Isotopic and Nonisotopic Assays for Measuring Somatotropin Compared: Re-Evaluation of Cutoff Value in Provocative Tests

Giuseppe Banfi, Marcello Marinelli, Erminia Casari, Michelangelo Murone, and Pierangelo Bonini

Measurement of human growth hormone (hGH; somatotropin) concentrations in serum after provocative tests is crucial for diagnosing deficiencies in production of this hormone. Serum hGH can be measured by various immunoassays, isotopic and nonisotopic, with monoclonal or polyclonal antibodies: a cutoff value of 10 μg/L after provocative testing is usually used to distinguish normal from hGH-deficient children. Previous studies demonstrated discrepancies in hGH measurement by different radioisotopic immunoassays. Here we evaluated the responses of six different commercial assays, radioisotopic and nonisotopic, with monoclonal or polyclonal antibodies in a series of 16 provocative tests (stimulation with clonidine) in short children. A wide range of discrepant values was obtained with the different kits. A cutoff of 10 μg/L produced discordance of diagnosis among assays for two children, whereas complete agreement was reached for a cutoff value of 7 μg/L. Parallelism tests performed with hGH international standard, pure recombinant hGH, and a serum with high hGH content suggest that heterogeneity of the antibodies used by the manufacturers, even among monoclonal antibodies, is the main source of discordant results. Cutoff values and reference values must be established separately for each method proposed for routine use.

Additional Keyphrases: analytical error · intermethod comparison · clonidine stimulation · monoclonal antibodies

Measurement of somatotropin (human growth hormone; hGH) concentrations in serum after a provocative test is crucial for diagnosing deficiencies and (or) disturbances in the production of this hormone (1).¹

hGH in serum can be measured by various methods, isotopic or nonisotopic. Radioimmunoassay (RIA), immunoradiometric (IRMa), and immunoenzymometric assay (IEMA) are commercially available for routine laboratory use. RIAs often involve use of polyclonal antibodies, whereas IRMAs and IEMAs are based on monoclonal ones (2). Some studies have reported disagreement among different methods (2–6), ascribing the origin of discordant results to differences among antibodies (monoclonal/polyclonal), heterogeneity and stochastic production of antibodies, different tracers (isotopic vs nonisotopic), and different international standardization (2, 6, 7). The use of different standard materials (2) and different molecular forms of hGH (molecular mass 20 kDa, the so-called 20K hGH, and molecular mass 22 kDa, “22K hGH,” as well as monomeric or dimeric forms) (2, 8) increases the complexity of hGH quantification.

In provocative tests, performed on short children, a crucial point is the definition of a cutoff value to distinguish between normal and hGH-deficient persons. This distinction may be of the utmost importance before considering long-term therapy with hGH. Various authors have set the cutoff value at 5 to 10 μg/L (9–11), the latter value being the most widely accepted in clinical endocrinology. The use of provocative tests appears to be a simple and useful way to diagnose hGH deficiency, in contrast to the alternative procedures (24-h measurements of pulsatile, spontaneous hGH release into serum and urine), which present technical, analytical, and interpretative problems.

Here we present a study of hGH quantification involving six commercial kits, radioisotopic and nonisotopic, based on competitive or sandwich methodology and monoclonal or polyclonal antibodies.

Materials and Methods

Immunoassays

The kits for assaying serum hGH were obtained from Hybritech (La Jolla, CA), Nichols Institute (San Juan Capistrano, CA), Pharmacia (Bromma, Sweden), Radioassay Systems Laboratories (RSL; Carson, CA), Eurogenetics (Tessenderlo, Belgium), and Medix Biotech (Foster City, CA). The Hybritech and Nichols kits are IRMAs, with two mouse monoclonal antibodies directed against two different hGH epitopes. The Pharmacia kit pairs one antibody derived from rabbit and one derived from sheep. The RSL kit is a classical RIA, involving competition methodology and polyclonal antibodies raised in rabbits. The Eurogenetics and Medix Biotech kits, IRMAs, make use of two monoclonal antibodies directed against different hGH epitopes.

All kits were used strictly according to the manufacturers' instructions. For quality control for all the kits, we used Lyphocheck Immunoassay Control Serum (BioRad, Anaheim, CA). The intra- and interassay CVs for all the kits used were <10%.

