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Análise do custo-efetividade da triagem neonatal para a hiperplasia adrenal congênita: comparação de duas populações diagnosticadas com e sem triagem neonatal

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RESUMO

Miranda MC. *Análise do custo-efetividade da triagem neonatal para a hiperplasia adrenal congênita*: comparação de duas populações diagnosticadas com e sem triagem neonatal [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2020.

Introdução: A hiperplasia adrenal congênita por deficiência da 21-hidroxilase (HAC) é uma doença de eleição para um programa de triagem neonatal. Entretanto, a implementação triagem para essa doença ainda não é um consenso mundial, devido a escassez de informação sobre a sua custo-efetividade. **Objetivo:** Determinar o custo-efetividade do programa de triagem neonatal (TN) para HAC no Brasil, avaliando seu benefício no incremento do diagnóstico precoce da doença, na diminuição da taxa de mortalidade e no impacto na redução dos custos em saúde pública em relação à diminuição de sua morbidade em longo prazo. **Metodologia:** Foi construído um modelo de decisão para comparar as duas estratégias, testado por análise de sensibilidade. Os dados dos pacientes não triados foram extraídos dos prontuários médicos do Hospital das Clínicas-São Paulo e os dados da triagem do Centro de referência de triagem de São Paulo, Instituto Jô Clemente. A perspectiva do custo é a do Sistema Único de Saúde. Os desfechos relacionados ao diagnóstico tardio foram medidos em ambas as coortes e a razão incremental de custo-efetividade (RICE) foi calculada. **Pacientes:** 195 pacientes clássicos, diagnosticados clinicamente e confirmados por análise hormonal/molecular, e 378.790 recém-nascidos submetidos à TN em 2017. **Resultados:** Na análise de custo da coorte não triada, na forma perdedora de sal a hospitalização ocorreu em 76% dos pacientes, comprometimento mental em 8,6% e 3% das mulheres foram criadas no sexo masculino. O custo total desses desfechos foi de US\$ 86.230/paciente com perda de sal. A leuprorelina foi utilizada em 28% dos pacientes com a forma virilizante simples e o hormônio do crescimento em 14%; 18% das meninas foram criadas no sexo masculino. O custo evitável foi de US \$ 4.232,74/paciente com virilização simples. Entre os 378,790 recém-nascidos (RN) triados, 25 eram afetados pela doença (incidência de 1:15.135). A taxa de falso-positivo da triagem foi de 0,22%. A taxa de mortalidade foi calculada em 11% nos não triados, enquanto não foram relatadas mortes pela doença na coorte triada. Comparando a coorte não triada versus a triada, o nível médio de sódio sérico foi de 121,2 mEq/L (118,3 to 124,1) x 131,8 mEq/L (129,3 to 134,5), a idade média no diagnóstico foi de 38,8 x 17 dias e a hospitalização ocorreu em 76% x 58% dos pacientes com a forma perdedora de sal, respectivamente. O custo incremental da triagem neonatal foi de US\$ 771.185,82 por morte evitada, o que gera uma RICE de US\$ 25.535,95 por anos de vidasalvo, considerada custo-efetiva. **Conclusão:** A realidade brasileira em relação a HAC é de

diagnóstico clínico ainda é deficiente e por tanto atrasado, levando a ocorrência de diversos desfechos adversos, que tem impacto econômico para o estado e psicossocial para os pacientes. Considerando esse contexto, a triagem neonatal para HAC é uma estratégia custo-efetiva e através do presente estudo tem respaldo científico para ser implementada em todo território nacional.

Descritores: Triagem neonatal; Custo-efetividade; Hiperplasia suprarrenal congênita; Doenças raras; Transtornos do desenvolvimento sexual; Custos e análise de custo

ABSTRACT

Miranda MC. *Cost-effectiveness analysis of newborn screening for congenital adrenal hyperplasia: comparison of two populations diagnosed with and without newborn screening* [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2020.

Introduction: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH) is a eligible disease for a newborn screening program (NBS). However, the implementation of NBS for this disease is not yet a worldwide consensus, due to the lack of information about its cost-effectiveness. **Objective:** To determine the cost-effectiveness of the NBS for CAH in Brazil, assessing its benefit in increasing the early diagnosis of the disease, in decreasing the mortality rate and in the impact on reducing public health costs in relation to decreased long-term morbidity. **Methodology:** A decision model was developed to compare the two strategies, tested by a sensitivity analysis. The data of the unscreened patients were extracted from the medical records of the Hospital das Clínicas-São Paulo and the NBS data from the São Paulo Reference Screening Center, Instituto Jô Clemente. The cost perspective is from the Public Health System. The outcomes related to late diagnosis were measured in both cohorts and the incremental cost-effectiveness ratio (ICER) was calculated. **Patients:** 195 classical patients, clinically diagnosed and confirmed by hormonal / molecular analysis, and 378,790 newborns screened in 2017. **Results:** The cost of illness analysis of the unscreened cohort revealed that in salt wasting form hospitalization occurred in 76% of patients, mental impairment in 8.6% and 3% of females were raised as males. These outcomes' total cost was US\$86,230/salt wasting patient. Leuporelin was used in 28% of simple-virilizing patients and growth hormone in 14%; 18% of females were raised as males. The preventable cost was US\$4,232.74/simple-virilizing patient. Among the 378,790 newborns screened, 25 were affected by the disease (incidence of 1: 15,135). The false-positive rate of the screening was 0.22%. The mortality rate was estimated at 11% in the unscreened, while no deaths from the disease were reported in the screened cohort. Comparing the unscreened versus screened cohort, the mean serum sodium level was 121.2 mEq/L (118.3 to 124.1) x 131.8 mEq/L (129.3 to 134.5), the mean age at diagnosis was 38.8 x 17 days and hospitalization occurred at 76% x 58 % of patients with the salt-wasting form, respectively. The incremental cost of neonatal screening was US\$ 771,185.82 for avoided death, which generates an ICER of US\$ 25,535.95 for years of life saved, considered cost-effective. **Conclusion:** The Brazilian reality in relation to CAH is that the clinical diagnosis is still deficient and therefore delayed, leading to the occurrence of several adverse outcomes, which have an economic impact for the state and psychosocial for the

patients. Considering this context, NBS for CAH is a cost-effective strategy and through this study has scientific support to be implemented throughout the national territory.

Descriptors: Neonatal screening; Cost-effectiveness; Adrenal; Rare diseases; Disorders of sex development; Costs and cost analysis hyperplasia, congenital

ABREVIATURAS E SIGLAS

17OHP	17 -hidroxiprogesterona
21-OH	21-hidroxilase
ACE	análise de custo-efetividade
ACTH	hormônio adrenocorticotrófico
A4	androstenediona
CID-10	Classificação Internacional de Doenças
<i>ELISA</i>	<i>enzyme-linked immunoabsorbent assay</i>
FIA	testes imunofluorimétricos
FP	Falso-positivo
GH	Hormônio do crescimento
GnRha	Análogo do hormônio liberador de gonadotrofinas
HAC	Hiperplasia Adrenal Congênita
K.	potássio
LC-MS / MS	cromatografia líquida- espectrometria de massa
LY	Anos de vida salvos
MS	Ministério da Saúde
Na	Sódio
PNTN	Programa Nacional de Triagem Neonatal
PS	Perdedor de sal
RIA	radioimunoensaio
RICE	Razão Incremental de Custo- Efetividade
RN	Recém-nascido
SIGTAP	Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos, Órgãos e Próteses do SUS,
SRTN-SP	Serviço de Referência de Triagem Neonatal de São Paulo
SUS	Sistema Único de Saúde
TN	Triagem neonatal
US\$	Dólar americano
UTI	Unidade de Terapia Intensiva
VS	Virilizante simples

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A doença é causada por mutações no gene que codifica a enzima 21-OH, *CYP21A2*, localizado no cromossomo 6p21.3. Este gene possui um pseudogene em cadeia altamente homólogo, *CYP21A1P*, situação esta que contribui para o emparelhamento desigual na meiose e, assim, para maior probabilidade tanto de rearranjos cromossômicos quanto transferências de mutações de ponto do pseudogene para o gene ativo. Mutações diversas levam a diferentes graus de comprometimento da atividade enzimática e, conseqüentemente, se correlacionam com formas clínicas de diferentes gravidades⁶⁻⁸.

As formas clínicas da HAC 21-OH são divididas em dois grandes grupos: forma clássica, com manifestações ao nascimento e forma não clássica, cujos sintomas se iniciam tardiamente, podendo ser na infância, puberdade ou vida adulta. A forma clássica é subdividida em perdedora de sal e virilizante simples⁹.

A forma perdedora de sal (PS) compreende 70 a 75% dos casos de forma clássica em países desenvolvidos¹⁰. Estes pacientes são carreadores de mutações, em homozigose ou em heterozigose composta, que resultam em comprometimento grave da atividade enzimática (atividade < 2%)^{11,12}. Uma vez que a 21-OH também é essencial para a síntese de mineralocorticoide, este comprometimento enzimático na síntese de aldosterona resulta em perda de peso seguida de desidratação hiponatrêmica nas primeiras semanas de vida e potencial evolução para choque hipovolêmico e óbito sem diagnóstico precoce¹³.

A forma virilizante simples (VS) caracteriza-se no sexo feminino por virilização pré-natal da genitália externa, que varia desde clitoromegalia isolada à fusão completa das pregas urogenitais, podendo ter aparência semelhante a genitália masculina normal. No sexo masculino, é descrito macrogenitossomia ao nascimento, não perceptível na maioria dos casos, o que contribui para maior dificuldade diagnóstica deste sexo. Esta forma clínica também causa virilização pós-natal em ambos os sexos, com aumento progressivo do clitóris ou pênis, aparecimento precoce dos pelos pubianos, aumento da massa muscular, acne e engrossamento da voz¹³. Ocorre também incremento da velocidade de crescimento, porém com maior avanço da idade óssea e, assim, fechamento epifisário precoce com baixa estatura final¹⁴. Os pacientes com esta forma clínica são carreadores de mutações que resultam em 3-7% de atividade residual da 21-OH, em homozigose ou em heterozigose composta com as mutações mencionadas anteriormente¹². Embora a síntese de aldosterona também esteja comprometida, essa atividade residual é suficiente para evitar a depleção volêmica verificada na forma perdedora de sal. As manifestações hiperandrogênicas, entretanto, são comuns a ambas as formas clínicas.

As manifestações da forma não clássica ocorrem mais tardiamente durante o desenvolvimento, com intensidades muito menores e complicações muito menos graves.

Compreendem basicamente aparecimento precoce de pelos pubianos em ambos os sexos, hirsutismo, irregularidade menstrual e/ou infertilidade¹⁵.

As formas clínicas da HAC-21OH de interesse para programas de triagem neonatal são a PS e VS. Quando não diagnosticadas precocemente, apresentam complicações potencialmente graves, tais como perda de sal com desidratação hiponatrêmica, virilização da genitália externa no sexo feminino causando erros de determinação do sexo ao nascimento e puberdade precoce no sexo masculino com comprometimento da altura final. Essas situações acarretam custos elevados para o sistema de saúde, inclusive pela necessidade do uso de medicações caras.

A Triagem Neonatal

Para incluir uma doença em um programa de triagem neonatal é preciso que esta preencha alguns critérios de elegibilidade, baseados nos princípios de Wilson e Junger. Dentre eles, destaca-se: a) necessidade da doença ter impacto na saúde da população com incidência elevada e taxa alta de morbi/mortalidade, b) manifestações clínicas não facilmente reconhecíveis por meios clínicos em fases iniciais, quando se é possível evitar as sequelas, c) exame diagnóstico com método de fácil execução e custo baixo, d) história natural conhecida e possibilidade de instituição de tratamento eficaz que mude sua evolução; desta forma o processo da triagem em uma população pode se tornar uma medida de saúde pública custo-efetiva¹⁶.

A HAC-21OH é considerada uma doença de eleição para inclusão em programas públicos de triagem neonatal, pois é potencialmente fatal, tem incidência elevada, sendo seu reconhecimento e tratamento precoces imprescindíveis para prevenir a sua morbi/mortalidade. O Alasca foi o primeiro estado a incluir a HAC no programa de triagem neonatal, pois a incidência desta doença é ainda mais elevada dentre os esquimós *Yupik*. Após a introdução da triagem, a incidência de indivíduos com a forma perdedora de sal nesse estado dobrou, passou de 1:490 para 1:282¹⁷. Atualmente, todos os 50 estados dos Estados Unidos, 35 outros países e porções de mais outros 17 já adotaram a triagem neonatal para HAC¹⁸. Entretanto, apesar de a *Endocrine Society* incluir em suas recomendações a adoção de um programa de triagem neonatal para a HAC, sua implementação nas políticas de saúde pública ainda não é um consenso mundial e alguns países, mesmo países desenvolvidos como Reino Unido e Canadá, não realizam universalmente a triagem neonatal para HAC em seu sistema público¹⁹. A incidência da HAC varia muito de acordo com a população estudada; Pang et al. coletaram dados de 29 programas de triagem existentes em 13 países. Dos casos confirmados, 68% só foram identificados pelos testes de triagem. A incidência mundial para a forma clássica a partir

dos dados de 6,5 milhões de recém-nascidos foi de 1:15.000 nascidos vivos, variando de 1:10.000 a 1:14.000 na maioria das populações caucasianas¹⁰. No Brasil, estudo com populações triadas revelaram uma incidência média parecida com a mundial variando de 1:10.000-15.000²⁰⁻²³.

O Programa Nacional de Triagem Neonatal (PNTN) brasileiro, implantado com a Portaria do Ministério da Saúde (MS) nº 822 de 06/06/2001, recomenda a realização da triagem neonatal para fenilcetonúria, hipotireoidismo congênito, doenças falciformes e outras hemoglobinopatias e fibrose cística²⁴. A Portaria MS nº 822 também considera a necessidade de definir e ampliar a gama de doenças congênitas a serem prioritariamente incluídas no programa, desde que levem em conta, entre outros aspectos, a sua frequência na população, a possibilidade de tratamento e os benefícios gerados à saúde pública.

No Brasil, o estado de Goiás foi o primeiro a adotar o programa de triagem da HAC, sendo seguido pelo estado de Santa Catarina. Alguns estados, como Minas Gerais e São Paulo têm implementado projetos pilotos e em dezembro de 2012, o Ministério da Saúde anunciou a inclusão da HAC e deficiência de Biotinidase no Programa Nacional de Triagem Neonatal (PNTN), que foi implantada inicialmente em 9 estados²⁵.

O processo de triagem consiste na coleta do sangue por meio de uma punção no calcanhar em cartões de papel-filtro, três a cinco dias após o nascimento. As amostras de sangue em papel-filtro são obtidas em unidades básicas de saúde ou em hospitais, para recém-nascidos pré-termo ou doentes, e enviadas ao local onde são processadas. É então realizada a dosagem da 17-OHP na amostra. As técnicas para medida da 17-OHP neonatal disponíveis incluem radioimunoensaio (RIA), testes imunoenzimáticos - *enzyme-linked immunoabsorbent assay* (ELISA) e testes imunofluorimétricos (FIA). O RIA foi o primeiro método desenvolvido, mas, como o ELISA, tem sido quase que completamente substituído pelo método imunofluorimétrico *dissociation-enhanced, lanthanide fluorescence immunoassay* (DELFA®), que é altamente automatizado²⁶.

Apesar de sua acurácia diagnóstica, o teste ainda apresenta considerável número de resultados falso positivos, relacionados, principalmente com prematuridade e baixo peso ao nascimento. Sabe-se que as taxas de resultados falso-positivos podem ser reduzidas através da implementação de valores corte para a 17OH-progesterona neonatal estratificados em categorias de peso ao nascer ou pela idade gestacional, minimizando o problema^{19,27,28}. Os pacientes com resultados positivos são convocados para avaliação clínica e realização de teste confirmatório mais específico. Ainda assim, 7-17% dos indivíduos não afetados permanecem com teste confirmatório alterado e são mantidos em acompanhamento até a definição

diagnóstica, o que aumenta os custos do programa de triagem, além de causar impacto psicológico nas famílias. Em estudo prévio realizado em conjunto com o programa de triagem do Estado de Goiás, evidenciou-se que 96 neonatos tiveram diagnóstico de HAC entre 1997-2007, outros 19 casos permaneceram em acompanhamento por período que variou de 2 até 18 meses por persistirem assintomáticos e com valores de 17OHP persistentemente elevados. A fim de se esclarecer o diagnóstico, foi realizado o teste molecular confirmatório (sequenciamento do gene da 21-hidroxilase) e observado que 3 meninos possuíam genótipo preditor da forma clássica virilizante simples, outros 2 meninos preditores da forma não clássica e 5 crianças apresentaram mutações em heterozigose, as quais receberam alta do programa por não serem afetadas. Mutações não foram identificadas nas amostras de DNA dos 9 pacientes remanescentes, os quais também receberam alta do programa²⁹.

Avaliação de custo-efetividade da triagem neonatal para HAC

Alguns estudos analisaram o benefício real do diagnóstico precoce da HAC através da triagem neonatal, mas, até hoje, os dados são conflitantes. Os resultados variam de acordo com a população e a metodologia do estudo, o que limita a implementação do programa em diversos países. Ao nosso ver, suas vantagens devem ser consideradas, sobretudo, em países em desenvolvimento, nos quais os programas de saúde são deficitários na realização do diagnóstico precoce por meios clínicos.

É sabido que em uma determinada população, a melhor maneira de se avaliar a eficiência de uma triagem seria a comparação de dados de coorte triadas e não triadas. Em estudo conduzido na Holanda, a análise dos dados de regiões com e sem triagem neonatal, evidenciou-se que a triagem permitiu redução significativa da idade (7 dias vs 14 dias) e do valor de hiponatremia (134,5 vs 124,5 mEq/L) ao diagnóstico de bebês com a forma perdedora de sal³⁰. Dados similares foram observados na população americana, a comparação de neonatos submetidos à triagem no Texas com os não triados no Arkansas e Oklahoma também identificou diminuição da idade ao diagnóstico, em torno de 2 semanas, nos meninos com a forma perdedora de sal. Embora sem diferença significativa, foi ainda evidenciado que os pacientes com a forma perdedora de sal do sexo masculino não triados tiveram uma permanência hospitalar de 7,5 dias a mais que o grupo triado³¹.

Para se estabelecer avaliação mais precisa de custo-efetividade da triagem para HAC, é preciso se obter uma estimativa da mortalidade secundária a crise de perda de sal, que, no entanto, apresentam estimativas variáveis em diversos estudos³². Por exemplo, na Finlândia

estimou-se que a HAC era responsável por 1 morte a cada 110-170 mil neonatos não triados, presumindo uma taxa de mortalidade entre 16-25%³³. Outros estudos realizados nos EUA têm citado taxas diferentes que variam de 9 a 13%, mas a maioria foi baseado em opinião de especialista. Em uma revisão sistemática conduzida para levantar dados para um estudo de custo-efetividade, Grosse *et al* encontraram taxas de mortalidade que variaram de 2 a 9%³². Em 2009, este mesmo autor avaliou o custo-efetividade do diagnóstico da HAC através de um programa de triagem comparando-o com o diagnóstico sem triagem. Foram utilizados dados da literatura sobre mortalidade pela doença com o diagnóstico clínico *versus* diagnóstico pela triagem, além de ter sido avaliado o custo adicional da implementação da triagem da HAC. Através do método de análise de sensibilidade, utilizando anos de vida salvos como a medida de benefício, os autores criaram dois cenários, um em que todas as variáveis seriam propícias à implementação da triagem e outro em que ocorreria o contrário. Observou-se que a razão custo-efetividade incremental era particularmente sensível às suposições a respeito da taxa de mortalidade para a forma perdedora de sal, em uma faixa de 2-9 %, sendo os custos-efetividade incrementais desse estudo para o melhor (maior mortalidade) e o pior caso-cenário (menor mortalidade) de US\$ 30.900 e US\$ 2,9 milhões por anos de vida salvo, respectivamente. Concluiu-se que, usando critérios de rentabilidade, que a triagem para HAC não seria considerada custo-efetiva³⁴. Brosnan *et al* também encontraram valor acima do considerado rentável na análise de coorte de neonatos no Texas em 1994, o custo incremental da inclusão da HAC em programa de triagem seria de US\$ 147.093 por criança diagnosticada (ano base 1994)³⁵. Porém, vale a pena ressaltar que estes dois estudos avaliaram populações americanas, na qual a taxa de diagnóstico precoce neonatal é elevada sem a triagem.

Similarmente, análise retrospectiva em 117 crianças com HAC, nascidas entre 1958 a 1985 no Reino Unido, identificou eficiência do diagnóstico sem a triagem. Nesta casuística, mais do que 90% dos casos foram diagnosticados no primeiro mês de vida e apenas 2 mortes por perda de sal foram registradas, ambas antes de 1970. Considerando-se os avanços no reconhecimento precoce da HAC por parte dos médicos e das tecnologias de saúde disponíveis no país, a triagem neonatal para HAC seria desnecessária no Reino Unido³⁶. Estudos que avaliaram tendências temporais, como os acima, evidenciam queda da mortalidade em coortes de nascimentos mais recentes; como na Hungria, a taxa de mortalidade para pacientes com HAC foi de 22,2% entre 1969-1983 e caiu para 4,5% durante o período entre 1983-1998. Nesse mesmo estudo foi observado que o tempo de diagnóstico dos casos perdedores de sal diminuiu significativamente ao longo dos 30 anos de observação, com a média de idade ao diagnóstico de 20 dias no período de 1969-1983 e 15 dias no período de 1983-1998³⁷.

O predomínio do sexo feminino sobre o masculino nos casos diagnosticados sem triagem é um fato que pode indicar indiretamente a subestimação da mortalidade da doença, levando à hipótese de que os meninos tiveram morte precoce, sem o diagnóstico¹⁹. Estudos de países desenvolvidos, no entanto, não costumam apresentar essa diferença. Na Holanda, no período entre 1998-1999, 17 crianças foram diagnosticadas em 8 províncias sem triagem e 15 crianças em 4 províncias com triagem. Não se observou diferença entre as taxas de incidência da doença entre os dois grupos, assim como da relação menina/menino e nenhuma morte foi reportada³⁰. Resultados semelhantes foram publicados em outros países como Itália, Suécia e Inglaterra, onde a razão menina/menino dentre os neonatos com a forma perdedora de sal não ultrapassou 1.00³².

Vale ressaltar que e a maioria desses estudos foram realizados em países desenvolvidos e em populações com características genéticas, demográficas e culturais diferentes da população brasileira. Consequentemente, a transferência dos resultados obtidos para o sistema de saúde brasileiro possui valor limitado. As diferenças dos níveis socioeconômicos e as características do sistema de saúde de cada país podem modificar as estimativas de custo- efetividade.

O fato acima discutido pode ser evidenciado na análise dos casos diagnosticados em outros países em desenvolvimento, nos quais a taxa de mortalidade neonatal pela HAC é significativamente maior. Na Índia, 86 crianças foram diagnosticadas entre 1996-2010, sendo 88,4% do sexo feminino. Apenas 18 crianças (21%) tinham a forma clássica perdedora de sal e destas, apenas 7 eram do sexo masculino, levando a uma relação menina/menino de 1,6:1. Adicionalmente 2/7 meninos apresentaram hiponatremia grave ao diagnóstico que resultou em déficit cognitivo grave. A idade média ao diagnóstico dos pacientes com a forma virilizante foi muito tardia, aproximadamente aos 9 anos e, duas crianças 46,XX foram registradas e criadas no sexo social masculino³⁸. Este estudo não relata o acompanhamento de longo prazo e não sabemos se as meninas com diagnóstico tardio possam também ter apresentado mudança de sexo social na idade adulta.

A Disciplina de Endocrinologia e Metabologia do Hospital das Clínicas-SP possui um centro médico multidisciplinar de acompanhamento de pacientes com HAC. Nos últimos 25 anos, temos registros do seguimento de aproximadamente 210 pacientes com a forma clássica da HAC, tendo sido todos eles diagnosticados previamente à introdução da triagem neonatal. Dados não publicados evidenciam razão desproporcional de meninas/meninos diagnosticados (~2,8:1), bem como proporção menor de pacientes com a forma perdedora de sal, correspondendo a apenas 55% dos casos com forma clássica. Adicionalmente, há possibilidade de que a perda de diagnóstico da HAC possa ser ainda maior nas regiões de menor

desenvolvimento econômico.

Até mesmo em países mais desenvolvidos, nos quais o diagnóstico clínico e conhecimento da doença seriam melhores, tem sido descrito uma relação desproporcional de acometimento entre meninas e meninos, sugerindo falha no diagnóstico clínico. Na Austrália, uma avaliação realizada entre 1990 e 2010 revelou 41 casos da doença, dos quais 26 eram meninas, levando a uma relação menina:menino de 1,7:1^{39,40}. Outro estudo realizado em uma província do Canadá revelou uma proporção de 2,2:1³⁶. Até mesmo na Inglaterra, já foi evidenciado uma preponderância do sexo feminino entre os pacientes afetados pela doença, sugerindo a importância da triagem neonatal, que até hoje ainda não é realizada no país⁴¹.

Outro ponto controverso na literatura na implementação da triagem da HAC é sobre a necessidade de se testar as meninas, considerando-se que a atipia genital é uma característica evidente ao nascimento, o que facilita o diagnóstico precoce. Interessantemente, no Texas, onde a HAC está incluída no programa de triagem, observou-se que o diagnóstico foi possibilitado apenas pelo resultado do teste neonatal em 8/15 crianças com as formas clássicas e em 3 destas 8 meninas a atipia genital não foi identificada ao nascimento³⁵. Na Austrália, entre as 26 meninas diagnosticadas com a doença entre os anos de 1990 e 2010, 4 foram registradas como meninos devido importante virilização da genitália e 4 não terem a atipia genital diagnosticada ao nascimento³⁹.

A atipia genital nem sempre é identificada ao nascimento e isso por vezes pode levar ao diagnóstico tardio, fazendo com que essas meninas cresçam no sexo social masculino. Estes pacientes 46,XX têm que ser submetidos a acompanhamento psicológico contínuo, genitoplastia masculinizante, mastectomia e pan-histerectomia total. Adicionalmente, necessitarão de reposição contínua com andrógenos por toda a vida. Estes dados sugerem que o registro social inadequado pode impactar significativamente na qualidade de vida de pacientes com HAC, além de aumentar os custos do tratamento⁴². No Estado de Goiás, que tem realizado a triagem da HAC e, conseqüentemente, os serviços de saúde já estão acostumados com este diagnóstico, o cenário é um pouco melhor no reconhecimento neonatal da atipia genital nas meninas; ainda assim, 28% delas só tiveram sua identificação pelo resultado do teste da triagem.

O custo da triagem para HAC inclui não somente a dosagem de 17-OHP na amostra no papel filtro como também os custos do acompanhamento de casos falso-positivos, para os quais a definitiva exclusão diagnóstica requer, em algumas situações, tempo longo de seguimento com a realização de consultas médicas e exames hormonais seriados e/ou testes confirmatórios moleculares^{29,43}. Embora se saiba que o custo do teste confirmatório molecular para a HAC seja

elevado, uma vez que o gene da 21-hidroxilase possui 3.700 pares de bases e suas as mutações estão distribuídas ao longo de todo o gene, deve-se levar em conta que o resultado deste teste prontamente elucida a situação diagnóstica e elimina o custo do seguimento de longo prazo dos casos indeterminados. Em universo de 6213 nascidos na *Mayo Clinic Rochester*, a análise do custo do seguimento 66 neonatos com resultados falso-positivos, que incluiu consultas médicas e testes laboratoriais, foi de aproximadamente US\$ 848,00/ paciente⁴⁴.

Geralmente, os benefícios de um programa de triagem são medidos principalmente através do número necessário de recém-nascidos para rastrear a fim de se diagnosticar um caso e salvar uma vida⁴⁵. Existem escassas análises de custo-benefício da triagem neonatal para HAC, as poucas disponíveis levam em conta apenas que o único resultado adverso de diagnóstico tardio seja a morte, especialmente nos meninos, e, portanto, que o benefício é melhor quantificado em anos de vida (crianças salvas por um diagnóstico precoce, multiplicado pela expectativa de vida). Os cálculos dos custos por ano de vida salvo são sensíveis à taxa de mortalidade assumida, e as estimativas variaram amplamente, entre US\$ 20.000⁴⁶ a US\$ 250.000,00 - 300.000,00⁴⁷. Tradicionalmente assume-se que a triagem para uma determinada doença é rentável apenas se apresentar custo de até US\$ 50.000 por ano de vida salvo⁴⁸. Por outro lado, esse valor costuma ser subestimado em populações não triadas, devido os casos de morte pela doença em neonatos não identificados como portadores de HAC.

Vale a pena ressaltar que os trabalhos referidos não consideraram os gastos elevados para o sistema público no acompanhamento de paciente com complicações decorrentes do diagnóstico tardio e, principalmente, o impacto dessas na qualidade de vida. Há evidências da ocorrência de déficits cognitivos, alterações neurológicas e, em casos extremos, até a paralisia cerebral consequentes à hiponatremia em neonatos^{49,50}.

Outro dado que merece atenção é que geralmente os pacientes com a forma virilizante simples não triados apresentam diagnóstico tardio, sobretudo o sexo masculino. Além da precocidade sexual, estes pacientes possuem avanço de idade óssea importante, o que predispõe à estatura final muito baixa. Nestes casos, além da tradicional terapia de reposição hormonal para a HAC, há a necessidade da adição de medicações de custo elevado, como hormônio do crescimento e análogos de GnRH para se bloquear a pseudopuberdade precoce, a fim de se melhorar o potencial de altura final^{51,52}.

