

ORIGINAL ARTICLE

The investigation of short stature: a survey of practice in Wales and suggested practical guidelines

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J Clin Pathol 2004;57:126–130. doi: 10.1136/jcp.2003.2238

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Accepted for publication
5 February 2003

Aim: To survey the investigation of short stature in Wales and suggest guidelines to improve practice.

Methods: Questionnaires were circulated to paediatricians and consultant clinical biochemists or consultant chemical pathologists at 13 Welsh hospitals where children with short stature are investigated.

Results: A 100% response was obtained from laboratory and clinical staff. Clinicians screened 1–50 patients each year (median, 10). Growth hormone (GH) deficiency was subsequently diagnosed in 0–30% (median, 10%) and GH treatment started in 30–100% (median, 100%) of patients. Five paediatricians and eight laboratories had written investigative protocols. Investigation of GH secretion was initiated in some centres before a complete clinical evaluation was carried out. Various screening tests for GH deficiency, including insulin-like growth factor 1 (IGF-1), random GH, and exercise tests were used. The clonidine stimulation test was used to assess the GH axis in most centres but eight different protocols were described. GH was measured in four Welsh laboratories using two automated immunoassay methods. However, nine different ranges of cutoff values for defining abnormal GH responses were quoted, and in three centres laboratories and paediatricians quoted different cutoffs.

Conclusion: This survey demonstrates the need for practical guidelines for the investigation and management of short stature in children, agreed by paediatricians and their laboratory colleagues. The guidelines should encompass the initial clinical investigation, assessment of the GH-IGF-1 axis (using standardised protocols), and provision for the transition to adult management. This article presents practical guidelines based on published points for good practice.

Recombinant growth hormone (GH) treatment is recommended for children with a clinical diagnosis of growth hormone deficiency (GHD) supported by auxological, biochemical, and radiological investigations.¹ However, for the child, dynamic tests of GH reserve are potentially dangerous² and unpleasant. For the clinician, a range of screening and diagnostic tests is available, none of which is recognised as the gold standard.³ The subject is also complicated by the heterogeneity of circulating human GH and the variation between assays used for diagnostic purposes.⁴

Here, we present a survey of the investigation of short stature in Wales and suggest practical guidelines for future use based on published points for good practice.

METHODS

Questionnaires were circulated to both the consultant paediatrician with an interest in childhood growth disorders and the consultant clinical biochemist or chemical pathologist at all 13 Welsh hospitals where children with short stature are referred for investigation. Performance was reviewed against GH Research Society consensus guidelines (2000) and other published articles.^{4–6}

RESULTS

All 13 laboratories and one paediatrician from each centre participated in the survey (table 1). Five of the 13 paediatricians and eight of the 13 laboratories had written protocols or guidelines for the investigation of short stature. Only two of 13 paediatricians and eight of 13 laboratories felt they had clearly defined written guidelines for interpreting the results of these tests. Centres screened between one and 50 patients each year (median, 10). GHD was subsequently diagnosed in 0–30% (median, 10%) of subjects after GH axis

testing. GH treatment was begun in 30–100% (median, 100%) of patients with GHD.

Clinical assessment and investigations done before assessment of the GH axis

In the initial clinical assessment of children with short stature all paediatricians assessed the growth rate for at least six months to demonstrate a height velocity lower than the 25th centile. They all examined the child for signs of other illnesses that could cause poor growth, such as hypothyroidism, Cushing disease, and coeliac disease. Nine of 13 paediatricians also assessed parental height, seven of 13 assessed the stage of puberty, and two of 13 investigated the social background of the child. Table 2 shows the laboratory and other investigations undertaken.

Screening tests for GHD

Various screening tests for GHD were used (table 3). Eight of 13 paediatricians used the measurement of serum insulin-like growth factor 1 (IGF-1), with or without serum IGF binding protein 3 (IGFBP3) or urine GH concentrations to screen for GHD. Three paediatricians proceeded directly to GH provocation tests and two others used measurement of random serum GH or post exercise serum GH concentrations to screen patients.

