UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

TESE DE DOUTORADO

ESTUDOS PROSPECTIVOS NO DIAGNÓSTICO DIFERENCIAL DA BAIXA ESTATURA E DA DEFICIÊNCIA DE HORMÔNIO DE CRESCIMENTO EM CRIANÇAS

LEILA PEDROSO DE PAULA

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Aos meus pais
Por todos estes anos de dedicação
Ao meu esposo
Pelo amor que aumenta a cada dia
Aos meus filhos
Pela renovada inspiração de vida.

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LISTA DE ABREVIATURAS

ALS: Subunidade Ácido-Lábil

ASC: Área Sob a Curva (AUC)

BE: Baixa Estatura (SS- short stature)

BEC: Baixa Estatura Constitucional (CSS- constitutional SS)

BEF: Baixa Estatura Familiar (FSS- Familial SS)

BEVN: Baixa Estatura Variante da Normalidade (NVSS- Normal Variant SS)

DC: Doença Celíaca (CD- Celiac Disease)

DP: Desvio Padrão (SD- standard deviation)

FSH: Hormônio Folículo-Estimulante

GH: Hormônio de Crescimento

GH Clo: teste de estímulo do GH pela Clonidina

GHRH: Hormônio Liberador do Hormônio do Crescimento

GHRPs: GH *Releasing Peptides* ou Peptídeos Liberadores do Hormônio do Crescimento

GnRH: Hormônio Liberador das Gonadotrofinas

HipoIns: Teste de estímulo do GH pela Insulina

IGF-1: Fator de Crescimento Insulina-Símile Número 1

IGF-2: Fator de Crescimento Insulina-Símile Número 2

IGFBP-1: Proteína Ligadora Número 1 do IGF-1

IGFBP-2: Proteína Ligadora Número 2 do IGF-1

IGFBP-3: Proteína Ligadora Número 3 do IGF-1

IGFBP-4: Proteína Ligadora Número 4 do IGF-1

IGFBP-5: Proteína Ligadora Número 5 do IGF-1

IGFBP-6: Proteína Ligadora Número 6 do IGF-1

IGFBP-rPs: Proteínas Correlatas de IGFBPs

KDa: kilodaltons

LH: Hormônio Luteinizante

Priming: Administração prévia de agente hormonal destinado a estimular resposta do GH ao teste de estímulo farmacológico

RCIU: Retardo do Crescimento Intra-Uterino (IGR- Intrauterine Growth Retardation)

RNA: Ácido Ribonucleico

ROC: *Receiver Operating Characteristics*

SNC: Sistema Nervoso Central

SOCS: *GH inducible suppressors of citokine signaling* ou
citoquinas que provocam insensibilidade ao GH

SPSS: *Statistical Package for the Social Sciences*

ST: Síndrome de Turner (TS- Turner Syndrome)

T4: Tiroxina

TSH: Hormônio Tireo-Estimulante

VC: Velocidade de Crescimento

VSG: Velocidade de Sedimentação Globular (ESG)

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INTRODUÇÃO

O crescimento é um processo complexo e multifatorial, determinado por fatores genéticos e também por influência do meio - incluindo aspectos nutricionais, psicológicos e ambientais - aliados a um conjunto de hormônios secretados harmonicamente e a uma boa saúde global. Apesar de ser multifatorial, o crescimento de uma criança usualmente ocorre de forma previsível e qualquer alteração na estatura ou velocidade do crescimento (VC) pode significar a existência de uma doença associada. Assim, no sentido de discutirmos a avaliação diagnóstica das causas da baixa estatura (BE) e da deficiência do hormônio do crescimento, é preciso que revisemos o eixo GH-IGFs (hormônio do crescimento e fatores de crescimento semelhantes à insulina), o crescimento normal nas diferentes idades, as causas de BE e a possível influência do eixo GH-IGF-1 nestas causas.

O GH, assim como os IGFs e suas proteínas carreadoras (BPs) formam a base do eixo responsável pela atuação do GH nos órgãos-alvo. Esse complexo eixo GH-IGFs atua em todas as fases do crescimento, sofrendo influência de outros hormônios e também de fatores externos (1, 2).

O entendimento deste complexo eixo hormonal, desde a embriogênese até sua regulação fisiológica, nos permite uma melhor compreensão dos processos normais e patológicos envolvidos com o crescimento. No sentido de introduzirmos os artigos que

compõem esta tese de doutorado, apresentamos a seguir uma revisão que procura compilar os principais aspectos atuais referentes ao eixo GH-IGFs e o crescimento.

1.Eixo GH-IGF:

Estudos recentes e, principalmente, a criação de inúmeros modelos animais com deleções de vários genes do sistema IGF, revelaram sua alta complexidade. Atualmente acredita-se que o crescimento seja mediado principalmente pela ação autócrina/parácrina do IGF-1 livre produzido localmente (na placa de crescimento, por exemplo) após estímulo pelo GH, porém com algum efeito direto do GH e um mínimo efeito do IGF-1 circulante, de origem hepática. Este IGF-1 circulante parece ter um maior efeito no *feed back* negativo da produção e secreção de GH pela hipófise. Além de sofrer influência dos níveis séricos de IGF-1, a secreção de GH é regulada pela inter-relação dos níveis de GHRH, Somatostatina, GHRPs (GH *Releasing Peptides*), Grelina, dentre outros. A hipófise secreta 70-75% do GH sob a forma ativa de 22kDa, cerca de 5-10% na forma de 20KDa, uma proteína de 176 KDa, com baixa afinidade pelo receptor de GH. Outras formas podem ser GH-desamino (8-13%) e uma variedade de oligômeros do GH, cujas bioatividades não estão bem estabelecidas (3). A concentração de IGF-1 varia com a idade, aumentando gradualmente durante a infância, atingindo pico em média aos 13 a 15 anos nos meninos e de 11 a 13 anos nas meninas. O IGF-1 aumenta gradativamente conforme o estágio puberal, alcançando pico no estágio IV da puberdade em meninos e III-IV em meninas (4,5). Os níveis de IGF-1 livre são

modulados também por um intricado sistema de proteínas carreadoras (IGFBPs), proteases e proteinas correlatas de IGFBPs (IGFBP-rPs) (6). Este sistema compreende 6 IGFBPs, onde a IGFBP-3 e, mais raramente, a IGFBP-5 podem ligar-se à subunidade ácido-lábil e ao IGF-1 formando o complexo ternário, de maior peso molecular 150Kda, o qual não atravessa o endotélio capilar. As IGFBPs aumentam a meia-vida das IGFs, inibindo sua proteólise e depuração e são responsáveis pelo transporte (IGFBP-3), além de modular a interação dos IGFs com seus receptores. A IGFBP-5 também é capaz de fixar as IGFs na matriz óssea aumentando seu armazenamento local e, aparentemente, as IGFBPs possuem também funções independentes de IGFs.

Os principais fatores implicados na regulação das IGFs são: nível de GH/ IGF-1, idade, estágio puberal, jejum e má-nutrição (reduzindo concentração de IGFBP3 e aumentando a de IGFBP1 e IGFBP2, reduzindo assim, a longo prazo, a meia-vida das IGFs, porém aumentando o transporte através do endotélio capilar e conseqüentemente aumentando o aporte de IGFs livres nos tecidos alvo), exercício (aumenta atividade da protease cálcio dependente o que aumenta o IGF livre no local e provoca efeito anabólico no tecido muscular) e outros hormônios como por exemplo os glicocorticóides (aumenta IGFBP-3 e diminui IGFBP-1 e IGFBP-2, reduzindo 60% da biodisponibilidade dos IGFs). Outro importante fator de regulação das IGFBPs é a existência de proteases específicas para IGFBPs, capazes de exercer diversos níveis de degradação destas proteínas, provocando desde uma leve redução da afinidade pelos

IGFs até impossibilidade total de ligação. Proteínas correlatas são proteínas inicialmente descobertas e caracterizadas em sistemas não envolvendo o eixo dos IGFs, mas com regiões semelhantes (1, 7).

Em ratos foi possível determinar a porcentagem de crescimento que é dependente de cada "passo do sistema", concluindo-se que 17% do crescimento é independente tanto do GH quanto do IGF-1, 14% dependente apenas do GH, 35% dependente apenas do IGF-1 e 34% dependente do GH e IGF-1 associados. Entretanto estas evidências são de estudos animais e ainda há poucos dados em humanos que comprovem com exatidão esta hipótese (1, 2).

2. Crescimento Normal e eixo GH-IGF

O período de vida intra uterina é uma fase de crescimento exponencial, sendo que já aos quatro meses de idade gestacional o feto desenvolve sua maior velocidade de crescimento, crescendo cerca de 11 cm/ mês. A partir daí se produz uma desaceleração que se prolonga até o momento do nascimento. O IGF-2 é importante no início da gestação, enquanto o IGF-1 parece influenciar os estágios mais tardios do desenvolvimento. A produção tecidual de IGF-1 com ação parácrina é crítica para o crescimento intra-uterino e sua regulação é quase totalmente GH independente e influenciada pelo estado nutricional do feto, principalmente pela disponibilidade de glicose e as conseqüentes mudanças na síntese de insulina fetal (8, 9). A placenta é origem de IGF-1 materno, o qual possui ação trófica no desenvolvimento fetal apesar de

não atravessar a barreira placentária. O IGF-1 materno aumenta o crescimento e a circulação placentária o que amplia o aporte nutricional ao feto e conseqüentemente aumenta a produção fetal de IGFs. A elevação da concentração de IGF-1 livre materno se deve também a um aumento de IGFBP-1 e IGFBP-2 e redução da IGFBP-3 por maior proteólise. Ratos mutantes, nulos para o receptor tipo IGF-1 nascem com 45% do peso normal, hipoplasia muscular, atraso de ossificação e pele translúcida. Ratos mutantes nulos para o gene de IGF-1 nascem com peso de 60% do normal e apresentam desenvolvimento pós-natal deficiente e infertilidade. Ratos nulos para o gene do IGF-2 também nascem com peso de 60% do normal, porém apresentam crescimento pós-natal normal (2).

Os níveis de IGF-1 e IGFBP-3 vão gradualmente se elevando a partir do primeiro/segundo ano de vida, na fase de crescimento considerada dependente de GH. O IGF-2 não varia significativamente com a idade. A nutrição permanece o mais importante regulador dos IGFs no início da vida.

Na infância o crescimento não é linear. Durante os três primeiros anos de vida extra-uterina, no período também denominado de primeira infância, a velocidade de crescimento é muito maior do que nos anos subseqüentes. No primeiro ano a criança cresce a uma velocidade média de 25 cm/ ano. Esta é uma etapa de risco, sensível a déficits nutricionais, infecções e outras doenças. A partir dos 3 até por volta dos 9 anos, na segunda infância, a VC é de 5 a 7 cm/ ano, alternando fases de crescimento rápido

com nenhum crescimento. Qualquer insulto, como uma doença aguda ou verminose, pode também interferir no ritmo do crescimento.

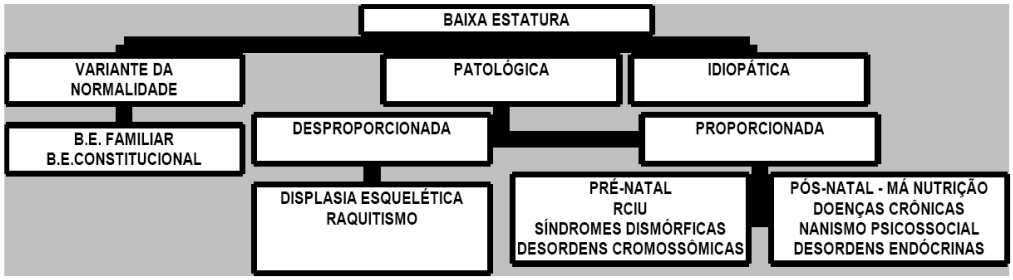
Entretanto, a maioria das crianças tem a capacidade de acelerar o crescimento no momento que a causa para a desaceleração prévia tenha desaparecido, caracterizando um crescimento compensatório para que a criança recupere seu tamanho normal ("*catch-up growth"*).