Dados como estes nunca foram publicados na literatura nas análises de custo-efetividade para a triagem da HAC. Nossa hipótese é que a triagem neonatal para Hiperplasia Adrenal Congênita é uma estratégia de saúde que pode salvar vidas, melhorar a qualidade de vida dos pacientes e, embora não seja mais barata que a estratégia de não triar, ela é sim custo-efetiva.

OBJETIVOS

Determinar o custo-efetividade do programa de triagem neonatal para HAC no Brasil, utilizando a comparação de duas populações, diagnosticadas com e sem triagem neonatal.

Objetivos específicos:

- Estimar a taxa de mortalidade da HAC na ausência de triagem neonatal.
- Realizar um estudo de custo da doença em pacientes com diagnóstico sem a triagem neonatal utilizando a população de pacientes com diagnóstico clínico acompanhados no HC-FMUSP. Objetivo é avaliar a ocorrência dos desfechos adversos relacionados ao diagnóstico tardio em ambas as formas clínicas e os custos relativos ao tratamento desses desfechos, bem como os custos do tratamento padrão da doença.
- Avaliar o custo da implementação da triagem para HAC em um programa estadual de triagem neonatal, utilizando a população de pacientes triados no ano de 2017 pelo programa nacional de triagem neonatal (PNTN) Estado de São Paulo.
- Avaliar a ocorrência de desidratação por perda de sal nos pacientes afetados triados e comparar taxas de hospitalização e gravidade da perda de sal com a população não triada.
- Comparar a avaliação de custos das duas populações para estabelecer a custo-efetividade da inclusão da triagem neonatal para HAC em um programa nacional.

METODOLOGIA

Aspectos éticos

As duas populações foram avaliadas exclusivamente através da observação de dados clínicos registrados nos prontuários de ambas as instituições envolvidas. Não houve contato direto com os pacientes e nem coleta de material biológico. O protocolo de estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade do São Paulo (número do registro *online* 14558) e estabelecido em respeito às diretrizes científicas vigentes.

Desenho do estudo

Estudo de análise de custo-efetividade para comparar duas estratégias: diagnóstico da HAC através da triagem neonatal universal ou através do reconhecimento clínico. A perspectiva do estudo foi a do Sistema Único de Saúde (SUS), considerando os custos diretos dos cuidados em saúde. A obtenção de evidências foi através de dados primários levantados retrospectivamente das duas populações em estudo (triados e não triados). O horizonte temporal usado na população de não triados foi do diagnóstico aos 19 anos e na população de triada até 1 ano.

População do estudo

Pacientes com o diagnóstico clínico

Os dados foram avaliados retrospectivamente por meio de uma revisão de prontuários de 195 pacientes com HAC (mulheres, n = 141; homens, n = 54), acompanhados no período de 1980 a 2016 no Hospital das Clínicas, em São Paulo, Brasil.

O diagnóstico clínico da forma de virilizante simples foi realizado de acordo com a suspeita clínica pela identificação de genitália atípica em mulheres e precocidade sexual em homens. Pacientes com forma perdedora de sal apresentaram desidratação hiponatrêmica nas primeiras semanas de vida. Todos os pacientes tiveram a suspeita clínica confirmada através da avaliação hormonal e diagnóstico molecular. Os níveis séricos de 17-OHP, androstenediona e renina plasmática foram medidos por radioimunoensaio. Os níveis séricos de cortisol, testosterona e gonadotrofinas foram medidos usando ensaios imunofluorométricos. O genótipo da HAC foi determinado através da análise do *CYP21A2*⁵³.

Pacientes com o diagnóstico através da triagem neonatal

Analizamos os dados do programa de triagem neonatal da HAC do estado de São Paulo, Brasil, de 1º de janeiro a 31 de dezembro de 2017. Um total de 378.790 recém-nascidos foram triados nesse período. O Serviço de Referência de Triagem Neonatal de São Paulo (SRTN-SP) é o laboratório O Instituto Jô Clemente. Os seguintes dados, arquivados no SRTN- SP, foram

registrados pelo pesquisador: peso ao nascer, sexo, data de nascimento e data da amostragem; os resultados da triagem neonatal 17-OHP; papel de filtro confirmatório para a dosagem da 17-OHP no RN de baixo risco e sangue periférico para a dosagem da 17-OHP nos de alto risco.

A coleta do sangue por meio da punção no calcanhar em cartões de papel-filtro foi realizada nos recém-nascidos entre os 2º e 7º dias de vida e depositado em papel de filtro S&S 903. As amostras foram enviadas diariamente ao laboratório de referência para análise de 17OHP. Todos os ensaios hormonais em papel de filtro foram realizados usando o mesmo kit com o mesmo anticorpo: o kit neonatal 17OHP em fase sólida, resolvido no tempo (B015-112- Auto DELFIA - 17OHprogesterona neonatal; Perkin Elmer Life and Analytical Sciences, Wallac Oy, Turku, Finlândia). As concentrações de 17OHP foram expressas como equivalência sérica (taxa de conversão: 1 nmol / l = 0,33 ng / ml no sangue total e 0,43 ng / ml na equivalência sérica).

Os recém-nascidos foram divididos em quatro grupos de acordo com o peso ao nascer (≤ 1500 g; 1501-2000g; 2001-2500g e >2500 g). Os valores de corte para o resultado da análise da 17OHP no teste do pezinho são determinados de acordo com o tempo de coleta e o peso ao nascer, utilizando o percentil 99,8 da normalidade^{23,27} (Tabela 1). Segundo o protocolo de triagem para HAC do estado de São Paulo, os valores abaixo do percentil 99,8 são considerados normais. Aqueles com valores acima de 2 vezes o percentil 99,8 são considerados de alto risco e receberão convocação de emergência; aqueles acima do percentil 99,8 porém abaixo de 2x o percentil 99,8 terão a dosagem no papel-filtro repetida e os critérios de avaliação serão os mesmos para a primeira amostra. Se o novo exame não apresentar valores normais, a criança deverá ser encaminhada para coleta de testes sérico e consulta médica de acompanhamento. (ver Figura 2 – fluxograma da triagem)

Os exames confirmatórios consistem na análise sérica de 17OHP, androstenediona e cortisol por cromatografia líquida-espectrometria de massa (LC-MS / MS) e testosterona por fluoroimunoensaio. Sódio e potássio também foram analisados. Os coeficientes intraensaio e interensaio foram $<8\%$ para o ensaio de papel de filtro. De acordo com o protocolo STRN-SP, todos os RN de baixo peso ao nascer foram submetidos a uma nova coleta de amostras em papel de filtro.

Tabela 1. Percentis dos valores de normalidade da 17-OHP (ng/ml) em soro-equivalência para cada faixa de peso²³.

Faixa de Peso	97,5 th	99 th	99,5 th	99,8 th
< 1500g	81,2	110,4	134,5	160,2
1500-2000g	36	43	53,7	55,2
2001-2500g	21,8	28,2	31,8	40,6
> 2500g	12,4	15,1	17,1	20

Figura 2. Algoritmo de triagem neonatal para HAC no Estado de São Paulo⁵⁴

Parâmetros Clínicos Avaliados

Na população não triada, os parâmetros foram definidos separadamente por forma clínica. Em todos os pacientes, foram avaliados dados relativos ao diagnóstico: média de idade, motivo do diagnóstico, identificação de atipia genital ao nascimento nas meninas, erro de registro do sexo social ao nascimento e média de idade do início do tratamento. Nos perdedores de sal, foi avaliado o número de internações devido desidratação até que o diagnóstico fosse estabelecido, o tempo médio de internação em enfermaria e Unidade de Terapia Intensiva (UTI), a média dos valores séricos de sódio ao diagnóstico, a proporção de pacientes que evoluíram com sequela neurológica devido a hiponatremia grave no período neonatal. Além disso, foi inferida

a taxa de mortalidade pela doença baseada na diferença entre a proporção de perdedores de sal na nossa população em comparação a proporção esperada de acordo com a literatura. Nos virilizantes simples, foi analisada a proporção de pacientes que apresentavam avanço de idade óssea e média de tempo desse avanço, a proporção de pacientes que apresentou pseudo-puberdade precoce, bem como a dos que tiveram que usar medicações de alto custo como análogo do GnRh (*Gonadotrophin Recombinant Hormone*) e Hormônio do Crescimento (GH). Nesse grupo, foi visto o tempo de tratamento e a média de dose de uso. Além disso, foi avaliada a proporção de meninas que tiveram erro de registro ao nascimento e a proporção das quais foram criadas como meninos sendo submetidas ao processo de masculinização, que envolve cirurgia de panhisterectomia e reposição hormonal com testosterona do início da puberdade até o os 19 anos (respeitando o horizonte temporal da análise).

Na população triada, também foram avaliados os parâmetros clínicos ao diagnóstico: média de idade, identificação de atipia genital, proporção e tempo de internação e média de sódio ao diagnóstico. Foram avaliados também dados referentes a triagem como média de tempo da coleta do teste do pezinho e o tempo entre a coleta e o resultado da triagem e convocação para consulta.

Foram avaliados no geral também os parâmetros referentes a triagem como taxa de falso-positivo, taxa de reconvocação, tempo médio de coleta do teste e tempo médio de liberação dos resultados.

Determinação dos custos

Foram computados como custos diretos aqueles resultantes das intervenções. Todos os recursos econômicos que envolvem procedimentos de saúde são iguais em todo o país. Todos os preços foram pesquisados diretamente através do Sistema de Gestão da Tabela SUS, conhecido como SIGTAP - Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos, Órgãos e Próteses do SUS, de acordo com os seguintes códigos da Classificação Internacional de Doenças (CID-10). Esta informação está disponível na plataforma online do Ministério da Saúde⁵⁵. A moeda norte-americana (US\$) foi usada como unidade monetária, para fins de comparação com outros estudos internacionais e publicação internacional; a conversão da moeda brasileira (R\$) para dólar americano foi realizada fixando a taxa de câmbio nos últimos dias de 2016: US\$ 1,00 correspondeu a R\$ 3,26⁵⁶. Os procedimentos de saúde utilizados para análise de custo e os respectivos valores extraídos do SIGTAP estão relacionados na Tabela 2.

Tabela 2. Procedimentos relacionados ao diagnóstico e tratamento da HAC e seus respectivos custos extraídos do SIGTAP⁵⁵

Procedimento	Real	US\$
Hospitalização (03.03.03.006-2 - tratamento de transtornos de outras glândulas endócrinas)	R\$230,76	\$70.77
Diária de UTI 08.02.01.007-5 - diária de unidade de terapia intensiva em pediatria	R\$ 508,73	\$155.99
Terapia hormonal no processo transexuallizador 03.03.03.009-7 - terapia hormonal no processo transexualizador	R\$50,0	\$15.33
Histerectomia e anexectomia bilateral no processo transexualizador 04.09.06.029-1 - histerectomia c/ anexectomia bilateral e colpectomia sob processo transexualizador	R\$1195,80	\$366.70
Prótese testicular 07.02.06.003-8 - prótese testicular em gel de silicone	R\$350,0	\$107.35
Genitoplastia 04.09.07.011-4 - construção de vagina	R\$398,05	\$122.09
Uretroplastia 04.09.02.013-3 - uretroplastia autógena	R\$469,55	\$144.02
Leuprorelina 11,25mg *preço por ampola 06.04.11.004-9 - leuprorrelina 11,25 mg injetável (por frasco-ampola)	R\$890,94	\$273.26
Somatropina 12ui *preço por ampola 06.04.61.002-5 - somatropina 12 ui injetável (por frasco-ampola)	R\$110,76	\$33.97
Consulta médica especializada 03.01.01.007-2 - consulta medica em atenção especializada	R\$10,0	\$3.07
Consulta médica na triagem neonatal 03.01.12.006-4 - acompanhamento em serviço de referência em triagem neonatal (SRTN) - hiperplasia adrenal congênita.	R\$ 27.5	\$ 8.43
Dosagem de 17OHP 02.02.06.004-7 - dosagem de 17-alfa-hidroxiprogesterona	R\$10,2	\$3.12
Dosagem da 17OHP no papel-filtro 02.02.11.009-5 - dosagem de 17 hidroxiprogesterona em papel de filtro	R\$8,00	\$2.45

Dosagem de sódio 02.02.01.063-5 - dosagem de sódio	R\$1,85	\$0,57
Dosagem de potássio 02.02.01.060-0 - dosagem de potássio	R\$1,85	\$0.57
Dosagem de renina 02.02.06.031-4 - dosagem de renina	R\$13,19	\$4.05
Dosagem de testosterona 02.02.06.034-9 - dosagem de testosterona	R\$10,43	\$3.19
Dosagem de androstenediona 02.02.06.011-0 - dosagem de androstenediona	R\$11,53	\$3.54
Rx p/ idade óssea 02.04.04.010-8 - radiografia de mão e punho (p/ determinação de idade óssea)	R\$ 6,00	\$1.84
Hidrocortisona 10mg * preço por comprimido	R\$0,49	\$0.15
Fludrocortisona 100mcg * preço por comprimido 06.01.34.001- 9 fludrocortisona 0,1 mg (por comprimido)	R\$0,38	\$0.12
Dexametasona 0.5mg * preço por comprimido	R\$0,17	\$0.05

Na população submetida à triagem, foi considerado o custo da análise da amostra para a dosagem da 17-OHP neonatal em um programa de triagem já em funcionamento, do reteste no papel de filtro e testes confirmatórios dos casos positivos e da avaliação médica dos casos suspeitos com testes laboratoriais adicionais. Os valores que utilizaremos para esses procedimentos foram previamente estabelecidos pelo Ministério da Saúde⁵⁵. Os custos da triagem estratificados por categoria de risco dos neonatos estão exemplificados na Tabela 3.

Tabela 3. Custos da triagem por paciente

Categorias	Risco de ser afetado	Descrição do custo	Custo por criança (US\$)
Não afetados pela HAC		A	2,45
Não afetados que tiveram que ser retestados		2A	4,9
Falso-positivos	Alto	A+B	24,91
	Baixo	2A+B	27,36
Afetados pela HAC	Alto	A+B	24,91
	Baixo	2A+B	27,36

A – 17OHP no papel de filtro = \$2,45

B – Testes confirmatórios (17OHP \$3,13, cortisol \$3,02, androstenediona \$3,54, testosterona \$3,20, sódio \$0,57 e potássio \$0,57) + Consulta médica (\$8,43) = \$22,46

Na população não triada, os custos foram separados por forma clínica da doença. A análise de custo nessa população foi realizada de duas formas. Na primeira, foi realizada estudo de custo da doença, levando em consideração todos os desfechos adversos encontrados nos 195 pacientes não triados e os custos relacionados ao tratamento desses desfechos e da própria doença desde o diagnóstico até os 19 anos. Na segunda análise, as proporções dos desfechos adversos e alguns custos relacionados ao tratamento foram utilizados para a construção do modelo de decisão para estabelecimento da análise de custo-efetividade da triagem.

No estudo de custo da doença, através da taxa de mortalidade pela doença inferida, foi calculado o valor monetário da morte prematura, a fim de destacar o impacto econômico, até então nunca mensurado, das mortes prematuras devido a ausência do diagnóstico precoce da HAC, que poderiam ser evitadas pela triagem. Foi utilizado o método do capital humano, que considera a perda de produtividade associada à mortalidade prematura⁵⁷. A vida produtiva esperada no Brasil foi estimada em 34,5 anos em 2010⁵⁸. Essa medida representa o número médio de anos que uma pessoa de 15 anos permanecerá no mercado de trabalho se, durante sua vida ativa, estiver exposto às taxas de mortalidade e atividade econômica por faixa etária em vigor em 2010. A taxa de mortalidade foi calculada comparando-se a frequência de perdedores de sal em nossa coorte não triada com a encontrada em outras coortes rastreadas brasileiras^{20,27}. O número de óbitos neonatal por ano foi calculado usando o número de nascidos vivos por ano⁵⁹, a incidência de perdedores de sal e a taxa de mortalidade calculada. O número de anos

perdidos foi multiplicado pelo Produto Interno Bruto (PIB) brasileiro per capita⁶⁰. Foi aplicada uma taxa de desconto de 5% à perda estimada de produção produtiva futura⁶¹.

Além disso, foi calculado também o valor relacionado ao acompanhamento de pacientes com sequelas neurológicas (retardo mental e paralisia cerebral) secundárias à hiponatremia severa neonatal. Como não foi encontrada uma fonte brasileira concreta para esses custos que envolvem tratamento e seguimento multidisciplinar, foram utilizados os dados publicados pelo *Center of Diseases Control and Prevention*, que relacionou os custos diretos e indiretos para o acompanhamento de pacientes com doenças neurológicas como retardo mental e paralisia cerebral⁶². Também foram calculados os custos relativos à hospitalização em enfermaria e UTI nos perdedores de sal e os relativos ao uso de GnRha e hormônio do crescimento, bem como aos custos da masculinização dos pacientes 46,XX criados como meninos, na forma virilizantes simples. Somando tudo, chegou-se a um valor final do custo total do paciente perdedor de sal e do virilizantes simples diagnosticados sem a triagem neonatal.

Na segunda análise, a de custo-efetividade, foi calculada a razão de custo-efetividade incremental pela divisão da diferença de custos diretos entre o diagnóstico clínico e por meio de triagem neonatal e pela diferença de efetividade (anos de vida salvos). Os custos utilizados para cada estratégia foram os descritos acima, multiplicados pela probabilidade de cada parâmetro ocorrer. (ver Figura 3 – Árvore de decisão). Para essa análise, não foi considerado o custo da morte nem o custo das sequelas neurológicas, a fim de não superestimar o valor da razão de custo-efetividade.

Modelo da análise de custo-efetividade

Foi realizada uma análise de custo-efetividade para comparar as estratégias de triagem universal para HAC com o reconhecimento clínico sem triagem; usando dados das duas coortes de pacientes com HAC, diagnosticados pela triagem e por meios clínicos. As frequências de resultados adversos foram avaliadas em ambas as populações e usadas, juntamente com dados sobre a triagem, para construir nosso modelo de decisão. O desenho do estudo seguiu as recomendações para avaliações econômicas em saúde⁶³. (Tabela 4)

Tabela 4. Características do estudo de avaliação econômica

PARÂMETRO	ESPECIFICAÇÃO
Tipo de estudo	Custo-efetividade
Tecnologia/intervenção	Triagem neonatal universal para HAC
Comparador	Diagnóstico por suspeição clínica, sem triagem
População-alvo	Nascidos vivos no Brasil
Desfecho	Complicações a curto e longo prazo do diagnóstico tardio da doença, incluindo mortalidade (Efetividade: Anos de vida salvos)
Horizonte temporal	Consideramos desfechos desde o nascimento até os 19 anos de vida
Taxa de desconto	3%
Perspectiva	Do SUS
Modelo	Árvore de decisão simples escolha
Tipos de custos	Diretos
Busca por evidência	Revisão sistemática
Origem dos dados econômicos	SIGTAP
Origem dos dados de efetividade	Análise retrospectiva de duas coortes de paciente com diagnóstico clínico e com diagnóstico por triagem

Foi calculada a razão de custo-efetividade incremental, que é a razão do custo incremental da triagem neonatal em comparação com a estratégia de não triagem por anos de vida salvos (LY). Foi suposto que a morte evitada de cada recém-nascido economizaria 30,2 anos de vida, aplicando a taxa de desconto de 3% à de expectativa de vida ao nascer no Brasil de 76 anos⁶⁴. A equação do cálculo do RICE é a seguinte: $RICE (US \$ / LY) = (\text{custos do braço da NBS} - \text{custos do braço não rastreado}) / (\text{número de mortes evitadas} \times 30,2 \text{ LY})$. As análises foram realizadas com o software de análise de decisão *TreeAge Pro Health Care* e *Excel*. A incidência de 1: 12.250 é uma média entre a incidência observada no presente estudo e outras descritas anteriormente^{20,21}. Consideramos a proporção de 75% dos pacientes com a forma PS, amplamente relatada na literatura e em estudo brasileiro de triagem neonatal^{19,20}. O

reconhecimento clínico foi a probabilidade de um caso ser detectado antes dos sintomas devido a atipia genital ao nascimento ou aos antecedentes familiares, portanto, antes da notificação dos resultados da TN. Essa probabilidade foi baseada na proporção de pacientes com a forma PS diagnosticados antes da desidratação e nos pacientes com a forma VS diagnosticados nos primeiros 30 dias de vida em nossa coorte não triada; a média entre essas proporções e a publicada anteriormente por *Yoo et al*⁴⁷ foi utilizada na análise. Os demais parâmetros foram extraídos dos dados das duas coortes analisadas. O modelo de decisão foi aplicado em uma população hipotética utilizando o número total de nascidos vivos em 2017 no Brasil, de acordo com os dados do Ministério da Saúde do Brasil⁵⁹. A Tabela 5 mostra os parâmetros utilizados na análise de decisão.

Considerando a incerteza inerente nos valores de várias suposições, a robustez do modelo foi testada aplicando uma análise de sensibilidade unidirecional para examinar como o RICE é influenciado pelo intervalo de cada parâmetro. Essa análise de sensibilidade envolveu quatro variáveis. Além disso, as análises de cenário 'melhor ou pior' selecionaram os valores mais e menos favoráveis para cada parâmetro, respectivamente.

Tabela 5. Parâmetros e estimativas utilizadas para análise de decisão do caso-base e dos melhor e pior cenários para a triagem neonatal para HAC

Parâmetro	Estimativa de probabilidade			Referencia
	Caso Base	Melhor Cenário	Pior cenário	
Expectativa de Vida no Brasil 2017 Anos de vida salvo com 3% de desconto	76 anos 30,2 LY	76 anos 30,2 LY	76 anos 30,2 LY	64
Incidência da HAC	1:12.250	1:10.000	1:15.000	²¹ estudo atual
% PS	75%	75%	75%	19,20
Taxa de falso-positivo no TP	0,2%	0,1%	0,5%	⁴⁷ estudo atual
FP de alto risco	25%	25%	25%	estudo atual
FP no teste confirmatório	8%	8%	8%	estudo atual
FP de baixo risco	75%	75%	75%	estudo atual
FP no reteste do TP	0,5%	0,5%	0,5%	estudo atual
Forma PS				
% Reconhecimento clínico	36%	16%	55%	⁴⁷ estudo atual
Mortalidade do PS sem TN	8%	11%	4,2%	⁴⁷ estudo atual
% Hospitalização com TN	58%	58%	58%	estudo atual
% UTI dentre os hospitalizados com TN	30%	30%	30%	estudo atual
Média de permanência em UTI	9 dias	9 dias	9 dias	estudo atual
% Hospitalização dos não triados	91%	91%	91%	estudo atual
% UTI dentre os hospitalizados não triados	36%	36%	36%	
Média de permanência em UTI	23 dias	23 dias	23 dias	
Forma VS				
% Reconhecimento clínico	28%	11%	45%	⁴⁷ estudo atual
% uso de GH	14%	14%	14%	estudo atual
Tempo de tratamento com GH	3,2 anos	3,2 anos	3,2 anos	
Média de dose diária de GH	5,9ui	5,9ui	5,9ui	
% de meninas submetidas ao processo de masculinização	15%	15%	15%	
% uso de GnRha	28%	28%	28%	
Tempo de tratamento com GnRha	3,8 anos	3,8 anos	3,8 anos	

Figura 3. Modelo da Árvore de Decisões

RESULTADOS

O presente estudo permitiu a confecção de dois artigos originais que serão apresentados na forma em que foram preparados para publicação. A confecção dos artigos foi realizada com a colaboração fundamental de outros pesquisadores, evidenciado na autoria dos manuscritos. A descrição específica dos resultados de cada um dos estudos que compõe a tese está no corpo dos textos apresentados.

CAPÍTULO 1 - *Adverse outcomes and economic burden of congenital adrenal hyperplasia late diagnosis in the absence of newborn screening*

O primeiro artigo compreende a análise de custo da Hiperplasia Adrenal Congênita diagnosticada em um contexto sem a triagem neonatal.

Compreender os custos em curto e longo prazo dos cuidados associados ao diagnóstico tardio de HAC é importante para a análise de custo-efetividade da implementação da TN para a doença. Em particular, informações sobre como os custos do tratamento são alterados quando a triagem evita a progressão da doença podem ser uma contribuição valiosa para futuras análises econômicas. Por exemplo, os custos diretos incrementais relacionados à hospitalização neonatal, terapias para erros na atribuição de sexo ao nascimento e puberdade precoce devem ser incluídos.

A frequência de desfechos adversos em uma grande população não rastreada por HAC e seus respectivos custos de tratamento nunca foram relatados na literatura. Nesse sentido, o objetivo deste artigo foi realizar um estudo de custo de doença de uma coorte de HAC diagnosticada clinicamente sem triagem neonatal, como um primeiro passo para realizar uma avaliação econômica abrangente da implementação da triagem para HAC, considerando não apenas seu benefício na redução da mortalidade, mas também seu impacto na redução de custos relacionados ao tratamento de resultados adversos a longo prazo.

A coorte compreende 195 pacientes com a doença seguidos no nosso ambulatório de Endocrinologia do Hospital das Clínicas, todos com diagnóstico clínico confirmado por biologia molecular. Dos 195, 105 são da forma perdedora de sal (54%) e 90 da forma virilizante simples. Como se tratava de uma coorte histórica dos últimos 25 anos, estratificamos os dados por décadas de nascimento, para avaliar se houve melhora do diagnóstico ao longo dos anos. Os desfechos medidos foram taxa de mortalidade, desidratação, frequências de sequelas neurológicas secundárias a desidratação neonatal e tempo de hospitalização na forma perdedora de sal; a frequência de pacientes do sexo genético feminino criadas no sexo social masculino em ambas as formas, e a frequência das terapias com análogo de GnRh e hormônio do crescimento na forma de virilizante simples. Os custos relacionados a esses resultados foram calculados.

O artigo foi publicado em 2019 no periódico *Journal of the Endocrine Society* e recebeu destaque sendo publicado e comentado na página da *Endocrine Society, MedPage Today*.

Adverse Outcomes and Economic Burden of Congenital Adrenal Hyperplasia Late Diagnosis in the Newborn Screening Absence

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Objective: To establish short- and long-term adverse outcome frequencies related to a late diagnosis of congenital adrenal hyperplasia (CAH) in the absence of newborn screening (NBS) and to determine respective treatment costs, which have never been reported.

Design: A retrospective analysis of a CAH cohort diagnosed without NBS.

Methods: We evaluated medical record data concerning 195 patients (141 females) diagnosed with CAH through clinical suspicion and confirmed using hormonal and *CYP21A2* analysis, who were followed from 1980 to 2016 at Sao Paulo University. We measured mortality, dehydration, mental impairment frequencies, and hospitalization length outcomes in the salt-wasting form; the frequency of genetic females raised as males in both forms, frequency of depot GnRh analog (GnRha) and GH therapies in the simple virilizing form, and related outcome costs were calculated.

Results: Mortality rates and associated costs, varying from 10% to 26% and from \$2,239,744.76 to \$10,271,591.25, respectively, were calculated using the Brazilian yearly live-births rate, estimated productive life years, and gross domestic product. In the salt-wasting form, 76% of patients were hospitalized, 8.6% were mentally impaired, and 3% of females were raised as males (total cost, \$86,230/salt-wasting patient). GnRha and growth hormone were used for 28% and 14% of simple virilizing patients, respectively, and 18% of females were raised as males (preventable cost, \$4232.74/simple virilizing patient).

Conclusions: A late CAH diagnosis leads to high mortality and morbidity rates, notably increasing public health costs, and may result in physical and psychological damage that is not easily measurable.

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Key Words: congenital adrenal hyperplasia, economic evaluation, newborn screening, delayed diagnosis, high morbi-mortality

Abbreviations: 17-OHP, 17-hydroxyprogesterone; CAH, congenital adrenal hyperplasia; CI, confidence interval; CP, cerebral palsy; GnRha, GnRh analog; HC, hydrocortisone; ICU, intensive care unit; NBS, newborn screening; Na, sodium; SUS, Brazilian public health system; SV, simple virilizing; SW, salt wasting; VSL, value of a statistical saved life

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Congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency (CAH) is a common autosomal recessive disease characterized as impaired cortisol and/or aldosterone synthesis and excessive adrenal androgen secretion [1]. The worldwide CAH incidence has been reported to range from 1:10,000 to 1:18,000 live births in screened populations [2], which is similar to the incidence in Brazil [3–6].

The disease presents a spectrum of clinical manifestations, classified as salt-wasting (SW), simple virilizing (SV), and nonclassical forms. Only SW and SV are the objective of screening programs because these forms present with severe manifestations in the neonatal period [7]. In the SV form, fetal androgen exposure leads to external genitalia virilization in females, often resulting in incorrect sex assignment at birth. If not treated, postnatal virilization continues in both sexes, resulting in pseudo-precocious puberty and short final height [8,9]. In the SW form, aside from the hyperandrogenic manifestations, the clinical presentation involves neonatal hyponatremic dehydration, followed by shock and death in the absence of treatment. Male newborns are more susceptible to severe SW crises in un-screened populations [1,7].

CAH is suitable for inclusion in public newborn screening programs because the measurement of 17-hydroxyprogesterone (17-OHP) levels involves an inexpensive screening test, CAH is a relatively frequent rare disease, and early diagnosis and treatment significantly reduces morbidity and mortality [7]. Although there have been reported false-negative cases [10], CAH newborn screening (CAH-NBS) has shown good efficacy in detecting classical patients. However, the high frequency of false-positive results remains a major issue [2, 11], especially in preterm newborns [12]. Neonates with positive screening results should undergo additional tests to confirm the diagnosis; however, this confirmation process increases screening costs. To date, the only rationale included in economic evaluations for CAH-NBS has been prevention of death resulting from SW crises. This mortality rate has not been well established; however, the risk has been reported to be very low in populations with high levels of health care. Therefore, CAH-NBS has not been considered to be a cost-effective diagnostic tool [13]. With modern ultrasonography, identification of atypical genitalia through routine gestational ultrasound can lead to a prenatal CAH diagnosis, which could reduce the need for CAH-NBS. However, diagnosis using ultrasound is only possible in affected female fetuses, and affected males cannot be diagnosed using this method [14].

