-3, and serum leptin levels in the aforementioned groups. Because serum leptin has sex dimorphism, the groups were divided into sexes when leptin was evaluated. As expected, the patients with active acromegaly had significantly higher mean values of plasma IGF-I and serum IGFBP-3 and lower mean values of serum leptin (only in women) than the control group. However, individual evaluation showed that 1 of 20, 9 of 20, and many patients with postadenomectomy active acromegaly patients had values that overlapped values of control subjects for plasma standard deviation score (SDS)–IGF-I, serum SDS–IGFBP-3, and sex-ajusted serum leptin, respectively. Application of the receiver operating characteristic (ROC) curves method shows that plasma IGF-I measurement has the best discriminatory power to differentiate patients with postsurgical active acromegaly from healthy people. Its area under the curve (AUC) was 0.95, with a sensitivity and specificity of 86% and 94%, respectively. Its positive and negative likelihood ratios were 14 and 0.15. Serum IGFBP-3 has certain discriminatory power, its AUC being 0.89, with a sensitivity and specificity of 83% and 77%. Its positive and negative likelihood ratios were 3.6 and 0.22. Serum leptin, both in women and in men, has a poor performance with sensitivity and specificity of 53% and 50% for women and 55% and 56% for men and positive and negative likelihood ratios of 1.06 and 0.94 for women and 1.26 and 0.8 for men. Application of the ROC curves method and the determination of positive and negative likelihood ratios in comparative evaluation of serum IGFBP-3 and serum leptin with plasma IGF-I as indicators of treatment success in acromegalic patients showed that neither serum IGFBP-3 nor serum leptin determinations have accuracy better than or similar to that of plasma IGF-I for monitoring treatment success in acromegaly patients. Serum IGFBP-3 is accurate but does not increase accuracy for age-ajusted plasma IGF-I, whereas determination of serum leptin level has no value in monitoring these patients.

CURRENTLY, growth hormone (GH) and insulin-like growth factor I (IGF-I) levels are used to monitor treatment success in acromegalic patients, the criteria being the same as those used for diagnosis. Using available GH to date, all controlled patients should experience normalization of GH values (<1 mg/L) for at least 50% of the points measured during the day.[1] This test, although reliable, is impractical for monitoring of disease cure and recurrence. Therefore, GH of <1 mg/L at baseline or within 2 hours after a glucose overload and normal age-adjusted IGF-I levels are the best standardized tests to diagnose acromegaly and define the patients who are controlled after treatment.[2,3] However, the search for other parameters that can be used to monitor success after application of therapeutic procedures is still open.

Insulin-like growth factor binding protein-3 (IGFBP-3) is the major circulating form of IGFBPs, is GH dependent but with a longer half-life than GH and does not exhibit pulsatility. It is also an integrated marker of somatotrope function. Unlike IGF-I, IGFBP-3 circulates in high concentrations and can be assayed reliably from a small sample volume. It has been used in the diagnosis of GH deficiency in children,[4,5] and it has also been shown that acromegaly patients have elevated levels.[6] Some studies have yielded conflicting results regarding whether it is more accurate[7-9] or improves the accuracy of the parameters currently used in monitoring these patients. Therefore, this issue needs to be clarified.

Leptin is secreted from adipose tissue, and its circulating concentrations reflect measurement of body fatness.[10,11] Furthermore, the amount of lean mass exerts a negative influence on circulating levels of leptin,[12,13] suggesting that serum leptin is an accurate measure of total body composition rather than just a measure of adiposity. In line with this finding, adult patients with GH deficiency had high levels of serum leptin, whereas patients with acromegaly showed decreased serum concentration of leptin levels,[14] and determination of serum leptin provided a strong metabolic marker for the growth response to GH treatment in children.[15] The fact that changes in serum GH levels that occur in GH deficiency and acromegaly are associated with modifications in serum leptin levels suggests that measurement of serum leptin may reflect the biologic effect of GH status.

The aim of the present study was to investigate the value of serum IGFBP-3 and serum leptin measurements as indicators of treatment success in patients with acromegaly compared with the plasma IGF-I determination currently used.

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MATERIALS AND METHODS

Thirty-five acromegaly patients (25 female, 10 male) aged 51.5 ± 3.4 years (range, 19 to 69 years) and 44 healthy volunteers matched for sex, age, and body mass index (BMI) (29 female, 14 male; aged 46.8 ± 1.5 years; age range, 18 to 75 years) were included in the study.