Por volta dos 12 anos nas meninas e 14 anos nos meninos, a VC aumenta, podendo passar para 10cm/ano ou mais. O crescimento na puberdade é determinado, em grande parte, pelo GH e os esteróides sexuais. Todos marcadores de maturação aparecem em média 2 anos mais cedo em meninas do que em meninos, como a maturação da idade óssea, a elevação dos níveis de IGF-1, IGFBP-3 e IGF-1 livre. Os níveis de IGF-1 e IGFBP-3 atingem pico no estágio IV de Tanner com declínio posterior. A retroalimentação negativa do GH é mais ativa na fase puberal em meninos, aumentando o padrão pulsátil, característico da puberdade (10, 11). Outro aspecto ainda a ser considerado é a dificuldade em interpretar os testes de estímulo do GH no período imediatamente peri puberal, uma vez que nesta fase do desenvolvimento os níveis de GH estão freqüentemente baixos (12). A secreção de GH aumenta fisiologicamente na puberdade, devido principalmente a uma maior amplitude dos pulsos do que a um número maior de pulsos (13, 14).

3. Desordens do Crescimento

O déficit de crescimento se caracteriza por estatura abaixo do 3º percentil ou abaixo de 2 desvios padrões para a média padronizada pela idade e sexo, ou VC abaixo do percentil 25. Como podemos observar no fluxograma abaixo, as desordens endócrinas são apenas algumas das diversas causas de déficit de crescimento, porém alterações do eixo GH-IGF-1 parecem estar implicadas, ao menos parcialmente, na etiopatogenia de causas consideradas não endócrinas. Apresentamos a seguir um breve resumo do papel do eixo GH-IGF-1 nestas situações clínicas.

Figura 1 - Diagnóstico Diferencial de Baixa Estatura



Adaptado da referência 15

Causas

Podemos reagrupar as diversas causas apresentadas na figura 1 em quatro grandes grupos, descritos abaixo:

1.**Variantes da Normalidade** Este é o grupo mais freqüente. Entretanto, classificar uma criança como portadora de BE que expressa a variação da normalidade exige que antes se exclua qualquer patologia que possa estar presente.

1.1.BE Constitucional - É um diagnóstico de exclusão. Os pacientes apresentam estatura abaixo do 3º percentil, peso e altura ao nascer normais, atraso de idade óssea, altura abaixo do canal de crescimento da altura alvo e possível atraso de desenvolvimento puberal. Na época esperada para a puberdade, por não terem atingido ainda o desenvolvimento puberal, estes pacientes possuem o padrão pulsátil de GH semelhante ao de crianças pré-púberes, ou seja, com amplitude baixa e respondem pouco aos testes de estímulo do GH. O IGF-1 e o IGFBP3 são baixos para a idade cronológica, mas não para idade óssea ou estágio puberal (16).

1.2. BE Familiar - Os pacientes apresentam peso e altura ao nascer normais, canal de crescimento compatível com altura alvo, sem atraso de idade óssea e velocidade de crescimento normal. Pode eventualmente estar relacionada com polimorfismos genéticos, produção inadequada de GH/IGF-1 ou resistência parcial ao GH.

2. Causas Patológicas Não Endócrinas

Relacionam-se a um grupo heterogêneo de patologias que podem ter como única manifestação a baixa estatura. Estas têm em comum VC anormal e atraso da idade óssea, com exceção das displasias ósseas e a maioria das síndromes genéticas.

2.1. Displasias esqueléticas – Baixa estatura com desproporção de segmentos corporais, aparentemente sem relação com o eixo GH-IGF-1, causada por anormalidades dos colágenos e outras proteínas que formam a matriz óssea.

2.2. Anormalidades cromossômicas e síndromes genéticas - Na sua maioria não apresentam alterações do eixo GH-IGF (exemplo: síndromes de Turner, Noonan, Silver Roussel).

2.3. Má nutrição - Infelizmente é uma causa muito freqüente de BE. Como característica principal os pacientes apresentam peso abaixo do esperado para a idade e menor ainda do que a altura esperada para a idade (idade-peso menor do que idade-estatura). Apresentam níveis elevados de GH e reduzidos de IGF-1. Deprivação alimentar de longo prazo reduz níveis de IGF-1 e eleva IGFBP-1 e IGFBP-2, sendo sugerido que este aumento de IGFBP-1 e IGFBP-2 iniba o *feed-back* negativo hipofisário na secreção de GH, provocando os níveis elevados de GH. Ocorre também redução dos níveis de IGFBP-3, especialmente por aumento da ação das proteases específicas para IGFBP-3. Este aumento de IGFBP-1 e 2 e redução de IGFBP-3 durante a má-nutrição reduz a meia-vida dos IGFs, porém tende a aumentar o transporte de IGFs através da parede endotelial o que aumenta a biodisponibilidade dos IGFs em nível tecidual, como uma forma de preservar o crescimento da melhor forma possível (6).

2.4. Doenças crônicas - Os mecanismos envolvidos no atraso do crescimento decorrente de doenças crônicas que podem estar relacionados ao eixo GH-IGFs podem depender de substratos insuficientes pelo jejum ou má-absorção, consumo excessivo de substratos em doenças como estados hiper catabólicos por infecção, doença cardíaca ou pulmonar importantes, e condições que aumentam a degradação muscular protéica

através da via da ubiquitina como acidose metabólica, uremia, queimaduras, sepse e outros. O jejum reduz a ligação do GH ao receptor enquanto a restrição protéica provoca defeito pós-receptor, ambos provocando resistência ao GH e reduzindo a expressão de IGF-1. Pode haver redução no RNA mensageiro do IGF-1 por jejum ou restrição protéica, não por diminuir a transcrição do gene do IGF-1 mas por retardar o "splicing" do pré-RNA mensageiro e prejudicar a estabilidade citoplasmática deste. Há alteração do perfil das proteínas ligadoras aumentando a depuração de IGF-1, pelas diferentes meias-vida dos IGFs ligados às diferentes proteínas. As citoquinas induzem SOCS (*GH inducible suppressors of citokine signaling)*, que provocam insensibilidade ao GH. Além disto ocorre resistência tecidual ao IGF-1 (17).

2.5. Nanismo Psicossocial - Pode estar associado à desnutrição ou distúrbios psiquiátricos. O GH pode estar diminuído, normal ou aumentado e o IGF1 diminuído. Parece haver um distúrbio hipotalâmico que interfere na secreção de GH. Em geral se identifica uma situação pessoal, familiar e/ou social que provoca grande alteração psicológica e/ou comportamental (18).

2.6. Restrição ao crescimento intra-uterino (RCIU) - Pode estar associado a síndromes genéticas, infecções intra-uterinas ou uso de drogas e/ou fármacos durante gestação. Sem atraso de idade óssea, sem atraso puberal, VC normal, aproximadamente 85 a 90% dos pacientes apresentam *catch up growth* nos primeiros anos de vida. Na RCIU o GH basal está elevado e a concentração de GH de 24 horas e o IGF-1 e IGFBP-

3 estão diminuídos (19).

3. Causas Endócrinas

Comparativamente às causas anteriores encontram-se entre as menos prevalentes, apesar de não menos importantes, especialmente pelas suas possibilidades terapêuticas.

3.1. Diabete Melito – quando cronicamente mal controlado, apresenta GH aumentado ou normal e níveis diminuídos de IGF1. Pacientes com diabete melito insulino-dependente possuem aumento nos níveis de IGFBP-1 e redução da IGFBP-3 (por aumento da ação de proteases) quando ainda não iniciaram o tratamento com insulina, talvez para tentar compensar o estado catabólico com o aumento da biodisponibilidade de IGF-1 (20).

3.2. Excesso de glicocorticóides endógenos ou exógenos - Glicocorticóides inibem o crescimento por uma série de fatores: alteração no perfil secretor do GH por estímulo do tônus somatostinérgico, alteração na atividade das IGFBPs, prejuízo na ação local das IGFs por redução de receptores e inibição direta da mitogênese dos condrócitos (6).

3.3. Hipotireoidismo congênito ou adquirido - A BE geralmente precede os outros sintomas, apresentando também atraso de idade óssea, piora do desempenho escolar e outros sinais e sintomas de hipotireoidismo. Tanto o hipo quanto o hipertireoidismo diminuem a síntese de GHRH e prejudicam o crescimento pós-natal

(21). Em relação ao T_3, sua ausência em células hipofisárias diminui a resposta do GH ao GHRH. No hipotireoidismo a secreção de GH está diminuída em resposta aos testes de estímulo fisiológicos e farmacológicos (22). O tratamento promove uma aceleração do crescimento e maturação esquelética.

3.4. Desordens do metabolismo do cálcio e da Vitamina D – Entre estas alterações incluímos as diversas formas de raquitismo ou os distúrbios decorrentes da má-absorção intestinal.

3.5. Deficiência de GH - A deficiência de GH pode ser congênita, incluindo anomalias do desenvolvimento do hipotálamo e da hipófise e anomalias da síntese e secreção de GH, ou ainda adquirida, secundária a processos neoplásicos, traumatismos, infecções ou doenças infiltrativas que acometam estruturas hipotálamo-hipofisárias (9). Dentre as alterações congênitas, podem ocorrer mutações nos fatores de transcrição, no gene GHRH, no gene do receptor do GHRH ou do próprio gene do GH ou ainda defeitos de linha média. As mutações nos fatores de transcrição e demais diagnósticos genéticos da deficiência de GH estão sumarizados na tabela 1.

3.6. Neuro disfunção Secretória - A criança apresenta BE e baixa velocidade de crescimento, resposta normal do GH aos testes de estímulo farmacológicos, mas na avaliação da secreção de 24h os níveis de GH são baixos, assim como a IGF-1 basal. Esses achados são mais comuns em crianças após radioterapia de crânio (15).

3.7. GH Bioinativo - Pode ser suspeitado em crianças com baixa estatura com GH imunorreativo (mensurável), mas de baixa biopotência. Estas alterações moleculares são raras, tendo sido descritos poucos casos efetivamente comprovados (23). O diagnóstico definitivo só pode ser estabelecido através de estudos moleculares. Na prática clínica pode ser presumido, porém, em uma criança com auxologia compatível com deficiência de GH, exclusão de outras causas, níveis elevados de GH basal ou responsivo aos testes de estímulo, nível muito baixo de IGF-1 que se eleva no teste de geração de IGF-1 e que apresenta uma resposta marcada no crescimento com o uso de GH exógeno (15).

3.8. Insensibilidade ao GH – Ocorre em crianças com fenótipo de deficiência de GH, mas com níveis de GH normais ou elevados, e diminuída produção de IGF-1 (deficientes de IGF-1). Classificada como insensibilidade ao GH primária ou nanismo de Laron, defeitos hereditários, como anormalidades do receptor de GH, anormalidades pós-receptor do sinal de transdução do GH, defeitos de biossíntese do IGF-1 e insensibilidade gênica à ação do GH (9, 22) Os níveis de IGF-1, IGF-2 e IGFBP-3 são baixos e as GHBPs são na maioria das vezes indetectáveis (23). Os pacientes não respondem ao GH exógeno, mas respondem á administração de IGF-1 (25).

3.9. Deleção do gene do IGF-1 - Existe apenas um único caso relatado no qual se observou intenso déficit de crescimento pré e pós-natal (- 6,9 DP) com microcefalia, surdez neurossensorial e retardo mental. O GH basal variava de 2,2 a 171 e o GH pós

estímulo com clonidina atingiu 98mcg/l, a IGFBP-3 era normal e o IGF-1 indetectável. Análise genética evidenciou deleção dos éxons 4 e 5 do gene IGF-1 e seus pais eram heterozigotos, com estatura no limite inferior da normalidade e IGF-1 discretamente reduzido.