Currently, CAH-NBS is undertaken across all 50 states in the United States, in 35 other countries, and in some regions of 17 additional countries. Although it is an Endocrine Society recommendation, there is no worldwide consensus on implementation within national public health systems, and some countries, even developed ones such as the United Kingdom and Canada, do not universally undertake CAH-NBS in their public health systems [15, 16]. There are limited data regarding the benefits of early CAH diagnosis [17]. Some studies have assessed long-term use of CAH-NBS and have evaluated the decline in mortality rates when comparing screened and unscreened populations [18–20].

Two cost-effective analyses concerning CAH screening have been conducted in the United States; both reached differing conclusions. Effectiveness was measured in terms of years of life saved and, through assuming varying mortality rates, 1 analysis concluded that CAH-NBS was cost-effective, whereas the other did not [13, 21]. Neither analysis considered other adverse outcomes of late diagnosis. It has been suggested that the cost-effectiveness of CAH screening might involve other benefits of early CAH detection, including correct sex assignment in cases of atypical genitalia; however, reliable estimates regarding long-term outcomes are still lacking [22].

Understanding the short- and long-term costs of care associated with delayed CAH diagnoses is important for analyzing the cost-effectiveness of NBS implementation. In particular, information concerning how treatment costs are altered when disease progression is prevented through NBS could be valuable for future economic analysis. For example, the incremental direct costs related to neonatal hospitalization in patients with the SW form, therapies undertaken because of erroneous sex assignment at birth, and precocious puberty should be included.

Table 1. Procedures Involved in CAH Diagnosis and Treatment and Their Respective Prices, Extracted from SUS Table of Procedures, Medications, and Orthoses, Prostheses and

Special Materials [25]	
Procedure	Price in US\$
Hospitalization of medium to high complexity (payment per hospitalization)	\$70.77
ICU diaries (payment per day)	\$155.99
Hormonal Therapy of transexualizing process (payment per month)	\$15.33
Hysterectomy with bilateral adnexectomy and colpectomy under transexualizing process	\$366.70
Testicular prosthesis	\$107.35
Genitoplasty	\$122.09
Autogenous urethroplasty	\$144.02
Leuporelin 11.25 mg (price per ampoule bottle)	\$273.26
GH (somatropin) 12 UI (price per ampoule bottle)	\$33.97
Specialized medical consultation	\$3.07
17-OHP laboratory measurement	\$3.12
Sodium laboratory measurement	\$0.57
Potassium laboratory measurement	\$0.57
Renin laboratory measurement	\$4.05
Testosterone laboratory measurement	\$3.19
Androstenedione laboratory measurement	\$3.54
Bone age radiography	\$1.84
Hydrocortisone 10 mg (price per tablet)	\$0.15
Fludrocortisone 100 mcg (price per tablet)	\$0.12
Dexamethasone 0.5 mg (price per tablet)	\$0.05

Abbreviations: 17-OHP, 17-hydroxyprogesterone; ICU, intensive care unit; SUS, Brazilian public health system.

The frequency of adverse outcomes in a large CAH unscreened population and their respective treatment costs have not been reported in the literature. In this study, we aimed to perform a cost of illness study comprising a CAH cohort diagnosed according to phenotype as a first step to conduct a comprehensive economic evaluation of CAH-NBS implementation.

1. Methods

A. Population

Data were retrospectively evaluated through a medical records review of 195 patients with CAH (females, $n = 141$; males, $n = 54$), who had been followed from 1980 to 2016 at the Hospital das Clínicas, Sao Paulo, Brazil. After the purpose and nature of all the procedures used had been explained, consent was obtained from each patient. This study was approved by the Ethics Committee responsible for research projects at the Hospital das Clínicas Medical School of Sao Paulo University.

A diagnosis of the SV form was made according to confirmation of atypical genitalia in females [23] and sexual precocity in males. Patients with the SW form also presented with hyponatremic dehydration in the first weeks of life. All patients had increased basal 17-OHP levels. Serum 17-OHP, androstenedione, and plasma renin activity levels were measured using radioimmunoassay. Serum cortisol, testosterone, and gonadotropins levels were measured using immunofluorometric assays. The CAH genotype was determined through *CYP21A2* analysis [24].

B. Cost analysis

The cost analysis was based on data derived from the Brazilian public health system (SUS). Prices were obtained directly from the SUS Table of Procedures, Medications, and Orthoses, Prostheses and Special Materials management system, in accordance with International Classification of Diseases, 10th revision [25] (Table 1), converted to United States dollars

Table 2. Characteristics of the Brazilian CAH Nonscreened Cohort Over Decades

	% SW Form	% Males	% Dehydration at Diagnosis	Mean Sodium% (mEq/L)	Genital Atypia Recognition at birth
<1989, group 1	42.6	25	87	119.9	64
1990–1999, group 2	62.7	28.6	81	121.8	87
>1999, group 3	66.7	41.7	88	121.4	79

% SW form: group 1 vs group 2, $P = .012$; group 1 vs group 3, $P = .014$; group 2 vs group 3, $P = .68$.

% Males: group 1 vs group 2, $P = 0.065$; group 1 vs group 3, $P = 0.08$; group 2 vs group 3, $P = 0.85$.

Abbreviations: CAH, congenital adrenal hyperplasia; SW, salt wasting.

from Brazilian reals (end of 2016, US\$1.00 = R\$3.26) [26]. Two prices were not available at SUS Table of Procedures, Medications, and Orthoses, Prostheses and Special Materials: hydrocortisone (HC) (10 mg/tablet = \$0.15) [27] and dexamethasone (0.5 mg/tablet = \$0.17).

C. Time horizon

The frequency of adverse outcomes was analyzed from the time of clinical diagnosis until patients reached 19 years of age.

D. Costs involving standard treatment

Standard treatment was set according to Endocrine Society Guidelines [7] and was calculated as the mean daily HC dose used until bone age maturation. Subsequently, we calculated the mean daily dexamethasone dose until a patient reached 19 years of age. The mean daily fludrocortisone dose was also calculated. Standard follow-up consisted of 4 consultations per year with serum hormone measurements [1, 7, 28]. The number of patients who had undergone feminizing genitoplasty was calculated (i.e., those with Prader III–V) [29].

E. Costs of adverse outcomes related to late diagnosis

E-1. Patients with the SW form

1. To determine early death cost, the human capital method was used, which considers the productivity loss associated with premature mortality [30]. The expected productive life in Brazil was estimated to be 34.5 years in 2010. This measure represents the average number of years that a 15-year-old person would remain in the labor market if, during his or her active life, he or she was exposed to mortality rates and economic activity levels according to the age group applicable in 2010 [31]. The mortality rate was calculated by comparing the SW form frequency in our unscreened cohort with those identified in other cohorts screened in Brazil [3, 5]. The number of infant deaths per year was calculated using Brazilian yearly live-births data [32], the SW form incidence, and the calculated mortality rate. The number of years lost was multiplied by the Brazilian gross domestic product *per capita* [33]. A discount rate of 5% [34] was applied to the estimated loss of future productive years.
2. Concerning hospitalization, the number of patients hospitalized because of dehydration was considered. The amount paid by the SUS has a fixed value for ward hospitalizations, regardless of duration. The costs concerning the intensive care unit (ICU) are paid for each day; therefore, the mean length of stay in the ICU was considered.
3. Regarding laboratory diagnosis for nonhospitalized patients, measurement data concerning serum 17-OHP, testosterone, androstenedione, renin, and electrolytes levels were determined.
4. To determine mental impairment resulting from an SW crisis, the number of patients with cerebral palsy (CP) or intellectual disability was identified. Total cost was extracted

from a United States Centers for Disease Control and Prevention survey that had been undertaken to determine costs associated with development disabilities [35].

5. Sex assignment error at birth concerned females who had been raised socially as males. This has been calculated as the costs of masculinization surgery and of androgen re- placement from puberty to 19 years old [29, 36].

E-2. Patients with the SV form

1. The use of expensive medications was determined according to the number of patients who had received GnRh analog (GnRha) and GH, as well as the mean doses and treatment duration.
2. Sex assignment error at birth was considered similarly to patients with the SW form.
3. The diagnosis workup was similar to patients with the SW form. For those diagnosed after the age of 2 years, this was added to the cost of bone age radiography.

G. Statistical analysis

Data are expressed as averages and confidence intervals (CIs) and frequencies in percentages. Comparisons between averages were made using a Student *t*-test and between frequencies using chi-square tests. Statistical significance was set at $p < 0.05$, and *SPSS Statistics* version 22 software was used.

3. Results

A. Estimating mortality and its costs

The cohort comprised 195 patients (SV, $n = 90$; SW, $n = 105$), of whom 54% were identified as having the SW form. Given clinical awareness of CAH has varied throughout recent decades, the results were stratified according to the year of birth, as follows: group 1, born before 1989; group 2, born between 1990 and 1999, and; group 3, born after 1999 (Table 2). Only group 1 presented a significantly lower SW frequency (43%); however, the female-to- male ratio was similar among the groups. The mortality rate varied from 10% to 26%, which was obtained through subtracting the SW frequency of groups 2 and 3 (64%) from the 75% to 90% found in cohorts screened elsewhere [3, 5].

We considered that, given there had been 3,017,668 yearly live births [32] and a varying CAH incidence reported of 1:10,320 [4] and 1:14,967 [5], a mortality rate of 10% to 26% would result in 15 to 68 newborn deaths yearly, respectively. This figure, multiplied by a Brazilian growth domestic product *per capita* of \$9001 [33] and 34.5 average productive years of work in Brazil [31] at a 5% discount rate resulted in a cost varying from \$2,239,744.76 to \$10,271,591.25 from lives lost annually.

B. Diagnosing and treating patients with the SW form

Atypical genitalia was recognized at birth in 54 of 74 females; however, this finding was associated with a precocious diagnosis in only 17 patients. Another 6 females were assigned as males at birth, of whom 2 were raised socially as males because their parents' decision and had undergone a masculinizing process following prolonged psychotherapy.

Neonatal SW crises occurred in 84% of patients (males, 100%; females, 77%). The mean sodium (Na) level was 117.7 mEq/L (95% CI, 113.2-122.2) in males and 123.2 mEq/L (95% CI, 120-127) in females ($P = .04$). The mean age at diagnosis was 38.8 days (31.1-46.4); 31 days (22-40) in females and 49 days (36-62) in males ($P = .036$). Nine patients (3 males, 6 females) developed severe neurological impairment requiring specialized care, of whom 2 presented with CP.

Hospitalization occurred in 91% of the dehydrated patients during a mean length of hospital stay of 38 days (30.3-47.4), and 36% had stayed in the ICU (mean, 23 days; 13.2-32.1). Six patients (5 males) had undergone multiple hospitalizations before diagnosis, resulting in a mean of 1.11 for hospitalizations/patient (1.02-1.2).

All costs related to adverse outcomes and treatment concerning the SW form are described in [Table 3](#).

The mean cost per SW patient, considering only hospitalization outcomes, was \$1087.14 (\$676.47-\$1497.81).

The costs of neurological sequelae calculated per person were \$1,014,000 for mental retardation and \$921,000 for CP [35]. The total cost of mental impairment was \$85,142.86 per patient with the SW form.

The mean cost concerning a patient with the SW form, considering outcomes resulting from a late diagnosis, was \$86,230 (95% CI, 85,819.32-86,640.67).

Regarding standard therapy, the mean HC dose was 14.4 mg/d (13.2-15.6), until 14.6 years of age (9.4-19.8). Subsequently, the mean dexamethasone dose until 19 years of age was 0.3 mg/d (0.27-0.34). The mean fludrocortisone dose was 57.4 mcg/d (55.2-59.5). Data regarding feminizing genitoplasty were obtained in 68 females, of whom 66 had undergone this procedure and 2 had slight genitalia virilization. The mean cost involving standard treatment from the time of diagnosis to 19 years of age was \$3119.04 per patient (\$2702.1-\$3626.74).

The total cost concerning the patients with the SW form, from the date of diagnosis to 19 years of age, including standard treatment and adverse outcomes, was \$89,349.04 (95% CI, 88,521.42-90,267.41), excluding costs related to neonatal death.

C. Diagnosing and treating patients with the SV form

Among the 90 patients with the SV form, 67 (73.6%) were females. Atypical genitalia was identified at birth in 28 (44%) patients. Sixteen of 67 females (25%) were assigned at birth as males, and 10 of 16 (63%) were raised as males and underwent a masculinization process after extensive psychological evaluation.

The mean age at diagnosis was 5.7 (3.9-7.4) years, and 58 patients (64%) were diagnosed after 2 years of age. Males were diagnosed through hyperandrogenic manifestations in 92% of patients, with only 2 diagnosed because of familial history. Atypical genitalia motivated the diagnosis in 34 of 67 (51%) females, and precocious pubarche was observed in 16 patients (24%).

Advanced bone age at diagnosis was found in 45% of patients (males, 90%; females, 33%). The mean difference between bone age and chronological age was 5.2 (4.4-5.9) years. GnRha was used in 25 (28%) patients, for a mean period of 3.8 years (95% CI, 3.08-4.5). GH was used in 13 (14%) patients for a mean period of 3.2 years (2.4-4.0) and the mean dose was 5.9 UI/d (4.9-6.8).

Considering adverse outcomes in patients with the SV form, such as the masculinization process, the laboratory and radiography examinations required to confirm the diagnosis, and the GnRha and GH therapies, a total cost of \$380,931.13 (\$265,485.90-\$660,903.48) was calculated, at \$4232.57 per patient (\$2949.84-\$7343.37). All costs related to adverse outcomes and treatment for the SV form are described in [Table 4](#).

Regarding standard therapy, the mean HC dose until bone maturation was 16.8 mg/d (14.8-18.6) and the mean chronological age at bone maturation was 13.2 years (10.6-18.5). The mean dexamethasone dose until 19 years of age was 0.38 mg/d (0.3-0.47). Among the females, 47 of 55 (85.5%) had undergone feminizing genitoplasty. The mean cost involving standard follow-up until 19 years of age was \$1689.74 per patient (95% CI, 1,160.79-2586.55).

The mean total cost for a patient with the SV form until 19 years of age, including diagnosis workup, standard treatment, and adverse outcomes, was \$5922.48 (95% CI, 4110.80-9930.09).

Table 3. Costs Description for the SW Form

105 Salt Wasters							
Costs of Late Clinical Diagnosis Outcomes							
	Point Estimate	Range (95% CI)		Unitary Value	Total Value	Range	
Girls with wrong sex assignment	2			Hysterectomy/bilateral oophorectomy	\$366.70	\$3812.50	
				Urethroplasty	\$144.00		
				Testicular prosthesis	\$107.30		
				Androgen Replacement (12–19 y)	\$1288.10		
					\$1906.20		
Mental retardation	7			\$1,014,000.00		\$7,098,000.00	
Cerebral palsy	2			\$921,000.00		\$1,842,000.00	
Hospitalization	80			\$70.80		\$6291.00	\$5765.70 \$6816.40
Average hospitalizations before diagnosis	1.11	1.0	1.2				
Hospitalization in ICU	29			\$155.90		\$102,466.50	\$59,871.20 \$145,061.80
Length of ICU hospitalization (d)	22.7	13.2	32.1				
Laboratory examinations to diagnosis	105			\$15.00		\$1579.60	
Total						\$9,054,149.60	\$9,011,028.90 \$9,097,270.30
Total per patient						\$86,230.00	\$85,819.30 \$86,640.70
Costs of Standard Clinical Follow-up							
	Point Estimate	Range (95% CI)		Unitary Value	Total Value	Range	
Medical consultations	4/y			\$3.07		\$24,474.70	
Laboratory	4/y			\$15.04		\$120,048.20	
Age at final height achievement	14.6 y	9.4 y	19.8 y				
Hydrocortisone	14.37 mg/d	13.2 mg/d	15.6 mg/d	\$0.15		\$120,837.50	\$71,162.80 \$177,685.60
Dexamethasone	0.3 mg/d	0.27 mg/d	0.34 mg/d	\$0.05		\$5,377.30	0 \$13,110.30
Fludrocortisone	57.39 mcg/d	55.2 mcg/d	59.5 mcg/d	\$0.12		\$48,704.50	\$46,867.20 \$50,541.90
Genitoplasty	66			\$122.08		\$8057.28	
Cost of standard treatment from diagnosis to 19 y in all patients						\$327,499.60	\$283,720.60 \$380,807.80
Cost of standard treatment from diagnosis to 19 y per patient						\$3119.00	\$2702.10 \$3626.70
Total costs from diagnosis until 19 y (outcomes and treatment)						\$9,381,649.20	\$9,294,749.50 \$9,478,078.10
Costs from diagnosis until 19 y per patient						\$88,349.00	\$88,521.42 \$90,267.41

Abbreviations: CI, confidence interval; ICU, intensive care unit; SW, salt wasting.

Table 4. Costs Description for the SV Form

90 Simple Virilizers							
Costs of Late Clinical Diagnosis Outcomes							
	Point Estimate	Range (95% CI)		Unitary Value	Total Value	Range	
Girls with wrong sex assignment	12			Hysterectomy/bilateral oophorectomy	\$366.70	\$22,874.97	
				Urethroplasty	\$144.00		
				Testicular prosthesis	\$107.35		
				Androgen replacement (12–19 y)	\$1288.14		
					\$1906.19		
Use of leuporelin	25			\$273.25 per ampoule bottle	\$103,493.93	\$84,144.06	\$122,843.80
Length of leuporelin treatment	3.79 y	3.08 y	4.5 y	of 11.75 mg			
Use of GH	13			\$33.97 per ampoule bottle of 12 U	\$253,101.56	\$157,006.21	\$513,724.05
Length of GH treatment	3.2 y	2.4 y	5.6 y				
Mean GH dose per day	5.9 U	4.9 U	6.8 U				
Laboratory examinations to diagnosis	90			\$15.04	\$1353.93		
Patients diagnosed after 2 y of age, submitted to bone age radiography	58			\$1.84	\$106.73		
Total cost of outcomes					\$380,931.10	\$265,485.90	\$660,903.50
Total costs of outcomes per patient					\$4232.60	\$2949.90	\$7343.40
Costs of Standard Clinical Follow-up and Treatment							
	Point Estimate	Range (CI 95%)		Unitary Value	Total Value	Range	
Medical consultations	4/y			\$3.07	\$20,978.30		
Laboratory (17-OHP, A4, testosterone)	4/y			\$9.86	\$67,466.20		
Age of bone maturation	13. 2y	10.6 y	18.5 y				
Hydrocortisone	16.8 mg/d	14.5 mg/d	18.6 mg/d	\$0.15	\$50,276.50	\$9,81.10	\$125,227.20
Dexamethasone	0.38 mg/d	0.3 mg/d	0.47 mg/d	\$0.05	\$7617.80	\$507.90	\$13,379.90
Genitoplasty	47			\$122.08	\$5737.80		
Total costs of standard follow-up and treatment					\$152,076.60	\$104,471.30	\$232,789.50
Costs of standard follow-up and treatment per patient					\$1689.70	\$1160.80	\$2586.50
Costs from diagnosis until 19 y, including outcomes, follow-up, and treatment					\$533,007.70	\$369,957.20	\$893,692.90
Costs from diagnosis until 19 y (outcomes, follow-up, and treatment) per patient					\$5922.30	\$4110.60	\$9929.90

Abbreviations: 17-OHP, 17-hydroxyprogesterone; A4, androstenedione; CI, confidence interval; SV, simple virilizing.

4. Discussion

Economic evaluations guide policy decisions through providing relevant data and projected estimations of long-term clinical and economic outcomes [37]. Costs and benefits have been assessed to establish the effectiveness of NBS in terms of reduced risk of death and/or of severe morbidity [38]; however, the cost-effectiveness evaluation of CAH-NBS has only focused on the mortality rate. It has been claimed that the lack of data on the costs of long-term disability resulting from late diagnosis should be addressed to improve the quality of evaluations for implementing NBS [22]. In developed countries, CAH is reasonably diagnosed by clinical means [17], which may not always be the case in developing countries [39–41]. This paper presents, for the first time to the best of our knowledge, the frequencies and costs of adverse outcomes in a population diagnosed using clinical means.

The primary goal of CAH-NBS is to prevent an SW crisis and death. The risk of death for patients with the SW form in the absence of NBS ranges widely across Europe and North America, from 0% to 9% [17], which differs markedly from the 10% to 26% range estimated in our cohort. However, those risk evaluations were performed among populations with high standards of CAH clinical awareness, and it is possible there was some underestimation in relation to undiagnosed cases. The observation of increased CAH incidence after CAH-NBS implementation and of unbalanced female-to-male and SW-to-SV ratios in some nonscreened populations [42–45] support our findings of increased CAH mortality from unrecognized SW crises. In our cohort, males corresponded to approximately 30% of patients in both SW and SV forms, indicating that especially males had likely not been diagnosed.

It is expected that a clinical diagnostic workup, knowledge of this disease, and patient care would likely improve with time [46, 47]. Our cohort comprised patients followed for at least 30 years and, when we compared data with regard to 3 different decades, only a partial improvement was found, suggested by the increasing SW form and male proportions in more recent decades (Table 2). However, no differences in the rates of dehydration, hospitalization, atypical genitalia recognition, and mean serum Na levels at diagnosis were observed, pointing out that a clinical diagnosis of CAH remains suboptimal.

Calculating the value of a statistical saved life (VSL) is challenging. In this study, we selected an income-based human capital method, as has been adopted in other cost-of-illness studies [30, 48]. Current United States regulatory agency practice uses a VSL estimate of approximately \$9 million to value an averted death [48]. Considering these data, our estimation of VSL was comparable.

SW crisis is life-threatening in the first 2 weeks of life; clinical diagnosis in our SW cohort occurred much later in both sexes. Males were diagnosed >2 weeks later than females. Similar results have been shown in other populations [18, 41, 42, 49]. It has been reported that NBS might be cost-effective only for males because females are clinically diagnosed through atypical genitalia [13, 50, 51]. Among our females with the SW form, only 21% were prevented from developing dehydration because of clinical recognition of the disease, highlighting that females are also at risk of death from a late diagnosis.

The patients with the SW form presented with low serum Na levels and a long length of hospitalization, suggesting severe SW crises among these patients. Severe salt loss may result in learning disabilities and/or cerebral lesions [52, 53]. We identified 8.6% of our cohort as having mental impairment, which also carries a considerable economic burden because disabled patients receive financial support and multidisciplinary specialized care that is state funded. Data concerning this total cost are not available for the Brazilian population; therefore, we used international data provided by the United States Centers for Disease Control and Prevention that has estimated the direct and indirect economic costs associated with 4 development disabilities [35]. Even though the costs in this study were calculated based on economic data published elsewhere, we emphasized the frequency of these outcomes and their role in accessing CAH-NBS cost-effectiveness evaluation.

In both Brazilian screened and nonscreened cohorts, errors in gender assignment were observed for 6 of 74 (8%) females in our cohort and for 1 of 24 (4%) females in a screened cohort [3]. NBS results provided early diagnosis and correction of this error [3], whereas, in its absence, 12 patients (SW, n = 2; SV, n = 10) were raised as males and underwent a masculinization process involving cost burdens, invasive surgical procedures, and psychological challenges such as poor self-image, infertility, and impaired sexuality [36].

Traditionally, the SW form has been the main concern of the NBS program; however, our data showed that the SV form also involves challenges concerning delayed diagnosis. The low male-to-female ratio suggests that, in this clinical form also, males are underdiagnosed and are, therefore, at risk of adrenal crisis during stressful conditions. The mean age at diagnosis of 5.7 years was sufficient to warrant signs of virilization and advanced bone maturation in almost 50% of patients. It has been reported that classical patients present with a loss of potential final height [8, 9], especially associated with increased androgen levels and advanced bone maturation during infancy [54]. This outcome is likely to have driven the frequent use of expensive therapies such as GnRha and GH in our cohort, which could have been avoided with an earlier diagnosis.

In this study, we used the Brazilian public health system's economic data to calculate the treatment costs of the relevant adverse outcomes. Large variations in costs may occur among different countries; however, we considered it important to describe the dramatic frequencies of late diagnosis-related adverse outcomes in the absence of NBS. These outcomes have a national economic impact and on patients' quality of life, which are likely to occur in differing nonscreened populations.

The findings in this study support the international endorsement of CAH-NBS. A clinical diagnosis of CAH is only possible in symptomatic neonates and cannot be made reliably in both sexes, which can lead to delayed treatment and severe sequelae. These consequences increase the costs of treatment, including measurable factors, such as longer hospitalizations for hyponatremic dehydration and expensive medications to improve final height. Furthermore, the absence of early diagnosis could result in unnecessary physical, psychological, and neurological damage to patients that is not easily measurable.

To date, the only rationale included in economic evaluations concerning CAH-NBS has been prevention of deaths from neonatal SW crises. We estimated that the mortality rate could be as high as 26% in our population; however, because the mortality rate varies widely in other populations, there is uncertainty concerning the cost-effectiveness of CAH-NBS. Considering our results, we recommend that the cost-effectiveness of CAH-NBS should be determined in relation to additional benefits of early detection, which have remained unassessed in the literature before this study. Further studies comparing costs and benefits of both clinical and screening diagnoses should be undertaken.

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Data availability: The data that support this study's findings are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

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CAPÍTULO 2 - *The Cost-effectiveness analysis of CAH newborn screening: a comparison between screened and unscreened cohorts from the same region*

Após o levantamento de dados dos pacientes não triados, foi realizada a análise de dados da triagem neonatal realizada pelo serviço de referência de triagem neonatal do estado de São Paulo, Instituto Jô Clemente. Foi feita a avaliação da triagem neonatal para a HAC realizado durante o ano de 2017, de 1 de janeiro a 31 de dezembro, período no qual foram triados 378.790 recém-nascidos. Foram levantados dados sobre data de coleta, peso ao nascer, valores da 17OHP neonatal e dos testes confirmatórios. Além disso, foi avaliado o percentual de pacientes confirmados pela triagem que apresentaram desidratação e que tiveram que ser internados, para comparar com o observado na população não triada.

Após o levantamento dos dados da população triada, foi possível a comparação com a população não triada e a aplicação do modelo de decisão para estabelecer a razão entre a diferença de custos entre as duas estratégias e a diferença de efetividade, que no caso foram as mortes evitadas pela triagem. Como conclusão do estudo, chegamos uma razão incremental de custo-efetividade considerada custo-efetiva baseada nos principais limites de custo-efetividade descritos em trabalhos de economia em saúde.

O artigo está em processo de submissão para publicação em periódico científico internacional.

The Cost-effectiveness analysis of CAH newborn screening: a comparison between screened and unscreened cohorts

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ABSTRACT

Context: The newborn screening for congenital adrenal hyperplasia (CAH-NBS) isn't yet a worldwide consensus due to the lack of data regarding its cost-effectiveness, whose analysis requires understanding short and long-term costs of care in delayed diagnosis. **Objective:** To conduct a cost-effectiveness analysis (CEA) comparing the strategies of CAH-NBS against clinical diagnosis. **Design:** A decision model comparing two strategies, tested by sensitivity analysis. Unscreened patients' data were extracted from medical records of Hospital das Clinicas-Sao Paulo and screened data were from the NBS Referral Center-Sao Paulo. The cost's perspective was the Public Health System. **Patients:** 195 classical patients, clinically diagnosed and confirmed by hormonal/*CYP21A2* analysis, and 378,790 newborns screened during 2017. **Main outcome measures:** The adverse outcomes related to late diagnosis were measured in both cohorts and the incremental cost-effectiveness ratio (ICER) was calculated. We hypothesized that CAH-NBS is cost-effective. **Results:** There were 25 screened NBs affected (incidence of 1:15,135). Mortality rate was calculated in 11% in unscreened while no deaths were reported in the screened cohort. Comparing unscreened versus screened cohort, mean serum sodium level was 121.2 mEq/L (118.3 to 124.1) x 131.8 mEq/L (129.3 to 134.5), mean age at diagnosis was 38.8 x 17 days and hospitalization occurred in 76% x 58% of SW patients, respectively. The NBS incremental cost was US\$ 771,185.82 per death averted, which yields an ICER of US\$ 25,535.95/LY. **Conclusions:** We demonstrated the NBS importance in preventing CAH mortality/morbidity and also in reducing costs associated to adverse outcomes, showing that performing CAH-NBS is cost-effective.

INTRODUCTION

Congenital adrenal hyperplasia due to 21-hydroxylase (CAH) is a common autosomal recessive disease, that results in impaired cortisol and/or aldosterone synthesis and excessive adrenal androgen secretion^{1,2}. It's worldwide incidence ranges from 1:10,000 to 1:18,000³ and in Brazilian screened cohorts the incidence is approximately 1:10,000^{4,5} live-births. The disease presents two clinical forms of newborn screening (NBS) interest: the salt-wasting (SW) and the simple virilizing (SV) forms. Female newborns (NBs) in both clinical forms present with virilized external genitalia at birth and in the SW form infants of both sexes are also at risk of life-threatening adrenal crisis in the absence of early treatment¹. Benefits of the SW form precocious diagnosis by NBS include reduced mortality and reduced length of hospitalization, while in the SV form the benefits are the correction of sex assignment errors in virilized females and the prevention of early advanced bone maturation⁶.