Diagnosis of acromegaly was based on typical clinical manifestations: elevated plasma age-adjusted IGF-I levels and nonsuppressible GH after oral glucose loading. A pituitary tumor was seen in all the patients by computed tomography or magnetic resonance imaging of the sellar area. The patients were surgically treated by the transphenoidal route. All had somatotrope adenoma revealed by pathologic study.

To compare the accuracy of age-adjusted serum IGFBP-3 and sex-adjusted serum leptin measurements with age-adjusted plasma IGF-1 values as indicators of treatment success in acromegaly patients, we divided the acromegaly patients after adenomectomy into those with "controlled" and "active" acromegaly according to serum immunoradiometric assay (IRMA)-GH suppression of <1 μg/L after oral glucose overload. We choose this parameter to classify the study subjects because it is a dichotomic variable and cannot be used for ROC curve analysis, whereas plasma IGF-I value is a continuous variable and can be used in ROC calculations. Twenty patients had active and 15 had controlled acromegaly after surgical treatment.

All patients with acromegaly were well nourished and without renal failure. None of them had overt diabetes mellitus or impaired glucose tolerance.

All subjects provided informed written consent before participation in the study protocol, which was approved by the ethics committee of Hospital Xeral-Cíes of Vigo.

The following groups of subjects were comparatively studied: (1) 20 (12 female and 8 male) patients with active acromegaly after adenomectomy, (2) 15 (13 female and 2 male) patients with controlled acromegaly after adenomectomy, and (3) 44 (29 female and 15 male) healthy subjects sex- and age-matched with acromegaly patients as a control group. These subjects were selected from a reference population of 198 healthy adults of both sexes, aged between 18 and 74 years. Because leptin differs according to sex, these groups were also divided into male and female groups when this variable was analyzed. Patients with acromegaly, both active and controlled, were evaluated at least 6 months after hypohysectomy.

The blood samples for assay of the biochemical parameters were obtained by standard venipuncture technique after overnight fasting (at 8:30 to 9 PM) and after at least 60 minutes of recumbent rest. Serum GH levels were measured by a highly sensitive 2-site monoclonal antibody IRMA (Nichols Institute Diagnostics, San Juan de Capistrano, CA). The sensitivity of the method is 0.02 μg/L. The mean intra-assay coefficients of variation (CVs) were 2.5% (2.6 μg/L), 3.8% (6.9 μg/L), and 2.6% (13 mg/L). The interassay CVs were 2.9% (2.6 μg/L), 3.4% (6.9 μg/L), and 2.5% (13 μg/L). Plasma IGF-I was estimated by a commercially available radioimmunoassay (RIA; Nichols Institute Diagnostics) after acid-ethanol extraction. The sensitivity of the assay was 0.06 μg/L, with an intra-assay CV of 2.4% to 3.8% for a range of 110 to 562 mg/L and an interassay CV of 5.2% to 6.8% for a range of 121 to 641 μg/L. Serum IGFBP-3 was determined by RIA (Mediagnost, Tübingen, Germany). The sensitivity of the assay was 0.06 mg/L and half-maximum displacement occurred at 6 mg/L, with an intraassay CV of 2.8% to 3% for a range of 2.2 to 4.3 mg/L and an interassay CV of 4.5% to 5.9% for a range of 2.6 to 4.5 mg/L. Serum leptin was measured by RIA (Linco Research, Inc, St Charles, MO) using recombinant human leptin. The detection limit was 0.5 μg/L, and the intra-assay and interassay CVs were 4.1% and 6.5%, respectively.

Our age-adjusted reference values of plasma IGF-I and serum IGFBP-3 were obtained from a population of 198 healthy subjects of both sexes with ages between 18 and 74 years. The reference values for plasma IGF-I were 100 to 403.5 μg/L, 78.5 to 305.3 μg/L, 46 to 284 μg/L, and 34.4 to 207 μg/L for subjects aged less than 35 years, 35 to 54 years, 55 to 64 years, and 65 years or older, respectively. Serum IGFBP-3 values are 2.9 to 6.0 mg/L, 2.4 to 5.0 mg/L, 2.5 to 5.0 mg/L, and 2.0 to 4.6 mg/L for the same age groups.

Statistical Analysis

Values are reported as means ± SEM. The differences in the mean values of the groups were obtained by the Student *t* test. Normal distribution of the variables was verified by the Kolmogorov-Smirnov test and differences in percent values by the χ^2 test. We used the age-specific standard deviation score (SDS) for IGFBP-3 and IGF-I when individual hormonal values were evaluated.