4- Baixa Estatura Idiopática Também se trata de uma classificação de exclusão. Caracteriza-se por estatura abaixo do percentil 3, estatura ao nascer normal, proporções corporais normais e maturação sexual adequada. Sem evidência de má-nutrição, desordem psicossocial, doença crônica ou endocrinopatia. Apresenta VC de 4 a 5 cm/ano, idade óssea normal ou atrasada, mas idade cronológica maior ou igual a idade óssea que é maior que a idade estatural. A etiologia não foi ainda completamente elucidada, porém existem algumas evidências que demonstram: produção inadequada ou resistência parcial a GH (polimorfismos do gene GH-1, mutações heterozigotas no gene do receptor de GH), menor biodisponibilidade do IGF-1 por elevação da IGFBP-1 e altos níveis teciduais de IGFBP-3 ou outros polimorfismos genéticos (16).

4. Avaliação Diagnóstica

Conforme podemos observar, frente a uma criança portadora de desordem do crescimento é necessário e fundamental seguir uma avaliação sistematizada na investigação da causa deste distúrbio. Neste sentido apresentamos a seguir dois artigos originais envolvendo aspectos diagnósticos e etiológicos da BE relatando achados obtidos a partir de estudo prospectivo de uma coorte de pacientes com BE atendidos em

ambulatório organizado especificamente para tal no Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre.

O primeiro artigo enfatiza a importância de uma investigação basal clínica prévia, analisa as causas mais prevalentes de baixa estatura (BE) assim como a utilidade dos exames laboratoriais basais nesta investigação. Como já discutido neste artigo clínico, é impressionante a escassez e até mesmo a ausência de dados publicados com relatos atualizados sobre a prevalência das causas de BE e sua avaliação em outros centros. Aparentemente, esta falta de informações clínicas não é decorrente de haver uma padronização na sistemática da investigação destes pacientes, pois um estudo de 2004 questionou a prática de 13 hospitais do sul da Inglaterra, especializados na avaliação de pacientes com BE (26). O surpreendente é que apesar dos especialistas destes hospitais diagnosticarem e tratarem até 12 casos de deficiência de GH ao ano em cada serviço, em apenas 9 dos 13 centros os pais destas crianças tiveram sua estatura aferida ou o estágio puberal da criança havia sido avaliado. Com relação à avaliação laboratorial, o hemograma era o único exame que era consenso em todos os serviços e apenas 8 centros submetiam os pacientes a um Raio X de mão e punho para idade óssea. Além disto, existiam 8 diferentes protocolos para a realização do teste da clonidina e alguns centros avaliavam o eixo GH-IGF-1 apenas pela medida do GH basal!

O segundo artigo compara diferentes testes diagnósticos, assim como define o ponto de corte com maior acurácia de cada um deles no diagnóstico da deficiência de

GH em crianças com BE. Baseado nos dados encontrados na análise desta Coorte com 851 pacientes com BE e na revisão da literatura, após os dois artigos, apresentamos nossa sugestão no que se refere à padronização na investigação de pacientes com BE.

Tabela 1. Genes associados com a deficiência isolada ou combinada de GH

Gene	Fenótipo humano	Herança
Hesx1/HESX1, *homeobox gene expression in embrionic stem cells*	Variável: displasia septo-óptica (defeito frontal, ocular e hipofisário), deficiência combinada de hormônios hipofisários, deficiência isolada de GH com ectopia de neuro-hipófise	Dominante ou Recessiva
Lhx3/LHX3 *LIM-homeobox*	Deficiência de GH, TSH, LH, FSH, com hipoplasia hipofisária. Sem deficiência de ACTH. Cervical curta e rígida, com dificuldade de rotação	Recessiva
Lhx4/LHX4 *LIM-homeobox*	Deficiência de GH, TSH, ACTH, persistência do conduto crânio-faríngeo e anormalidade de tonsila cerebelar	Dominante
Prop1/PROP1	Deficiência de GH, TSH, LH, FSH (variável) e prolactina. Deficiência de ACTH. Hipófise aumentada (tumor-*like*), com involução tardia	Recessiva
*Pit1/POU1F1 (*PIT1) (POU-family)	Deficiência de GH, TSH, prolactina variável. Hipófise anterior de tamanho variado (normal/hipoplásica)	Dominante ou Recessiva
Ghrhr/GHRHR	No Brasil, na cidade de Itabaianinha pacientes homozigotos para mutação no gene do receptor do GHRH apresentam baixa estatura extrema e não respondem aos testes de estímulo do GH	Recessiva
	Deficiência de GH Tipo 1B com hipoplasia de hipófise anterior - fenótipo heterogêneo diminuição da síntese ou bioatividade GH.	Recessiva
Gh-1/GH1	Deficiência de GH **GHD 1 A**- retardo crescimento no 1º ano pode desenvolver anticorpos anti-GH **GHD II:** molécula mutada com disposição espacial alterada. **GHD III:**Não há alteração do gene GH. É uma doença ligada ao X, associada a hipo ou agamaglobulinemia e deficiência de GH	Recessiva (tipo 1A, 1B) Dominante (tipo II) Ligada ao X (tipo III)

Adaptado das referências 27 e 28.

Tabela 2. Exames recomendados na investigação da BE.

<table>
<tr><th colspan="2">EXAMES</th></tr>
<tr><th>Gerais para todos</th><th>Específicos + comuns</th></tr>
<tr><td>Hemograma</td><td rowspan="3">IGF-1
-abaixo do 3º percentil de altura ou
-baixa velocidade de crescimento</td></tr>
<tr><td>Glicemia</td></tr>
<tr><td>Potássio</td></tr>
<tr><td>Cálcio, fósforo, albumina</td><td rowspan="3">Cariótipo sangue periférico (bandas G)
-meninas abaixo do 3º percentil
-sem outra causa de BE identificada</td></tr>
<tr><td>Fosfatase alcalina</td></tr>
<tr><td>Aspartato aminotransferase</td></tr>
<tr><td>Creatinina, exame comum de urina</td><td rowspan="6">IgA e Anticorpos anti-Transglutaminase IgA
-se perda ou dificuldade de ganho de peso,
-anemia persistente,
-evidência metabólica de má-absorção ou
-qualquer sintoma gastrointestinal</td></tr>
<tr><td>Bicarbonato, pH urinário</td></tr>
<tr><td>Velocidade de hemossedimentação</td></tr>
<tr><td>TSH, T4</td></tr>
<tr><td>Exame Parasitológico de fezes</td></tr>
<tr><td>Raio X de mão e punho p/ idade óssea</td></tr>
<tr><th colspan="2">Específicos Incomuns</th></tr>
<tr><td colspan="2">Ecografia abdominal, pélvica ou cardíaca, Raio X de coluna e ossos longos,
17OH progesterona, Testes e demais dosagens hormonais</td></tr>
</table>

Adaptado das referências 29 e 30.

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ARTIGO ORIGINAL 1

Clinical and laboratory evaluation of 851 short-stature children referred to an endocrine outpatient clinic

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ABSTRACT

Slower growth may be the first and only sign of several chronic diseases and should be investigated to distinguish healthy children from those who are ill. The objective of this study was to show the importance of auxological and complementary baseline investigation, to validate a protocol for the evaluation of SS, and to quantify the prevalence of the final diagnosis of 851 children evaluated prospectively. All the patients were evaluated by a complete history and physical examination followed by X-rays for bone age determination and laboratory tests.

Referrals to our Center included 511 boys and 340 girls ($p<0,05$). More boys than girls (62.6% vs 42.6%, $p<0.001$) were of normal stature or variants of normality. On the other hand, the percentage of girls (56.6% vs 46.0% $p<0.001$) with any organic disease or genetic syndrome diagnosed before referral was significantly higher than that of boys. Anemia (25%), eosinophilia (36.4%) and helminthic diseases (20.1%) were prevalent. Despite the hight prevalence of suspicious tests (elevated ESR-17%, low bicarbonate-8%, hight TSH-10,6% and altered urinalysis-14.1%), we diagnosed only a few cases of celiac disease (n=2), renal tubular acidosis (n=3), primary hypothyroidism (n=19) and renal failure (n=4). Growth hormone deficiency was diagnosed in 52 children and Turner Syndrome in 43 girls. The reported stature, both of mothers and fathers, was on average 2.2 centimeters higher than the measured stature ($p<0,001$).

These data demonstrate that even in a specifically endocrine outpatient clinic, one must be aware of the most common causes of SS and recalls the importance of an adequate baseline evaluation before any endocrine test. Finally, the target height should always be calculated considering the measured, not the reported, stature of the parents.

Key Words: short stature, growth, Turner Syndrome, celiac disease, short stature causes, growth hormone deficiency, target height.

Introduction

By monitoring the growth of a child, the parents and attending physician can observe a slowdown of growth velocity. This may be the first sign of several disorders, congenital or acquired. Since growth is a marker of several disorders, the same importance must be given to evaluating patients of both sexes, although generally there is greater concern regarding the stature of boys (1).

Short stature (SS) may have many causes, and appropriate treatment and stature of the child in adult life depend on the right etiological diagnosis. Endocrine disorders are only some of the different causes of growth deficit, the most frequent being variants of normality such as familial SS and constitutional SS (3). However, classifying a child as having an SS that expresses variation of normality requires that several diseases be excluded in advance, and physical examination and laboratory tests must be used for this purpose (4, 5).

In this study we demonstrate the importance of the auxological and complementary baseline investigation in diagnosing the causes of SS and we quantified their prevalences in 851 children prospectively evaluated at a specific pediatric endocrine outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil. In these cases we also evaluate the usefulness of the complementary baseline investigation, establishing a protocol which tries to be appropriate to our reality as a tertiary care hospital in a developing country.

Patients and Methods

Design: Cohort study

Patients

Eight hundred and fifty-one children were evaluated for SS according to a pre-established protocol at an endocrine outpatient clinic at Hospital de Clínicas de Porto Alegre between 1994 and 2006. These children were referred from the Primary Care Units of public health system of the city of Porto Alegre RS Brasil, after a pediatric consultation, or else they were sent by pediatric specialities from the Hospital itself.

Initial Evaluation

All the children were evaluated according to a predetermined protocol (figure 2), by a complete history and physical examination. In the history we emphasize perinatal data, the prior existence of some disease, hospitalization or use of medication, neuropsychomotor development, any other complaint associated or not with SS, socioeconomic conditions, nutritional profile and family history. Besides the basic physical examination, a systematic, detailed evaluation was performed of the limbs and face looking for phenotypical alterations suggesting genetic disorders. The stature was measured using a Harpender stadiometer and plotted in the National Center for Health Statistics (NCHS) growth charts or the Centers for Disease Control and Prevention 2000 Growth charts improvements to the NCHS version. Height under the third centile was considered short stature. Span and body segments were measured and evaluated

according to the reference (6,7). Pubertal stage was evaluated and classified according to Marshall and Tanner (8,9), and testicular volume was evaluated by Prader orchidometer. The target height was calculated using the Tanner method, and the height of the parents was obtained by verbal information and then measured, thus obtaining the informed and measured target height.

The protocol was reviewed and approved by the Ethics in Research Committee at Hospital de Clínicas de Porto Alegre. After informed consent by the parents or legal guardian, the patients were submitted to initial evaluation with X-rays of the left hand and wrist for bone age, analyzed according to the Greulich-Pyle method (10) and to laboratory evaluation by tests analyzed mostly by modular equipment – Roche R: blood count (light absorbance method/impedance/flow cytometry), glycemia (colorimetric-oxidase enzymatic method), erythrocyte sedimentation rate (ESR-capillary kinetic photometry method), ferritin (electrochemical luminescence method), urea (enzymatic UV method) , creatinine (Jaffé method without deproteinization), sodium and potassium (ion selective electrode method), bicarbonate (indirect potentiometric method), calcium (o-cresolphthalein colorimetric method), phosphorus (UV –phosphomolybdate method), albumin (bromocresol green colorimetric method), alkaline phosphatase and gamma glutamyltransferase (colorimetric kinetic method), aspartate aminotransferase and alanine aminotransferase (UV kinetic method), thyroxine and TSH (electrochemoluminescence method, Urisys 2400 and UF 100i equipment), three

samples for stool examination for parasitic infection and fecal steatocrit. One hundred and twenty-five girls had a karyotype (peripheral blood, G cell-bands) corresponding to 36.7% of the total number of girls. When tests showed any abnormalities, the patients were submitted to a specific evaluation in order to establish a definitive diagnosis of the different associated disorders and followed to have their growth pattern observed. Median follow-up was 2,4 years (0- 12,8 years). After initial evalution and in suspected cases, growth hormone (GH) deficiency, alone or associated with hypopituitarism, was diagnosed using auxological criteria, a baseline measure of IGF-1, clonidine and insulin tolerance tests , tests to evaluate hypophyseal function and an sella turcica image (CT, MRI) (11, 12).