Although there have been reported false-negative cases^{7,8}, CAH newborn screening (CAH-NBS) has demonstrated good efficacy in detecting classical CAH patients, and its implementation is an Endocrine Society recommendation⁶. However, the high frequency of false-positive (FP) results remains a major issue, especially in preterm NBs⁹. Neonates with positive screening results should undergo additional tests to confirm the diagnosis and this process ends up increasing screening costs^{9,10}.

The CAH-NBS implementation isn't yet a worldwide consensus, what reflects the lack of data regarding the cost-effectiveness of early CAH diagnosis through NBS¹⁰. The scarcity of comprehension in this matter is highlighted by the recent CAH clinical guideline⁶. Long-term experience with CAH-NBS has been described, but just a few studies compared screened and non-screened populations¹¹⁻¹³. Two cost-effectiveness analyses (CEA) concerning CAH-NBS have been conducted in the United States, and,

by assuming varying mortality rates, both reached differing conclusions: one concluded that CAH-NBS was cost-effective, while the other did not^{14,15}. A recent CEA was performed in Canada, reaching the conclusion favor to NBS¹⁶. None of these analyses considered the long-term adverse outcomes secondary to late diagnosis and all these studies were performed in developed countries, in which CAH is reasonably precociously diagnosed by clinical means.

The CAH clinical suspicion is due to the genital atypia identification in females or due to family history of CAH. Thus, most of males could only be clinically diagnosed during a neonatal salt-wasting crisis in the SW form or due to hyperandrogenic manifestations during early infancy in the SV form. The delayed SW form diagnosis may incur in more severe hyponatremic dehydration with neurological sequels, longer hospitalizations and some patients may die without the correct diagnosis^{17,18}. In the SV patients, the delayed diagnosis leads to precocious pubarche and/or precocious pseudo-puberty, culminating in short final height and hyperandrogenic manifestations in females^{19,20}. It is known that genital atypia at birth can go unnoticed and the most virilized females could also be miss assigned at birth as males^{18,21}.

Our perspective is that cost-effectiveness analysis requires understanding short and long-term costs of care secondary to CAH delayed diagnosis. The frequencies and costs of these adverse outcomes remained unassessed in the literature so far. Previous data of our large unscreened cohort diagnosed by clinical suspicion showed, for the first time, not only a high mortality rate, but also a high prevalence of adverse outcomes that resulted in important economic burden to the health system¹⁷. We hypothesized that CAH-NBS not only leads to important benefits as it is also cost-effective.

The major goal of our study is to assess the cost effectiveness of CAH-NBS in terms of the prevention of early childhood death and comorbidities with a set of

evidence-based assumptions. This study contributes by offering a comprehensive cost-effectiveness evaluation of CAH-NBS implementation by using a decision-tree framework allowing variation in the model parameters, considering the costs related to the screening compared to the costs related to treatment of short and long-term adverse outcomes of CAH late diagnosis.

METHODS

A cost-effectiveness analysis (CEA) was conducted to compare the strategies of CAH-NBS against the clinical recognition; using data of two CAH cohorts, diagnosed by NBS and by clinical means. Frequencies of adverse outcomes were evaluated in both cohorts and used, along with data about CAH-NBS, to construct our decision model. The study design followed the recommendations for health economic evaluations²².

The study was approved by the Ethics in Research Committees of the Universidade de São Paulo (online register number 14558) and of the Jô Clemente Institute, the entities involved in providing data. The unscreened cohort information was retrospectively extracted from medical records of patients followed in Hospital das Clínicas, the main tertiary Hospital of São Paulo State. Data about screened population were granted by the Neonatal Screening Referral Center of São Paulo.

The cost's perspective was the Brazilian Public Health System (SUS), which includes health care costs, but not the families' costs. It was used the values of reimbursements paid by the Brazilian Government for each health procedure, surveyed directly from the Management System of the Brazilian Public Health System Table (*SIGTAP*). These information are available on the Brazilian Ministry of Health online Platform²³. The monetary unit was North American currency (US\$), converted from Brazilian currency (R\$) by the 2016 exchange rate of US\$ 1.00 = R\$ 3.26²⁴, since this conversion was used in the published cost analysis of our unscreened cohort¹⁷.

The unscreened population consisted of 195 patients (141 F) diagnosed by clinical suspicion and confirmed by hormonal and molecular analysis. Mortality rate, dehydration parameters and hospitalization length were measured in the SW form. The number of deaths due to diagnosis absence was calculated applying the calculated mortality rate to the proportion of SW patients who wouldn't have clinical recognition. The amount paid by the SUS has a fixed value for ward hospitalizations, regardless length, but the one for Intensive Care Unit (ICU) is paid for each day. Therefore, the costs related to dehydration were related to the number of ward hospitalizations and the number and length of ICU hospitalization. In the SV form, it was evaluated the frequency of 46,XX patients raised in the male social sex and the frequency of Gonadotropin-Releasing Hormone Analog (GnRha) and Growth Hormone (GH) therapies for precocious pseudopuberty in both sexes²⁵. The costs related to therapies for 46,XX males were masculinization surgery and androgen replacement from puberty to 19 years and the ones related to GnRha and GH use was calculated according to the number of patients treated, the mean doses and treatment duration.

In the screened population, it was used data of the CAH-NBS program of the Sao Paulo State, during 2017, in which 378,790 NBs were screened. It was recorded data about: birth date and weight, sex, date of sampling, neonatal 17-OHP and serum confirmatory tests. NBS was performed by heel prick capillary blood collection from NBs between 2th to 7th days of life. The blood was deposited on S&S 903 filter paper; dried samples are shipped to the referral laboratory for 17OHP analysis, using the time-resolved, solid-phase fluoroimmunoassay 17OHP neonatal kit (B015-112 –Auto DELFIA – Neonatal 17OHprogesterone; Perkin Elmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The intra-assay and interassay coefficients were <8% for the filter paper assay. The cut-off for 17-OHP levels on filter paper were established

according to birth weight and age at sample collection, using the 99.8th 4. NBs with increased 17OHP levels, but less than twice the reference value (low risk), were submitted to a new sample collection on filter paper; those with 17OHP levels higher than twice the 99.8th percentile (high risk) were submitted to serum confirmatory tests and direct referred to a specialist consultation. Serum confirmatory testes included measurements of 17OHP, androstenedione and cortisol levels by liquid chromatography-mass spectrometry (LC-MS/MS), testosterone by eletroquimioluminescence, sodium and potassium by selective ion electrode. Costs considered the 17OHP measurement on the filter paper in addition to confirmatory tests and medical follow-up for the all positive cases. It was calculated the cost for measuring 17OHP from the same filter paper collected for the other screening tests performed by the running national NBS program. The costs by category of screened NBs were showed in Table 1. In the NBs with confirmed CAH diagnosis it was also calculated the frequency and length of hospitalization due to dehydration and their additional costs were used in our decision model. It was considered that under screening strategy, long-term outcomes wouldn't occur, since all patients would be diagnosed early. Additionally, no deaths due to salt-wasting crisis were reported during NBS.

Figure 1 displays the decision tree constructed. It was calculated the incremental cost-effectiveness ratio (ICER), which is the ratio of the incremental cost of CAH-NBS compared to non-screening strategy per life years saved (LY). Following a model of CEA of *Yoo & Grosse*¹⁴, our analyses assumed that each NBs death avoided would save 30.2 LYs, applying the 3% discount rate to 76 years of life expectancy at birth in the 2017 Brazilian life Table²⁶. The equation of ICER calculation used was: $ICER (US\$/LY) = (\text{costs of NBS arm} - \text{costs of non-screened arm}) / (\text{number of deaths averted} \times 30.2 LY)$. The analyses were conducted using the decision analysis software

TreeAge Pro Health Care and the results were confirmed by an Excel analysis. Table 2 lists all parameters used in the CEA, including point estimates for the base case. The CAH incidence of 1:12,250 is an average among the incidence we found in this study (1:15,135) and different ones described by others Brazilian centers previously^{5,27}. We considered the 75% of SW patients proportion, which is largely reported in the literature and in a Brazilian NBS study^{6,28}. The clinical recognition was the probability of a case being detected before symptoms due to genital atypia at birth or family history, therefore prior to the NBS results. This probability was based on the proportion of SW patients diagnosed before dehydration and the SV patients diagnosed in the first 30 days of life in our unscreened cohort¹⁷; the average between these proportions and the one previously published by *Yoo & Grosse et al* was used in the base case analysis¹⁴. The mortality rate considered in the base case analysis was also an average between the one calculated in our unscreened cohort and other published before¹⁴. The decision model was applied in a hypothetical population using the 2017 total number of live-births in Brazil, surveyed from the Brazilian Ministry of Health database²⁹ (see Table 3).

Considering the inherent uncertainty in the values of various assumptions, the robustness of the model was tested by applying a one-way sensitivity analyses to examine how the ICER is influenced by the range of each parameter. This sensitivity analysis involved four variables, listed in Table 4. In addition, ‘best-case’ and ‘worst-case’ scenario analyses selected the most and least favorable values for each parameter, respectively. The values for the ranges were extracted from published literature.

Statistical analysis

Data are expressed as average and 95% confidence interval. Student *t*, chi-square and Fisher exact tests were used. Statistical significance was established by $p < 0.05$. The software *Openepi* was used, available at <https://www.openepi.com/>.

RESULTS

Unscreened CAH cohort

The cohort consists of 195 patients (90 SV/ 105 SW); SW form comprises 54% of patients. Pondering that CAH clinical awareness could vary along the decades, the results were stratified according to the year of birth: Group 1 until 1989, Group 2 from 1990 to 1999 and Group 3 after 1999. Just group 1 presented significantly lower SW frequency (43%), but the imbalanced female/male ratio was similar among groups. The mortality rate considered was 11%, obtained by subtracting the mean SW frequency of groups 2 and 3 (64%) from the 75% found in a Brazilian screened cohort²⁸. Salt wasting crisis occurred in 84% of patients (100% of males and 77% of females). Therefore, the probability clinical recognition before symptoms for the SW form was calculated in 16%. Mean serum sodium (Na) level was 121.2 mEq/L (CI 95% 118.3 to 124.1), mean age at diagnosis was 38.8 days (CI 95% 31.1 to 46.4). Hospitalization occurred in 91% of the dehydrated patients (76% of all SW patients), during mean length of 38 days (CI 95% 30.3 to 47.4); 36% have been in ICU (mean=23 days, 13.2 to 32.1). Table 5 illustrated the main clinical differences between screened and unscreened SW patients.

Among the 90 SV patients, 67 (73.6%) were females. The atypical genitalia was identified at birth in 28 patients (44%). Only 11% of all SV patients were diagnosed with CAH during neonatal period; Therefore, that was the considered probability of clinical recognition. Ten out of 67 females (15%) were assigned at birth as males and were reared in the male social sex, being submitted to the masculinization process, after extensive psychological evaluation. Mean difference between bone age and chronological age at diagnosis was 5.2 years in both genders (CI 95% 4.4 to 5.9). Treatment with GnRHa (11.25mg/3mo) was used in 25 patients (28%), during a mean time of 3.8 years (CI 95% 3.08 to 4.5). The GH therapy was used in 13 patients (14%),

during a mean time of 3.2 years (CI 95% 2.4 to 4.0) and mean dose was 5.9 UI/day (CI 95% 4.9 to 6.8).

Newborn Screening in Sao Paulo

A total of 378,379 NBs was screened in 2017. Among them, 25 were affected by classical forms of CAH, reaching an incidence of 1:15,135 live-births. Recall was performed in 4,677 NBs (1,23%), being in 3,810 NBs due to extreme low birthweight (<1500g) and in 435 NBs due to neonatal transfusion. For the cost-effectiveness analysis, it was considered only the recall motive of altered neonatal 17OHP results. The mean time of neonatal sample collection was 5.25 days of life (CI 95% 4.87 – 5.82) and the mean time between sample collection and NBS result was 5.16 days (CI 95% 4.93 to 5.40). A total of 843 non-affected NBs presented with altered 17OHP levels on filter paper, reaching a 0.22% false positive rate of the first tier. Death due to prematurity and neonatal complications occurred in 321 NBs, of which 22 had 17OHP levels on filter paper altered; but information about their hospitalization and laboratory exams ruled out the CAH diagnosis. Among the false positive cases, 75% were considered low risk and retest on filter paper was performed; 99.5% of them were dismissed after the negative retest result, the other 0.5% were dismissed after serum confirmatory tests and medical consultation. Among the 25% considered high risk, 92% were dismissed after a negative serum confirmatory test and 8% remained inconclusive and needed a second confirmatory test.

Considering the 25 CAH cases, there were 10 males and 15 females. Despite NBS, 12 patients presented with dehydration and 11 were hospitalized, 30% in ICU with mean length of 9 days (CI 95% 1.8 to 16.2). The mean sodium level among the patients who dehydrated was 131.8 mEq/L (CI 95% 129.3 to 134.5). Mean sodium level at diagnosis among all patients was 133.7 mEq/L (CI 95% 131.5 to 135.8). The mean

time between filter paper sample collection and first medical consultation was 12 days (CI 95% 7.8 to 16.1) and the mean age at first medical consultation was 17.2 days (CI 95% 11.3 to 23.3). Among females, 8 out of 15 were hospitalized due to dehydration. Since the patients weren't followed at the same center and it was not performed molecular diagnosis, we cannot precise the SW proportion. If we consider the expected SW form proportion of 75%²⁸, 19 patients would be salt-wasters, so the hospitalization rate would be 58% (11/19).

Cost-effectiveness analysis

In a hypothetical population of 2,923,535 live-births in a year, using the Brazilian live-birth rate of 2017²⁹, applying the incidence of 1:12,250, we would have 239 CAH NBs, 179 (75%) salt-wasters. Under the NBS strategy, considering the above-mentioned frequencies of positive and negative results and also the frequencies of affected patients' hospitalizations, there would be a cost of US\$ 7,247,683.00 related to screening (US\$ 30,368.75 per CAH patient diagnosed). We pondered that in this scenario, would be no deaths related to CAH undiagnosed cases. In a non-screened scenario, 36% of SW and 28% of SV patients would have their diagnosis prior to symptoms, so the cost of this endpoint is only related to the diagnostic laboratory exams (serum 17OHP \$3.13, renin \$4.04, androstenedione \$3.54, testosterone \$3.20, sodium \$0.57 and potassium \$0.57). Among the 64% of SW patients who would not have their diagnosis prior to dehydration, 8% would die without CAH diagnosis and the rest would be dehydrated, being 91% hospitalized, 36% of them in ICU in a mean length of 23 days. The cost related to dehydration without hospitalization was also only related to diagnostic laboratory exams. It was not included in this analysis the cost of mental impairment due to severe neonatal hyponatremia and the cost of death that were previously calculated for SW patients¹⁷, because it was considered that would

overestimate the ICER for NBS. Among SV patients, 72% of patients would have a delayed diagnosis, 28% of them would need GnRha treatment and 14% GH therapies; 15% of females would have been reared as males, being submitted to the masculinization process. For them, it was also included the costs of diagnostic laboratory exams. The total cost in this non-screened scenario would be US\$ 307,010.60, and there would be 9 neonatal deaths. Therefore, the incremental cost of screening was US\$6,940,672.40 and the effectiveness was 9 deaths averted, US\$ 771,185.82 per death averted. Dividing that number by 30.2 discounted LY yields an ICER in the base-case analysis of US\$ 25,535.95 /LY saved. The CEA analysis is explained in Table 3.

The ICER variation according to each variable is displayed on Table 4. With a set of assumptions favoring CAH screening, the best-case analysis yielded an ICER of US\$ 11,223.81 /LY. The worst-case analysis showed an ICER of US\$ 78,427.24. The SW mortality and clinical recognition variables had the greatest influence on the ICER. The 4.2% to 11% mortality variation led to the ICER from US\$ 46,387.70 to US\$ 17,870.08, respectively; whereas the assumption of 55% clinical recognition for SW and 45% for SV led to an ICER of US\$ 38,959.02, while the assumption of 16% and 11% respectively led to an ICER of US\$ 19,189.90.

DISCUSSION

Recommendations for NBS are primarily based on evidence of the importance and certainty of net benefit³⁰. The benefit to affected NBs is emphasized in terms of reduced risk of early neonatal death or severe morbidity. While NBS saves lives and also improves the quality of life of affected children and families, an analysis of its economic impact is mandatory in order not to overload the public health system³¹.

The analysis of 1-year CAH-NBS in Sao Paulo State could exemplify these benefits. Although half of screened patients still had to be hospitalized due to dehydration, the length of hospitalization and severity of dehydration, inferred by sodium levels, were significantly lower compared to the unscreened cohort. This fact demonstrates that NBS could prevent more severe dehydration and therefore neurological sequelae^{12,16,32,33}. The probable reason for CAH screened patients still had to spend time in hospital was the delay in getting the NBS results. The mean age at diagnosis in the screened cohort was 17.2 days, period in what salt-wasting crisis may already occur. Other NBS programs also faces similar problems with delayed results^{16,32,34}. Clearly, this issue presents implications in cost-effectiveness of screening and its improvement must be targeted by NBS programs.

Until now, studies about CAH-NBS were focused in its benefit to prevent SW crises and didn't accessed the adverse outcomes related to the SV late diagnosis, which also represents an economic impact^{17,35}. In the NBS absence, NBs with the SV form generally goes unrecognized at birth and can develop, in early infancy, precocious pseudo-puberty with increased growth velocity and rapid bone age maturation³⁶. This explains the frequent use of expensive therapies, such as GnRha and GH, in our cohort, which could have been avoided with an earlier diagnosis. The mean age at diagnosis of 5.7 years was sufficient to warrant advanced bone maturation in almost 50% of patients¹⁷. For the first time, a CEA about CAH-NBS considered these outcomes.

Traditionally, NBS is assumed to be cost-effective if its ICER are up to US\$ 50,000/LY³⁷. The World Health Organization (WHO) considers a strategy very cost-effective if the ICER is lower than the country's per capita gross domestic product (GDP) and cost-effective if it is up to three times the GDP³⁸, which would be US\$ 29,294.63 in 2017³⁹. Thus, our study has shown that CAH-NBS is cost-effective, in a

developing country context. The worst-case analysis suggested that in a set of assumptions not favorable to NBS, like low mortality, high standards of clinical awareness and lower CAH incidence, CAH-NBS wouldn't be considered cost-effective. However, we found this scenario less likely to occur, especially in an underdeveloped and developing country reality.

We observed that the two variables that most impacted the ICER were mortality rate and the ability to clinically recognize the disease in a pre-symptomatic phase. Interestingly, these two variables are exactly the ones that differ most between developed, underdeveloped or developing countries, demonstrating the importance of carrying out economic studies respecting the population characteristics of each region. CAH-NBS it's not performed in United Kingdom as no evidence suggested increased mortality in NBS absence⁴⁰. On the opposite side, some other developed countries that do not perform CAH-NBS currently have assumed that this strategy can be reliable, sensitive and effective in reducing morbidity and mortality^{16,41,42}. The risk of death for SW patients in the absence of NBS ranges widely across Europe and North America, from 0% to 9%¹⁰. However, even though those evaluations were performed among populations with high standards of CAH clinical awareness, it is possible there was some underestimation in relation to undiagnosed cases. The observation of increased CAH incidence after CAH-NBS implementation and of unbalanced female-to-male and SW/SV ratios in some non-screened populations support our findings of increased CAH mortality due to unrecognized SW crises⁶. Although we previously describe that the mortality rate may reach 26%, we decided to be more conservative and used the lowest mortality rate calculated previously (11%) and pondered it with a mortality rate used in another CEA carried out in a developed country (4.2%)^{14,17}.

Clinical recognition of CAH was considered as the case detection before symptoms due to genital atypia at birth or family history. In an ideal situation, all female NBs would be diagnosed at birth due to genitalia atypia, but we showed that only 21% of SW females were prevented to dehydrate due to early recognition. These data explains why our clinical recognition probabilities are lower than other assumed in previously studies¹⁴. Other studies with screened CAH populations in developed countries also described that up to 20% of females were diagnosed only by NBS, highlighting the importance to perform screening in both genders^{12,16,34}.

Surely, our study has limitations. One is that data regarding the parameters of our decision model are derived from a study performed in a specific State of Brazil and we used the Brazilian public health system's economic data to calculate the reimbursement costs, therefore large variations may occur among different countries. These reimbursement values could be underestimated, but this would be equal in both unscreened and screened cohorts. This fact in addition to some methodologies variations explains why our ICER result is not comparable to others previously published¹⁴⁻¹⁶. To deal with the uncertainty in variables, we conduct the sensitivity analysis and used data published in other studies about CAH.

Other limitation was that in our one-year analysis of CAH-NBS it's not possible to assured that no case was missed out. False negative results have been reported in other screened populations⁷, that could be related to the early sample collection. In our cohort heel prick collection is performed after 48h of life and the values cut-offs are in accordance with birth-weight, what have guaranteed a good sensibility and specificity for screening results^{4,27}. The incidence observed in our screened cohort of Sao Paulo in 2017 was a little higher than previously reported^{5,27}. Although no missed case was reported to our knowledge, the female to male ratio suggests otherwise. It is possible

that some SV males could be missed, possibly due to the antenatal use of glucocorticoid, which is a frequently practice in São Paulo obstetrician centers. It is discussed that NBS could be less sensitive to detect the SV form, motivating a second tier in the Texas NBS program⁴³. It could also be possible that this female to male imbalance be due to a sampling error, because it is only a one-year analysis and if we increase the sample this would be corrected. Anyway, even if a few cases of the SV form have been lost by screening, the reality of SV patients without NBS would be much worse¹⁷.

The main strengths of the study are the fact that we were able to access long-term benefits of NBS and use them in CEA analysis, what had never been done before. We are aware that it could be argued that we have a biased cohort of unscreened patients. *Grosse & Van Vliet* advised researchers to the risks of biases in data from historical cohorts¹⁰. In Brazil, unlike some developed countries, patients with CAH are followed in Tertiary Centers and not in primary care units, therefore the referral bias may not be applied in our case. Even so, we believe that the large size of our unscreened patients and the stratification by time period minimize potential bias. As an expert center of CAH treatment in Brazil, we know that the reality in North/Northeast regions of the country are even worse than the one we described⁴⁴.

In conclusion, this study was able to emphasize the importance of NBS in reducing CAH mortality and morbidity, showing that even under an economic point of view performing CAH-NBS can be worth. We believe that our study provides a useful economic assessment to inform decisions on the adoption of CAH screening in federal NBS programs. On second thought, we strongly encourage that more CEA about CAH-NBS be conducted in different economies adapted to their reality.

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Table 1. Costs for different categories after CAH screening until the age of one year old.

Categories	Risk at screening	Costs description	Cost per child (US\$)
Non-affected by CAH		A	2.45
Non-affected by CAH retested		2A	4.9
False positive	High	A+B	24.91
	Low	2A+B	27.36
CAH affected	High	A+B	24.91
	Low	2A+B	27.36

A – 17OHP on filter paper = \$2.45

B – Confirmatory lab (serum 17OHP \$3.13, cortisol \$3.02, androstenedione \$3.54, testosterone \$3.20, sodium \$0.57 and potassium \$0.57) + medical consultation (\$8.43) = \$22.46

Table 2. Parameters and its point estimate assumption in modeling the base, best- and worst-case scenarios in decision analysis for CAH-NBS

Parameters	Point of estimate			Referen ce
	Base case	Best case	Worst case	
2017 Brazilians life expectation and LY saved after 3% discount	76 – 30.2	76 – 30.2	76 – 30.2	26
CAH Incidence	1:12,250	1:10,000	1:15,000	27, Actual study
% SW	75%	75%	75%	6,28
Screening false positive rate	0.2%	0.1%	0.5%	14, Actual study
High risk false positive	25%	25%	25%	Actual study
False positive confirmatory	8%	8%	8%	Actual study
Low risk false positive	75%	75%	75%	Actual study
Retest false positive	0,5%	0,5%	0,5%	Actual study
SW form				
% Clinical recognition	36%	16%	55%	14
SW mortality without screening	8%	11%	4.2%	14,17
% hospitalization even with screening	58%	58%	58%	Actual study
% ICU hospitalization even with screening (among hospitalized patients)	30%	30%	30%	Actual study
Screened length of ICU (days)	9	9	9	Actual study
% hospitalization unscreened	91%	91%	91%	17
% ICU of unscreened, among hospitalized patients.	36%	36%	36%	
Unscreened length of ICU (days)	23	23	23	
SV form				
% Clinical recognition	28%	11%	45%	14,17
% use of GH	14%	14%	14%	17
Length of GH treatment (years)	3.2	3.2	3.2	
Mean GH dose (ui)	5.9	5.9	5.9	
% Females submitted to Masculinization process	15%	15%	15%	
% use of GnRha	28%	28%	28%	
Length of GnRha treatment (years)	3.8	3.8	3.8	
Monetary reimbursements values	US\$			
Nursery hospitalization	70.8			23
ICU diary	155.9			
Neonatal 17OHP screening test (A)	2.45			
Confirmatory laboratory + medial consultation (B)	22.46			
GH ampole 12ui	33.97			
GnRH ampole 11.75mg	273.75			
Masculinization process	1,906.19			
Laboratory diagnosis (17OHP, andro, testo, Na, K, renin)	15.05			

Table 3. CEA for CAH-NBS in Brazil during the year of 2017

Decision model on a hypothetical population	Base case	Best case	Worst case
Yearly live-births (N)	2,923,535	2,923,535	2,923,535
Yearly CAH affected NBs (N)	239	292	195
SW NBs (N)	179	219	146
SV NBs (N)	60	73	49
Screening strategy			
SW form			
Clinical Recognition (N)	64	35	80
Costs (A+B) (US\$)	1,594.24	871.85	1,992.80
Deaths (N)	0	0	0
Hospitalizations (N)	0	0	0
<i>Total Costs (US\$)</i>	<i>1,594.24</i>	<i>871.85</i>	<i>1,992.80</i>
No Clinical recognition (N)	115	184	66
Costs (A+B) (US\$)	2,864.65	4,583.44	1,644.06
Deaths (N)	0	0	0
Hospitalizations (N)	67	107	38
ICU (N)	20	32	11
ICU length (days)	9	9	9
Hospitalizations costs (US\$)	32,805.60	52,474.80	18,124.50
<i>Total Costs (US\$)</i>	<i>35,670.25</i>	<i>57,058.24</i>	<i>19,768.56</i>
SV form			
Costs (2A+B) (US\$)	1,641.60	1,997.28	1,340.64
Deaths	0	0	0
Late adverse outcomes (N)	0	0	0
<i>Total Costs (US\$)</i>	<i>1,641.60</i>	<i>1,997.28</i>	<i>1,340.64</i>
Non-affected (N)	2,923,296	2,923,243	2,923,340
True negatives (N)	2,917,449	2,920,320	2,908,723
<i>Costs (A) (US\$)</i>	<i>7,147,750.05</i>	<i>7,154,784.00</i>	<i>7,126,371.35</i>
False positives (N)	5,847	2,923	14,617
High Risk (N)	1,462	731	3,654
Confirmatory true negative (N)	1,345	673	3,362
<i>Costs (A+B) (US\$)</i>	<i>33,503.95</i>	<i>16,764.43</i>	<i>83,747.42</i>
Confirmatory false positive (N)	117	58	292
<i>Costs (A+2B) (US\$)</i>	<i>5,542.29</i>	<i>2,747.46</i>	<i>13,832.04</i>
Low risk (N)	4,385	2,192	10,963
Retest true negative (N)	4,363	2,181	10,908
<i>Costs (2A) (US\$)</i>	<i>21,378.70</i>	<i>10,686.90</i>	<i>53,449.20</i>
Retest false positive (N)	22	11	55
<i>Costs (2A+B) (US\$)</i>	<i>601.92</i>	<i>300.96</i>	<i>1,504.80</i>
Total cost of screening strategy (US\$)	7,247,683.00	7,245,211.12	7,302,006.81

Deaths (N)	0	0	0
Non-screening strategy			
SW form			
Clinical Recognition (N)	64	35	80
Deaths (N)	0	0	0
Hospitalizations (N)	0	0	0
Diagnostic lab (US\$)	963.20	526.75	1,204.00
<i>Total costs (US\$)</i>	<i>963.20</i>	<i>526.75</i>	<i>1,204.00</i>
No Clinical recognition (N)	115	184	66
Deaths (N)	9	20	3
Hospitalizations (N)	96	149	57
ICU (N)	35	54	21
ICU length (days)	23	23	23
Hospitalization costs (US\$)	132,296.30	204,177.00	79,335.30
Dehydration without hospitalization (cost of lab exams) (US\$)	150.05	225.75	90.30
<i>Total costs (US\$)</i>	<i>132,446.80</i>	<i>204,402.75</i>	<i>79,425.60</i>
SV form			
Clinical Recognition (N)	17	8	22
Costs with lab diagnostic (US\$)	255.85	120.40	331.10
No Clinical recognition (N)	43	65	27
Number of 46XX reared as males (N)	3	5	2
Masculinization process (US\$)	5,718.57	9,530.95	3,812.38
Number of patients with GH treatment (N)	6	9	4
Use of GH cost (US\$)	117,047.03	175,570.55	78,031.35
Number of patients with GnRh treatment (N)	12	18	8
Use of GnRha cost	49,932.00	74,898.00	33,288.00
Costs with lab diagnostic (US\$)	647.15	978.25	406.35
<i>Total costs (US\$)</i>	<i>174,160.23</i>	<i>260,977.75</i>	<i>115,538.08</i>
Total cost of non-screening strategy (US\$)	307,010.60	466,027.65	196,498.78
Deaths (N)	9	20	3
Incremental cost of screening (US\$)	6,940,672.40	6,779,183.47	7,105,508.03
Deaths averted (N)	9	20	3
Incremental cost per death averted (US\$)	771,185.82	338,959.17	2,368,502.68
Incremental cost per life years saved (US\$)	25,535.95	11,223.81	78,427.24

SW= salt-wasting form SV=simple virilizing form LY= years of life saved N= number

US\$ = American dollar

Table 4. CEA sensitivity analysis

One-way sensitivity analysis of base-case analysis	Range	ICER (US\$/LY)
1) CAH incidence	1:10,000	20,972.42
	1:15,000	33,337.73
2) Clinical Recognition SW/SV	16%/11%	19,189.90
	55%/45%	38,959.02
3) SW mortality without NBS	11%	17,870.08
	4.2%	46,387.70
4) False positive rate	0.1%	25,423.64
	0.5%	25,872.61

CEA = Cost-effectiveness analysis SW= salt-wasting form SV=simple virilizing form LY= years of life saved NBS = newborn screening

Table 5. Clinical differences between unscreened and screened patients.