GH axis stimulation tests

Various tests for the assessment of the GH axis were used (table 4). The clonidine stimulation was the test of choice in

Abbreviations: GH, growth hormone; GHD, growth hormone deficiency; IGF-1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor binding protein 3

Table 1 Summary of investigation and management of patients with growth hormone deficiency (GHD)

Centre	Number of patients screened annually	% Screened patients with GHD	% Of patients with GHD treated with GH	% Of patients re-tested at the end of childhood growth
1	50	25	100	90
2	25	<15	100	Those with severe GHD and additional pituitary hormone deficiency
3	<20	<5	50	0
4	3–4	None so far	Not stated	Unknown
5	10	<10	100	0
6	40–50	5	90	Unknown
7	2–3	30	30	Leave this to regional centre
8	8–10	5	100	0
9	10–12	<1	<1	<2
10	2–3	Very few	100	Unknown
11	1–2	25	Probably all after discussion	Unknown
12	10–12	<10	100	0
13	4	7	100	0

most centres throughout Wales. Although eight different test protocols were provided, sampling at 0, 15, 30, 60, 90, 120, and 150 minutes was the most popular protocol provided by paediatricians ($n = 4$) and 0, 30, 60, 90, and 120 minutes ($n = 4$) the most popular protocol provided by the laboratories. One centre recommended prolonging the test for 180 minutes and another to only 90 minutes. The use of the more dangerous insulin and glucagon stimulation tests was largely restricted to the regional tertiary referral centre. However, some district general hospital laboratories reported that they were still in use; one centre, which used clonidine and arginine tests for older children, used the glucagon stress test for children under the age of 2–3 years. In all centres, provocative GH testing was carried out by experienced teams and patients were carefully monitored, although the number of patients investigated each year was very low in some centres.

Measurement of growth hormone

Serum GH concentrations were measured “in house” in four Welsh laboratories (table 5) using two different automated two site immunoassay methods. Both methods use a monoclonal and polyclonal antibody pair and are standardised against the pituitary derived World Health Organisation 80/505 standard. Interassay coefficients of variation between 4% and 11% were reported. All four laboratories subscribed to an external quality assurance scheme. The other nine laboratories referred samples to another laboratory that used one or other of these methods.

Interpretation of GH axis stimulation tests

Five of the 13 centres quoted a maximum serum GH concentration of 20 mU/litre or above following a GH stimulation test to exclude a diagnosis of GHD. In other centres, a range of other cutoff values was quoted as suggestive of GHD (table 5). In three centres, the laboratory and the paediatrician quoted different serum GH concentrations to exclude a diagnosis of GHD. Four laboratories that referred samples elsewhere for the measurement of GH did not use the cutoff value quoted by the analysing laboratory. One laboratory using the DPC Immulite method stated that serum GH concentrations below 15 mU/litre were indicative of GHD, whereas the other two laboratories using this assay quoted a cutoff value of 20 mU/litre. Some of the cutoff values of GH responses to provocation tests used to define GHD were taken from published literature ($n = 8^{7,8}$), national guidelines ($n = 1$), or guidelines of uncertain origin supplied by the laboratory ($n = 4$). Others were of long standing use in the unit concerned ($n = 1$) or were of unknown or unspecified origin ($n = 12$).

Re-evaluation of GH secretion at the end of growth

At the end of childhood growth there was little consistency between units regarding the re-testing of GH secretory reserve in those previously diagnosed as having GHD (table 1). Eight of 13 paediatricians reported that they had not arranged re-testing of GH secretion in their patients, whereas two reported arranging re-evaluation of GH secretion, one in 90% of patients previously diagnosed with GHD and another only in those with multiple hormone deficiency.

DISCUSSION

This survey of the clinical and laboratory investigation of short stature in Wales has revealed the need for practical guidelines that encompass the initial clinical investigation

Table 2 Investigations done before proceeding to assess the growth hormone axis

Test	Paediatricians
Thyroid function tests	12
Full blood count and film	13
Urea, electrolytes, and creatinine	11
Antibodies for coeliac disease	10
Karyotype (girls)	9
Bone age x ray	8
Liver function tests	6
Calcium and phosphate	5
Urine microscopy and culture	5
Urine glucose and protein	3
Glucose	3
Erythrocyte sedimentation rate	2
Immunoglobulins	2
C reactive protein, ferritin, chest x ray	1 each

Table 3 Screening tests for growth hormone (GH) deficiency

	Paediatricians	Laboratories
Random serum GH	1	7
Urine GH	2	2
Serum IGF-1	6	6
Serum IGFBP3	4	3
None	4 (3 proceeded direct to provocation tests)	1
Serum GH after Bovril	0	1
Serum GH after exercise	1	2

IGF-1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor binding protein 3.