The diagnostic performances of the different biochemical parameters studied were assessed by receiver operating characteristic (ROC) curves statistics using a parametric model. ROC curves were drawn after normalization of the variables by plotting the sensitivity or true-positive fraction (TPF) against 1 − specificity or false-positive fraction (FPF). Overall, the cut-off points obtained with the test indicate the effectiveness of the test, considering how close the plot is to the upper left corner. Thus, a perfect test would coincide with the upper left corner of the box, and a nondiscriminating test would follow the diagonal line of the figure.[16,17] By simple observation of the figures, we can obtain a qualitative comparison between several tests. The area under the curve (AUC)-ROC is a global measure of the accuracy of a diagnostic test, defined as the probability of correct classification of a pair of subjects; healthy and ill, selected at random from a population by the results obtained from the application of a diagnostic test. The Rockit 0.9B beta version (Charles E. Metz, University of Chicago, Chicago, IL) software program was used for the ROC calculations.

The likelihood ratio (LR) for each biochemical parameter studied was also determined by the formulas LR^+ = Sensitivity/(1 − Specificity) and LR^- = (1 − Sensitivity)/Specificity. An LR^+ of >10 virtually guarantees disease, and an LR^- of <0.1 virtually rules out the chance that a patient has the disease. Statistical significance was defined at $P < .05$. Statistical calculations were performed using the SPSS software (SPSS, Chicago, IL).

RESULTS

As expected, the active group had significantly higher mean values of plasma IGF-I and serum IGFBP-3 than the other groups. Furthermore, the control and controlled groups had similar mean values of both variables (Table 1). All the study groups had similar mean values for serum leptin.

When individual hormonal values were evaluated, a visual inspection showed that only 1 of 20 patients with postadenomectomy active acromegaly had overlapping values of serum SDS–IGF-I with their matched controls; whereas 9 out of 20 of these patients had overlapping values of serum SDS–IGFBP-3 with their matched controls (Fig 1). Likewise, a considerable number of patients with active acromegaly had overlapping serum leptin levels with their matched controls, both in women and in men (Fig 2). Futhermore, 1 of 20, 10 of 20, and almost all patients with postadenomectomy active acromegaly also had overlapping values with patients with controlled acromegaly for SDS–IGF-I, SDS–IGFBP-3, and leptin, respectively (Figs 1 and 2). These data show that plasma IGF-I measurement seems to be the best biochemical parameter to differentiate between patients with postoperative active acromegaly and healthy subjects.

The accuracy of the biochemical parameter for differentiating healthy subjects (normal GH secretion) from patients with

Table 1. Comparison of Parameters Among the Study Groups

	Control (n = 44)	Controlled (n = 15)	Active (n = 20)
Age (yr)	46.80 ± 1.56	50.50 ± 4.09	52.81 ± 2.80
IGF-I (μg/L)	180.50 ± 6.94*	175.27 ± 21.72*	537.64 ± 86.26*†‡
IGFBP-3 (mg/L)	3.72 ± 0.10*	3.67 ± 0.22*	5.06 ± 0.24*†‡
Leptin (μg/L)			
Female	20.41 ± 2.34	25.44 ± 5.80	15.53 ± 1.51
Male	7.85 ± 0.97	12.27 ± 2.44	6.74 ± 1.03

NOTE. Results are presented as means ± SEM.

*$P < .05$ *v* active acromegaly.

†$P < .05$ *v* control.

‡$P < .05$ *v* controlled acromegaly.

acromegaly was also estimated by analysis of the ROC curve. In the present study, a parametric model was used because of because of the kinds of variables studied. In Fig 3, we show the comparison of the biochemical parameters. By changing the decision threshold of the test, ie, the cut-off values, a complementary set of sensitivity and specificity pairs were deducted constructing the ROC curve. In this curve, the point closest to the upper left corner of the box corresponded to 100% sensitivity and 0% false-positives (100% specificity). Figure 3 shows that the behavior of the plasma IGF-I test in the ROC curves was the best among the study parameters, with an AUC value of 0.95 and a sensitivity and specificity of 86% and 94%, respectively, for discriminating between patients with active acromegaly after surgical treatment and healthy subjects.

Figure 3 shows that serum IGFBP-3 measurement follows IGF-I in accurracy, with an AUC of 0.89 and a sensitivity and specificity of 83% and 77%, respectively. Serum leptin in women had an AUC of 0.57, with a sensitivity and specificity of 53% and 50%, respectively, whereas serum leptin in men had a AUC of 0.52, with a sensitivity and specificity of 55% and 56%. The sensitivity and specificity values were significantly different among the biochemical parameters studied (Fig 3).