Variables	Unscreened	Screened	<i>p value</i>
Age at SW diagnosis	38.8 days	17.3 days	<0.0001
Mean Na level at diagnosis	121.2 mEq/L	131.8 mEq/L	<0.0001
% hospitalization (of all SW patients)	76%	58%	0.058
% ICU	29%	36%	0.041
Length of ICU	22.9 days	9 days	0.042

SW= salt-wasting form SV=simple virilizing form Na = sodium ICU=intensive care unit

Figure 1. The design of the decision tree analysis

DISCUSSÃO

As avaliações econômicas orientam as decisões políticas, fornecendo evidências e estimativa de resultados clínicos e econômicos a longo prazo⁶⁵. Tanto os benefícios quanto os custos são necessários para estabelecer a efetividade da triagem neonatal para determinada doença em termos de redução de morbi-mortalidade⁶⁶. As avaliações de custo-efetividade para triagem neonatal para HAC têm sido focadas na redução de mortalidade. Tem sido sugerido que a falta de dados sobre os custos dos desfechos adversos a longo prazo devido ao diagnóstico tardio deva ser abordada para melhorar a avaliação da implementação da triagem neonatal da HAC³⁴. Enquanto nos países desenvolvidos a HAC é adequadamente diagnosticada por meios clínicos³², a realidade nos países subdesenvolvidos e em desenvolvimento é completamente diferente^{38,39,67}. O presente estudo apresentou, pela primeira vez, as frequências e os custos de resultados adversos em uma população diagnosticada por meios clínicos e utilizou tais dados para a construção de um modelo de decisão completo que avaliou o custo-efetividade da implementação da triagem neonatal para HAC no Brasil.

O principal objetivo da triagem neonatal para a HAC é a prevenção da morte neonatal secundária a desidratação hiponatrêmica; dessa forma, consideramos que a efetividade considerada no estudo seriam os anos de vida salvos. Determinar a taxa de mortalidade pela doença foi o principal desafio do nosso trabalho, já que sabemos que muitas mortes ocorrem sem que o diagnóstico da doença seja feito¹⁹. Através da análise de sensibilidade do nosso modelo de decisão, observamos que as duas variáveis que mais tiveram impacto na análise de custo efetividade foram a taxa de mortalidade e sensibilidade do reconhecimento clínico, que são justamente as características que mais diferem entre países desenvolvidos e em desenvolvimento/subdesenvolvidos. Isso ressalta a importância de se conduzir um estudo como esse no nosso país, considerando as características da nossa população, para que a decisão sobre a implementação de uma tecnologia em saúde como a triagem neonatal seja feita baseada em dados primários da nossa população.

O risco de morte para pacientes com a forma PS na ausência de triagem neonatal varia amplamente na Europa e América do Norte, de 0% a 9%⁴⁷. No entanto, embora essas avaliações tenham sido realizadas em populações com alto padrão de reconhecimento clínico da HAC, é possível que essas taxas estejam subestimadas devido aos casos não diagnosticados. A observação do aumento da incidência de HAC após a implementação da triagem e da predominância de pacientes do sexo feminino e de uma menor proporção de perdedores de sal

em populações não triadas sugerem fortemente taxas da mortalidade maiores devido a crises de perda de sal não diagnosticadas como HAC ^{41,68-70}. Na nossa coorte não triada, os pacientes do sexo masculino correspondem a aproximadamente 30% em ambas as formas clássicas da doença, indicando que o diagnóstico é perdido espacialmente no sexo masculino. Para o cálculo da mortalidade, nós buscamos englobar os casos perdidos, sem, contudo, superestimarmos o cálculo, pois isso poderia implicar que a triagem fosse inadequadamente considerada custo-efetiva. Dessa forma, utilizamos a diferença entre a proporção de perdedores de sal na nossa coorte não triada e em outras coortes triadas. Para reduzir a possibilidade de viés devido tratar-se de uma coorte histórica, nós estratificamos os resultados por década de nascimento e só consideramos a proporção de PS nas décadas mais recentes. Embora em um primeiro momento tenhamos descrito que a taxa de mortalidade poderia chegar a 26%, esse cálculo foi feito considerando que em um estudo de triagem brasileiro realizado em São Paulo anteriormente a proporção de PS foi de 90%²³. Na literatura, a proporção mais descrita é de 75%¹⁹, logo ponderamos que seria possível que no trabalho anterior alguns pacientes com a forma VS tenham sido perdidos pela triagem e por isso a proporção de PS foi maior. Por isso, durante a análise de custo-efetividade, decidimos ser mais conservadores e usar a menor taxa de mortalidade calculada anteriormente (11%) e fizemos uma média com uma taxa de mortalidade usada em estudo de custo-efetividade realizado em um país desenvolvido (4,2 %) ⁴⁷.

Além de realizar a análise de custo-efetividade, nosso objetivo era mostrar o impacto econômico do diagnóstico tardio da doença e mais uma vez a mortalidade se mostrou como um fator importante. Foi um desafio calcular o valor de uma vida salva; neste estudo, optou-se pelo método do capital humano baseado na renda, conforme adotado pela maioria dos pesquisadores de economia em saúde^{57,71}. A prática atual da agência reguladora dos EUA usa uma estimativa do valor da vida salva de aproximadamente US\$ 9 milhões para avaliar uma morte evitada⁷¹, o que foi comparável ao que calculamos. Não utilizamos esse valor no modelo de decisão, pois já que utilizamos as vidas salvas como medida de efetividade (denominador da equação do cálculo da custo-efetividade), se computássemos o custo da morte no numerador estaríamos superestimando o RICE e favorecendo a decisão em relação a triagem.

A alternativa para a triagem neonatal é confiar no reconhecimento clínico precoce da HAC. A sensibilidade dessa estratégia é a probabilidade de um caso ser detectado clinicamente com base nos sintomas ou histórico familiar antes da notificação dos resultados da triagem. Espera-se que o diagnóstico clínico, o conhecimento e os cuidados da doença melhorem ao longo de décadas^{37,72}. Nossa coorte de paciente não triados compreende pacientes acompanhados no

Hospital das Clínicas há pelo menos 30 anos e quando comparamos os resultados ao longo das décadas, percebemos que a melhora no diagnóstico clínico da doença foi parcial: embora as proporções de perdedores de sal e a relação menino:menina tenham melhorado e se aproximado do esperado para a doença, não houve diferença nas taxas de desidratação, hospitalização, reconhecimento da atipia genital e média dos valores de sódio ao diagnóstico. Isso significa que o diagnóstico clínico da HAC ainda é deficiente, o que tem implicação direta na importância da triagem neonatal para o reconhecimento precoce da doença. A realidade que mostramos é a de um centro de referência no estado de São Paulo, sabemos que a de outras regiões do país pode ser ainda pior⁷³. Em uma situação ideal, todos as RNs do sexo feminino seriam diagnosticadas no nascimento devido à atipia genital, mas mostramos que apenas 21% das pacientes com a forma PS do sexo feminino não desidrataram devido ao reconhecimento precoce. Esses dados explicam por que nossas probabilidades de reconhecimento clínico são menores do que as assumidas em estudos anteriores⁴⁷. Outros estudos com populações triadas de HAC em países desenvolvidos também descreveram que até 20% das mulheres foram diagnosticadas apenas pela triagem, destacando a importância de realizar a triagem em ambos os sexos⁷⁴⁻⁷⁶, ao contrário do que alguns outros autores tem discutido que a triagem seria custo-efetiva apenas para os meninos^{47,77,78}.

Os pacientes com a forma PS apresentam hiponatremia grave e longos períodos de internação, sugerindo maior gravidade da crise de perda de sal. A perda de sal grave e prolongada pode resultar em lesões neurológicas que vão desde dificuldades de aprendizado até paralisia cerebral^{49,50}. Identificamos 8,6% dos pacientes com a forma PS que apresentam deficiência mental. Estes pacientes recebem apoio financeiro e atendimento especial multidisciplinar, o que também onera em um enorme custo para o Estado. Dados sobre esse custo total não estão disponíveis para a população brasileira, portanto, foram utilizadas evidências estrangeiras fornecidas pelo *Centers for Disease Control and Prevention (CDC)* que estimaram os custos econômicos diretos e indiretos associados a quatro deficiências de desenvolvimento⁷⁹. Como esse custo foi calculado com base nos dados econômicos de outro país, não o incluímos na análise de custo-efetividade, mas consideramos importante enfatizar a elevada frequência desses eventos.

Quando comparamos os dados clínicos entre os pacientes com perda de sal entre as coortes triadas e não triadas é ainda mais perceptível o benefício da triagem neonatal. Embora metade dos pacientes submetidos a triagem neonatal ainda tenham tido que ser hospitalizados, eles apresentaram níveis de sódios melhores e tempo de hospitalização significativamente menor

que os não triados. Essa observação demonstra que a triagem neonatal previne crises de perda de sal mais graves e, dessa forma, previne possíveis sequelas neurológicas secundárias à desidratação hiponatrêmica grave^{30,31,74,75}.

A provável causa para os pacientes triados terem sido internados foi o atraso na obtenção dos resultados da triagem neonatal. A média de idade ao diagnóstico na população triada foi de 17,2 dias, período no qual já pode ocorrer a crise de perda sal. Outros programas da NBS também enfrentam problemas semelhantes com resultados tardios^{30,74,76}. Claramente, esta questão apresenta implicações na relação custo-benefício da triagem e sua melhoria deve ser objetivada pelos programas da triagem neonatal.

O erro de atribuição de sexo pode ocorrer em paciente do sexo feminino e mambas as situações de triagem ou diagnóstico clínico²⁷, mas com o resultado da triagem neonatal este erro pode ser corrigido a tempo. Já na ausência de triagem, essas pacientes podem permanecer no sexo masculino, devido a ausência do diagnóstico correto precoce, o que foi observado na nossa coorte de pacientes não triados, na qual 12 pacientes (2 PS/10 VS) foram criadas no sexo masculino e submetidas ao processo de masculinização, o que envolve procedimentos cirúrgicos caros e invasivos e acarreta questões psicológicas envolvendo problemas de autoimagem, infertilidade e sexualidade prejudicada⁸⁰.

O diagnóstico precoce da forma PS tem sido o principal objetivo da triagem neonatal e até o momento os estudos em custo-efetividade têm focado no benefício da prevenção da crise perdedora de sal e não têm considerado os desfechos adversos relacionados ao diagnóstico tardio da forma VS⁸¹, o que, como demonstramos nesse estudo, também representa um impacto econômico. A baixa proporção de homens dentre os pacientes com a forma VS sugere que também nessa forma clínica os meninos são subdiagnosticados e, portanto, correm risco de crise adrenal durante condições de estresse. A idade média no diagnóstico de 5,7 anos foi suficiente para justificar sinais de virilização e maturação óssea avançada em quase metade dos pacientes. É descrito que pacientes clássicos apresentam perda potencial de altura final^{51,82}, principalmente associada a altos níveis de andrógenos e maturação óssea avançada durante a infância⁵². Isso motivou a alta frequência de terapias caras, como o uso de GnRha e o GH, em nossa coorte, que poderiam ser evitadas com o diagnóstico precoce através da triagem neonatal. Pela primeira vez, uma análise de custo-efetividade sobre triagem neonatal para HAC considerou esses desfechos.

Em estudos de custo-efetividade, orienta-se não incluir os custos relacionados ao seguimento a longo prazo daqueles pacientes que morreriam, mas sobreviveram devido a

triagem, pois se o custo evitado de uma hospitalização associada a uma morte infantil é mais baixo do que o custo do tratamento de HAC a longo prazo, o custo-efetividade dessa proporção seria menos favorável para essa triagem⁴⁷. No nosso estudo, foi possível também calcular o custo do tratamento padrão em ambas as formas clínicas do diagnóstico aos 19 anos, considerando a média de dose de cada medicação em ambas as formas clínicas. O resultado desse cálculo nos mostrou que o custo do tratamento padrão é acessível e inferior ao custo para o tratamento das complicações dos desfechos tardios, o que ressalta que vale a pena a busca pelo diagnóstico precoce, em ambas as formas clínicas.

Tradicionalmente, supõe-se que a triagem seja rentável se o seu RICE for de até US\$ 50.000/LY⁴⁸. A Organização Mundial da Saúde (OMS) considera uma estratégia muito rentável se o RICE for menor que o produto interno bruto per capita do país (PIB) e como custo efetivo se for até três vezes o PIB, o que seria US\$ 29.294,63 no Brasil em 2017⁶⁰. Assim, nosso estudo mostrou que a triagem neonatal para HAC é econômica, principalmente em um contexto de país em desenvolvimento. A análise do pior caso sugeriu que, em um conjunto de suposições não favoráveis à triagem, como baixa mortalidade, altos padrões de consciência clínica e menor incidência de HAC, a triagem não seria considerada custo-efetiva. No entanto, consideramos que esse cenário é bem menos provável de ocorrer, especialmente em uma realidade de países subdesenvolvidos e em desenvolvimento.

A incidência da doença observada em nossa coorte triada de São Paulo em 2017 foi um pouco maior do que o relatado anteriormente^{21,23}. Embora nenhum caso perdido tenha sido relatado ao nosso conhecimento, a proporção desbalanceada de mulheres e homens sugere o contrário. É possível que alguns homens com a forma VS tenham sido perdidos, possivelmente devido ao uso pré-natal de glicocorticoide, que é uma prática frequente nos centros obstétricos de São Paulo. Discute-se que a triagem neonatal poderia ser menos sensível para detectar a forma VS, o que motivou o estabelecimento de uma segunda coleta no protocolo de triagem no Texas⁸³. Também é possível que o desequilíbrio entre mulheres e homens seja devido a um erro de amostragem, porque se trata de uma análise de apenas um ano e, se aumentássemos a amostra, isso seria corrigido. De qualquer forma, mesmo que alguns casos da forma VS tenham sido perdidos pela triagem, a realidade dos pacientes com VS sem triagem neonatal seria muito pior.

A questão propulsora para a realização do presente estudo era de que não existiam no Brasil estudos que avaliassem a custo-efetividade da incorporação da HAC no programa nacional de triagem e que os estudos internacionais sobre o assunto não abordavam os desfechos

a longo prazo do diagnóstico tardio. Dessa forma, um dos pontos fortes do nosso trabalho foi ser capaz de acessar os benefícios de longo prazo da triagem neonatal para a doença e usá-los na análise de custo-efetividade, o que nunca havia sido feito antes. Este estudo adiciona mais argumentos para o endosso internacional triagem neonatal para HAC. O diagnóstico clínico da HAC só é possível em neonatos sintomáticos e não pode ser feito de maneira confiável em ambos os sexos, o que leva a um atraso no tratamento e permite a ocorrência de sequelas graves. Essas consequências aumentam os custos do tratamento, incluindo fatores mensuráveis, como internações mais longas por desidratação hiponatrêmica e uso de medicamentos caros para melhorar a altura final. Além disso, a ausência de diagnóstico precoce pode acarretar aos pacientes danos desnecessários físicos, psicológicos e neurológicos, que não são tão facilmente mensuráveis.

Em conclusão, este estudo foi capaz de enfatizar a importância da triagem na redução da mortalidade e morbidade por HAC, mostrando que, mesmo sob um ponto de vista econômico, a sua realização pode valer a pena. Acreditamos que nosso estudo fornece uma avaliação econômica útil para subsidiar decisões sobre a adoção da triagem para a HAC em programas federais de triagem neonatal.

CONCLUSÕES

- O diagnóstico clínico pela doença é, na grande maioria dos casos, atrasado, permitindo a ocorrência de eventos adversos importantes que além de prejudicarem a qualidade de vida do paciente, têm um impacto econômico importante para o Estado.
- O Estudo de custo da doença evidenciou que o tratamento padrão para HAC tem baixo custo, mas o tratamento para as complicações pelo diagnóstico tardio acrescenta valor significativo.
- A mortalidade secundária a HAC não diagnosticada na ausência de triagem pode variar de 11 a 26%, o que é um número maior que o observado em países desenvolvidos.
- A qualidade do diagnóstico clínico da doença parece ter melhorado ao longo dos anos, mas ainda não é suficiente para garantir um acesso universal ao diagnóstico precoce e evitar a ocorrência de complicações.
- Apesar da atipia genital de ser um sinal clínico evidente ao nascimento, que possibilitaria o diagnóstico precoce em pacientes do sexo feminino, ela ainda é subdiagnosticada em nosso meio, o que justifica a realização da triagem em ambos os sexos.
- A triagem neonatal no Brasil ainda tem alguns problemas a serem enfrentados como o tempo para a obtenção dos resultados, o que possibilita que alguns pacientes ainda tenham que ser internados, mesmo com a triagem.
- A análise de custo-efetividade chegou-se a um RICE que coloca a triagem neonatal para HAC como uma estratégia custo-efetiva e através do presente estudo tem respaldo científico para ser implementada em todo território nacional.

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ANEXOS

Outras produções científicas relacionadas ao diagnóstico da Hiperplasia Adrenal Congênita

Durante o período de execução do presente trabalho, tive a oportunidade de participar de outros trabalhos na área de Hiperplasia Adrenal Congênita e Triagem Neonatal para a doença, com publicação de artigos e capítulos de livros na área:

Artigos:

1. de Carvalho, D., Miranda, M., Gomes, L., Madureira, G., Marcondes, J., Billerbeck, A., Rodrigues, A., Presti, P., Kuperman, H., Damiani, D., Mendonca, B., and Bachega, T. (2016). Molecular CYP21A2 diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction. *European Journal of Endocrinology* 175, 2, 107-116, available from: < <https://doi.org/10.1530/EJE-16-0171> >
2. Hayashi, G.Y., Carvalho, D.F., de Miranda, M.C., Faure, C., Vallejos, C., Brito, V.N., Rodrigues, A.D.S., Madureira, G., Mendonca, B.B. and Bachega, T.A. (2017), Neonatal 17-hydroxyprogesterone levels adjusted according to age at sample collection and birthweight improve the efficacy of congenital adrenal hyperplasia newborn screening. *Clin Endocrinol*, 86: 480-487. doi:10.1111/cen.13292
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4. Riccardo Pofi, Alessandro Prete, Vivien Thornton-Jones, Jillian Bryce, Salma R Ali, S Faisal Ahmed, Antonio Balsamo, Federico Baronio, Amalia Cannuccia, Ayla Guven, Tulay Guran, Feyza Darendeliler, Claire Higham, Walter Bonfig, Liat de Vries, Tania A S S Bachega, Mirela C Miranda, Berenice B Mendonca, Violeta Iotova, Màrta Korbonits, Nils P Krone, Ruth Krone, Andrea Lenzi, Wiebke Arlt, Richard J Ross, Andrea M Isidori, Jeremy W Tomlinson, Plasma Renin Measurements are Unrelated to Mineralocorticoid Replacement Dose in Patients With Primary Adrenal Insufficiency, *The Journal of Clinical Endocrinology & Metabolism*, Volume 105, Issue 1, January 2020, Pages 314–326, <https://doi.org/10.1210/clinem/dgz055>
5. Iervolino, L.L., Ferraz-de-Souza, B., Martin, R.M. *et al.* Real-world impact of glucocorticoid replacement therapy on bone mineral density: retrospective experience of a large single-center CAH cohort spanning 24 years. *Osteoporos Int* (2020). <https://doi.org/10.1007/s00198-019-05268-0>

Capítulos:

- 1- MIRANDA, Mirela C.; BACHEGA, Tania A.S.S. Triagem neonatal da hiperplasia adrenal congênita — como interpretar um resultado alterado? In: Proendócrino. 9. Ed. Porto Alegre: Artmed Panamericana, 2017. p.
- 2- MIRANDA, Mirela C; BACHEGA, Tania. A.S.S; KOPACEK, Cristiane. Hiperplasia Adrenal Congênita - Triagem Neonatal. In: Guia Prático de Atualização - Departamento Científico de Endocrinologia da Sociedade Brasileira de Pediatria. 2019. p.1-16. Disponível em: https://www.sbp.com.br/fileadmin/user_upload/22106c-GPA_-_Hiperplasia_Adrenal_Congenita-TriagemNeonatal.pdf

- 3- MIRANDA, Mirela C; HAYASHI, Giselle Y.; BACHEGA, Tania. A.S.S. Hiperplasia Adrenal Congênita da 21-hidroxilase – Interpretando um teste positivo. In: RIBEIRO, Antonio F; GOTO, Maura M. F; GLINDER, Carmela M; LEMOS-MARINE, Sofia H. V. Triagem Neonatal e Doenças Raras. 1.ed. Rio de Janeiro:Thieme Revinter Publicações. 2019.

Molecular *CYP21A2* diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction

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Abstract

Background: Most congenital adrenal hyperplasia (CAH) patients carry *CYP21A2* mutations derived from conversion events involving the pseudogene, and the remaining carry new mutations.

Objective: To review causal mutations and genotype–phenotype correlation in 480 Brazilian patients.

Methods: DNA was extracted from 158 salt-wasters (SWs), 116 simple virilizing (SV), and 206 nonclassical (NC) patients. Fourteen point mutations were screened by allele-specific PCR, large rearrangements by Southern blotting/MLPA, and sequencing was performed in those with incomplete genotype. The gene founder effect was analyzed by microsatellite studies. Patients were divided into six genotypes (Null; A: <2%; B: 3–7%; C: >20% of residual enzymatic activity (EA); D: unknown EA; E: incomplete genotype).

Results: Targeted methodologies defined genotype in 87.6% of classical and in 80% of NC patients and the addition of sequencing in 100 and 83.5%, respectively. The most frequent mutations were p.V281L (26.6% of alleles), IVS2-13A/C>G (21.1%), and p.I172N (7.5%); seven rare mutations and one novel mutation (p.E351V) were identified. Gene founder effect was observed in all but one (p.W19X) mutation. Null, A, B, and C genotypes correlated with SW (88%), SW (70%), SV (98%), and NC forms (100%), respectively. In group D, the p.E351V mutation correlated with classical form and group E comprised exclusively NC-patients. ACTH-stimulated 17OHP level of 44.3 ng/mL was the best cutoff to identify NC-patients carrying severe mutations.

Conclusions: We identified a good genotype–phenotype correlation in CAH, providing useful data regarding prediction of disease's severity; moreover, we suggest that ACTH-stimulated 17OHP levels could predict carrier status for severe mutations. Sequencing is essential to optimize molecular diagnosis in Brazilian CAH patients.

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Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is the most common inborn metabolism error, which presents an autosomal recessive inheritance and comprises 90–95% of CAH cases. A spectrum of clinical manifestations is observed and, traditionally, it is divided into classical (simple virilizing – SV and salt wasting – SW) and nonclassical

(NC) forms. The SV form is characterized by prenatal external genitalia virilization in females and postnatal virilization in both sexes. In the SW form, in addition to the prior listed symptoms, patients also present with hyponatremic dehydration in the first weeks of life. In the NC form, postnatal hyperandrogenic manifestations initiate at infancy, adolescence, or even at adult life (1).

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The classical form frequency is approximately 1:10 000 to 1:15 000 live births, whereas the NC form is much more frequent and is estimated to have a frequency of 1 in 100–1000 in the general population (2, 3, 4, 5).

The 21-hydroxylase coding gene (*CYP21A2*) and its pseudogene (*CYP21A1P*) extend over a long region in the short arm of chromosome 6, alternating in *tandem* with C4A and C4B genes. Because of the high homology between *CYP21* genes and the tandem-repeat organization of the *CYP21/C4* locus, this genomic region is vulnerable to gene conversion events; thus, 90–95% of patients carry *CYP21A2* mutations derived from the pseudogene, instead of new casual mutagenic events (6, 7, 8, 9).

The great variability in phenotypic expression of CAH results from mutations leading to different degrees of enzymatic impairment, from mild to severe, and the phenotype correlates with the allele harboring the less severe mutation in a compound heterozygous patient (10, 11, 12).

A high genotype–phenotype correlation of up to 90–95% has been described in several studies (9, 13, 14, 15, 16, 17). Mutations resulting in up to 2% of residual enzymatic activity correlate with the SW form, 3–10% correlate with the SV form, and 20–50% correlate with the NC form (6, 7, 8, 9, 11, 16). These findings have emphasized the use of molecular diagnosis in clinical practice, especially in situations that require phenotype prediction. For example, molecular analysis could elucidate the diagnosis in male asymptomatic newborns with positive neonatal tests or in premature females in whom true clitoromegaly can be overestimated (4, 18). In Brazil, CAH newborn screening (CAH-NBS) is being implemented, and, considering the usefulness of molecular diagnosis to confirm/exclude CAH diagnosis, its use has been approved as a confirmatory test (19).

The aim of this paper is to review our 20 years of experience in diagnosis of CAH due to 21-hydroxylase deficiency. We determined the causing mutations in a cohort of 480 Brazilian patients to obtain a panel of the most prevalent mutations and evaluated their phenotype prediction. This information would be helpful for improving diagnostic accuracy and genetic counseling.

Subjects and methods

Patients

The study was approved by the Ethical Committee of Faculdade de Medicina da Universidade de São Paulo and written consent was obtained from all the participants.

Between 1993 and 2013, a total of 480 CAH patients from 429 families were diagnosed (158 SW, 116 SV, and 206 NC). All patients were followed at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, most of them were from São Paulo state, but few were from other Brazilian States, especially from Northeast region. The cohort included 359 females and 121 males, and the female/male ratios in classical and nonclassical forms were 1.9/1.0 and 6.7/1.0, respectively.

Clinical and hormonal-criteria defined phenotypic classification is as follows: SW, SV, and NC forms (6). In the SV form, females were referred by atypical genitalia at birth according to Prader genital scores (PGSs) or by clitoris enlargement during early childhood; males were diagnosed by precocious pubarche and penis enlargement. In both genders, bone age was advanced. In the SW form, in addition to the virilizing signs, patients also failed to thrive and demonstrated hyponatremic dehydration in the first weeks of life. All classic patients presented with extremely increased basal 17OHP levels. The mean chronological age at diagnosis in the SW form was 66 ± 121 days, and in the SV form, it was 4.0 ± 4.5 years in girls and 4.2 ± 2.0 in boys. The mean bone age at diagnosis in SV girls was 4.2 ± 5.3 years and in SV boys was 8.5 ± 6.6 years. The NC form included female patients with late-onset hyperandrogenic manifestations such as precocious pubarche, menstrual abnormalities, hirsutism, and/or infertility. Nonclassical males were referred by precocious pubarche. All NC patients demonstrated basal or 1-h after ACTH-stimulated 17OHP levels clearly increased above 10 ng/mL (20). NC females were tested during the follicular phase of the menstrual cycle or with progesterone levels ≤ 3 ng/mL. The mean age at diagnosis in NC females was 18.3 ± 13.7 years and in NC males was 7.1 ± 3.6 years.

Hormone assays

Serum 17OHP and androstenedione levels and plasma renin activity were measured by radioimmunoassays, and the intra- and interassay coefficients of variation were 12%, 18%, and 18%, respectively. Serum cortisol and testosterone levels were analyzed by immunofluorometric assays, and the intra- and intervariation coefficients were $<8\%$ for both variables. The 17OHP values were expressed in ng/mL ($1 \text{ ng/mL} = 3.18 \text{ nmol/L}$). All steroid hormones were measured before treatment introduction.

Mutation analysis of the *CYP21A2* gene

DNA samples from patients and at least one parent were obtained from peripheral leukocytes. The *CYP21A2* gene was PCR-amplified, and the most frequent 14-point mutations were screened by allele-specific PCR (10, 11). Large gene rearrangements (LRs) were screened by Southern blot followed by *CYP21* probe hybridization or by multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA kit P050B CAH; MCR Holland, Amsterdam, the Netherlands) (21, 22).

When at least one allele remained with no mutation identified, entire *CYP21A2* sequencing was performed by dideoxynucleotide terminator methodology as described previously. The new allelic variant identified was also screened in at least 100 alleles of *CYP21A2* and *CYP21A1P* genes from normal Brazilian subjects (10, 23, 24), and also assessed in online known genome databases like ExAC (Exome Aggregation Consortium) and 1000 Genome. *In silico* prediction analysis of the novel p.E351V mutation was assessed using Mutation Taster (www.mutationtaster.org), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2>), and SIFT (Sorting Intolerant from Tolerant Human Protein) (http://sift.jcvi.org/www/SIFT_enst_submit.html) tools.

Two microsatellite loci (D6S273 and TAP 1), flanking the *CYP21A2* gene, were studied in all patients and their relatives who carried new mutations, as described previously (23, 24).

Genotype categories

After mutation segregation analysis, patients' genotypes were divided into six different groups. Group Null included patients who carried two mutations that predicted 0% overall activity. Group A included patients who were homozygous for the IVS2-13A/C>G mutation (<2%) or in compound heterozygosis with the group Null mutations. Group B included patients who were homozygous for the p.I172N mutation (3–7%) or in compound heterozygosis with mutations from groups Null or A. Group C included patients who were homozygous for the p.P30L, p.V281L, and p.P453S mutations (20–50%) or in compound heterozygosis with the former mutations (12). Finally, group D included patients with novel mutations for which enzymatic activity impairment had not been assessed and group E with at least one allele without identified mutations.