Statistical Analysis

The database was stored and analyzed using SPSS (*Statistical Package for the Social Sciences*) version 14.0. The quantitative variables were described by mean and standard deviation, or median and range of variation (maximum-minimum) in the case of asymmetry. The categorical variables were described by absolute and relative frequencies. The Student t test was used to compare the groups as to the quantitative variables of normal distribution. The Mann-Whitney test was applied for asymmetry. The level of significance adopted was 5%, and $p \leq 0.05$ values were considered stastistically significant.

Results

Analysis of the clinical and laboratory data of the initial evaluation shows that mean age at the first visit was 9.8 years. Referrals to our center included 511 boys and 340 girls ($p<0.05$), and considering as a null hypothesis that the percentage of SS diagnoses in both sexes should be similar, a tendency is perceived to refer more boys than girls. More boys than girls (62.6% vs 42.3%, $p<0.001$) were diagnosed as being of normal stature or as variants of normality. On the other hand, the percentage of girls (56.6% vs 46.0%, $p<0.01$) with any organic disease or genetic syndrome already diagnosed before referral was significantly greater than that of boys.

Basal clinic characteristics are described at table 1. The reported stature of the mothers was on the average 2.48 centimeters higher than their stature measured ($p<0.001$), and the reported stature of the parents was average 1.84 centimeter higher than their measured stature ($p<0.001$). Thus, if we were to use the information reported by the parents to calculate the target-height, we would have a significantly greater target height.

At the first visit, 39.8% of the parents mentioned that their children had a low weight associated with their SS, although in fact only 26 children (3%) presented a Z-score of weight to stature below 2 standard deviations. The most frequent diseases diagnosed before the first visit were genetic syndromes in girls and asthma or allergic rhinitis in boys.

Among the children born at term, 15% (80/539) had a birth weight of less than 2.5 Kg, and in 5.4% of all pregnancies the mothers had not been followed in antenatal care.

At physical examination, a high percentage of alterations was detected, a few highly suggestive of a given pathology such as, for instance, Turner syndrome stigmata, and other unspecific ones, the most prevalent being delayed puberty, alterations of the extremities or of the fascies, alterations at cardiac auscultation or of the genitalia. The measurement of disproportionate body segments caused a radiological investigation of long bones, spine and skull in 27 children and allowed the diagnosis of conditions ranging from epiphysiolysis of the femur to diseases such as rickets and osteodystrophies in 16 children (59%). Some of these children had clear phenotypic alterations, but others had discrete manifestations, and this diagnosis could only be performed by systematically measuring the body segments of all children.

As to bone age, 55.8% of the patients presented more than two standard deviations of retardation. Anemia (25%), eosinophilia (36.4%) and helminthic disease (20.1%) were also prevalent. In the baseline laboratory evaluation, there was a high index of small alterations of the liver transaminases, several times related to the use of medications, but only two cases of chronic hepatitis were diagnosed after complete investigation of the liver function. Despite the high prevalence of suspicious tests (elevated ERS, 17%, low bicarbonate 8% and altered urinalysis, 14.1%, we diagnosed

few cases of celiac disease (n=2), renal tubular acidosis (n=3) and renal failure (n=4). We observed elevated TSH levels in 10.6% of the children (90/851), mostly discretely above normal, and the diagnosis of primary hypothyroidism was confirmed in only 19 cases (2.8%).

Among the 851 patients, 9.17% (78/ 851) withdrew from the study, and were then excluded from the analysis of cause of SS, but not from the general characteristics. Patients with less than six months of follow up of growth velocity were not included, nor were those who are still undergoing baseline exams or following a specific diagnostic investigation. Thus, the total number of patients classified for the final diagnosis of SS is 670 (table 2).

As we can see, even when it is a tertiary hospital, our cases were mainly normal variant short stature (NVSS). However a non-negligible number of diagnoses of endocrine disorders, chronic diseases and genetic syndromes were established during the evaluation of growth deficiency.

Growth hormone (GH) deficiency, alone or associated with panhypopituitharism was diagnosed in fifty-two children (52/670; 7.8%). These patients presented auxological criteria, low or normal-low IGF-1, GH peak after clonidine and insuline stimulation tests lower than 5 ng/ml using a specific 22 Kda GH assay and variable sela turcica images (12). Among “other diagnoses” are classified patients with

idiopathic SS, those with psychosocial dwarfism (13), as well as the three cases of suspected biologically inactive GH.

The patients who presented altered baseline exams were submitted to further evaluation, and were often referred to the specialist responsible for this, such as the pediatric nephrologist, etc. In this sense it should be mentioned that children with an elevated ESR, and no other evident cause, altered fecal steatocrit or persistent difficulty in gaining weight, were referred to the gastropediatrics service, where they were submitted to digestive endoscopy with duodenal biopsy. This strategy was used in thirty-seven patients, and two were observed with complete atrophy of the villi, seven with inflammation, six of them without any degree of atrophy and one with partial atrophy of the villi.

Based on these approaches chronic disease could be diagnosed as the cause of SS in 43/670 (6.4%) children, they are listed in Table 2 and are similar to those observed in other series in the literature (14, 15, 16, 17, 18). The children classified as having SS resulting from recurrent helminthic disease could be considered short due to multiple needs, both for food and habits of environmental hygiene. For a few children, calcium metabolism disorders were considered the cause of chronic disease, but even a few children classified as having idiopathic SS, presented hypocalciuria that could only be reversed with elevated doses of calcium and calcitriol supplementation. Recent studies have correlated the capacity to absorb calcium with stature in children and

adolescents (19, 20), and haplotypes of the vitamin D receptor gene and stature difference in adults (21). These findings suggest a connection between calcium and vitamin D metabolism which can modify the stature of a child more often and subtly than the classical cases of rickets and hypoparathyroidism.

Although more than 80 children had low birth weight, restricted intrauterine growth without growth recovery was considered responsible for SS in only 29 (36%) of them. This finding results from the fact that in the others the diagnosis of other pathologies or associated genetic syndromes was established. Thus, these data support the strategy that, when a child had low birth weight and its growth did not catch up, we must rule out the presence of various disorders and/or genetic syndromes, performing additional investigations, depending on the clinical suspicion.

Systematic karyotyping of all girls with SS without any other known cause, in this study, as currently recommended (22, 23), led to the diagnosis of 43 girls with Turner syndrome (TS). Eightteen of these girls had already been referred with the diagnosis of TS, and the other 25 did not have this suspected diagnosis, although most of them also presented a few suggestive clinical stigmata. Among our cases, the proportion of patients with mosaicisms was higher in those in whom we observed less TS stigmata. Eleven of the eighteen girls (61.1%) already referred with a diagnosis of TS had karyotype 45 X0 and only ten of the 25 remaining ones (40%) did not present any degree of mosaicism detected at karyotype. The other genetic syndromes in children

seen at the outpatient clinic coincide with those usually associated with SS, and account for 18.42% of our cases (Table 3). Even after diagnosis we continue to follow these children, optimizing clinical care according to each syndrome, seeking to develop the potential for growth as far as possible, according to up-to-date reviews and protocols (23, 24, 25, 26, 27).

Discussion

Concerning the objectives of this study, we above all managed to demonstrate the importance of the auxological and basal complementary investigation to diagnose the causes of SS. Even at a tertiary center, endocrine disorders are not the most prevalent causes. When we compare the frequencies between different groups, it is important to consider where the work was done. The prevalence studies on causes of SS are distinguished mainly according to the level of care at which they were performed. In population base studies, the proportion of short normal children is always greater than in those performed at specialized centers, to which the more complex cases are referred, with a higher probability of presenting associated diseases.

As to the comparison with the prevalence observed at other centers, the absence of recently published data with up-to-date reports is noticeable. Even with the discovery of new diagnostic tools, and new diagnoses involving SS, the literature of the last few years has set aside the clinical characteristics in favor of new molecular discoveries. The clinical reports published are on specific pathologies, such as the

auxology of haploinsuficiency of gene SHOX (28, 29). Apparently this gap in clinical information is not due to the fact that the system used to investigate these patients is already standardized, since a 2004 study questioned the practice of 13 hospitals in Wales, specialized in evaluating patients with SS. The surprising fact is that although the specialists at these hospitals diagnose and treat up to 12 cases of GH deficiency a year at each service, the parents were measured or the pubertal stage of the child evaluated at only 9 of the 13 centers. In this study the complete blood count was the only consensus examination at all services, and only 8 centers submitted the patients to an X-ray to find out bone age (30).

In this study we evaluated prospectively 851 children with SS, at an outpatient clinic specifically for pediatric endocrinology, and we found that 54.3% of them were of normal stature or had a short variant of normality, 7.8% had GH deficiency, 9.2% of the patients had treatable organic diseases and 18.1 % had genetic syndromes. These numbers are mostly no different from those found at services with the same profile of care provided at ours. During the 70s, Vimpani et cols (2) examined 449 children with a stature over two standard deviations below the average in Scotland and found 41% of familial or constitutional SS, 24% organic disease, 8% GH deficiency, 7.5% due to intrauterine retardation and 19% from an undetermined cause.

On the other hand, in a population base study performed on children of lower social classes, in Chile, the most prevalent cause of SS was an association of

malnutrition, bad environmental hygiene, SS at birth and a short period of exclusive breastfeeding (31). This study supports the importance of environmental factors in the etiology of SS. Among our patients, even considering that we evaluate children previously seen at basic health units, 1/5 had helminthic infestations, and ¼ presented some degree of anemia. These findings are certainly also related to the patients' socioenvironmental factors. We already knew this, but it is increasingly demonstrated that calorie intake, iron deficiency and even hypermetabolism, even at a low level, interfere in the growth velocity of children with SS (32, 33, 34). The fact that few of our patients presented low weight related to stature does not exclude this possibility, since studies of Latin American populations of a low socioeconomic level showed high prevalences of stature for age deficit, while there is no evidence of major deficits of weight for height. A Brazilian study, analised 386 children aged 6 to 59 months and found 26%, 14% and 3.5%, respectively, of stature deficits for age, weight for age, and weight for stature (35).

As to the prevalence of clinical and subclinical hypothyroidism, a group that evaluated 2067 children with SS found a prevalence similar to ours (1,88% vs 2.8%) (36).

When we compared our numbers with those reported by studies evaluating children with a similar profile, we perceive a greater discordance in relation to cases of celiac disease (CD) and TS. Analyzing the differences in the evaluation system used for

these children, the cause of this discordance appears clear. A given disease will be more frequently diagnosed depending on the greater accuracy and frequency with which the specific exams for this pathology are performed. In this study, the systematic karyotyping of all girls with SS, without any other known cause, led to diagnosing a high percentage of cases of this pathology. On the other hand, we observed only two diagnosed cases of CD in thirty-seven patients submitted to endoscopy with a biopsy after clinical suspicion. Very few children were submitted to serological tests and since these tests are easier to perform as screeening, even in asymptomatic patients, apparently they would allow a greater number of diagnoses, as suggested in the literature, where the routine investigation of CD by IgA antiendomysium and antitransglutaminase antibodies (ATA) is one of the factors responsible for the high prevalence of CD in other countries (1.7 to 8%) (17,18). Anyhow, it is striking that even in a selected group of patients, in whom digestive endoscopy with a biopsy was performed, only 2 out of 37 patients were seen with typical findings of the disorder, suggesting that in our population, CD is not a frequent cause of SS. On the contrary, there is a study, also by a Brazilian group, that studied 106 asymptomatic children with SS, and found a similar prevalence of CD to that in the European literature, of 4.7% (37).