Patients' Sera

The analyzed 96 sera obtained from provocative tests (clonidine 150 μg/m² of body surface orally) (12) in 16 short children (~1.5 to −3 SD for height when compared with normal growth tables; ages 6−13 years, 10 boys, six

¹ Nonstandard abbreviations: hGH, somatotropin (human growth hormone); IRMA, immunoradiometric assay; IEMA, immunoenzymometric assay; RSL, Radioassay Systems Laboratories; and IRP WHO, International Reference Preparation, World Health Organization.

Received June 29, 1990; accepted November 30, 1990.
girls) diagnosed by pediatric endocrinologists at our Institute. Blood samples were collected in plain evacuated collection tubes (Vacutainer Tubes; Becton Dickinson, Rutherford, NJ) 30 min before and 0, 30, 60, 90, and 120 min after clonidine administration, and then centrifuged at 1500 × g for 10 min at 4 °C. The resulting serum was divided into six aliquots and stored frozen at −20 °C until assayed.

Parallelism Tests

Three parallelism tests were performed on all the kits evaluated. In the first, we used the World Health Organization First International Reference Preparation (1st IRP WHO 80/505), extracted from human pituitaries, supplied courtesy of Dr. G. B. Romelli, Sclavo, Milan, Italy. A secondary biosynthetic GH standard, purified by high-performance liquid chromatography (a gift of Mr. F. Rosseel, Hybritech, Florence, Italy; lot no. RS0066), was used for the second parallelism test. For the third test, we used serum from a seven-year-old girl with an hGH-producing pituitary adenoma, whose hGH concentration was ~200 μg/L. These hGH standards were reconstituted according to the suppliers’ instructions, and then diluted with phosphate-buffered saline (10 mmol/L, pH 7.5), containing bovine serum albumin, 5 g/L, and stored frozen until assayed.

Data Analysis

An MDA 312 gamma counter (Kontron, Geneva, Switzerland) was used to measure the radioactivity of the RIA and IRMA standards and unknowns, whereas the results of the IEMA methods were quantified with a microtiter plate reader (Eurogenetics). Linear–linear and logit–log approaches were used to extrapolate results from standard curves. Linear-regression statistical analysis and analysis of variance were applied to parallelism tests (13). A computer-assisted study of results of 300 provocative tests (stimulation with arginine or clonidine) of short children to evaluate the cutoff value for hGH as measured with our routinely used kit (RSL) was carried out with use of Application System software—a fourth-generation, user-friendly computer program that can provide reports and graphics of nonparametric and parametric statistics—and an IBM 3990 computer, before the start of the present study.

Results

A wide range of hGH values was obtained by the various methods. Discrepancies among methods are evident, particularly in the high range of values after stimulation. RSL RIA (polyclonal) results are two- to threefold higher than those with the Eurogenetics IEMA (monoclonal); RSL RIA and Nichols IRMA present the highest values. An important discord between the two methods is the range of hGH values obtained by the two manufacturers' kits in the case of the girl with the pituitary adenoma.

![Fig. 1. hGH curves obtained with the six kits, after the clonidine stimulation test, in a six-year-old short boy (left) and in a 10-year-old girl affected by Turner's syndrome (right). All the kits, with 10 μg/L as the cutoff value, support diagnoses of normal production and release of pituitary hGH in the boy and hGH deficiency in the girl.](image-url)
IRMA with monoclonal antibodies is the fact that the Nichols assay values are consistently higher than those with the Hybritech.

In 12 children evaluated with a cutoff value of 10 μg/L, the discrepancies did not affect the diagnosis of normal hGH secretion and, consequently, of constitutional short stature. An example of these results is shown in Figure 1 (left). Moreover, in two cases, lack of hGH release by the pituitary gland after stimulation is clearly shown by all six methods, which give, however, different absolute values. These data allowed a diagnosis of isolated hGH deficiency and of hGH deficiency in Turner’s syndrome, the latter illustrated in Figure 1 (right). Conversely, in two cases the discrepancies among kits affected diagnosis and prognosis. A child with puberty stage G1 P1 A1 (Tanner method) and hyposomy could be diagnosed as having hGH deficiency by four kits, dubious by one (other provocative tests or a 24-h pulsatile test is necessary), and normal by another (Figure 2). The second hyposomic case, puberty stage G2 P2, could be diagnosed as hGH-deficient by four kits and as normal by the other two.