Statistical analysis

Data are presented as median and interquartile range of variables for which the normality test failed. The Fisher's test was used to analyze the association of mutations with clinical forms and also to verify the association of the new mutations with their respective haplotypes. Correlation between genotype groups and 17OHP levels was verified using either Mann–Whitney or Hodges–Lehmann nonparametric tests. Statistical significance was considered if $P < 0.05$. All of the statistical analyses were performed using SPSS software version 22.

Results

Disease-causing allele's distribution

Mutations were identified in both alleles using allele-specific PCR followed by Southern blot or MLPA methodologies in 88.6% of SW patients, 86.3% of SV, and 80% of NC patients. The addition of *CYP21A2* sequencing allowed for the identification of mutations in all of the classical patients and in 83.5% of NC patients. The rate of compound heterozygosis was 65.3%.

Mutations were observed in 95.7% of the 856 unrelated alleles; 86.7% had point mutations and 9% carried large gene rearrangements. The identified mutations were segregated in samples from at least one of the parents, and 6% of the alleles carried two or more point mutations. The most frequent point mutations were p.V281L (26.6% of alleles), IVS2-13A/C>G (21.1%), and p.I172N (7.5%) (Table 1).

Mutations not derived from gene conversion events

The previously described mutations p.G424S, p.R408C, IVS2-2A>G, p.Ser170fs, p.R426H, p.H365Y, and p.W19X were observed in 35 nonrelated patients (23, 24, 25, 26) (Table 2). Among them, the most frequent were the IVS2-2A>G, p.G424S, and p.R426H mutations. Microsatellite analysis was performed for six former mutations using markers flanking the *CYP21A2* gene and disclosed the same haplotype for each new mutation, suggesting a gene founder effect.

Additionally, a new allelic variant was observed: the p.E351V, in three nonrelated patients (1SW/2SV) (Table 2). This substitution was not found in 100 alleles of *CYP21A2* and *CYP21A1P* genes from normal subjects, and neither in Human Genome databases, ruling out the possibility

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Table 1 Frequency of *CYP21A2* mutations in Brazilian CAH patients and in other countries.

	Country or region (reference)									
	Brazil (this study)	Argentina (13)	Portugal (29)	Spain (17)	Chile (27, 28)	Germany (14)	Sweden (9)	USA (15)	Mexico (30)	Mid-Europe (35)
Number of unrelated alleles	856	866	112	354	164	310	186	3005	94	864
Mutations										
<i>CYP21A2</i> deletion or conversion	9.0	11.2	25.9	19.2	19.5	27.4	29.8	20.0	1.0	30.6
p.P30L	0.6	0.7	1.8			2.6	1.6	2.6	8.5	3.7
IVS2-13A/C>G	21.1	20.6	9.8	17.5	15.8	30.3	30.0	22.9	47.9	31.2
p.G110EfsX21 (E3Δ8bp)	1.8	0.8	2.7	3.3		1.6	1.2	2.1	2.1	1.0
p.I172N	7.5	8.2	9.8	3.7	7.3	19.7	20.3	8.2	11.7	14.5
p.I235N, p.V236E, p.M238K (E6 cluster)	1.2	2.0			1.8	1.0	1.1	2.1	0.0	0.3
p.V281L	26.6	26.2	25.9	33.9	2.4	2.9	7.0	23.9	8.5	3.4
p.G291S	0.1	*					0.5		0	*
p.Leu307fs (InsT)	2.2	*	4.4	1.4	0.0	0.3	0.5		1.1	1.6
p.Q318X	6.1	6.7	6.3	9.3	7.9	4.8	3.2	3.5	4.2	2.6
p.R356W	5.4	4.2	1.8		9.7	4.5	4.3	3.6	7.4	2.4
p.P453S	1.4	1.4				0.3	0.5		2.1	0.7
p.R483P	0.0									0.1
p.R483PfsX58	0.0	1.5								0.9
% of mutations involving events with pseudo-gene	82.9	82	88.4	93.9	64.4	98.1	100	88.9	94.5	92
% of alleles with no mutations identified	4.3	11.9	0.9	9.3	23.7	1.3			21.3	

*Not screened.

of polymorphism. All of the carriers disclosed the same haplotype, suggesting a gene founder effect. *In silico* analysis by Polyphen and Mutation Taster revealed scores of 0.811 and 121, respectively.

Genotype–phenotype correlation

The overall genotype–phenotype correlation was 97.3%. Genotype accurately predicted phenotype in 88.2% of the

patients in group Null, 70% of the patients in group A, 98% of the patients in group B, and 100% of the patients in group C. The Null and A genotypes comprised mainly SW patients; the B genotype was mainly associated with the SV form, but one patient presented with the NC form (LR, p.P30L/IVS2-2A>G). The C genotype was exclusively associated with the NC form. The D genotype comprised four classical patients. Finally, the E genotype was observed in 34 NC patients (Table 3).

Table 2 *CYP21A2* mutations not derived from pseudogene events: mutations with gene founder effect and a novel mutation.

ENSP00000408860	ENST00000418967	Frequency (%)	Patients [§] (n)	Founder effect	Microsatellites	
					D6S273	TAP-1
p.G424S	c.1273G>A	0.6	6	+	1	5
p.Ser170fs	c.511_512insA	0.5	4	+	3	6
p.R408C	c.1225C>T	0.5	4	+	5	5
IVS2-2A>G		1.2	10	+	3	1
p.H365Y	c.1096C>T	0.2	2	+	2	5
p.R426H	c.1280G>A	0.8	7	+	6	3
p.W19X	c.59G>A	0.2	2	–	–	–
p.E351V	c.1055A>T	0.3	3	+	4	5

[§]Number of unrelated patients.

In the SW form, the most frequent mutation was IVS2-13A/C>G (33.2% of alleles), in the SV form the p.I172N (32.0%) followed by IVS2-13A/C>G (27.8%), and in the NC form the p.V281L (61.4%).

Prader genital score was available in 101 classical females. The most common PGS in Null, A, and B genotypes was Prader III (49.5%). Severe virilization (Prader IV and V) was observed more frequently in genotypes Null and A than in B ($P=0.03$), although there was no difference between groups Null and A (Fig. 1). The PGSs were similar in all classical female siblings (5 families). Interestingly, 8% of the NC females had a slight clitoromegaly (Prader I).

Median basal 17OHP levels were significantly different among genotype groups Null, A, B, and C. The lowest basal 17OHP level in a classical patient was 38 ng/mL. Among NC patients from group C, median basal 17OHP levels varied from normal (7.2%) to those that overlap with the classical form. The median basal 17OHP level was significantly higher in genotype A/C (median 17.5 ng/mL, range 0.4–80 ng/mL) than in C/C (median 7.6 ng/mL, range 0.3–58 ng/mL) as was ACTH-stimulated 17OHP levels ($P=0.005$) (Table 4). Data of basal and stimulated-17OHP levels from pre- and postpubertal NC patients were analyzed together, since their values did not significantly differed ($P>0.05$). The lowest ACTH-stimulated 17OHP level in an NC patient from group C was 11 ng/mL. ACTH-stimulated serum 17OHP level of 44.3 ng/mL was the cutoff with higher sensitivity (0.674) and specificity (0.702) to identify NC patients with compound heterozygosity for severe mutations (area under ROC curve = 0.701 (0.597–0.804) ($P=0.001$).

Even after *CYP21A2* sequencing, 16.5% of the NC patients persisted with an incomplete genotype (group E). Among these, 8 patients presented basal 17OHP level above 15 ng/mL, 8 presented ACTH-stimulated 17OHP level between 10 and 15 ng/mL, and 20 presented ACTH-stimulated 17OHP level above 15 ng/mL. These patients had their *CYP21A2* sequencing revised though a new PCR product and allelic drop out was ruled out.

Discussion

In the present study, we explored the clinical and molecular features of one of the largest Latin American CAH cohorts. We identified the nine previously described *CYP21A2* point mutations, derived from gene conversion events, in 74% of unrelated alleles and large gene

rearrangements in 9% of the alleles. Compared with other ethnic groups, the overall results were similar, especially concerning the high frequency of IVS2-13A/C>G mutation. Our lower frequency of LR was probably because many SW patients died without precocious neonatal diagnosis (6, 13, 15, 16, 17, 26, 27, 28, 29). The lower SW:SV ratio (1.3:1.0) than that found in countries where CAH neonatal screening is routinely performed (3-4:1), in addition to the lower proportion of males than females (1.0/1.9) among classical patients, illustrates the importance of introducing CAH newborn screening (4). In Argentina (13) and Mexico (30), countries in which CAH-NBS has been implemented more recently, the patient cohort characteristics are similar to ours.

The widely used site-directed mutation analysis and Southern blot/MLPA techniques failed to identify mutations more often than expected. Considering the significant proportion of mutations that were not derived from the pseudogene (4.4% of the alleles), we suggest the addition of sequencing to propose an accurate diagnostic molecular panel in our population. *CYP21A2* sequencing increased the identification of mutations to 100% of classical alleles, similar to what was described previously (9, 16).

Remarkably, analysis of the Brazilian cohort found eight rare/novel mutations. The gene founder effect was observed in all carriers of the p.R408C, p.Ser170fs, p.H365Y, IVS2-2A>G, and p.G424S, as described previously (23, 24, 26), and in the present work, we also observed in carriers of the p.E351V and p.R426H mutations. For instance, the p.H365Y, p.G424S, and p.R426H mutations were already described in Spanish and Portuguese patients, which are the two main parental populations responsible for Brazilian population constitution, suggesting a common origin (29, 31). Although the majority of our patients was from São Paulo state, we have no information about their ancestry, which may come from other places of Brazil. For instance, we also identified these mutations in screened patients from Goiás state, suggesting that they are present in Brazilian population overall (32).

The codon 351 is a highly conserved residue of P450 cytochromes and belongs to the E351/R354/R408 triad that stabilizes the three-dimensional protein structure (33). A missense mutation p.E351K was described in the same codon leading to severe enzyme activity impairment (34). Although we did not perform *in vitro* studies, *in silico* analysis suggested deleterious effect of this mutation; the p.E351V mutation was found in classical patients confirming the severity of the mutation *in vivo*.

Table 3 Genotype–phenotype correlation and gender distribution of patients with point mutations or large rearrangements.

Group	CYP21A2 genotype	CAH phenotype				Sex	
		SW	SV	NC	Total	F	M
Null	Del or conv./Del or conv.	9	1		10	6	4
	Del/p.Leu307fs	1			1	1	
	Del or conv./p.Q318X	5	1		6	1	5
	Del or conv./p.R356W	5			5	2	3
	Del/p.W19X	1			1	1	
	p.Leu307fs/E6 cluster	2			2	1	1
	E3 Δ8bp/E3 Δ8bp	3			3		3
	E3 Δ8bp/p.Q318X	3			3		1
	E3 Δ8bp/p.R356W	1			1	1	
	E6 cluster/E6 cluster	1			1		1
	E6 cluster/p.R356W	1			1	1	
	p.Q318X/p.Q318X	7			7	4	3
	p.Q318X/p.Leu307fs	1			1	1	
	p.R356W/p.R356W	5	1		6	5	1
	p.R356W/p.Q318X	4	1		5	1	4
	p.R356W/p.Leu307fs	5			5	3	2
	p.H365Y/IVS2-13A/C>G	1			1	1	
	p.Ser170fs/p.Leu307fs	1			1	1	
	p.Ser170fs/p.Q318X	1			1		1
	IVS2-2A>G/IVS2-2A>G	1			1	1	
	p.Ser170fs/p.R408C	1			1		1
	p.R408C/p.Q318X		1		1		1
	p.R408C, IVS2-13A/C>G/IVS2-13A/C>G	1			1	1	
A	IVS2-13A/C>G/Del or conv.	18	3		21	19	2
	IVS2-13A/C>G/IVS2-13A/C>G	23	15		38	25	13
	IVS2-13A/C>G/E3 Δ8bp	2	1		3	2	1
	IVS2-13A/C>G/E6 cluster	1			1	1	
	IVS2-13A/C>G/p.G291S	1			1	1	
	IVS2-13A/C>G/p.Leu307fs	4			4	2	2
	IVS2-13A/C>G/p.Q318X	9	4		14	10	4
	IVS2-13A/C>G/p.R356W	3	3		6	3	3
	IVS2-13A/C>G/IVS2-2A>G	3			3	2	1
	IVS2-13A/C>G/p.W19X, p.V281L	1			1	1	
	IVS2-13A/C>G/p.Ser170fs	1			1	1	
B	p.I172N/Del or conv.		4		4	3	1
	p.I172N/IVS2-13A/C>G		14		14	12	2
	p.I172N/E6 cluster		3		3	2	1
	p.I172N/p.I172N		16		16	12	4
	p.I172N/p.Q318X		5		5	4	1
	p.I172N/p.R356W		4		4	3	1
	p.I172N/p.Leu307fs		1		1	1	
	p.I172N/Conv P30L		2		2	2	
	p.I172N/IVS2-2A>G		2		2	1	1
	p.I172N/p.G424S		1		1		1
	p.I172N/p.R408C		1		1	1	
	p.G424S/IVS2-13A/C>G		2		2	2	
	p.G424S/p.R356W		2		2	1	1
	p.R426H/Del		1		1	1	
	p.R426H/p.Q318X		2		2	1	1
	p.R426H/p.H365Y		1		1		1
	p.R426H/p.R426H		1		1	1	
	Conv P30L/IVS2-13A/C>G		1		1	1	
	Conv P30L/p.R356W		1		1	1	
	Conv P30L/IVS2-2A>G			1	1	1	
C	p.V281L/Del or conv.			13	13	11	2
	p.V281L/IVS2-13A/C>G			21	21	19	2
	p.V281L/p.I172N			7	7	5	2

Continued

Table 3 (Continued).

Group	CYP21A2 genotype	CAH phenotype				Sex	
		SW	SV	NC	Total	F	M
	p.V281L/p.V281L			73	73	64	9
	p.V281L/p.P30L			1	1	1	
	p.V281L/E3 Δ8bp			3	3	2	1
	p.V281L/E6 cluster			1	1		1
	p.V281L/p.Leu307fs			3	3	3	
	p.V281L/p.Q318X			3	3	3	
	p.V281L/p.R356W			8	8	7	1
	p.V281L/Conv P30L			1	1	1	
	p.V281L/p.R426H			1	1		1
	p.V281L/p.G424S			1	1	1	
	p.V281L/IVS2-2A>G			1	1	1	
	p.V281L/p.P453S			8	8	6	2
	p.P30L/IVS2-13A/C>G			2	2	1	1
	p.P30L/p.P30L			1	1	1	
	p.P453S/E3 Δ8bp			1	1	1	
	p.P453S/IVS2-13A/C>G			2	2	2	
D	p.E351V/E6 cluster		1		1	1	
	p.E351V/IVS2-13A/C>G	2			2		2
	p.E351V/p.G424S		1		1		1
E	IVS2-13A/C>G/-			3	3	3	
	p.V281L/-			20	20	19	1
	-/-			9	9	9	

A strong positive genotype–phenotype association was verified, similar to that found in other ethnic groups (9, 13, 14, 15, 16, 17, 27, 28, 29, 30, 35). The salt-waster frequency was higher in the Null group than in group A, which is also important for clinical decisions in asymptomatic patients (18) during the neonatal period. When the most frequent mutation IVS2-13A/C>G was present in the less severe allele (group A), carriers has an increased chance (71%) to develop the SW form. Its presence in some simple virilizing patients can be explained by alternative splicing, which leads to a minimal residual enzyme activity, thus avoiding salt loss (36).

High concordance between genotype and phenotype was also observed in group B. This was different from other studies, which demonstrated a wide phenotypic spectrum with the p.I172N mutation, varying from SW to SV forms (7, 13, 15, 16). Another positive genotype–phenotype correlation was observed in group C, where all patients presented with the NC form except for those carrying the p.P30L mutation, since some patients also presented the SV form. It is important to evaluate whether p.P30L mutation represents a microconversion event involving the exon 1 of the pseudogene or a macroconversion event involving the promoter region plus exon 1 of the pseudogene. For the latter cases, in our experience, the phenotypic expression of SV form prevails, since the promoter region of the pseudogene carries mutations that

reduce the transcriptional activity five-fold, resulting in a more severe phenotype (37).

Despite the above-mentioned positive genotype–phenotype correlation, we observed few cases with less severe phenotypes than what was predicted by the genotype. This divergence has been observed in some

Figure 1

Frequency of Prader genital scores (PGSs) according to genotype groups.

Clinical Study	D F Carvalho and others	CAH molecular diagnosis before NBS	175:2	114
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Table 4 Serum basal and ACTH-stimulated 17OHP levels according to genotype in CAH patients.

Genotype group	Patients (n)	Basal 17OHP (ng/mL)			ACTH-stimulated 17OHP (ng/mL)		
		Mean	Median	Range	Mean	Median	Range
Null	85	227.5	188.3	50–801	–	–	–
A	115	225.8	180.1	50–1549	–	–	–
B	71	166.8	145.7	38–393	–	–	–
C	171	17.6	12.8	0.6–80	49.2	43.6	11–255
NC genotypes							
A/C	74	23.1	17.5	0.4–80	60.3	55	11–255
B/C	10	23.2	19	8–43	64.5	60	20–118
C/C	87	12	7.6	0.3–58	39.8	36	15–121
C/– or –/–	34	10.7	3.5	0.9–58.9	24	19	10.8–83.7

studies worldwide (14, 15); this may be due to a role of modifier genes involving 21-hydroxylase transcription/translation or even the presence of extra-adrenal 21-hydroxylation (21, 38).

Data available regarding genotype correlation with the degree of external genitalia virilization reveal conflicting results (9, 14, 39). We observed severe genitalia virilization more frequently in genotypes Null and A than in B; however, due to the wide overlap of this manifestation, genotype could not be used as a predictor.

Median basal 17OHP levels were significantly different among genotypes Null, A, B, and C; however, a great overlap among genotype groups has been observed (7, 11). Basal and ACTH-stimulated 17OHP levels were significantly higher in NC genotype A/C than C/C. These findings are in accordance with other series that included a significant number of NC patients (2, 20, 40).

Different ACTH-stimulated 17OHP levels (10–15 ng/mL) have been used for NC diagnosis (2, 3). Most of our NC patients presented with highly increased stimulated 17OHP levels, but 3.3% of them presented with levels lower than 15 ng/mL. Among these latter patients with incomplete genotype, we cannot rule out the polycystic ovary syndrome diagnosis. In this way, it was observed that some obligate heterozygotes for severe mutations presented with ACTH-stimulated 17OHP levels that overlapped with those of NC diagnosis (41). All these data indicate that hormonal nonclassical diagnosis criteria should be revised taking into account molecular data. However, we also observed that most patients with incomplete genotype (group E) presented ACTH-stimulated 17OHP levels higher than 15 ng/mL. Nevertheless, this finding also points out to the possibility of mutations in other sites like distal regulatory regions (42).

Due to our large number of NC patients, we investigated the peak 17OHP cutoff that better predicts the risk of compound heterozygosis with severe mutations

(genotype A/C), which is important for genetic counseling purposes. The ACTH-stimulated 17OHP value of 44.3 ng/mL presented the highest sensitivity and specificity to identify this subgroup of patients; a previous study found a similar value (49.8 ng/mL) (43).

CAH molecular diagnosis is one of the most complicated of all monogenic disorders. Through our large CAH cohort, we were able to designate the complexities in their genotypes. We provided mutation prevalence in Brazil, which enables the development of a mutation panel for molecular diagnosis in our population in the clinical practice, such as newborn screening.

The CAH-NBS is not universally adopted, mainly because of its high false-positive results rate and of the good healthcare professionals' performance in detecting CAH during neonatal period in developed countries (44, 45). Unfortunately, the scenario is different in emergent nations. Data of this unscreened Brazilian cohort highlight the pitfalls of clinical diagnosis, illustrating the importance of CAH-NBS introduction nationwide. Recent data from NBS in São Paulo indicated that it is highly efficient to promote early diagnosis, preventing neonatal deaths in our state (32). In NBS, the molecular study is particularly useful to set the precise diagnosis, avoiding unnecessary treatment and follow-up of oligo/asymptomatic patients with persistently increased 17OHP levels.

In conclusion, we demonstrated a good genotype-phenotype correlation and that *CYP21A2* sequencing is essential to achieve adequate CAH molecular diagnosis, although 16.5% of the NC patients persisted with an incomplete genotype. We also suggest that stimulated 17OHP levels could be predictive of carrier status for severe mutations, which is important for genetic counseling. Genotyping is a useful complementary tool for confirming or excluding CAH diagnosis and predicting the clinical manifestations in patients who are diagnosed before the symptomatic phase.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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ORIGINAL ARTICLE

Neonatal 17-hydroxyprogesterone levels adjusted according to age at sample collection and birthweight improve the efficacy of congenital adrenal hyperplasia newborn screening

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Summary

Introduction The primary concern related to congenital adrenal hyperplasia (CAH) newborn screening (NBS) is the high rate of false-positive results (FPR) associated with prematurity; false-negative results (FNR) can also occur due to precocious sample collection.

Objective To determine the neonatal 17-hydroxyprogesterone (N17OHP) normal range in newborns in São Paulo using different references according to age and birthweight (BW) and to establish the optimal NBS cut-off levels.

Methods Neonatal 17-hydroxyprogesterone levels from 271 810 newborns (NBs) according to sample collection time (G1: 48–<72 h and G2: ≥72 h) and BW (≤1500 g, 1501–2000 g, 2001–2500 and >2500 g) were evaluated. N17OHP was measured by an fluoroimmunoassay, and serum 17OHP was measured by liquid chromatography-mass spectrometry. Affected and asymptomatic NBs with persistently increased 17OHP levels were submitted to CYP21A2-sequencing.

Results Neonatal 17-hydroxyprogesterone levels in G1 were lower than G2 in all BW groups ($P < 0.001$). The FPR rate in G1/G2 was 0.2% using the 99.8th and 0.5% using the 99.5th percentile. The 99.8th percentile N17OHP value was the best cut-off for distinguishing between unaffected and affected NBs. Forty-four salt wasters, and five simple virilisers were diagnosed; N17OHP levels ranged from 93.3 to 2209.8 nmol/l, and no affected neonates with FNR were identified. The positive predictive value in G1 and G2 using the 99.8th percentile was 5.6%

and 14.1%, respectively, and 2.3% and 7%, respectively, using the 99.5th percentile. Molecular tests identified two NBs with the nonclassical form among the 29 FPR.

Conclusion Neonatal 17-hydroxyprogesterone levels adjusted to sample collection age and birthweight reduced the FPR, and the use of N17OHP values based upon the 99.8th percentile improved the NBS efficacy.

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Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a common genetic disorder caused by CYP21A2 mutations. The impairment of 21-hydroxylase activity results in decreased cortisol and/or aldosterone synthesis and leads to a spectrum of clinical manifestations, ranging from a simple virilising (SV) type, with prenatal external genitalia virilisation in females, to the severely affected salt-wasting form, a life-threatening condition.^{1–6}

Congenital adrenal hyperplasia is an eligible disease for newborn screening (NBS) programmes, which aim to establish an early diagnosis in order to prevent significant morbidity and mortality.^{4,7–9} The pilot newborn screening for congenital adrenal hyperplasia (CAH-NBS) in Brazil revealed a high frequency of classical forms, approximately 1:10 000 live-births in São Paulo and Goiás states,¹⁰ justifying the introduction of CAH in our National Newborn Screening Program in November 2013.^{11,12}

Congenital adrenal hyperplasia-NBS shows high efficacy of detection in severe cases; however, the false-positive results (FPR) rate of 0.4–9.3% from these tests is a significant concern.^{3,13–15} Approximately 70% of the FPR are due to prematurity and/or low birthweight (BW); other causes include stressed conditions and methodological assay specificities.^{14,16–18} Therefore, protocols

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that consider the physiological variation in neonatal 17-hydroxyprogesterone (N17OHP) values have been suggested, adjusting the 17-OHP cut-off values for gestational age and/or BW.^{19–21} However, data concerning the optimal cut-off values of blood 17-OHP in CAH-NBS are conflicting. Additionally, it is well known that age at sample collection also influences neonatal 17-OHP levels,^{21,22} and a previous study indicated that adjusting 17-OHP cut-off values for sampling age might further reduce recall rates.²³ These aforementioned studies were published before the implementation of a new antibody in the fluoroimmunoassay, the most widely used methodology, which improved its specificity and likely altered the 17OHP cut-off levels.

In the light of this variability in N17OHP levels, standardization of the cut-off levels for fluoroimmunoassay could improve the CAH-NBS efficacy. After 18 months of CAH-NBS in São Paulo, and taking into account that sample collections are performed at different ages across the country, we evaluated whether N17OHP levels differed in samples collected at the third day of life from those collected thereafter. We also established different N17OHP cut-offs according to BW to determine which one results in the lowest FPR.

Patients and methods

The study was approved by the Ethical Committee of Faculdade de Medicina da Universidade de São Paulo and of APAE DE SÃO PAULO.

We performed a retrospective evaluation of data from 271 810 newborns from São Paulo state who underwent NBS routinely performed by the National Newborn Screening Program, in which CAH was introduced in November 2013. Heel prick capillary blood was collected from newborns and deposited on S&S 903 filter paper; dried samples were shipped daily to the APAE laboratory for N17OHP analysis. Newborns were divided into two groups: samples collected between 48 and <72 h of life (G1) and those after 72 h (G2). G1 and G2 groups were subdivided into four groups according to BW (BW1: ≤1500 g; BW2: 1501–2000 g; BW3: 2001–2500 g; BW4: >2500 g), as outlined in a previous study.¹⁷

All filter paper hormonal assays were performed using the same kit with the same antibody: the time-resolved, solid-phase fluoroimmunoassay 17OHP neonatal kit (B015-112 – Auto DELFIA – Neonatal 17OHprogesterone; Perkin Elmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). N17OHP concentrations were expressed as serum equivalence (conversion rate: 1 nmol/l = 0.33 ng/ml in whole blood and 0.73 ng/ml in serum equivalence).

Newborns with increased N17OHP levels according to the 99.5th percentile (current protocol of São Paulo state) were submitted to a new sample collection on filter paper; those with N17OHP levels higher than twice the 99.8th percentile were submitted to a serum analysis of 17OHP, androstenedione and cortisol by liquid chromatography-mass spectrometry (LC-MS/MS) and testosterone by fluoroimmunoassay. Sodium and potassium were also analysed. The intra-assay and interassay coefficients were <8% for the filter paper assay.

For this study protocol, after written informed consent was obtained from the parents, DNA samples from peripheral leucocytes were obtained from affected newborns and from those that were asymptomatic but presenting with persistently increased serum 17OHP levels. The *CYP21A2* gene was PCR-amplified with specific primers, and the products were submitted to direct automatic sequencing. For all patients whose DNA was analysed, the whole gene was sequenced including the promoter and the intronic regions, as previously described.²⁴ DNA samples from at least one parent were collected to perform mutation segregation analysis.

Statistical analysis

Numerical data are presented as the means, standard deviations, medians, interquartile intervals (IQ), ranges and percentages. The N17OHP levels were non-normally distributed (Kolmogorov–Smirnov test, $P < 0.05$) in all groups, and the Mann–Whitney test was used to compare data of samples collected between G1 and G2 of each BW group. Different N17OHP cut-off values were determined for each BW group, G1 and G2, using the 99.5th and 99.8th percentiles.

The number of cases that would be recalled as FPR using different cut-off levels for each BW group and age at sample collection was evaluated. Sensitivity (S) and specificity (E) were determined for each cut-off value, as well as positive and negative predictive values. Statistical significance was established at $P < 0.05$. The software SPSS Statistics (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp), version 22 was used.

Results

A total of 271 810 newborns were screened; 214 092 samples were collected between 48 < 72 h of life (G1) and 57 718 were collected after ≥72 h of life (G2). The number of newborns in each group is described in Table 1.

Median N17OHP levels in BW1, BW2, BW3 and BW4 in both G1 and G2 are shown in Table 1. The N17OHP levels were significantly lower in the G1 than in the G2 group in all BW groups ($P < 0.001$). Subsequently, N17OHP cut-offs were determined according to each BW group of G1 and G2 using both the 99.5th and 99.8th percentiles. The results are displayed in Table 1.

To determine the accuracy of these N17OHP cut-offs, data from affected newborns were also evaluated (Table 2). Twenty-five newborns (12 M, 13 F) presented with the salt wasting (SW) form in the G1 group, and their N17OHP levels ranged from 93.3 to 1572.6 nmol/l. Nineteen newborns (8 M, 11 F) presented with the SW form in the G2 group, and their N17OHP levels ranged from 294.9 to 2209.8 nmol/l. Five (two M, three F) newborns with the SV form were diagnosed in the G1 and G2 groups, and their N17OHP levels ranged from 109.8 to 159.3 nmol/l (Table 2, cases 8, 21, 27, 35 and 45).

During the study period in São Paulo state, no CAH diagnosis with negative screening was reported. Even one SW case whose

Table 1. Neonatal 17-hydroxyprogesterone (nmol/l) levels according to BW and sample collection age

Weight	G1 (<72 h)				G2 (≥72 h)				P*
	NB (n)	Median (IQ)	Range	Cut-offs 99.5th/99.8th	NB (n)	Median (IQ)	Range	Cut-offs 99.5th/99.8th	
BW1	466	38.7 (18.9–79.8)	(1.5–155.5)	330/360	3583	40.2 (23.1–75.3)	(0.7–200.2)	441/519	<0.001
BW2	1763	24.6 (15.9–40.2)	(0.8–122)	168/213	6502	26.7 (16.8–44.4)	(0.4–262.6)	207/270	<0.001
BW3	13 527	20.1 (14.4–28.5)	(0.8–125.3)	96/117	6813	20.7 (13.5–33.3)	(0.4–138.7)	144/198	<0.001
BW4	198 336	15.6 (11.7–21)	(0.5–169)	51/60	40820	11.7 (8.1–16.5)	(0.3–187.0)	60/75	<0.001

IQ, interquartile interval; BW, birthweight; BW1: ≤1500 g; BW2: 1501–2000 g; BW3: 2001–2500 g; BW4: >2500 g.