As to systematic ATA in all patients with SS, at our hospital, this exam only became available a short while ago, and even now we recommend it only when there is

a clinical suspicion, even though minimal. This situation includes patients with SS and low weight or persistent difficulty in gaining weight, persistent anemia, metabolic evidence suggesting malabsorption (for instance: hypocalciuria with adequate calcium and vitamin D intake), or any gastrointestinal symptom. This management was recently justified by a cohort of 2000 patients. Apparently, establishing a "rule" to investigate CD, which includes: evaluation of the degree of clinical suspicion, ATA dosage and performing biopsy only on the patients with positive antibodies or IgA deficiency, increases the sensitivity and specificity of the diagnostic method (38).

These findings led us to rediscuss our baseline complementary investigation, in order to evaluate the usefulness of the exams performed and establish the simplest protocol possible. In this review of the protocol, it was decided to exclude a few baseline exams whose results were not useful for the differential diagnosis of SS, namely, sodium, urea, gamma glutamyltransferase, alanine aminotransferase and fecal steatocrit. On the other hand the evaluation of our cases suggested the inclusion of the IGF-1 dosage for children with a stature less than 2 standard deviations from the average height for age and/or low growth velocity. Although no major prevalence of CD occurred in our cases, the review of literature creates a foundation for measuring IgA ATA, in children with the characteristics described previously, even if they do not have gastrointestinal symptoms. Our data recommend also that karyotyping be performed in girls without any other known cause of SS, as already emphasized in the

literature.

Conclusions

These data showed that even in a specific endocrine outpatient clinic there is a large number of patients with a short stature varying from normality, or short stature resulting from diseases that are simpler to manage, who are referred for specialized evaluation. This population, with a high incidence of common clinical problems, depending on the approach to them, adds a very significant potential health cost. Apparently this initial evaluation may be long and expensive, but the hormonal investigations would be much more expensive. And if wrongly interpreted due to ignorance of the baseline situation of the child, they would lead to unnecessary treatments, at a high cost with doubtful results.

Herein lies the importance of our results, which call attention to a careful, detailed evaluation of general factors associated with growth, including the importance of taking a complete history and detailed physical examination, and general baseline tests (39), before carrying out specialized investigations and endocrine treatments. As part of the physical examination, it is important to measure the parents, since their reported stature, compared with the one measured in our outpatient clinic was an average of 2.2 centimeters taller, interfering in the target-height calcule.

Most chronic diseases and even a few genetic syndromes beside endocrine diseases are currently treatable (23, 40) and for satisfactory treatment we need, above all

a correct diagnosis.

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Table 1: Basal Clinical Characteristics				
	Total	Boys	Girls	Significance (between sexes)
Number	851	511	340	
Age at first medical visit Mean (SD) Minimum-Maximum	9.82 (4.4) 0.35 – 20.5 years	10.13 (4,4) 0.45 – 20.2 years	9.36 (4.41) 0.35 – 20.5 years	NS
Z-score of height for age Median (SD) Minimum-Maximum	-2.77 (1.33) -9.98- +3.18	-2.69 (1.33) -9.98 - + 3.18	-2.87 (1.28) -9.98 - +0.26	NS
Z-score of weight for age Median (SD) Minimum-Maximum	-1.7 (1.12) -6.68 - +4.13	-1.73 (1,14) -6.68 - + 4.13	-1.67 (1.10) -4.69 - +3.82	NS
Z-score of weight for height Median (SD) Minimum-Maximum	0.15 (1.66) -5.36 - +9.98	0.12 (1.63) -4.65 - + 9.98	0.19 (1.7) -5.36 - +6.44	NS
Sougth care previously	63.5%	63.8%	63.6%	NS
Birth order	35% only child 33% second or third child 32% 4th to 10th child			Not different and NS
Prematurity	16.8%	17%	16%	NS
Delivery by C-Section	36%	34.9%	37.9%	NS
Percentile of target height	41.9% < = p 5 88.9% < = p 25 3.15% > = p 75	38.7% < = p 5 90.3% < = p 25 3% > = p 75	47% < = p 5 87.6% < p 25 3.3% > = p 75	NS

NS – not significant, SD – standard deviation

Table 2. Diagnosis of the Cause of Short Stature in the 670 patients with a defined diagnosis (General , 396 boys and 274 girls).

DIAGNOSIS	General (%)	Boys (%)	Girls (%)
No Short Stature	56 (8.3%)	36 (9.0%)	20 (7.3%)
CSS+ FSS –Variant of normality	308 (46.0%)	212 (53.6%)	96 (35.0%)
Hypothyroidism	19 (2.8%)	8 (2.0%)	11 (4.0%)
GH deficiency	52 (7.8%)	35 (8.8%)	17 (6.2%)
Chronic disease	43 (6.4%)	28 (7.2%)	15 (5.5%)
Genetic syndrome	121 (18.1%)	37 (9.3%)	84 (30.7%)
IUGR	29 (4.3%)	17 (4.3%)	12 (4.4%)
Others	42 (6.3%)	23 (5.8%)	19 (6.9%)

CSS- Constitutional short stature, FSS- Familial short stature, IUGR-Intra-uterine growth restriction

Table 3. Chronic diseases and genetic syndromes associated with short stature observed in this case list	
Chronic diseases	Genetic syndromes
Celiac Disease Chronic Liver Disease Diabete Mellitus Hemosiderosis Hirschprung Disease Histiocytosis X Hypoparathyroidism Juvenile Rheumatoid Arthritis Medullary Aplasia Recurring Helminthic Infections Renal Tubular Acidosis Severe Asthma Sickle Cell Anemia Thalassemia Major Thyroid Carcinoma Vitamin D Deficiency	Aaskorg Achondroplasia Aicardi Albright Blackfan Diamond Cornélia de Lange Chromosomal translocations Dandywalker Dent Di-George Down Dubowitz Duchene Muscular Dystrophy Fetal Alcohol Friedrich Ataxia Hadju-Cheney Homocysteinuria Mckusick chondrodysplasia Neurofibromatosis Noonan Rubinstein-Taybi Schmidt Dysplasia Seckel Septo-optic Dysplasia Silver Roussel Turner Type I Tricorhinofalangian William

Table 4. Tests recommended to investigate SS.

<table>
<tr><th colspan="2">TESTS</th></tr>
<tr><th>General for all</th><th>Most common specific ones</th></tr>
<tr><td>Blood count</td><td rowspan="3">IGF-1
-below 3rd percentile of height or
-slow growth velocity</td></tr>
<tr><td>Glycemia</td></tr>
<tr><td>Potassium</td></tr>
<tr><td>Calcium, phosphorus, albumin</td><td rowspan="3">Karyotype of peripheral blood (G bands)
-girls below the 3rd percentile
-no other cause identified for SS</td></tr>
<tr><td>Alkaline phosphatase</td></tr>
<tr><td>Aspartate aminotransferase</td></tr>
<tr><td>Creatinine, urinalysis</td><td rowspan="6">IgA and IgA anti-transglutaminase antibodies
-if weight loss or difficulty in gaining weight,
-persistent anemia,
-metabolic evidence of malabsorption or
-any gastrointestinal symptom</td></tr>
<tr><td>Bicarbonate, urinary pH</td></tr>
<tr><td>Erythrocyte sedimentation rate</td></tr>
<tr><td>TSH, T4</td></tr>
<tr><td>Stool test for parasitic infections</td></tr>
<tr><td>X-ray for bone age</td></tr>
<tr><th colspan="2">Uncommon specific ones</th></tr>
<tr><td colspan="2">Abdominal, pelvic or cardiac ultrasound, X-ray of the spine and the long bones, 17OH progesterone, Tests and other hormone dosages.</td></tr>
</table>

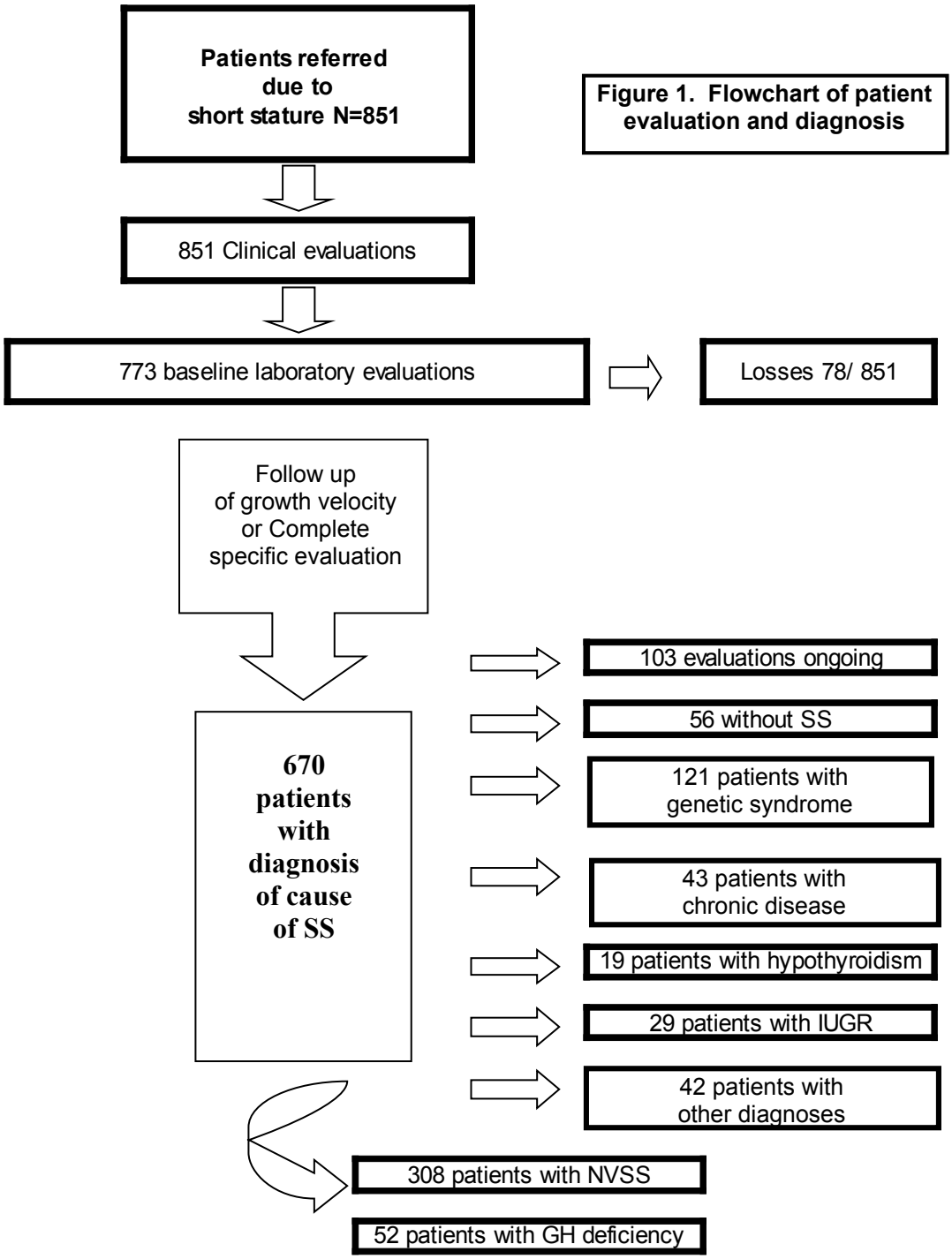

Figure 1. Flowchart of patient evaluation and diagnosis

IUGR – Intra-uterine growth restriction, SS – short stature,

NVSS – normal variant short stature

ARTIGO ORIGINAL 2

Comparison of the IGF-1 and GH peak after clonidine or insulin in the diagnosis of growth hormone deficiency in short children.

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Short Title: ROC Curve of IGF-1 and GH peaks after GH deficiency tests

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ABSTRACT

The current recommendation is to submit children with auxological criteria characteristic of GH deficiency and/or with IGF-1/ IGFBP3 below 2 standard deviations (SD) from normality for sex and age to GH secretion stimulation tests

Using the ROC (*Receiver Operating Characteristics*) curve approach, the present study evaluates two tests for the diagnosis of GH deficiency in patients with short stature (SS), mainly to determine clear, objective cutoff points that could be useful in clinical practice.