Table 4 Growth hormone (GH) axis stimulation tests

	Paediatricians	Laboratories
Clonidine stimulation test	12	12
Arginine infusion followed by clonidine, or glucagon in children under 2–3 years	1	0
Insulin stress test (IST)	2 (1 referred for IST)	3
Glucagon stimulation test	1	5
GH releasing hormone stimulation test	4	1
Exercise test	1	3
None	1	1
Bovril/arginine	2	2

and assessment of the GH–IGF-1 axis in children suspected to have GHD. Provision also needs to be made for the longterm follow up of individuals with GHD once their growth is complete.

Twelve of the 13 paediatric units in Wales investigated children with short stature for GHD (one unit referred patients to the regional centre). Ideally, a thorough clinical evaluation should be carried out before proceeding to the evaluation of the GH–IGF-1 axis.⁹ However, investigations were often initiated despite incomplete initial clinical assessment. Eight of the 13 paediatricians measured serum IGF-1 with or without serum IGFBP3 or urine GH concentrations to screen for GHD. However, two used the measurement of random serum GH or post exercise GH concentrations and three proceeded straight to GH provocation testing without previous biochemical screening. Of those children investigated for GHD, the overall proportion eventually diagnosed with the condition was low (median, 10%), but varied greatly from one unit to another (range, 0–30%). This suggests more stringent previous clinical assessment and investigation of these individuals is required before the investigation of GH secretion is considered. GH provocation testing should be reserved for those children in whom there is a high degree of clinical suspicion of GHD.

There was poor agreement between laboratories and paediatricians as to which tests of GH secretion should be used. The clonidine stimulation test was the most popular GH provocation test, although various test protocols were submitted. The original description of the clonidine stimulation test¹⁰ describes the measurement of GH at 0, 30, 60, 90,

120, 150, and 180 minutes after the administration of clonidine, with peak GH concentrations observed between 60 and 120 minutes. One of the protocols supplied for the clonidine stimulation test continued for only 90 minutes, which might result in failure to detect the GH peak. Several protocols recommended sampling beyond 120 minutes. Prolonging the test is unpleasant for the patients and there is no evidence to suggest that this improves diagnostic performance. Some laboratories thought that insulin induced hypoglycaemia stimulation tests were still being performed, but the survey suggests that, in line with current guidelines,^{6, 11} this test was only used by paediatricians in the tertiary referral centre. In one district general hospital, the glucagon stimulation test was reported to be the test of choice in children aged less than 2–3 years. However, even this test has been associated with fatalities,² and should be limited to use in centres with regular experience of performing this investigation. Non-recommended tests such as the exercise test were still in use. However, it was reassuring that all centres reported careful supervision of GH provocation tests by experienced clinical staff.

There is debate regarding the usefulness of GH stimulation tests for the diagnosis of GHD,³ these tests having been abandoned in Australia, where auxological tests are used to select patients who require GH treatment. However, this is not the case in the UK, where the recent National Institute for Clinical Excellence guidelines for the use of GH in children with growth failure recommend that biochemical testing should be used to support a clinical diagnosis of GHD.¹ Our survey has demonstrated that a variety of GH

Table 5 Growth hormone (GH) cutoff values and methods

Centre	Paediatricians' GH cutoff	Laboratory GH cutoff	GH method
1	20 mU/l	20 mU/l	Nichols Advantage
2	20 mU/l	20 mU/l	DPC Immulite
3	20 mU/l	20 mU/l	DPC Immulite
4	>15 mU/l excludes GHD, <7 mU/l suggests GHD	>20 mU/l GHD unlikely, <10 mU/l suggests GHD	DPC Immulite*
5	Not stated	15 mU/l	DPC Immulite
6	15 mU/l	Not stated	Nichols Advantage*
7	20 mU/l	20 mU/l	DPC Immulite*
8	15–20	5 mU/l	Nichols Advantage*
9	Not used	Not used	N/A
10	Not stated	>20 mU/l excludes GHD, 15–20 mU/l need further tests, <15 mU/l suggests GHD	Nichols Advantage*
11	20 mU/l	20 mU/l	Nichols Advantage*
12	Not stated	Not stated	Nichols Advantage*
13	>15 mU/l excludes GHD, <7 mU/l suggests GHD	15–20 mU/l	Nichols Advantage*

Manufacturers suggested cutoff values for the interpretation of GH provocation tests: Nichols Advantage human GH, 7 ng/ml which is equivalent to 18.2 mU/l; DPC Immulite, laboratories should establish their own reference ranges.