*Statistical significance

mother received 12 mg of betamethasone injection 24–48 h before delivery would be diagnosed using the new 99.8th cut-off level.

Among CAH females, only case 11 was promptly diagnosed at delivery due to the presence of atypical genitalia. Surprisingly, atypical genitalia were not recognized at delivery in two 46XX newborns. Patient 9 (Table 2) was assigned as male due to severe external genitalia virilisation (Prader V). Patient 13 presented with less severe external genitalia virilisation (Prader II), so she was correctly assigned at birth as female. The remaining CAH females were under diagnostic investigation for atypical genitalia by the time of the NBS results. In these females, the CAH diagnosis was established only through NBS-CAH, highlighting its importance to prevent salt-wasting crises even in females.

Among the salt wasters, two were hospitalized with severe hyponatraemic dehydration before the NBS result. Fourteen salt wasters (six males) immediately received gluco- and mineralocorticoid therapy due to a filter paper result associated with hyponatraemia, but admission at the emergency care unit was avoided. Mean sodium levels were 127.5 ± 10.6 mmol/l, and mean potassium levels were 4.8 ± 0.1 mmol/l. These findings were likely due to some basic health units sending the filter paper samples to the NBS laboratory only once a week. However, no deaths due to salt-wasting crises were reported.

Taking into account the N17OHP cut-offs according to the 99.5th percentile, 1143 (0.53%) newborns would present with FPR in the G1 group, and 290 (0.5%) newborns would present with FPR in the G2 group. Using cut-offs established by the 99.8th percentile, 470 (0.21%) and 121 (0.2%) newborns would present with FPR in the G1 and G2 groups, respectively (Table 3). Among cases with FPR, eight presented with extremely increased N17OHP levels; in 4/8, N17OHP levels ranged from 1495.2 to 2099.7 nmol/l. This was identified as a methodological sample collection error due to the use of EDTA blood tubes, which interfere with the immunofluorometric assay. In the remaining four cases, N17OHP levels ranged from 489.9 to 1152.6 nmol/l. These newborns were in the intensive care unit due to sepsis. N17OHP levels of cases with FPR according to the 99.8th percentile are reported in Fig. 1.

The sensitivity, specificity, positive and negative predictive values of N17OHP values according to the 99.5th and 99.8th percentiles are described in Table 4.

With regard to the confirmatory serum tests of the affected newborns, the mean 17OHP value in the SW form was 693.4 ± 512.5 nmol/l and ranged from 219.3 to 351.9 nmol/l in the five cases with the SV form. Sixteen of 44 SW newborns and four of five SV newborns from which DNA samples were collected had mutations in both alleles (maternal and paternal) in the *CYP21A2* gene. The SW newborns carried mutations predicting the total or almost total (I2 splice) impairment of enzymatic activity in both alleles; the SV newborns carried mutations predicting the severe impairment (I172N) of enzymatic activity in compound heterozygotes with the former mutations. A good correlation between genotype and phenotype was observed in all classical cases, as previously described.⁶ DNA sample collection of the remaining affected patients was not permitted by their parents, and the classical form diagnosis was confirmed through hormonal analysis in a private centre, where they are being followed by paediatric endocrinologists.

Among the cases with neonatal FPR, 29 newborns retained increased serum 17OHP levels, nine of whom normalized serum 17OHP levels during follow-up (<6 nmol/l). The remaining 20 cases were followed up at different medical centres but only in six cases did parents agree to collect DNA samples. In 2/6 newborns (one M, one F), complete *CYP21A2* sequencing revealed genotypes predicting the nonclassic (NC) form, namely mutations predicting moderate impairment of enzymatic activity (V281L mutation) in compound heterozygosity with those predicting almost total or near total impairment (Q318X, R356W and R426H mutations). In the remaining four cases, no *CYP21A2* mutation was identified in the exonic, intronic or promoter regions, and newborns were discharged from further follow-up.

Discussion

Despite the high miscegenation of our population, previous studies observed a high frequency of CAH in Brazil.^{17,25,26} The present study demonstrates the importance of NBS implementation in Brazil, as it was effective in identifying the salt-wasting form, increasing the SW/SV proportion as well as normalizing the male/female ratio in comparison with data from the unscreened Brazilian cohort.²⁷ The data from Sao Paulo CAH-NBS are in accordance with those in the literature.^{10,13,14}

Table 2. Phenotype and genotype of congenital adrenal hyperplasia patients

Pt.	Clinical Form	Sex	Weight (g)	Age NBS (h)	N17OHP (nmol/l)	Age serum (days)	Na (meq/l)	Serum 17OHP (nmol/l)	Genotype
1	SW	F	2470	48:00	126.6		—	>60*	
2	SW	F	3155	48:00	232.5		132	91.2**†	
3	SW	F	4430	48:00	826.8	3	133	169.5	LGR/LGR
4	SW	F	2880	48:00	1407.6		—	>60*	
5	SW	M	3990	48:00	1572.6	14	136	569.1	Q318X/I2 splice, V281L
6	SW	M	2910	48:03	664.5	81	126	74.1**†	I2 splice/Q318X, R356W
7	SW	M	3765	48:06	776.7	13	122	768.6	LGR/LGR
8	SV	M	2950	48:10	109.8	31	135	312	I172N/G424S
9	SW	F	3470	48:12	828.0	6	129	407.4*	I2 splice/IVS2–2A>G
10	SW	M	3335	48:13	286.2	19	131	80.1†	
11	SW	F	3620	48:18	83.1‡			91.5**†	
12	SW	M	3200	48:20	990.0			>60*	
13	SW	F	2950	48:20	93.3	13	131	636.0*	I172N/IVS2–2A>G
14	SW	M	2360	48:23	967.8		131	42.3†	
15	SW	M	3270	48:29	599.4	14	121	391.5	I2 splice/R356W
16	SW	M	3200	48:30	1182.9	45	124	1357.5	I2 splice/CYP21A2 Del
17	SW	M	2540	48:36	912	22	126	22.2†	CYP21A2 Del/CYP21A2 Del
18	SW	F	3855	49:00	541.2	16	115	744	
19	SW	F	3985	49:00	381	—	—	125.4**†	
20	SW	M	3205	49:40	413.1	—	—	>60*	
21	SV	M	3620	49:51	158.4	17	133	>60	I172N/InsT, Q318X, R356W
22	SW	M	3580	51:06	1232.7	—	—	>60*	
23	SW	F	3610	56:15	782.4	10	105	288.0	I2 splice/Q318X, Cluster, I236N
24	SW	M	3650	56:25	693.6	32	122	582.0	I2 splice/I2 splice
25	SW	F	4020	57:20	793.5	8	143	108†**	
26	SW	F	3740	61:21	359.7		126	>60*	
27	SV	F	3340	65:08	155.4			>60	
28	SW	F	3340	65:35	1191	23	140	859.2	
29	SW	M	3650	78:30	1440.9			573.0*	
30	SW	F	3540	79:39	493.2	7	139	76.5†	LGR/LGR
31	SW	M	2170	81:06	656.7	49	135	66.9†	I2 splice/Q318X
32	SW	F	3445	82:36	666	50	136	102.9†	LGC/I2 splice
33	SW	M	1825	84:12	1138.8			>60*	
34	SW	F	2655	85:00	1330.5			>60*	
35	SV	F	3835	85:11	159.3	10	136	219.3	I172N/I2 splice
36	SW	F	3095	90:46	1188.3			>60*	
37	SW	M	2690	92:05	294.9			>60*	
38	SW	F	3440	92:05	2209.8	23	115	1552.2	
39	SW	F	3150	92:16	1468.2	44	136	2004.3	R483W, Cluster, I236N/LGC
40	SW	F	3885	92:19	489.3			>60†	
41	SW	F	2090	101:10	1109.4		126	345	
42	SW	M	3465	98:54	1111.5	13	124	990.3	
43	SW	M	2430	103:27	345			>60*	
44	SW	M	3350	154:39	1152			>60*	
45	SV	F	2860	165:00	146.4	57	135	351.9	I2 splice/R426H
46	SW	F	2260	175:25	1161.3		129	53.4†	
47	SW	M	2455	191:45	1482		94	>60**†	
48	NC	F	3255	192:05	61.8			>60	V281L/Q318X, R356W
49	SW	F	2600	317:42	1312.2			38.6**†	
50	SW	F	3195	391:02	1356.3	12	132	198.0	Q318X/Q318X
51	NC	M	4094	696:00	285	222	140	45.3	V281L/R426H

SW, salt wasting; SV, simple virilising; NC, nonclassical; NBS, newborn screening; age NBS, age at filter paper sample collection; age Serum, age at confirmatory serum collection; LGR, large gene rearrangement; LGC, large gene conversion; Del, deletion.

*Radioimmunoassay.

†NB on glucocorticoid therapy.

‡Mother received betamethasone injection before delivery.

Table 3. Percentage of false-positive results according to age at sample collection and birthweight using the 99.5 and 99.8th percentiles for neonatal 17-hydroxyprogesterone levels

Birthweight	G1 (<72 h)			G2 (≥72 h)		
	Newborns (n)	99.5 (%)	99.8 (%)	Newborns (n)	99.5 (%)	99.8 (%)
BW1	466	0.64	0.21	3583	0.21	0.08
BW2	1763	0.51	0.22	6502	0.53	0.21
BW3	13 527	0.48	0.22	6813	0.51	0.19
BW4	198 336	0.53	0.21	40 820	0.51	0.22
Total	214 092	0.53	0.21	57 718	0.5	0.2

BW1: ≤1500 g; BW2: 1501–2000 g; BW3: 2001–2500 g; BW4: >2500 g.

Fig. 1 N17OHP (nmol/l) values of false-positive results according to birthweight and age at sample collection using the 99.8th percentile as the cut-off. *Indicates the cases in whom 17OHP value was outside the interquartile range (IQ 25–75).

Additionally, our preliminary data suggest that in Brazil both males and females should be screened, in contrast to the recommendation of screening only male newborns to improve cost-effectiveness.²⁸ In some series, and confirmed in our study, atypical genitalia is widely underdiagnosed.²⁹

The main concern of CAH-NBS is the high rate of FPR mainly related to prematurity and/or low BW.^{30–32} Similar to other reports, we observed that N17OHP values increased significantly with decreasing BW. Consequently, we established cut-offs for each BW and sample collection time using the values of the 99.5th percentile, as suggested by the current NBS protocol of São Paulo state.³³ However, the FPR rate related to the 99.8th percentile seems more appropriate for our programme, in accordance with other studies.^{17,23,34,35}

Using the 99.8th percentile as a cut-off adjusted to the two groups of sample collection times and to the four BW groups,

Table 4. Sensitivity (%), specificity (%), positive and negative predictive values (%) were defined for G1 and G2 groups, according to birthweight, using the 99.5 and 99.8th percentiles

	G1 (<72 h)				G2 (≥72 h)			
	S	E	PPV	NPV	S	E	PPV	NPV
99.5								
BW1	NA	99.3	NA	100	NA	99.7	NA	100
BW2	NA	99.4	NA	100	100	99.4	2.7	100
BW3	100	99.5	2.9	100	100	99.4	12.5	100
BW4	100	99.4	2.3	100	100	99.4	7.0	100
99.8								
BW1	NA	99.7	NA	100	NA	99.9	NA	100
BW2	NA	99.7	NA	100	100	99.7	6.6	100
BW3	100	99.7	6.2	100	100	99.8	27.7	100
BW4	100	99.7	5.6	100	100	99.7	14.1	100

BW1: ≤1500; BW2: 1501–2000; BW3: 2001–2500; BW4: >2500; S: sensitivity; E: specificity; PPV: predictive positive value; NPV: negative predictive value; NA: not applicable.

we observed that there was significant improvement in the positive predictive value and in the specificity of a positive test with the fluoroimmunoassay, in agreement with other studies.^{13,36} These cut-offs were efficient in identifying salt wasters, with a sensitivity of 100%. However, we could not determine these values for the extremely premature group as no affected newborn was diagnosed due to the low number of babies screened. Nevertheless, it remains controversial whether this group should be screened as the positive predictive value of a positive test is low, varying from 0.4% to 5.9%.¹³ Considering the extended duration that these babies spend in intensive care units, they could be diagnosed by clinical means.

The main problem of using a high cut-off such as the 99.8th percentile is that it increases the possibility of false-negative results (FNR). Screening for CAH is primarily designed to detect the severe life-threatening SW and SV forms; therefore, the expectation is that the less severe SV and NC forms may be missed. Most studies of NBS for CAH have described a very small FNR that is most frequently associated with the SV form.¹⁵ One study found the FNR to be associated with the SW form in Minnesota, USA, where samples were collected 24–48 h after birth.³⁷ As shown in our study and in others, early collections of dried blood display lower 17-OHP levels and could thus lead to a greater FNR. Although we cannot entirely rule out FNR in our pilot study, we consider this unlikely. During the study period in São Paulo state, no CAH diagnosis with negative screening was reported. A widely considered cause of false-negative screening results is perinatal treatment with corticosteroids;³⁸ however, the newly proposed NBS cut-off level was able to identify the one salt waster that received antenatal glucocorticoid therapy. We recognize that this study has some limitations and that the false-negative rate could be underestimated due to patients with CAH that remain undiagnosed, patients who moved out of the state, or infants who passed away with unidentified CAH.

It is known that sample collection before 24–48 h of life could impair the diagnostic precision of NBS.^{9,21} In our pilot NBS study, we found that some of the severely affected newborns presented with moderately increased N17OHP values and that all of them were submitted to a sample collection at 48 h of life. Cut-off values for 17-OHP have mostly been based on a fixed recommended sample collection time; however, in practice, age at sample collection varies. In Brazil, most samples in other states are collected between the 4th and 7th days of life, while in Sao Paulo state, they were collected on the third day of life.

We observed that samples collected on the third day of life had significantly lower N17OHP levels in all BW groups than those collected thereafter, highlighting the need to create specific N17OHP cut-offs for each age at sample collection. A previous study in Brazil determined N17OHP cut-offs, but we cannot compare our data as a different methodology was used to measure N17OHP levels and because neonatal samples were collected at approximately 7 days of life.³⁹ Using time-resolved immunofluorescence with the AutoDelfia Neonatal 17-OHP test kit, Olgemoller *et al.*⁴⁰ showed a 35% reduction in the total recall rate (from 1.13% to 0.74%) and an increase in the positive predictive value from 0.84% to 1.29% without reducing the diagnostic sensitivity, using 17OHP cut-off levels adjusted for both age and BW at the time of sampling.²³ Our findings, which were undertaken after improvements in the immunoassay, are similar and reinforce the suggestion that the efficiency of CAH screening can be improved using easily obtainable information on sampling age to adjust 17-OHP cut-off values. This methodology allows CAH-NBS to proceed with a rational follow-up workload, even when early blood sample collection is necessary.

All salt-wasting newborns had extremely increased N17OHP levels. The only five newborns diagnosed with the SV form had moderately increased N17OHP levels; however, all were diagnosed using the both the 99.5th percentile and 99.8th percentile cut-offs. As we diagnosed few SV newborns, we cannot rule out a sample size effect due to precocious NBS onset. It has been suggested that NBS may not identify all SV newborns; hence, some programmes perform a second sample collection at approximately 2 weeks of life.²² However, in developed countries, this group of patients is not the main objective of NBS as they are usually promptly diagnosed in infancy due to signs of postnatal virilisation.^{1,7}

In the present study, we did not evaluate the performance of confirmatory tests as they were performed by LC-MS/MS and/or radioimmunoassay and were used to confirm the neonatal diagnosis.^{41,42} Similarly, the molecular tests were performed to confirm the result of an altered neonatal test. Although CAH molecular analysis is a time-consuming and expensive methodology, it has been noted that it is useful for confirming the neonatal diagnosis, especially in asymptomatic newborns with persistently increased serum 17OHP levels. This prevents unnecessary follow-up in unaffected newborns,^{31,43} as occurred in four normal newborns and in the two NC patients with positive screening in our cohort.

In this study, we were able to identify some issues concerning our NBS programme. The main problem was the delay in

sending the samples for analysis from basic health units out of the city centre. This resulted in late diagnosis and explains why a significant number of newborns presented with hyponatraemia, some severe, at the first consultation. Although no deaths due to salt-wasting crises were reported, this emphasizes the need for a continuous education programme for the health professionals engaged in our NBS programme.

Congenital adrenal hyperplasia-NBS is an important advance in establishing an early diagnosis of CAH and preventing morbidity and mortality. However, the NBS programme still faces problems that mainly relate to the economic and psychosocial costs associated with FPR. Considering these factors, we conclude that adjusting N17OHP levels by sample collection time and BW allows a reduced FPR and improves NBS-CAH efficacy. To prevent late diagnosis and the development of salt-wasting crises, we recommend that the sample collection should be performed between 48 h and 72 h. Additionally, we suggest that adoption of N17OHP values based on the 99.8th percentile further improves NBS-CAH efficacy.

Disclosure

The authors have no conflict of interest to disclosure.

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Impact of Long-Term Dexamethasone Therapy on the Metabolic Profile of Patients With 21-Hydroxylase Deficiency

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Context: No consensus has been reached regarding the glucocorticoid (GC) to use for congenital adrenal hyperplasia (CAH) during adulthood. Dexamethasone (DEX), because of its longer half-life, could improve compliance; however, no data are available regarding the long-term effects of DEX therapy.

Objective: To analyze the metabolic effect of DEX therapy for adults with CAH.

Design: Retrospective analysis of a CAH cohort receiving DEX therapy.

Setting: Medical School Hospital, São Paulo University, Brazil.

Participants: Sixty patients with well-controlled classic CAH (41 women; 30 with salt-wasting) receiving DEX after achievement of final height.

Interventions: None.

Main Outcome Measures: Clinical, laboratory, and metabolic data were compared immediately before DEX and at the last evaluation.

Results: The mean age at the last evaluation was 31.9 ± 9.6 years, and the duration of DEX therapy was 11.5 ± 4.9 years. The mean DEX dose was 0.18 ± 0.07 mg/m²/d. The body mass index SD score (1.6 ± 1.6 vs 1.5 ± 1.5 mg/m²; $P = 0.65$) and obesity prevalence (27% vs 27%) did not differ between evaluations. However, the waist/height ratio (WtHR) had increased from 0.54 ± 0.08 to 0.56 ± 0.1 ($P = 0.001$). An increase in the homeostatic model assessment for insulin resistance index (2.5 ± 1.3 vs 2.8 ± 1.7 ; $P = 0.03$) was observed and positively correlated with the WtHR ($r = 0.54$). The prevalence of metabolic syndrome (7% vs 10%; $P = 0.7$) and hypertension (15% vs 13.3%; $P = 0.8$) did not differ significantly between the two evaluations.

Conclusions: Long-term and low-dose DEX therapy did not lead to increases in obesity or metabolic syndrome, although it was associated with an increased WtHR and greater homeostatic model assessment for insulin resistance observed with chronic use of GCs. DEX appears to be an acceptable option to treat adult CAH.

Abbreviations: 17OHP, 17 hydroxy-progesterone; BMI, body mass index; CAH, congenital adrenal hyperplasia; DEX, dexamethasone; GC, glucocorticoid; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; NC, nonclassic form; SDS, SD score; SV, simple virilizing; SW, salt-wasting; TG, triglycerides; VAT, visceral adipose tissue; WC, waist circumference; WtHR, waist/height ratio.

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Freeform/Key Words: congenital adrenal hyperplasia, waist circumference, dexamethasone, obesity, metabolic syndrome

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a common autosomal recessive disorder caused by mutations in the 21-hydroxylase gene (*CYP21A2*). These mutations result in a complex imbalance of adrenal steroids with decreased glucocorticoid (GC) secretion, accompanied by mineralocorticoid deficiency and androgen excess [1, 2]. Different mutations will cause variable degrees of residual enzymatic activity, leading to a spectrum of clinical manifestations. These manifestations have been classified into the classic form, including the salt-wasting (SW) and simple virilizing (SV) forms, and the nonclassic form (NC). The classic forms have an incidence of 1:10,000 to 1:20,000 live births [3, 4], and the NC has an estimated prevalence of 1:1,000 of the general population [5].

The aims of CAH therapy are to replace deficient hormones, prevent an adrenal crisis, and control androgen excess. A substantial interindividual variability in GC dose requirements make the achievement of these therapeutic goals a challenging task, and patients are frequently exposed to under- or overtreatment. No consensus has yet been reached regarding which type of GC and daily regimen should be used for adult patients with CAH [6–8].

Short-acting GCs such as hydrocortisone are indicated for the treatment of children with CAH because these will have less effect on the children's growth [9]. These can also be used in adults. However, they require at least two to three daily doses and, for a life-long chronic disorder such as CAH, adherence can be an issue. Long-acting GCs such as dexamethasone (DEX) are another option for treating adults according to the Endocrine Society guidelines [8]. However, some investigators have reported that its use has been related to a greater frequency of adverse metabolic outcomes [6, 7, 10]. Therefore, hydrocortisone has been suggested as a first-choice therapy by many investigators, because it will result in fewer metabolic side effects. However, no reported studies have compared both regimens.

Relatively few studies have been performed of adults with CAH receiving long-term treatment, and many of these have reported a greater prevalence of obesity, hypercholesterolemia, insulin resistance, osteopenia, and/or hypertension than in the normal population [6, 11–14]. However, these were usually cross-sectional studies and involved patients using distinct types and regimens of GCs, which impaired the correlation of these findings with a specific type of GC. At present, no longitudinal studies with a homogeneous cohort of patients with classic CAH using DEX to evaluate its long-term metabolic effects have been performed. The objective of the present study was to analyze the metabolic effects of DEX therapy in adult patients with well-controlled CAH followed up in a single tertiary center after the diagnosis.

1. Subjects and Methods

A. Subjects

We selected 60 adult patients with classic CAH (41 women). Of the 41 women, 21 had the SV form and 20 had the SW form. All the patients had basal 17 hydroxy-progesterone (17OHP) levels >50 ng/mL at diagnosis and molecular genetic test results predicting for classic form phenotypes [15]. The inclusion criteria were the exclusive use of a short-action GC during childhood and puberty (cortisone acetate, 18 to 20 mg/m²/d); introduction of DEX therapy in elixir (0.1 mg/mL) or tablet (0.5 and 0.75 mg) formulations only after the achievement of final height; good compliance with treatment during adulthood; and adequate hormonal control [normal serum electrolytes, normal androgen levels according to sex and age, and no suppression of serum 17OHP levels (<5 ng/mL) in the previous 5 years]. The SW patients also

received fludrocortisone ($55.7 \pm 10.6 \mu\text{g/d}$). Mineralocorticoid replacement was monitored by the blood pressure and plasma renin activity. We excluded patients who had developed SW crises during the study period and those receiving cytochrome P450 inducers.

The local ethics committee approved the present study, and all individuals gave their written informed consent.

B. Methods

We compared the clinical and laboratory data immediately before the beginning of DEX therapy and at the last evaluation. The clinical data included the body mass index (BMI), waist/height ratio (WtHR), and blood pressure. The BMI data of all the patients were converted to the SD score (SDS) according to the World Health Organization growth reference graphs [16], because 28 of the 60 patients had initiated DEX therapy before they were 19 years old. The clinical data were compared with data extracted from a paired population matched by age and sex available from a Brazilian national health research study conducted in 2013 [17].

The laboratory data included measurements of glucose, insulin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) and calculation of the homeostatic model assessment for insulin resistance (HOMA-IR) index. The serum 17OHP and androstenedione levels were measured at baseline using a radioimmunoassay and at the end of the study using liquid chromatography-mass spectrometry. The intra-assay and interassay coefficients of variation ranged from 5% to 10%.

The presence of metabolic syndrome (MetS) in adults was defined in accordance with the National Cholesterol Education Program Adult Treatment Panel III as the presence of three or more of the following abnormalities: waist circumference (WC) ≥ 102 cm in men and ≥ 88 cm in women, blood pressure $\geq 130/85$ mm Hg, fasting glucose > 110 mg/dL, fasting serum TG ≥ 150 mg/dL, and low HDL-C levels (men, < 40 mg/dL; women, < 50 mg/dL) [18]. For patients aged < 19 years, the modified Adult Treatment Panel III criteria [19] were used, defined as the presence of three or more of the following abnormalities: WC ≥ 90 th percentile for age and sex, blood pressure ≥ 90 th percentile for age and sex, fasting glucose ≥ 100 mg/dL, fasting serum TG ≥ 90 th percentile for age and sex, and HDL-C levels < 40 mg/dL.

C. Statistical Analysis

Data are presented as the mean $\pm$ SD, unless otherwise stated. Analyses of the metabolic profile before and after DEX therapy were undertaken using the paired Student *t* test or Wilcoxon signed-rank test for numerical continuous variables, as appropriate. The categorical variables were compared between groups using the χ^2 test or Fisher exact test, as appropriate. Linear and multiple regression analysis were performed to evaluate the correlation between the metabolic data and DEX doses and/or the duration of treatment. Statistical analysis was performed using Minitab, version 17, software, and a *P* value < 0.05 was considered to indicate statistical significance.

2. Results

The mean patient age at the transition from the short-acting GC to DEX therapy was 20.5 ± 9.8 years. The mean patient age at the last evaluation was 31.9 ± 9.6 years. The mean duration of GC therapy since the diagnosis, including the short-acting GC, was 27.1 ± 7.7 years, and the mean duration of DEX therapy was 11.5 ± 4.9 years. DEX was administered once daily at bedtime, and the mean daily dose in the previous 5 years was $0.18 \pm 0.07 \text{ mg/m}^2/\text{d}$. The patients with SW also received fludrocortisone ($55.7 \pm 10.6 \mu\text{g/d}$). The differences in the baseline and last hormonal 17OHP levels were not statistically significant (Table 1). However, the levels of androstenedione were lower at the last evaluation, which could suggest better compliance.

Table 1. Metabolic and Hormonal Parameters at Baseline and Last Evaluation of Dexamethasone Therapy

Data	Baseline	Last Evaluation	P Value
BMI-SDS	1.6 ± 1.6	1.5 ± 1.5	NS
Obesity, %	27	27	NS
WC, cm			
Women	78.6 ± 10.3	80.8 ± 11.5	NS
Men	92.6 ± 15.3	97.4 ± 17.9	NS
WtHR	0.54 ± 0.08	0.56 ± 0.1	<0.01
Hypertension, %	15	13.3	NS
Glucose, mg/dL	83.6 ± 7.7	82.6 ± 10.8	NS
HOMA-IR	2.5 ± 1.3	2.8 ± 1.7	0.03
TG, mg/dL	84.2 ± 40.5	94.2 ± 51.1	0.051
HDL-C, mg/dL			
Women	59.8 ± 13.1	58 ± 10.9	NS
Men	51.9 ± 12.8	49.9 ± 20	NS
Low HDL-C, %	22	25	NS
LDL-C, mg/dL	108.3 ± 28	109.9 ± 31.6	NS
MetS, %	7	10	NS
17-OHP, ng/mL	53.2 (31.8–79)	45.3 (34.9–59.1)	NS
Androstenedione, ng/mL	5.9 ± 6.3	2.8 ± 1.2	0.001

Data presented as percentage, mean ± SD, or median (interquartile range), as appropriate.

Abbreviations: LDL-C, low-density lipoprotein cholesterol; NS, not significant.

When we compared the metabolic variables immediately before DEX therapy and at the last evaluation (Table 1), we observed a small but statistically significant increase in the WtHR, from 0.54 ± 0.08 to 0.56 ± 0.1 ($P = 0.001$). The individual values and increase in the WtHR during DEX therapy are shown in Fig. 1. Furthermore, a statistically significant increase occurred in the HOMA-IR index (from 2.5 ± 1.3 to 2.8 ± 1.7 ; $P = 0.03$), which correlated positively with the WtHR ($r = 0.54$). The difference in the BMI-SDS was not statistically significant during the DEX period (1.6 ± 1.6 kg/m² at the transition to DEX and 1.5 ± 1.5 kg/m² at the last clinical visit; $P = 0.65$). Moreover, the prevalence of obesity according to the BMI was 27% in both evaluations, and 33.3% of patients were overweight immediately before the introduction of DEX and 35% at the last visit ($P = 0.842$). The mean DEX dose did not differ between those of normal weight and those who were overweight or obese (0.17 ± 0.07 vs 0.18 ± 0.08 mg/m²/d; $P = 0.7$).

The comparison of the WtHR between the CAH cohort and the reference Brazilian population paired by age [17] showed a similar prevalence of an increased WC at the beginning of the DEX therapy in the first group (18.3% vs 14.3%, respectively) and at the last evaluation (26.7% vs 29.6%, respectively; Fig. 2).

No other metabolic parameter was significantly different during DEX therapy, including HDL-C according to sex, low-density lipoprotein cholesterol, fasting glucose, TG, or blood pressure. MetS was observed in four patients (7%) at the transition to DEX and in six (10%) at the last evaluation ($P = 0.743$). The results of the linear and multiple regression analysis showed no correlation between the metabolic variables and DEX therapy (duration and doses), sex, or clinical form of CAH.

3. Discussion

CAH is the most common genetic endocrine disorder and, with the availability of treatment with GCs, most of these patients have been reaching adulthood. However, no standard regimen has been determined for treating adults [6, 13].