Eight hundred and fifty-one children referred to an endocrine outpatient clinic because of SS during the 1994-2006 period were evaluated prospectively. Three hundred and thirty-five children in this population participated in a cross-sectional study of a diagnostic test nested in a cohort. One hundred and fourteen had at least a baseline dosage of IGF-1 and two hundred and twenty-one children were submitted to at least one GH secretion stimulation test, either with clonidine (GH-Clo), with or without pretreatment with sexual steroids (priming), or with insulin hypoglycemia, a total of 306 stimulation tests. Fifty-one patients were diagnosed as GH-deficient. The other patients were classified as having normal variant SS. The GH was measured using the IMMULITE–DPC assay by chemoluminescence.

As to the ideal cutoff point of the standard deviation of IGF-1, the sensitivity of the value of -2 SD usually used, although quite specific, after excluding chronic

diseases and malnutrition, is only 14.3%. When a ROC curve was constructed for the value of the SD of IGF-1 with the best accuracy to diagnose GH deficiency, 92.9% sensitivity was found, and 84.2% specificity for the cutoff point of about -1 SD.

New ROC curves were also constructed for the GH peak value in the GH–Clo tests. without and with priming and for the higher response value of GH that was presented by a same patient, independent of the type of stimulation used in the test (total GH). Ninety-five percent sensitivity and 93.4% specificity were found in the GH-Clo tests without priming for the cutoff point of 4 ng/ml. In the GH-Clo test with priming, a sensitivity of 96.3% and a specificity of 100% were found for the 5 ng/ml cutoff point. In the total GH tests a sensitivity of 97.9% and a specificity of 97.6% were found for the cutoff point of about 5ng/ml of GH.

Thus our data show that a child without a chronic disease, genetic syndrome or malnutrition, that has compatible auxology plus an IGF-1 dosage below -1 SD from the normal for its age and sex, should be submitted to a GH stimulation test. The child must be diagnosed as GH deficient if it presents a GH-Clo peak response value without priming below 4ng/ml and below 5ng/ml (in the GH Clo tests with priming or Insulin test), when the latter was measured by specific chemoluminescent assay for form 22 KDa.

Key words: Growth hormone (GH), IGF-1, GH stimulation tests, Clonidine, ROC Curve, GH deficiency

Introduction

Currently, the base of GH deficiency (GHD) diagnosis is still clinical according to auxologic criteria and hormonal and laboratory criteria including the evaluation of the GH-IGFs axis (1-12). The current recommendation is that children with characteristic auxological criteria and/or IGF-1/IGFBP3 below 2 standard deviations (SD) be submitted to GH secretion stimulation tests. Most countries have established diagnostic criteria for GHD based on maximum "normal" arbitrary GH responses (peaks), so that a peak greater than 10 ng/ml is currently accepted as a cutoff point to consider a normal response to stimulation (13, 14, 15). However it was found that even children without SS and with normal growth could present responses lower than 10 ng/ml in the stimulation tests (16, 17).

Despite the use of new assays in which the values of measured GH are lower than those of the previous assays, because they measure predominantly biologically active GH (22KDa) in the circulation, this cutoff point was not modified (18, 19, 20). This controversy led to several studies with initially very different results (2, 10, 16, 17). In our group and based on a previous prospective study, we demonstrated the best accuracy of a peak cutoff point, different from the historically and empirically accepted one (21). In constructing an ROC curve, considering the GH peaks in response to clonidine administration, a rate of 46.8% of false positives was obtained with peak > 10

ng/ ml and greater accuracy with a cutoff point of 4 ng/ ml. Recent publications have ratified this peak value as adequately discriminant (22, 23, 24).

There are a number of studies in the literature that compare the sensitivity and specificity of these two diagnostic methods of GHD, baseline dosages of IGF-1 versus GH stimulation tests. However, most of these studies used the empirical cutoff point of 10, and because we found equal sensitivity but higher specificity with the cutoff point of 4, we consider the new comparison between the two diagnostic methods, using these new data, extremely useful to obtain a more accurate diagnosis of GHD.

Using the ROC (*Receiver Operating Characteristics*) curve approach which allows comparing test sensitivity and specificity in a large range of cutoff points, the study was done to determine the performance of these two tests in diagnosing GHD in patients with short stature (SS), and especially to determine clear, objective cutoff points which may be useful in clinical practice.

Patients and Methods

In an endocrine outpatient clinic, 851 children referred because of SS during the 1994-2006 period were evaluated prospectively. During the first years of follow up we did not have the IGF-1 dosage available at our service, only the GH stimulation tests. A total of 345 children, out of this population fulfilled the inclusion criteria: stature below the third percentile, growth velocity measured at our clinic during at least 6 months, exclusion of chronic diseases and genetic syndromes. One hundred and

fourteen had at least one baseline dosage of IGF-1 and two hundred and twenty-one children were submitted to at least one test to stimulate GH secretion, either with clonidine, with or without pretreatment with sexual steroids (priming) or with insulin hypoglycemia, a total of 306 stimulation tests in these children alone. The tests were performed according to a detailed protocol and according to literature (25, 26).

Fifty-one patients were diagnosed as GH deficient considering, for diagnosis, a set of factors besides SS and the exclusion of chronic diseases and genetic syndromes: growth velocity below the 25th percentile; delayed bone age, stature below the growth channel in relation to the target height; presence of risk factors such as previous radiation of the CNS, chemotherapy, tumor or another hypothalamo-hypophyseal disease; IGF-1 values; value of the peak response of GH to at least two pharmacological stimuli (clonidine and insulin); hormone tests to evaluate the integrity of the other hypophyseal hormones and alteration in the hypothalamo-hypophyseal region image. The other patients were classified as having normal variant SS (NVSS).

Initial Evaluation

All the patients were evaluated by history and complete physical examination, the target height was calculated using the Tanner formula, according to the parents' stature, preferably measured or else reported. After informed consent by parents or legal guardians, the patients were submitted to initial evaluation with an X-ray of the left

hand and wrist for bone age, according to the Greulich-Pyle atlas (27) and laboratory evaluation by complete blood count, thyroid function and biochemistry (28).

Design

Cross-sectional study of a diagnostic test nested in a cohort

Endocrine Evaluation

GH- Clonidine (GH-Clo) test performed at 8 am, after fasting. A blood sample was drawn and clonidine was administered in a 0.15 mg/m2 dose orally, and additional samples were drawn at 30-minute intervals until 2 hours had elapsed. The patients who did not reach the value of 10 at the initial test were submitted to a test with priming, consisting of testosterone 50 mg intramuscularly, 7 and 2 days before the boys' test and conjugated estrogens 1.2 mcg/m2/day for 3 days before the girls' test.

Insulin hypoglycemia (HipoIns): this was performed administrating insulin intravenously, after the same precautions described previously, at a dose of 0.05-0.1 IU/KG and blood samples were drawn at baseline time and every 30 minutes until 2 hours had elapsed.

Hormone Dosages

GH was dosed using the IMMULITE–DPC assay, by chemoluminescence, which uses specific monopolyclonal antibodies for GH form 22kDa calibrated with recombinant GH, with a sensitivity of up to 0.01 ng/ml, intraassay coefficient of variation ranging from 5.3% (1.7 ng/ml) to 6.5% (31 ng/ml) and interassay coefficient of variation of 5.5 %(3 ng/ml) to 6.2% (18 ng/ml).

The IGF-1 concentrations were determined by immunoradiometric assay (IRMA), DSL-5600 Active (Diagnostic System Laboratories Inc, Webster, Texas), with intra-assay and inter-assay coefficient of variation of 3,9% and 4,2%.

Statistical Analysis

The data obtained were stored in an Excel database and exported to SPSS version 14.0. The categorical variables are described by the percentage relative frequency and the quantitative variables by the mean and SD when the distribution is symmetrical, and median and interquartile range (p25-p75) when asymmetrical. Comparison between the groups of patients with and without GHD considering the IGF-1 values using the Student t test for independent samples, or the Mann-Whitney test according to the distribution of the (asymmetrical) variable. The categorical variables were compared using the Chi-square test with Yates correction. Considering the sensitivity and specificity of different cutoff points of the GH response peak and of the cutoff points of the IGF-1 SD levels, it was possible to construct a ROC curve for each diagnostic method. The level of significance adopted was 5%, and $p \leq 0.05$ values were considered stastistically significant.

Results

The distribution by sex was equal among the two groups, as well as the mean age at first consultation, and age when growth reduction was observed for the first time (table 1). It should be pointed out that the weight/height ratio was similar in both groups

and much closer to normality than the height and weight for age. Other group characteristics can be seen at table 1.

All exams of the GH-IGF-1 axis were statistically different among the groups. It should however be emphasized that there was a greater variance of the values in the group of patients with NVSS, as viewed in figure 1.

Sensitivity and specificity of different cutoff points of GH tests can be seen at table 2 and this curves are represented graphically in figure 2. As to the ideal cutoff point of the SD of IGF-1, in table 3 and in figure 2 we can see that the value of -2SD usually used, although quite specific, after excluding chronic diseases, has a sensitivity of only 14.3%. When a ROC curve was constructed for the SD value of IGF-1, with the best accuracy to diagnose GH deficiency, a 92.9% sensitivity and a 84% specificity were found for the cutoff point of about -1 SD. In other words, in cases in which another cause of IGF-1 reduction was excluded, such as chronic disease or malnutrition, our data suggest that one should use the stimulation tests to evaluate all the children who have a compatible auxology and a screening exam with IGF-1 below -1 SD from the normal for their age and sex.

New ROC curves were also constructed for the peak value of GH in the GH-Clo tests without and with priming and for the higher response value of GH that a same patient presented, independent of type of stimulation used in the test (total GH). The cutoff points that presented the best diagnostic accuracy for GH deficiency were very

close among the tests without and with priming, because, although the priming amplifies the GH response to stimulation in patients with NVSS, it did not modify the response in GH deficient patients in any major way.

Discussion

Usually a diagnostic test value is considered abnormal when the latter is at least two standard deviations from the mean observed in normal people. However, only 2 out of 15 deficient subjects presented an IGF-1 value below -2SD, which showed that this criterion was not appropriate, especially due to the fact that any screening test should prioritize sensitivity. A series of studies compared the sensitivity of the IGF-1 measure with the GH stimulation test response in diagnosing GHD and found percentages that vary from 50 to 100% (on average 71% sensitivity), using the cutoff points of -2 SD of IGF-1 and 7 to 10 ng/ml in the GH response to the stimulation test. (29). Our data, showing lower sensitivity with the IGF-1 cutoff point of -2SD, are similar to the group of Nunez et cols, 1996 , who had also found the IGF-1 cutoff point of -1 SD as most appropriate in investigating GHD (30). These authors had compared the SD of IGF-1 with the peak response of GH to stimulation by arginine and insulin hypoglycemia, besides the nocturnal concentration of GH in 104 children with SS and found a sensitivity of 50 and 21% as the IGF-1 cutoff point of -2DP to diagnose the complete or partial GHD. These data are different from those described here, especially by the type of GH assay used (polyclonal) and by the cutoff point considered diagnostic

for deficiency in the GH stimulation tests (7-10 ng/ml). Juul et cols evaluated 108 patients previously diagnosed as deficient, at the time of the retest, and compared the usefulness of the IGF-1 measure in reevaluating these patients (31). Comparing it with the cutoff point of 7.5 ng/ml of GH to the stimulation test, they found 75.9% sensitivity and 72% specificity for the IGF-1 measure. The only group in which all the patients presented IGF-1 values below -2 SD was the group with panhypopituitarism and a deficiency of 3 or more pituitary hormones, besides the GH. This study also calculated the sensitivity, specificity and accuracy of different cutoff points of GH and the SD of IGF-1, and manipulating these cutoff points, it found the highest concordance index using a GH cutoff point of approximately 3 to 4 ng/ml and -2 SD for IGF-1. Using a different strategy, but with a similar population, we were able to observe almost the same values. The IGF-1 reference values are defined by age and sex, and, as suggested by Saenger (32) it might be more appropriate to compare the result of IGF-1 with the bone age pattern, because children with growth disorders often have delayed physical and/or pubertal development.