The first parallelism tests were performed by using IRP WHO 80/505. The results obtained by various kits are illustrated in Figure 3. We emphasize dispersion of the results at the highest hGH values. This dispersion corresponds to the major discrepancies also exhibited by provocative tests. The second parallelism test was performed with use of pure hGH; the results are presented in Figure 3. The third parallelism test involved assaying dilutions of serum with a high content of hormone. The trend of results obtained in the second and third parallelism tests is similar to that of the first; the variation of slopes shown by the different kits (descending for Pharmacia, Medix, and Nichols; ascending for RSL, Hybritech, and Eurogenetics) is not significant: P > 0.05.

Discussion

Discrepancies in hGH measurement by different immunoassays were reported in previous studies (2–6). This variability, which could induce serious consequences in the differential diagnosis of short children, has been attributed to matrix effects, different standard materials, and differences in specificity among antibodies. Reiter et al. (3) confirmed the results of Blethen and Chasalow (4) and Levin et al. (5), who found low values of hGH in serum by IRMA. IRMAs involve use of monoclonal antibodies directed to peculiar epitopes of the hGH molecule, and show greater specificity, but can
yield discrepant results when compared with RIAs involving monoclonal antibodies. In most cases, the values of monoclonal IRMAs are lower than those obtained by polyclonal RIAs. On the other hand, different IRMAs present monoclonal antibodies directed against different epitopes; in fact, a monoclonal IRMA (Nichols) gave results for serum hGH that were two- or threefold higher than those by the other monoclonal IRMA (Hybritech) (2, 15). These evident discordances are probably explained by the different specificities of antibodies for individual hGH epitopes: 20K hGH is not recognized by the Hybritech assay (2), and the dimeric form of hGH interferes with several kits but not with the Hybritech (8). In addition, matrix effects of the kits' standards are important: horse serum in the Nichols IRMA kit induces an overestimation (2, 14, 15).

Nonisotopic assays for hGH have been proposed (6, 11), involving sandwich methodology and monoclonal antibodies. Discrepancies have been confirmed by using these novel assays, which have a specific tracer activity different from that of isotopic ones (6). In previous studies, however, discordant hGH results observed by using various, but exclusively radioisotopic, commercial kits have been studied and sometimes explained only by using comparison tests (3–6) or by performing dilution tests with use of recombinant hGH (2). We performed parallelism tests with different materials (international standard, pure hGH, and serum with high hGH content) to ensure that the kits, although presenting discrepant absolute values, give the same performance for three diluted matrices. Indeed, each kit presented a peculiar but constant behavior in these experiments. Thus, matrix differences did not affect or only slightly affected the kits' performances.

Conversely, the relevance of heterogeneity of the antibodies must be emphasized. The commercial use of monoclonal antibodies against different hGH epitopes could be affected by stochastic and heteroclitic production (7) and by various forms of hormone (2, 8). The mix of carefully selected monoclonal antibodies could provide an elegant solution to the problem (11). The difficulties induced by heterogeneity could reduce the clinical impact of monoclonal IRMAs, because the increased specificity is accompanied by other interferences conducive to misclassifications and confusions. This could have potentially serious consequences for diagnosis, as was described for two of the 16 children examined.

At present, it is difficult to recommend a method of choice for measuring the "real" hGH, although the Hybritech IRMA kit seems to be influenced the least by extraneous variables (2) and the RIA RSL seems to be the nearest to bioassayable hGH (16).

It is important, however, that cutoff values adopted by the laboratory be well grounded in experience and clinically approved. We established the validity of a 10 \( \mu g/L \) cutoff value for our routinely used kit (RSL) by a computer-assisted study of 300 tests (arginine or clonidine provocative tests in children). In addition, the cutoff values and reference values of each commercially available kit must be clearly defined by the manufacturers. For example, the two curves with discrepant absolute hGH values could be brought into conformity by adopting a discriminant value of 7 \( \mu g/L \). However, all of the manufacturers indicated 10 \( \mu g/L \) as the decision value for hGH in provocative tests.

Another proposal could be that the international hGH standards be unified, thereby simplifying the manufacturers' production. In addition, standardization of monoclonal antibodies, with specificity studies and performance descriptions, can be made and acknowledged.

References