*Samples referred for assay to another laboratory.
GHD, GH deficiency.

provocation tests were in use, with lack of general agreement as to how they should be performed.

Four laboratories in Wales measured GH using two commercial automated methods. All participated in an external quality assurance scheme and reported acceptable coefficients of variation for the performance of the assay. The assays in use are standardised against the pituitary derived IS 80/505. However, the GH Research Society recommends that assays are standardised against recombinant 22 kDa GH. Although the use of 22 kDa GH alone has limitations, in that it does not reflect the mixture of GH peptides in serum, it is unclear which other GH variants should be included for clinical relevance at the current time. Calibration using recombinant 22 kDa GH will allow calibration of the GH assay in mass units, which is not possible with the present standard, and should result in reduction in the bias differences between methods.^{4 12} Cooperation from the manufacturers of GH immunoassays is required to meet this guideline.

Both GH assays used by Welsh laboratories make use of a monoclonal/polyclonal antibody pair. The GH Research Society recommends the use of an assay for 22 kDa GH using monoclonal antibodies. However, there are concerns that this assay design would be too specific, and although well standardised, this could be at the expense of clinical relevance because these assays could fail to detect some biologically active forms of GH.⁴

“Growth hormone provocation testing should be reserved for those children in whom there is a high degree of clinical suspicion of growth hormone deficiency”

In response to GH provocation tests, several serum GH cutoff values were quoted to exclude or suggest GHD. The discrepancies of most concern were those between laboratories and clinicians at the same centres and between referring laboratories and the laboratories to which they sent samples for assay. Defining a normal cutoff value for use in these provocation tests is difficult because of the continuous spectrum of GH secretion in childhood.¹ For a child with clinical criteria for GHD, a peak GH concentration below 20 mU/litre¹ in a GH provocation test has traditionally been regarded to support a diagnosis of GHD. However, although the original radioimmunoassays used for the measurement of GH have been replaced by commercially available automated immunoassays, revised normative data to reflect the technological advances have not been adopted,¹³ with cutoffs derived from the earlier assays being transferred uncritically for use with the newer assays.³ The diagnosis of GHD is fraught with difficulties in interpreting the results of tests. Variation in the performance of GH assays in different laboratories is such that a serum sample with a mean GH concentration of 18 mU/litre would be reported by different UK laboratories to have a GH concentration between about 13 and 27 mU/litre.¹² Therefore, clinicians and laboratories should be aware of the GH assay method they are using, its limitations, and the manufacturer's recommended GH cutoff values for use with these new assays.

Given the difficulties of diagnosing GHD, it is of concern that in some centres some patients identified as having GHD were not then treated with GH. This raises questions regarding the rationale for undertaking these expensive and unpleasant investigations in the first place.

In summary, the survey has shown that various screening and provocation tests of the GH axis were in use with a paucity of clearly defined written protocols. There was a lack of agreement between paediatricians and their laboratory colleagues as to which tests were in use, how they should be

performed, and how their results should be interpreted. Although no gold standard tests are available, the survey has demonstrated the need for practical guidelines that encompass the initial clinical evaluation and assessment of the GH–IGF-1 axis, and which include a defined interpretation of the results of these tests.

RECOMMENDATIONS

General approach

There should be a protocol for the investigation and further management of GHD, agreed between the local paediatrician with an interest in childhood growth disorders and the clinical biochemistry laboratory. GHD is primarily a clinical diagnosis, supported by measurements of height, together with biochemical and radiological findings. Assessment of the GH–IGF-1 axis should only be undertaken when other causes of growth failure (for example, hypothyroidism, chronic systemic disease, Turner syndrome, and skeletal disorders) have been excluded by careful history taking, clinical examination, and initial investigations. Assessment of the GH–IGF-1 axis requires GH provocation testing. The measurement of serum IGF-1 or IGFBP3 concentrations may also be helpful.

Initial clinical assessment and investigations

If there is clinical concern about a child's short stature or height velocity,⁹ and the bone age is significantly delayed compared with chronological age, the following initial laboratory tests are recommended:

- Blood sample for the measurement of the erythrocyte sedimentation rate or C reactive protein, full blood count and film, and concentrations of glucose, urea, creatinine, electrolytes, calcium, phosphate, thyroid stimulating hormone, free T4, coeliac disease antibodies, and in girls, assessment of the karyotype.
- Urine testing for protein and glucose.