As to the GH peak response to the stimulation test, in this study we see that the cutoff point of 10 ng/ml, widely recommended in the literature, produces an extremely elevated number of false positive tests (46.1%) in the GH-Clo tests without priming, and a relatively low specificity of 53.9 to 76.5%. This had already been described when children who were not of SS were tested. Marin et cols, when they evaluated normal

children and adolescents with 3 GH stimulation tests (exercise, arginine and insulin), considering a cutoff point of 7ng/ml, observed that, as puberty advances, the percentage of normal children who failed to reach a GH level of 7 ng/ml during any of the tests, diminished from 61% during the prepubertal stage to 0% in the stages 4 and 5 (33). If in normal children without SS it is already difficult to define a normal standard of evaluation of the GH-IGF-1 axis, in patients with constitutional SS, this difficulty is even more marked. They are very often clinically indistinct from the GH deficient ones, and at the time expected for puberty, because they have not yet reached pubertal development, they have a GH pulsatile pattern similar to that of pre-pubertal children, i.e., low range and low response to GH stimulation tests. Besides, they may present a low IGF-1 for their chronological age, but not for their bone age or pubertal stage.

It has become increasingly clear that the diagnosis of isolated GHD in childhood may not be confirmed in the retest after the end of growth, and this appears to reflect the use of inappropriate diagnostic criteria, including the GH cutoff point to the stimulation test (33, 34, 35). Carel et cols retested 208 young adults who had been treated in childhood for GHD, demonstrating that among the patients with this prior diagnosis, but with the complete or partial idiopathic form, 44% and 89%, respectively, presented a GH peak greater than 10 ng/ml at retest, while this fact occurred among less than 5% of those with a previous diagnosis of GHD due to organic cause or CNS radiation (36).

A study of 2,852 children with isolated idiopathic GHD found an interesting item: the children who interrupted GH treatment early grew to a final height similar to those who took the whole course of treatment, suggesting that the previous diagnosis of GH was mistaken. Based on their data, they suggested that the peak value of GH, considered responsive, should be about 2-4 ng/ml, and that the priming strategy should be used before the GH test (37).

A paper published in 2004 used questionnaires sent to physicians and laboratories at 13 hospitals in Wales, where children with SS were habitually investigated, asking about their current practices (38). Although these centers evaluate up to 50 new cases of SS a year, and diagnose GHD in 10 to 25% of them, a few did not even perform the prior clinical and laboratory evaluation, such as a bone age X ray or basic biochemical exams. Several screening tests for GHD were used, including IGF-1, baseline GH and GH-stimulating test with exercise. Clonidine was used at most of the centers to stimulate GH secretion in diagnostic investigation, but 8 different protocols were described and 9 different cutoff points were considered indicative of deficiency, sometimes even varying at a same center. The need for appropriate standardization is thus clear, to define which child should be tested and the diagnostic tests to be used, the protocol of times and sample collections, which essays are to be used, and the points considered diagnostic for GHD.

ROC curves are particularly useful to validate diagnostic tests and to determine a cutoff point of the response to this test more accurately, but their use assumes that there is a comparison test that will be a faithful indicator of whether a disease really exists (gold standard). In clinical research, however, this gold standard is often difficult to find. The difficulty is particularly significant in SS patients, in whom the precise diagnosis is habitually based on the association of different criteria, including the GH pharmacological stimulation tests. Thus, comparing GH secretion tests with the definitive diagnosis of its deficiency implies that the test itself should be used as one of the diagnostic criteria. In order to get around this difficulty, in the present study our gold standard is a set of clinical and laboratory variables from a same patient, and they were diagnosed as GH deficient or not according to medical evaluation.

Using the assay described previously, and the cutoff point of 4ng/ml, 95% sensitivity and 93.4% specificity were found for tests without priming. Martinez et cols studied 15 pre-pubertal patients with GHD, and 44 patients with idiopathic SS who were submitted to the arginine-clonidine test for GH, randomizing them to receive placebo or priming with estradiol 1 or 2 mg/day for 3 days, and found 73% sensitivity and 95% specificity, with a cutoff point of 3.7 mcg/l after placebo, and 87% sensitivity and 98% specificity for a cutoff point of 8.3 mcg/l after priming (22).

Chalera et al used the SD score of growth velocity in the first year of treatment with GH as a biological marker to confirm the diagnosis of deficiency and validate the

GH cutoff point in response to two stimulation tests with arginine and clonidine, dosed by two different assay, one specific for the 22KDa form and the other called total assay, since it dosed all isoforms of GH (23). Also using the ROC curve approach, this group defined the SD of growth velocity in the 39 GH-deficient patients and in the 11 patients with NVSS, and considering this information the gold standard, constructed new curves for the GH values and found a cutoff point of 10.8 ng/ml when the total assay was used, and 5.4 ng/ml for the specific 22KDa assay. In a prior paper, these authors had found the cutoff points of 10.0 and 4.8 ng/ml, respectively for total GH and the specific 22KDa, using biochemical validation (39).

Our data, associated with a review of the literature, confirm the need to modify the cutoff point of the GH response for values around 4-5 ng/ml, when the latter is dosed using a monopoliclonal assay. They also demonstrate that this value previously validated in response to clonidine coincides with the value when the stimulation used is insulin hypoglycemia The main novelties were related to priming and to IGF-1 value. On the contrary of previous studies, where the value of the cutoff point in response to GH almost doubled when it was preceded by sexual steroids, in our study we observed a significant amplification of the GH response only in the group of patients with NVSS and not in the deficient ones. Because of this, the cutoff point in the test without and with priming were not much different, and it showed that, when we reduce the cutoff point to 5 ng/ml there is mostly no longer any need for priming to mark this difference.

Conclusions

As established by the ROC curve and using the IGF-1 IRMA assay, the most accurate cutoff point was about –1 SD adjusted for sex and age in patients with SS resulting from different etiologies. As also established by the ROC curve and using the chemoluminescent GH assay (Immulite-DPC), the most sensitive and specific cutoff point of the GH peak response to stimulus by clonidine without priming was 4 ng/ml, while the cutoff point of the stimulation tests with clonidine preceded by priming or by insulin was 5 ng/ml in patients with SS.

Despite all the studies, there is no exclusive diagnostic study for GHD, and we decided to maintain the diagnosis by analyzing the same set of clinical factors previously described, plus IGF1 value below -1 SD and value of the GH response peak to at least two pharmacological stimulations (clonidine and insulin) below 4-5 ng/ml when the specific assay for the 22KDa form was used.

In clinical practice, our findings are important because they suggest that one should: 1) perform a clinical and laboratory evaluation of a child with SS (40); 2) dose baseline IGF-1 in children with a stature below the third percentile, or when there is a reduction in the growth velocity below the 25th percentile; 3) if the child does not have any other cause of IGF-1 reduction (chronic disease, malnutrition) and presents an IGF-1 value below -1 SD for age, submit it to a GH secretion stimulation test (clonidine or glucagon); 4) children who in two GH stimulation tests have no response higher than 4-

5 ng/ml, when a similar assay is used, should be diagnosed as deficient and treated; 5) children with IGF-1 below -1 SD and one non-responsive stimulation test associated with the presence of risk factors such as prior irradiation of the CNS, chemotherapy, tumor or another hypothalamo-hypophyseal disease, alteration in the imaging exam of the hypothalamo-hypophyseal region or deficiency of more hypophyseal hormones must also be diagnosed as GH deficiencient; 6) children with IGF-1 greater than -1 SD are unlikely to be deficient, but they must be followed to evaluate their growth velocity which, if very low, may indicate the need to reevaluate the GH-IGF-1 axis.

Thus, considering the medical and public health aspects, particularly in a country like ours, in which GH therapy for SS should be recommended only when the diagnosis of GHD is confirmed, the results of this study are important, because they support the importance of clinical characteristics and they demonstrate a cutoff point with good sensitivity and with a small number of GH stimulation test “false-positives”, enabling a precise, accurate diagnosis of GHD.

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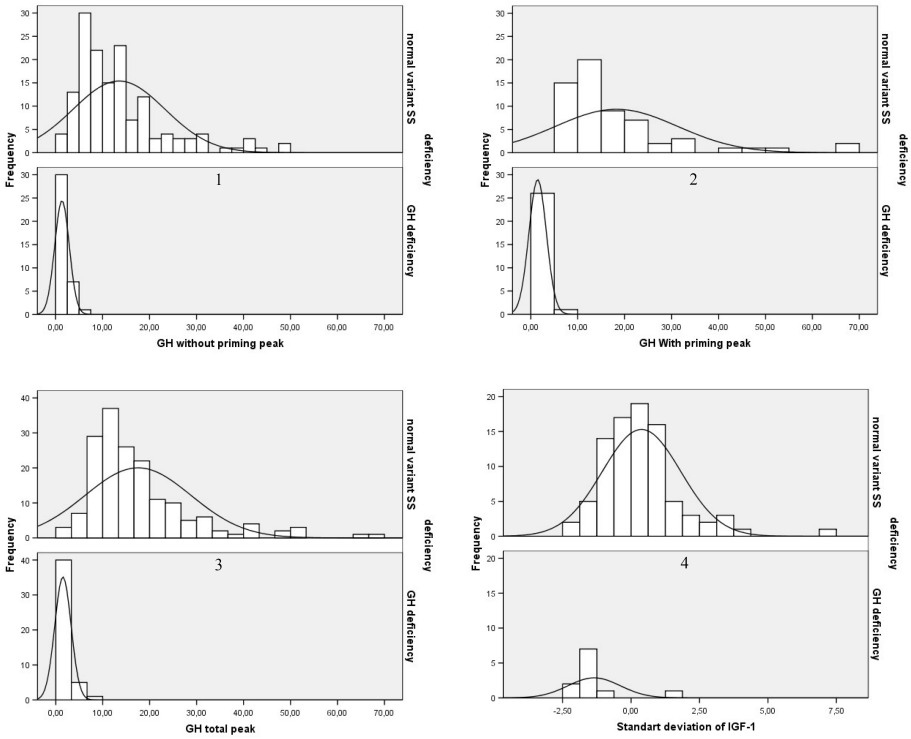
Table 1. Comparison of characteristics of patients

.

	GH deficiency (n= 51)	Normal Variant Short Stature (n=294)	Significance
Male	66.7%	69.7%	p=0.785 NS
Age at first medical visit Mean (SD)	10.3 anos (4.8)	10.6 anos (4.0)	p=0.662 NS
When noticed growth deviation Median and p25-75	4.5 years 1-7years	5 years 3 months- 9 years	p=0.523 NS
Sougth care previously	87.8%	63.8%	P=0.005
Events during pregnancy (%)	37.3%	36.4%	p=1 NS
Delivery by C-Section	29.5%	34.8%	p=0.609 NS
Birth weight Mean (SD)	3019 grams (655)	2971 grams (628)	p=0.640 NS
Delayed puberty (%)	43.8%	24.5%	P=0.012
Height different from the growth channel of target height (%)	100%	58.3%	P<0.001
Z-score of height for age Median (SD) Percentile 25-75	-3.73 (1.37) -4.80/ -3.10	-2.44 (0.96) -2.88/ -1.96	P<0.001
Z-score of weight for age Median (SD) Percentile 25/ 75	-2.09 (1.18) -3.18/ -1.46	-1.76 (0.84) -2.31/ -1.26	P=0.006
Z-score of weight for height Median (SD) Percentile 25/ 75	0.17 (1.35) -0.79/ 0.80	-0.08 (1.20) -0.67/ 0.64	P=0,354 NS
Delayed bone age	87.0%	59.4%	P= 0.001
Growth velocity Mean and SD	3.6 (1.75) cm/year	6.2 (2.0) cm/year	P< 0.001
Percentile of target height	<5 - 33.3% 5-25 - 46,7% >=50 - 20.0%	<5 - 43.2% 5-25 - 46.8% >=50 - 10.0%	
Glycemia (mg/dl) Mean (SD)	78.4 (11.8)	86.1 (10.5)	P< 0.001

Figure 1. Percentage frequency of the GH peak response values in the stimulation tests and standard deviation of IGF-1 in patients with normal variant SS and GH deficiency.