The determination of LR^+ and LR^- showed the following results: 14.05 and 0.15 for plasma IGF-I, 3.64 and 0.22 for IGFBP-3, 1.06 and 0.94 for leptin in women, and 1.26 and 0.80 for leptin in men (Fig 3).

To know if determinations of plasma IGF-I and serum IGFBP-3 together improved the performance of each measured separately, we applied a logistic regression analysis between the state of the subjects (healthy or ill) as a dependent variable and plasma IGF-I and serum IGFBP-3 as independent variables. The study showed that plasma IGF-I values had a probability of 96% ($P = .00001$) to properly classify the subjects as healthy or ill, whereas serum IGFBP-3 had a probability of 89% ($P = .001$). Measurements of both parameters together does not improve their individual diagnostic accuracy.

DISCUSSION

The present work was undertaken to investigate the value of the serum IGFBP-3 biochemical parameter used in the management of patients with acromegaly and serum leptin levels, which are highly correlated with body fat mass and can express modifications of body composition associated with changes in GH secretion[13,15,18] in comparison with plasma IGF-I. This measurement is a currently used biochemical parameter in the management of acromegalic patients. Serum IGFBP-3 and serum leptin measurements might be good markers of GH status and be useful in monitoring treatment success of these patients.

As expected, significantly higher mean values of plasma IGF-I and serum IGFBP-3 were seen in postsurgical active acromegaly patients than in the normal volunteers. However, the analysis of individual values showed that determination of age-adjusted plasma IGF-I levels in patients with postoperative active acromegaly shows considerably fewer overlapping values than age-adjusted serum IGFBP-3 compared with their matched controls. Results of the present study are in conflict with previous observations[7,19] observing that determination of serum IGFBP-3 did not have overlapping values between untreated patients with acromegaly and normal subjects. However, the present data coincide with other reports[2,8] that found considerably more overlapping values of serum IGFBP-3 than of plasma IGF-I between acromegaly patients and normal con-

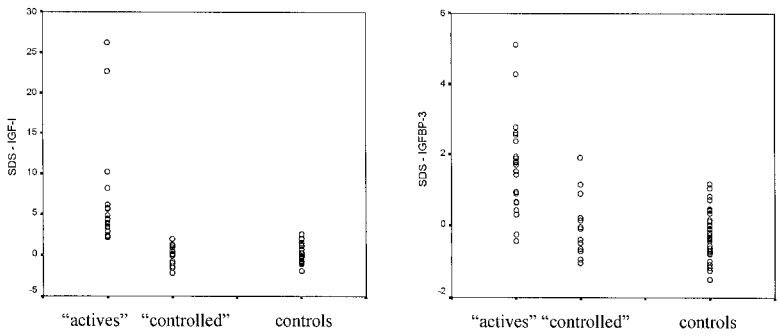


Fig 1. Comparison of individual values of plasma SDS–IGF-I and serum SDS–IGFBP-3 between patients with postoperative active acromegaly, patients with postoperative controlled acromegaly, and matched controls. Only 1 patient with active acromegaly has SDS–IGF-I value overlapped with that of controls, whereas 9 patients with active acromegaly have overlapped values of SDS–IGFBP-3 with their matched controls. Similarly, 1 and 10 patients with active acromegaly have overlapped values of SDS–IGF-I and SDS–IGFBP-3, respectively, with patients with controlled acromegaly.