IGF-1–IGFBP3 measurements

- Serum IGF-1 and/or IGFBP3 results should be interpreted against bone age using age and sex related reference ranges.⁹
- Decreased concentrations of IGF-1 and/or IGFBP3 strongly suggest an abnormality in the GH axis if other causes (such as poor nutrition and liver disease) have been excluded.⁹
- IGF-1 and IGFBP3 results within the reference range can occur in children with GHD.⁹

GH provocation testing

- In suspected isolated GH deficiency, two GH provocation tests are recommended.¹ GHD should only be diagnosed if both tests demonstrate inadequate GH responses. The tests should be performed sequentially. The second test is only required if there is an inadequate GH response in the first. Evaluation of other aspects of pituitary function should be undertaken as clinically indicated.
- In children with defined central nervous system pathology, history of irradiation, multiple pituitary hormone deficiency, or a genetic defect affecting the GH axis, one GH provocation test will suffice.¹
- Clonidine, arginine, glucagon, or insulin are suggested provocative agents and should be used after an overnight fast in a well standardised protocol.⁹ Insulin induced hypoglycaemia should not be used in children aged less

than 5 years, in whom the glucagon test may be more appropriate.

- Clonidine (0.15 mg/m²) should be given orally, with blood samples for GH measurement collected at 0, 30, 60, 90, and 120 minutes.¹⁰ The patient should be monitored for possible hypotension.
- Arginine HCl (0.5 g/kg body weight, maximum 30 g) should be infused intravenously (10% arginine HCl in 0.9% NaCl at a constant rate over 30 minutes). Intravenous patency should be frequently assessed and there should be limited movement of the patient during the infusion. Blood samples for GH measurement should be collected at -30, 0, 30, 60, 90, and 120 minutes. As a precaution, an antihistamine and adrenaline should be available for treatment of potential allergic reactions to arginine. Importantly, arginine should not be used in patients with electrolyte or acid base disturbance, uraemia, diabetes, or in those with renal or liver disease.
- Great care should be exercised when performing insulin induced hypoglycaemia or glucagon stimulation tests. These tests require very careful supervision and should only be undertaken by experienced staff working in a specialist unit where these tests are frequently undertaken. Given the risks of tests involving hypoglycaemia, it is recommended that in non-specialist units clonidine and arginine tests should be used to test for GHD.
- Sex steroid priming is not routinely recommended given the absence of a consensus on whether it is necessary.

Analytical considerations

- The definition of a normal response remains arbitrary because there is a continuous spectrum of GH secretion in childhood. In a child with clinical criteria of GHD, peak GH concentrations below 20 mU/litre have traditionally been used to support the diagnosis.¹ However this value will vary depending on the GH immunoassay used. Clinicians and laboratories should be aware of GH assay methods in use, their limitations, and the cutoff values.
- Laboratories referring samples elsewhere should quote the same cutoff limits as the laboratory performing the assays.
- Each laboratory performing GH, IGF-1, and IGFBP3 assays should be Clinical Pathology Accreditation (UK) accredited and should ensure that appropriate internal quality control and external quality assessment procedures are in place.

Further management

- If a diagnosis of GHD is confirmed, the case should be discussed with a specialist in paediatric endocrinology and consideration given to testing other pituitary hormones. Magnetic resonance imaging of the brain, with particular attention to the hypothalamic-pituitary region, should be

carried out in any child diagnosed as having GHD, to exclude the possibility of a tumour.¹

- Treatment with recombinant GH is recommended if GHD is confirmed.¹ GH treatment should, in all circumstances, be initiated and monitored by a paediatrician with special expertise in the management of children with GH disorders.¹ Continuation of treatment can be maintained under an agreed shared care protocol with a general practitioner.
- After attaining adult height, re-testing of the GH-IGF-1 axis should be undertaken after discussion with an adult physician with expertise in endocrinology, because GHD may persist.¹

ACKNOWLEDGEMENTS

We would like to thank the members of the All Wales Clinical Biochemistry Audit Group for advice and support, and the paediatricians, clinical biochemists, and chemical pathologists who completed the questionnaires.

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This article was prepared at the invitation of the Chemical Pathology Best Practice Committee of the Association of Clinical Pathologists.

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