1. stimulation test with clonidine without priming in normal variant SS and GH deficiency; 2. stimulation test with clonidine with priming in normal variant SS and GH deficiency; 3. GH total - all stimulation tests that have been performed on the patient: clonidine stimulation test without and with priming and insulin hypoglycemia; 4. standard deviation of the baseline IGF-1 in patients with normal variant SS and GH deficiency.

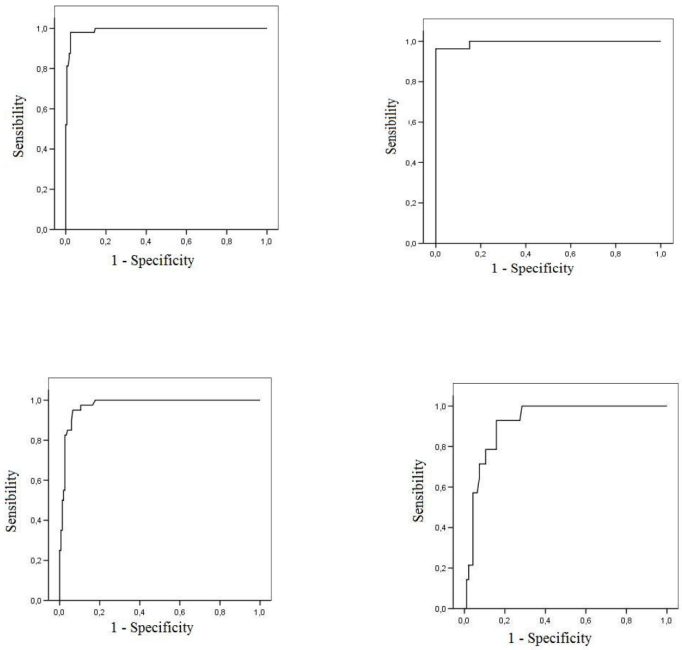
Table 2. Sensitivity, specificity and percentage of false positives tests from different cutoff points in the GH response to the clonidine stimulation tests, preceded (P+) or not (P-) by priming and to all stimulation tests (clonidine and insulin).

Cutoff point ng/ml	Sensitivity %			Specificity %			False Positive %		
	GH Clo P -	GH Clo P +	Total GH	GH Clo P -	GH Clo P +	Total GH	GH Clo P -	GH Clo P +	Total GH
1	55	59.3	52.1	97.4	100	99.4	2.6	0	0.6
4	95	92.6	89.6	93.4	100	97.6	6.6	0	2.4
5	97.5	96.3	97.9	88.2	100	97.6	11.8	0	2.4
7	100	96.3	97.9	73	93.3	92.,4	27	6.7	7.6
10	100	100	100	53.9	75	76.5	46.1	25	23.5

Table 3. Sensitivity and specificity of different cutoff points of the standard deviation of IGF-1

Cutoff points	Sensitivity %	Specificity %	False-positive %
- 2	14.3	98.9	1.1
- 1.5	6.3	93.7	7.4
- 1	92.9	84.2	15.8

Figure 2. ROC curve of the peak response of GH to clonidine stimulation test without and with priming and insulin hypoglycemia in the diagnosis of GH deficiency and ROC curve of the standard deviation of IGF-1 in diagnosing GH deficiency.



From left to right: ROC curve of the peak response value of GH to the stimulation test by clonidine without priming in the diagnosis of GH deficiency area under the curve (AUC) of the ROC curve = 0.975; ROC curve of the peak response value of GH to the stimulation test by clonidine with priming (AUC = 0.995); ROC curve of the GH total (AUC = 0.992); ROC curve of the standard deviation of IGF-1 in diagnosing GH deficiency(AUC = 0.887).

COMENTÁRIOS FINAIS

PROTOCOLO SUGERIDO PARA INVESTIGAÇÃO DE BAIXA ESTATURA

1- Anamnese completa

Antecedentes perinatais e mórbidos

Doenças crônicas ou uso de medicações

Anamnese nutricional e psicossocial

Desenvolvimento neuro psicomotor

História familiar

2- Exame físico

Medida de altura do paciente e dos pais

Criança até 24 meses deve ter seu comprimento aferido em posição supina, medida em uma superfície plana, deitada de costas. Mantém-se a cabeça da criança em contato com uma prancha fixa ao longo da mesa e desliza-se a prancha até os calcanhares.

Criança acima de 24 meses deve ser medida em posição ortostática, de pés descalços, com o dorso e os calcanhares em contato com uma parede, a cabeça deve ser segura de modo que a criança olhe diretamente para frente, a prancha deslizada até o topo da cabeça e feita a leitura.

Medida de segmentos corporais para obtenção do segmento inferior e da envergadura

Desenvolvimento puberal- Conforme classificação de Tanner e medida de volume testicular com orquidômetro de Pradder.

Exame físico completo.

Cálculo da altura alvo (AA) = A pai + A mãe +- 13 / 2.

Preenchimento dos dados na curva de crescimento.

Deve-se investigar na anamnese e exame físico indícios que possam sugerir o diagnóstico de deficiência de GH, quais sejam:

- No neonato presença de hipoglicemia, icterícia prolongada, micro pênis ou parto traumático.
- Irradiação craniana
- Traumatismo ou infecções do SNC.
- Consangüinidade e/ou um membro da família afetado
- Anormalidades da linha média craniofacial.
- BE severa, com mais de 3 desvios padrões (DP) abaixo da média para idade.
- Estatura com mais de 1,5 DP abaixo do percentil da altura alvo.
- Estatura com mais de 2 DP abaixo da média e velocidade de crescimento (VC) em 1 ano abaixo da média em pelo menos 1 DP, ou diminuição de 0,5 DP da altura em 1 ano em crianças maiores de 2 anos.
- Na ausência de BE, uma VC pelo menos 2 DP abaixo da média em 1 ano

ou 1,5 DP sustentados em 2 anos.

3- Raio X de Mão e Punho para determinação da Idade Óssea

O Raio X de mão e punho esquerdo para determinação da idade óssea deve ser interpretado através de métodos como o de Grewlich-Pyle e o de Tanner-Whitehouse.

4- Exames de triagem de doença crônica

Uma série de exames gerais deve fazer parte da avaliação inicial da baixa estatura, conforme está representado na tabela 2. Mesmo onerando a avaliação inicial, através desta avaliação sistematizada é possível diagnosticar uma série de outras patologias não endócrinas implicadas na gênese da BE, tratá-las adequadamente, revertendo o quadro, muitas vezes. Cabe lembrar também que as alterações laboratoriais do eixo GH-IGF-1 nestas outras patologias podem mimetizar as alterações diagnósticas de deficiência de GH, e que pulando esta etapa inicial da avaliação por economia, pode-se cair no extremo oposto, testando um maior número de crianças com avaliações hormonais dispendiosas, ou o que é pior, diagnosticando erroneamente algumas crianças como deficientes de GH e submetendo-as a tratamentos longos, caros e de resultado medíocre.

5- Avaliação do Eixo GH-IGF1

Várias abordagens têm sido utilizadas para confirmar a suspeita clínica de deficiência de GH em uma criança, e as mais comuns são apresentadas na tabela 3,

assim como também os “prós” e os “contras” de sua utilização. Uma vez que todos os métodos possuem “falsos-positivos”, é importante que se defina com exatidão quais as crianças que necessitam avaliação da suficiência de GH, tendo como base a análise prévia dos critérios auxológicos relatados acima, e somente após exclusão de doenças crônicas e síndromes genéticas.

Uma proposta de utilização na prática clínica de nossos achados é: 1) avaliar clínica e laboratorialmente a criança com BE; 2) dosar o IGF-1 basal nas crianças com estatura abaixo do terceiro percentil ou quando houver diminuição da velocidade de crescimento abaixo do percentil 25; 3)caso a criança não tenha outra causa para redução do IGF-1 (doença crônica, desnutrição) e apresentar valor de IGF-1 abaixo de -1 DP para a idade, submetê-la a um teste de estímulo da secreção do GH (clonidina ou glucagon); 4) crianças que em dois testes de estímulo do GH não tenham resposta superior a 4-5 ng/ml, quando utilizado ensaio monoclonal do GH, devem ser diagnosticadas como deficientes e tratadas; 5) crianças com IGF-1 abaixo de -1 DP e 1 teste de estímulo não responsivo + presença de fatores de risco como irradiação prévia de SNC, quimioterapia, tumor ou outra doença hipotálamo-hipofisária, alteração de exame de imagem da região hipotálamo-hipofisária ou deficiência de mais hormônios hipofisários também devem ser diagnosticadas como deficientes de GH; 6) crianças com IGF-1 maior do que -1 DP dificilmente serão deficientes, porém devem ser acompanhadas para avaliar sua velocidade de crescimento, que se muito baixa pode

indicar uma reavaliação do eixo GH-IGF-1.

5- Avaliação Adicional

Nos pacientes com deficiência de GH comprovada são realizados também testes para avaliação dos outros eixos hipotálamo-hipofisários e avaliação do Sistema Nervoso Central, especialmente a região hipotálamo-hipofisária, por Ressonância Magnética ou Tomografia Computadorizada. Em meninas, caso não se encontre outra causa de baixa estatura, mesmo sem estigmas que sugiram síndrome de Turner, deve-se realizar cariótipo.

Tabela 1- "Prós" e "Contras" dos Métodos Diagnósticos Utilizados na Avaliação do Eixo GH- IGF-1.

MÉTODO DIAGNÓSTICO	"Prós"	"Contras"
GH basal	--------	❖Não reflete a suficiência de GH – secreção pulsátil
IGF-1 e IGFBP-3	❖Possuem níveis mais estáveis do que o GH, tornando a coleta mais fácil ❖Têm maior reprodutibilidade ❖Existem tabelas padronizadas de níveis normais para cada faixa etária em ambos os sexos	❖Variam conforme: Idade, idade óssea, estágio puberal, sexo estado nutricional e doenças orgânicas crônicas ❖Não evidencia "o nível" do defeito do eixo ❖Não existe preparação de referência aceitável
Concentração integrada GH em 12 ou 24 horas (amostras de sangue de 20/20' por 12/ 24 hs)	❖Sensibilidade e especificidade semelhantes aos testes de GH ❖Pesquisas científicas ❖Indicado na discrepância entre os testes de estímulo de GH e o IGF-1	❖Necessário hospitalização ❖Custo elevado ❖Inconveniente provocado ao paciente.
Testes de estímulo da secreção de GH Manipulação farmacológica do tônus somatostinérgico ou GHRH*érgico* hipotalâmicos ou das condições fisiológicas que afetam a secreção de GH **arginina** **clonidina** **glucagon** **insulina** **L-dopa**	❖Prática clínica comum na avaliação da causa de baixa estatura ❖Confirmam a DefGH quando o diagnóstico é suspeito em bases auxológicas ou em um fator predisponente ❖Menor dependência de fatores nutricionais ou doenças crônicas do que o IGF-1 ❖GH clonidina apresenta resultados tão confiáveis quanto o teste da hipoglicemia insulínica, sendo, além disso, mais seguro	❖Diferentes protocolos e estímulos farmacológicos ❖Baixa reprodutibilidade ❖Depende de: Idade, peso corporal, estágio puberal ❖Picos de GH "normais" arbitrários (5, 7 ou 10 ng/ml?) ❖Variação do GH depende: Tipo de ensaio empregado, preparação de referência, pureza do traçador de GH, anticorpo monoclonal X policlonal método básico de medida ❖Não há consenso quanto ao "priming" ❖Respostas normais ao reteste
Avaliação Genética Técnica promissora	mutação./ del. gene do GH, gene PIT-I - GH, TSH e PRL, gene PROP-I - GH, TSH, LH, FSH, ACTH e PRL outros	❖ Custo elevado ❖Inacessibilidade
GH urinário **IGF-II** **IGFBP-2** **Sub-unidade ácido-lábil**	Podem ser úteis quando utilizados em combinação com outros testes	❖não são diagnósticos