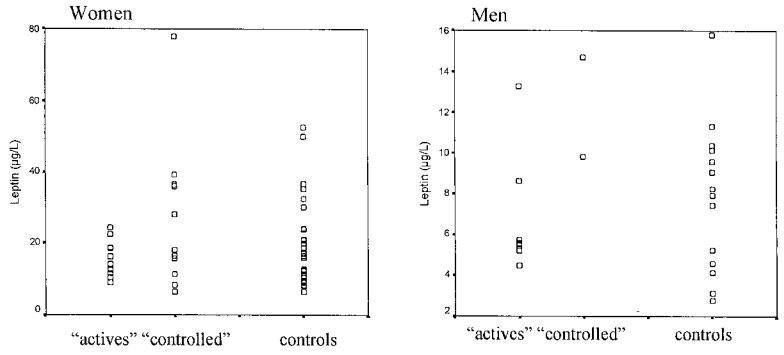
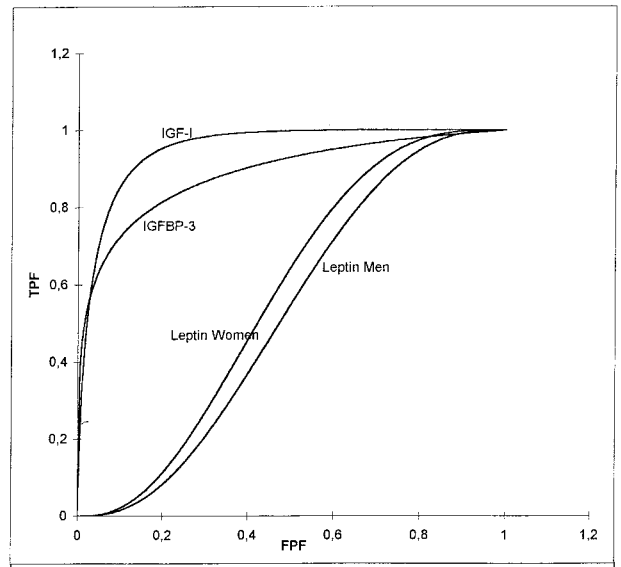


Fig 2. Almost all patients with postadenomectomy active acromegaly had overlapped values of leptin with controls in both women and men.

trols. In a previous study by our group,[9] we also observed overlapping values of serum IGFBP-3 but not of plasma IGF-I between patients with postoperative active acromegaly and healthy subjects, which is in line with the present study. Morever, we cannot forget that as indicated by our reference and previous studies,[20,21] the concentration of serum IGFBP-3 has less dependence on age than that of plasma IGF-I.

The recognized fact of sexual dimorphism in serum leptin concentrations is the reason this parameter was studied separately in women and men. Patients with postsurgical active acromegaly had significantly lower mean levels of serum leptin than the control group, but individual analysis of serum leptin values between patients with postadenomectomy active acromegaly and their matched controls showed considerably overlapping values in both women and men.

The validation of a given diagnostic test should not be carried out by visual inspection and simple correlation analysis alone, as is customary, but through methodogic analysis to permit the objective comparison of the tests; therefore, we used ROC curves analysis. The ROC method is based on statistical decision theory and was developed in the context of electronic signal detection and problems with radar.[13,22,23] Currently, ROC plots provide a unifying concept in the process of test evaluation and in the definition of diagnostic accuracy as a measure of test performance.[24] In the present study, we applied the ROC method to compare diagnostic accuracy of serum IGFBP-3 and serum leptin with plasma IGF-I determinations.



	AUC	CI 95% AUC Lower	Upper	TPF	FPF	LR +	LR -
IGF-I	0.95	0.86	0.99	0.86	0.06	14.05	0.15
IGFBP-3	0.89	0.76	0.96	0.83	0.23	3.64	0.22
Leptin Women	0.57	0.39	0.74	0.53	0.50	1.06	0.94
Leptin Men	0.52	0.29	0.74	0.55	0.44	1.26	0.80

Fig 3. Comparison of the ROC curves of plasma IGF-I, serum IGFBP-3, and serum leptin (both in women and in men).

The simple inspection of the graph followed by evaluation of sensitivity and specificity of each test showed that plasma IGF-I is the best test, with the best sensitivity and specificity. Determination of serum IGFBP-3 showed acceptable performance but did not improve accuracy when measured with plasma IGF-I. The diagnostic accuracy of serum leptin measurement in both women and men was poor.

The LR is the likelihood that a given test result would be expected in a patient with the target disorder compared with the likelihood that the same result would be expected in a patient without the target disorder. It is used to assess how good a diagnostic test is and to help in selecting an appropriate diagnostic test or sequence of tests.[25] Application of LR^+ and LR^- analysis to the studied parameters objectively showed that plasma IGF-I is clearly superior to serum IGFBP-3 measurement for monitoring patients with postsurgical acromegaly and that serum leptin determination shows poor performance in distinguishing between patients with active acromegaly and healthly subjects.

In conclusion, application of the ROC curve method and LR ratios in the evaluation and comparison of the tests currently used in the management of acromegalic patients, and in serum leptin measurement as a marker of GH effect on body composition, showed that determination of age-adjusted plasma IGF-I levels is the best test for monitoring treatment success of acromegalic patients. Serum IGFBP-3 measurement, which has less age dependence, has a diagnostic value but does not improve effectiveness of plasma IGF-I when measured together. Serum leptin determination in both women and men showed poor performance in monitoring these patients.

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