DEX offers the advantage of a once-daily dose, which could improve treatment compliance. However, its use has been feared because it has been suggested that its use could be related to a greater risk of the development of an adverse metabolic profile [7, 20]. In addition to the

Figure 1. (A) Individual values of WHtR in our cohort during 20 y of DEX therapy. (B) Boxplots showing the evolution of WtHR for every 5 y of DEX treatment.

lack of longitudinal studies, one drawback in the available data on metabolic disturbances in these patients has been the scarcity of homogeneous cohorts to evaluate the specific effects of the disease and each GC regimen. To focus on the metabolic effects of long-term DEX therapy,

Figure 2. Prevalence of increased WC in our CAH cohort and the Brazilian reference population.

we selected only patients with classic CAH forms and adequate compliance and who had used exclusively DEX since the attainment of their adult height.

According to the World Health Organization, the prevalence of obesity in the Brazilian population was 17% in men and 24% in women and the prevalence of overweight was 38% in men and 29% in women. In our group, 27% of patients were obese and 33.3% were overweight immediately before DEX introduction. These rates did not increase during the use of DEX and were similar to, or even lower than, that in other CAH cohorts receiving diverse types of GC. For instance, in a cross-sectional study conducted in the United Kingdom [CaHASE (United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive)] [6], which evaluated 165 adult patients with CAH (103 women), 41% had a BMI in the obese range and 37% in the overweight range. Moreover, in a French cross-sectional study [21], which had included 70 adult patients with CAH (45 women; 53 with SW), 44% had a BMI >25 kg/m². Finally, in an American cross-sectional study [13], which had include 44 adults with CAH (25 women; 26 with SW), one third of the patients were obese.

Although the BMI did not change with DEX, we observed a small, but substantial, increase in the WtHR, compatible with an undesirable body fat distribution. The WtHR is an effective anthropometric indicator and correlates better than the WC alone with cardiovascular health [22]. The WHtR cutoff value of 0.5 was proposed after an analysis of data from 14 different countries and was a stronger predictor of the development of diabetes and cardiovascular disease than the BMI [23]. Furthermore, the final WC in our female patients was lower than that from the CaHASE cohort (80.8 cm and 99.5 cm, respectively), in which 26% of the patients had received hydrocortisone, 43% prednisolone, 19% DEX, and 10% a combination of GCs [6]. Only one study evaluated visceral adipose tissue (VAT) [24]. They evaluated 28 subjects with CAH (aged 15.6 ± 3.2 years), including 8 treated with DEX, and increased VAT was found compared with paired healthy controls [24].

A substantial increase in the HOMA-IR index was also observed during the use of DEX, with 38% of patients having a HOMA-IR >2.5 (compatible with insulin resistance) at our last evaluation. Moreover, in our sample, the HOMA-IR presented a moderate positive correlation with WtHR ($r = 0.54$), consistent with the correlation seen between WtHR and VAT in the American cohort ($r = 0.84$) [24].

The prevalence of MetS and hypertension remained unchanged during DEX therapy in our series. These results have varied among studies. For example, in a Norwegian study of 64

adult patients with CAH [25], a higher diastolic blood pressure was observed compared with that in the normal population. In the CaHASE study [6], an increase in diastolic blood pressure in female patients with CAH was also observed. Regarding the prevalence of MetS, in the American cohort [13], it was identified in 18% of adults.

It is possible that we saw few substantial metabolic effects with DEX treatment in our patients because they were still young (mean age, 31.9 ± 9.6 years). This cohort of patients was organized within the previous 20 years and did not have enough older patients from which to draw further conclusions about elderly patients with CAH. Continued follow-up of the cohort will hopefully result in more information as the population ages. However, they have received DEX treatment for a considerable time (11.5 ± 4.9 years). In addition, our results are similar to those from other young CAH cohorts receiving mixed GC regimens. We could not rule out that the undesirable effects observed for the WHtR could be related to aging and long-term GC exposure and not exclusively related to the use of DEX.

One limitation of the present study was the absence of a control group using other GC regimens. This was because our study was a real-life setting study in a tertiary center in which we had opted to improve compliance by the use of a once-daily dose of DEX, which is more accessible in Brazil. The lack of an age-matched control group was partially resolved through a comparison with data from a large Brazilian population and with data from patients with CAH described in other studies. Second, some concerns exist regarding the long-term DEX effects on bone health. However, a recent study reported the 10-year follow-up data for patients with CAH who had been receiving mixed GC regimens (89% of the patients receiving DEX therapy) [26]. They found no correlation between bone mineral density and the cumulative dose of GC. Therefore, the investigators suggested that the risk of osteoporosis in this cohort could be similar to that of the normal population [26]. Finally, the use of DEX should not be used during pregnancy, because it crosses the placenta and reaches the fetal circulation.

4. Conclusions

These preliminary data have indicated that DEX therapy appears to be an acceptable option to treat adult patients with CAH, because it was not associated with an increase in the prevalence of obesity, MetS, or hypertension. Furthermore, DEX could be an interesting option to improve patient compliance. Prospective studies are still necessary to compare the long-term effects of each GC formulation.

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Data Availability: The datasets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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Plasma renin measurements are unrelated to mineralocorticoid replacement dose in patients with primary adrenal insufficiency.

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Abstract

Context: No consensus exists for optimization of mineralocorticoid therapy in patients with primary adrenal insufficiency.

Objective: To explore the relationship between mineralocorticoid replacement dose, plasma renin concentration (PRC) and clinically important variables to determine which are most helpful in guiding mineralocorticoid dose titration in primary adrenal insufficiency.

Design: Observational, retrospective, longitudinal analysis.

Patients: 280 patients (with 984 clinical visits and plasma renin measurements) with primary adrenal insufficiency recruited from local databases and the international congenital adrenal hyperplasia (CAH) registry (www.i-cah.org). Thirty-seven patients were excluded from the final analysis due to incomplete assessment. Data from 204 patients with salt-wasting CAH (SW-CAH) (149 adults and 55 children) and 39 adult patients with Addison's disease (AD) were analysed.

Main outcome measures: PRC, electrolytes, blood pressure (BP) and anthropometric parameters were used to predict their utility in optimizing MC replacement dose.

Results: PRC was low, normal or high in 19%, 36% and 44% of patients, respectively, with wide variability in mineralocorticoid dose and PRC. Univariate analysis demonstrated a direct positive relationship between mineralocorticoid dose and PRC in adults and children. There was no relationship between mineralocorticoid dose and BP in adults, while BP increased with increasing mineralocorticoid dose in children. Using multiple regression modelling, sodium was the only measurement that predicted PRC in adults. Longitudinally, the change in mineralocorticoid dose was able to predict potassium, but not BP or PRC.

Conclusions: The relationship between mineralocorticoid dose and PRC is complex and this may reflect variability in sampling with respect to posture, timing of last mineralocorticoid dose, adherence and concomitant medications. Our data suggests that mineralocorticoid titration should not primarily be based only on PRC normalization, but also on clinical parameters as BP and electrolyte concentration.

Précis: Plasma renin concentration is not related to mineralocorticoid dose. Serum electrolytes are associated with MC dose and should be considered to optimize MC dose in primary adrenal insufficiency.

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Introduction

The renin-angiotensin system plays a crucial role in the regulation of fluid volume status and electrolyte balance. Renin is released from the juxtaglomerular cells in the kidney in the presence of renal hypoperfusion and cleaves angiotensinogen to produce inactive angiotensin I. Angiotensin I is then converted to active angiotensin II by endothelial angiotensin converting enzyme. Angiotensin II causes vasoconstriction of arteriolar vessels through inhibition of nitroxide synthetase and sodium retention, acting both in the proximal tubule (1,2) and through stimulation of adrenal aldosterone release (3). Aldosterone, synthesized and released from the adrenal zona glomerulosa, acts through the nuclear mineralocorticoid (MC) receptor and enhances epithelial sodium channel activation, causing sodium and water retention with renal potassium loss and is a crucial mechanism for maintaining blood pressure (BP) and electrolyte balance.

Primary adrenal insufficiency (PAI) is a life-threatening disease resulting from diseases directly involving the adrenal cortex. The clinical spectrum is characterised from deficient production or action of glucocorticoids (GCs), with or without concomitant deficiency of MC and adrenal androgens. In the majority of cases, PAI is caused by autoimmune adrenalitis (Addison's disease, AD)(4,5), and the commonest symptoms include weakness, fatigue, anorexia, abdominal pain, weight loss, orthostatic hypotension, and salt craving. Congenital Adrenal Hyperplasia (CAH) is a different form of PAI caused by a group of rare autosomal recessive diseases resulting to mutations in genes encoding enzymes in pathways critical for adrenal steroid biosynthesis (6). The commonest form is caused by mutations in the *CYP21A2* gene, accounting for approximately 95% of cases of CAH (7). Defective 21-hydroxylation can lead to decreased GC and MC synthesis. Specifically, salt-wasting CAH (SW-CAH) is characterized by both GC and MC deficiency. In SW-CAH and PAI, both GC and MC treatment are essential to avoid life-threatening adrenal crises (8). However, much attention has focused mainly on optimization of GC replacement in AI (9-11): so far only a few, small studies have investigated MC replacement in patients with primary AI (12). Tailored and accurately titrated MC replacement therapy may be of crucial importance in patients with MC deficiency to improve long-term outcomes. MC replacement, usually in the form of fludrocortisone, is often administered with the aim of achieving plasma renin concentration (PRC)

within the upper limit of the local reference range (5,6). The most recent CAH Endocrine Society guidelines suggests a fludrocortisone replacement dose of 50-200 µg/day (13). MC requirements in infants and children decrease with age, reflecting changes in the capacity of the renal tubules to reabsorb sodium over time. In adults, current guidance advocates titrating MC doses (and/or salt supplementation) according to BP, serum sodium, potassium, and PRC appropriate for age.

Taking into account the complex regulation of PRC, for example with posture, as well as the variability of timing of blood sampling with respect to the last fludrocortisone dose, we aimed to explore the relationship between MC dose regimens and clinical and biochemical variables in *real-life* clinical practice to determine whether they can usefully guide appropriate MC dose titration.

Patients and Methods

Patient selection

We performed a retrospective observational analysis of data from the International CAH Registry (www.i-cah.org) collected from 1982 to 2018, alongside that from local adrenal patient databases.

The I-CAH Registry contains pseudoanonymized information on patients with CAH and for this study we included patients from 14 centers in 7 countries (United Kingdom, Brazil, Italy, Turkey, Israel, Bulgaria and Germany). Salt-wasting CAH was diagnosed on clinical grounds and / or on genetic testing. Patients were included if they had a diagnosis of CAH and were taking MC replacement. Records without MC replacement dose, or patients under salt replacement were excluded from the analysis.

A total of 984 visit records of 280 patients with PAI were recorded. 249 visits from 37 patients were excluded from the analysis due to incomplete medical records. The remaining 735 assessments of 243 patients were selected for the final analysis: 204 patients had SW-CAH (149 adults, 55 children) and 39 had AD (Figure 1). The analyses were performed separately for adults (age ≥ 16 years) and children, a subsequent analysis was stratified by underlying disease etiology. A longitudinal analysis was performed in 112 patients (90 with SW-CAH and 22 with AD) (Figure 1). Patient demographics are presented in Table 1.

Seven variables were considered in the final multivariate models: serum sodium (Na^+), serum potassium (K^+), mean arterial blood pressure (MAP), PRC, MC replacement dose, age and body mass index (BMI). Sitting mean arterial pressure (MAP) was calculated using the formula: diastolic blood pressure (DBP) + $1/3$ of differential blood pressure (SBP-DBP). 17-OH Progesterone (17-OHP) and androstenedione of patients with SW-CAH were also considered in the baseline and univariate analysis, as indirect parameters of adherence to steroid replacement. For longitudinal analyses, data are expressed as median unless otherwise stated and change (Δ) for any variable was calculated by the difference: follow-up minus baseline. For the analysis in children, SDS (standard deviations scores) for BMI (sBMI), centiles for systolic blood pressure (cSBP) and diastolic blood pressure (cDBP) were calculated and the MC dose corrected by body surface area (MC_{BSA}). Data and samples were collected as part of 'real-life' clinic consultations. Standard

laboratory biochemical analyses were undertaken to measure electrolytes. No data was recorded with regards to the timing of the last fludrocortisone dose or adherence, and no centre adopted a standardised posture protocol prior to sampling for PRC.

Plasma renin concentration and renin assays

Different renin assays and units of measurement were used across the multiple centres that enrolled patients into the study ($\mu\text{IU/mL}$, ng/mL/h , nmol/L/h , pg/mL , ng/L). Every centre provided local reference range, which we used to categorise results as 'low', 'normal' or 'high'. Subsequently, all results were standardized according to the most frequently used reporting units ($\mu\text{IU/mL}$), using the following procedure: a) ng/L ($n=57$), ng/mL/h ($n=70$) and pg/mL ($n=3$) values were converted using a factor of $\times 1.67$, $\times 12$ and $\times 5.26$ respectively as recommended within the Endocrine Society guidance (14); b) plasma renin activity values expressed as nmol/L/h ($n=33$) were converted using a factor derived from a polyfit 3rd grade equation generated with MatLab (version 2017, MathWorks® Inc.) using the reference range of the different assay as intersection points.

Statistical Analysis

A Spearman's rank-order correlation was run to assess the relationship between individual variables. A $p < 0.05$ was considered indicative of a statistically significant difference. A Kruskal-Wallis test was conducted to determine if there were differences between groups that differed in their level of renin at baseline (the "low", "normal" and "high" PRC groups according to local reference range) or ΔMC dose for longitudinal analysis ("unchanged", "decreased", and "increased" dose). Distributions were similar for all groups, as assessed by visual inspection of a boxplot. When statistical significance was found, pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. In the longitudinal analysis, a sign test with continuity correction was also conducted to determine the difference (within each group) between follow-up and baseline.

A first multiple regression model was run to assess the utility of clinical and biochemical variables to predict PRC. Six variables were initially inserted into the model: total MC daily replacement

dose, Na^+ , K^+ , MAP, age and BMI. All significant variables in the model were then tested as dependent variables in the subsequent multiple regression analyses. In order to have a linear relationship between all variables inserted into the models, a Log_{10} of PRC was computed and used for the multiple regression analysis.

In all the models generated, there was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values. There was independence of residuals, as assessed by a Durbin-Watson value of approximately 2; there was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values; there was no evidence of multicollinearity, no studentized deleted residuals greater than ± 3 standard deviations, no leverage values greater than 0.2, and values for Cook's distance above 1; the assumption of normality was met, as assessed by a Q-Q Plot; finally no outliers were found.

Statistical analyses were performed using SPSS (version 24, Chicago, IL, USA) and GraphPad Prism 7.0 software package (GraphPad Software, Inc. La Jolla, CA, USA).

Results

Patient characteristics including clinical and biochemical variables are presented in Table 1. A total of 243 patients with PAI currently taking MC replacement were included in the study. The analyses were performed in adult patients (n=188) and children with SW-CAH (n=55). Separate subgroup analyses were performed in adults with SW-CAH (n=149) and AD (n=39). No children affected by AD were included in the analysis.

The distributions of MC doses in adults (stratified by underlying disease) and children are presented in Figure 2. There was large variability in PRC, ranging from 0.6 to 3166 $\mu\text{UI/mL}$ in adults (median 86 $\mu\text{UI/mL}$) and 0.1 to 5090 $\mu\text{UI/mL}$ in children (median 66 $\mu\text{UI/mL}$). When stratified according to local reference ranges, 8%, 31% and 61% of adults and 31%, 43% and 26% of children had low, normal and high PRC values, respectively.

Baseline correlations and univariate analysis - Adults

Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. Univariate analysis demonstrated positive correlations between MC daily dose and BMI ($r=0.233$, $p<0.001$), age ($r=0.116$, $p=0.023$), and PRC ($r=0.135$, $p=0.051$), while there was no relationship with Na^+ , K^+ or MAP (Figure 3a-d). When adjusted to the local reference ranges, those patients with high PRC had lower serum Na^+ concentrations and higher concentrations of K^+ in comparison with those patients with low PRC. There was no relationship with the total MC replacement dose (Figure 3e-h).

Baseline correlations and univariate analysis – Children

Analysis of data from children showed a correlation of MC_{BSA} daily dose with sBMI ($r=-0.166$, $p=0.023$), age ($r=-0.761$, $p<0.01$), cSBP ($r=0.364$, $p<0.001$), cDBP ($r=0.281$, $p=0.005$), PRC ($r=0.228$, $p=0.002$), K^+ ($r=0.308$, $p<0.001$), and Na^+ ($r=-0.130$, $p=0.035$) (Figure 4a-e). When adjusted to the local reference ranges, as with the adults, those children with high PRC had lower serum Na^+ and higher K^+ concentrations in comparison to those with low PRC. Patients with low and high PRC were younger and took higher MC_{BSA} dose compared to those with normal PRC (Figure 4f-j).

Plasma renin concentration, 17-OH progesterone and androstenedione

In order to determine if elevated PRC might be a reflection of under-treatment or non-adherence across both mineralocorticoid and glucocorticoid replacement, we examined the 17-OHP and androstenedione levels in SW-CAH patients with low, normal or high PRC. In adult patients with SW-CAH, 17OHP levels were similar in those patients with low, normal or high PRC (low PRC: 29.2 nmol/L (1.6-117.9); normal PRC: 38.2 nmol/L (1.2-1000); high PRC: 46.3 nmol/L (0.8-862.4), median (min-max), $p=0.39$). Data were similar for androstenedione levels (low PRC: 3.35 nmol/L (0.7-18.5); normal PRC: 4.9 nmol/L (0.9-49.2); high PRC: 7.5 nmol/L (0.4-86.2), $p=0.06$). However, in children with SW-CAH, both 17-OHP and androstenedione were lowest in those individuals with low PRC (17OHP: low PRC: 0.7 nmol/L (0.3-423.65); normal PRC: 48.41 nmol/L (6.35-1716); high PRC: 131 nmol/L (1.21-1424.1), $p<0.01$. Androstenedione: low PRC: 0.98 nmol/L (0.28-38.4); normal PRC: 5.58 nmol/L (0.35-34.91); high PRC: 4.75 nmol/L (1.01-45.39), $p<0.01$).

Multiple regression models

All mineralocorticoid deficient patients - Adults

When considered individually as dependent variables, our 6-variable multiple regression model was able to predict PRC ($p<0.001$) and MC total daily dose ($p=0.017$). Na^+ was the only variable weakly related to PRC ($B=-0.091$, $p<0.001$). MC total daily dose was directly related to BMI ($B=2.812$, $p=0.001$), but not MAP ($B=0.566$, $p=0.34$) or PRC ($B=5.846$, $p=0.51$). All the computed and relative coefficients generated by the models are summarized in Table 2.

Salt-wasting congenital adrenal hyperplasia - Adults

When data from adults with SW-CAH were analysed separately using the same strategy, results were comparable with the complete adult cohort analysis. The data are summarized in Table 3. The multiple regression model significantly predicted PRC ($p=0.008$) MC total daily dose ($p<0.001$). Na^+ was the only variable that was weakly associated (negatively) with PRC ($B=-0.097$, $p<0.001$). Moreover, as physiologically expected, K^+ was strongly and inversely related to MC daily dose ($B=-41.180$, $p=0.007$) (Table 3).

Salt-wasting congenital adrenal hyperplasia - Children

The subgroup analysis on CAH children showed a similar pattern; Na^+ ($B=-0.142$, $p=0.005$) and K^+ ($B=-0.697$, $p=0.004$) were related to PRC; MC_{BSA} total daily dose, as expected, was inversely related to age ($B=-7.397$, $p<0.001$), but not cSBP or cDBP ($B=0.810$, $p=0.2$ and $B=-0.405$, $p=0.3$) or PRC ($B=6.697$, $p=0.5$) (Table 4).

Addison's disease - Adults

In the subgroup analysis on patients with AD, the multiple regression model significantly predicted PRC ($p=0.050$) and Na^+ ($p=0.004$). Once again, serum Na^+ was able to predict PRC ($B=-0.115$, $p=0.002$). The model was not significant for prediction of MC total daily dose and serum K^+ (data not shown).

Longitudinal follow-up in adults with SW-CAH

Longitudinal analysis was performed in 112 adult patients (90 patients with SW-CAH and 22 patients with AD; median time between the assessments 433 days, range 33-2082). At follow-up, MC dose remained unchanged in 80 (67%) patients (*group A*) whilst in 9 (6%) patients (*group B*) MC dose was decreased (ΔMC dose $-100\mu\text{g}/\text{day}$, range -50 to -200) and in 23 (19%) patients (*group C*) MC dose was increased (ΔMC dose $50\mu\text{g}/\text{day}$, range 25 to 100). Within each group, there was no significant change in ΔPRC ($\Delta\text{PRC}_{\text{group A}}$ $5\mu\text{UI}/\text{mL}$, $z=0.783$, $p=0.434$; $\Delta\text{PRC}_{\text{group B}}$ $0.1\mu\text{UI}/\text{mL}$, $p=1.000$; $\Delta\text{PRC}_{\text{group C}}$ $-61\mu\text{UI}/\text{mL}$, $p=0.405$) (Figure 5). In addition, ΔPRC compared across groups were not different ($p=0.560$).

Multiple regression modelling significantly predicted ΔPRC ($p=0.015$). Only Na^+ concentration at final follow-up visit was strongly associated with ΔPRC ($B=59.465$, $p<0.001$). There was no relationship between ΔPRC and final MAP, K^+ or MC replacement dose. Finally, as expected, ΔMC dose was inversely related to ΔK^+ ($B=-3.104$, $p=0.002$). No correlations were found between ΔMC dose ΔPRC , ΔNa^+ or ΔMAP (data not show).

Discussion

In patients with adrenal insufficiency, there is an absolute requirement for lifelong steroid hormone replacement therapy. Almost 70 years have now passed since the introduction of MC replacement therapy for patients with PAI (15). MC treatment strategies have only been examined in a small number of studies and, to date, there are limited data regarding dose optimization. Current standard replacement usually consists of fludrocortisone 50 to 200 µg (13), given once daily in the morning reflecting the circadian rhythm of aldosterone, which is similar to that of cortisol (16). Guidance suggests that MC replacement dose should be tailored clinically by measuring BP, evaluating salt cravings and presence of peripheral edema (17). However, these are not always reliable and markers that are more objective are often used in addition. Compelling data to support the use of serum Na⁺, K⁺ and PRC levels is lacking (12).

Oelkers and colleagues found that, when targeted to the upper limit of the reference range, plasma renin activity (PRA) correlated more closely with MC dose than with Na⁺ and K⁺ levels alone (18). Conversely, *Thompson et al.* showed that PRA was unable to distinguish between adequate and over-replacement and therefore raised doubts about its utility in MC dose optimization (19). Current expert consensus suggests that MC replacement should aim at normotension, normokalemia, and try to achieve PRC in the upper normal reference range (13,18,20,21).

In patients with AI, much attention has focussed on optimization of GC replacement, but it can be hard to differentiate clinically between GC and MC under-replacement. It is important to avoid overtreatment with GCs, which is associated with significant adverse effects (22-24). Bearing in mind the MC activity of commonly used GCs (hydrocortisone, prednisolone), it is possible that increased doses of GCs are actually treating relative MC deficiency, therefore, highlighting the possibility that many patients with PAI may actually be under-replaced with MC. This may well be an important contributing factor to the lack of relationship between MC dose and PRC that we observed in our data.

In our cohort, PRC was weakly related to Na⁺, but had no relationship to other clinical variables (including BP) or, importantly, MC daily replacement dose. Furthermore, our longitudinal analysis suggests that MC dose changes are not associated with subsequently measured PRC. In contrast,

serum electrolytes (notably K^+) are most closely and strongly related to MC dose both at baseline and in the longitudinal analysis. Our observations may well reflect underlying physiology in that those patients with the highest PRC had lower Na^+ levels, (Na^+ was also the only variable associated with future change in PRC in the longitudinal analysis), perhaps suggesting relative MC under-replacement and consequent Na^+ loss, although it should be noted that we did not control for GC dose and therefore cannot assess the relative contribution of GCs to Na^+ balance. In parallel, the association between MC daily dose and K^+ suggested that higher MC doses were associated with lower serum K^+ concentrations, as expected.

The utility of PRC and aldosterone measurements in the diagnoses of MC deficiency is not in doubt. However, significant challenges arise when they are used for MC dose adjustment. There are challenges that relate to difficulties in sample collection and handling, with no internationally accepted standard reference range and interpretation of results that is dependent upon local reference intervals (17). In addition, there are many other factors that have a profound influence on PRC including volume status, salt intake, pregnancy, posture, ambient temperature, antihypertensive and non-steroidal anti-inflammatory drugs (25). Salt replacement is also a major confounding factor when PRC is evaluated and, for this reason, patients under salt supplementation were excluded from our analysis. In most centres, samples taken for PRC measurement are not standardised with respect to posture or timing of last MC replacement dose and therefore meaningful interpretation and comparison of the results is difficult. Furthermore, there are cost implications that need to be considered if PRC are routinely requested that cannot meaningfully help guide replacement strategies. Adherence to medication is a further factor that needs to be considered and in many cases, '*prescribed*' doses are not necessarily reflective of what is actually being taken. The effects of differing levels of salt consumption, together with a different mineralocorticoid sensitivity and treatment adherence, all could potentially contribute to explain the findings of higher PRC in patients under higher MC doses, especially in children. This is particularly true in patients with CAH in whom up to one third of adult patients are non-adherent (26). However, in our cohort of adult patients with SW-CAH, there was no relationship between PRC and disease control (as measured by 17-OHP and androstenedione levels) suggesting that

global non-adherence (of both GC and MC replacement) may not be occurring and our data might potentially point towards specific mineralocorticoid under-replacement. This contrasts with the analysis in children, where we observed concordance between 17-OHP / androstenedione and PRC levels. Taken together, these factors will undoubtedly contribute to the wide variability in PRC values that we observed and the lack of relationship with MC replacement dose and biological relevant clinical variables and endorses observations made in much smaller studies (27).

Our study does have limitations. It is a retrospective analysis from multiple centres, so there is the potential for selection bias as well as high heterogeneity in our study population, and detailed, extensive medical records were not available in many patients. Similarly, plasma renin has not been measured centrally but was analysed by different assays in the participating centres. In addition, we were unable to estimate the impact of glucocorticoid replacement therapy due to a lack of information about preparation and dose. Also, we excluded the small number of patients (n=7) who were taking salt supplementation as precise data on salt intake was not reported in the records. The data we have analysed are from 'real-life' clinic consultations and are not from a standardized controlled clinical trial. This is particularly true for the longitudinal analysis where titration of MC dose was made by physician preference rather than an established specific algorithm. Prospective trials designed to reduce the effects of confounding factors through a dedicated and rigorous approach are needed to clarify the contribution of different clinical and biochemical variables on PRC and subsequently on MC dosage titration. While our study design is clearly a limitation, this does offer a true reflection of the variables that are presented to clinicians when trying to optimize the management of patients with PAI.

In conclusion, routine monitoring of serum electrolytes (alongside clinical assessment of symptoms and BP) provides the most informative approach to add to PRC when MC replacement needs to be adjusted. However, in the absence of the ability to standardise accurately the collection of samples used to measure PRC, its routine measurement may conflict with other tools used to assess the adequacy of MC replacement and decisions to modify MC dose should not be solely based on PRC. There are many other questions that need to be addressed including under- or over-replacement with MC and its clinical impact in patients with PAI. Dedicated large scale prospective

studies will be required to conclusively determine the role of PRC in monitoring MC replacement in PAI patients.

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Figure Legends

Figure 1

Flow chart for patient selection for the analysis of optimization of mineralocorticoid replacement. (SW-CAH=salt-wasting congenital adrenal hyperplasia; AD=Addison's disease).

Figure 2

Distribution of mineralocorticoid replacement dose in 188 adults with adrenal insufficiency. Black bars refer to patients with salt-wasting congenital adrenal hyperplasia (n=149) and white bars to patients with Addison's disease (n=39) (a). Distribution of mineralocorticoid replacement dose in 55 children with salt-wasting congenital adrenal hyperplasia (b).

Figure 3

Baseline correlations of mineralocorticoid daily dose with clinical and biochemical variables in adult patients with adrenal insufficiency (solid lines represent the regression analysis; shaded areas within dotted lines represent the 95% confidence intervals; n=number of individual clinical assessments included in the analysis; PRC=plasma renin concentration; Na⁺=serum sodium; K⁺=serum potassium; MAP= mean arterial pressure) (a-d).

When PRC is expressed as '*low*' (white bars), '*normal*' (grey bars) or '*high*' (black bars) according to local reference ranges, those patients with '*high*' PRC have lower Na⁺ concentrations in comparison with individuals in whom PRC is '*normal*' or '*low*' (e). K⁺ is lower in individuals with '*low*' PRC in comparison with individuals in whom PRC is '*normal*' or '*high*' (f). There is no difference in MAP or mineralocorticoid dose in groups when stratified by local PRC reference range (g and h) (**p<0.001).

Figure 4

Baseline correlations of mineralocorticoid daily dose corrected for body surface area (MC_{BSA}) with clinical and biochemical variables in children with adrenal insufficiency due to salt-wasting congenital adrenal hyperplasia (solid lines represent the regression analysis; shaded areas within dotted lines represent the 95% confidence intervals; n=number of individual clinical assessments

included in the analysis; PRC=plasma renin concentration; Na⁺=serum sodium; K⁺=serum potassium; cSBP and cDBP=centile-corrected systolic and diastolic blood pressure) (a-e).

When PRC is expressed as '*low*' (white bars), '*normal*' (grey bars) or '*high*' (black bars) according to local reference ranges, those children with '*high*' PRC have the lowest Na⁺ concentrations (f). K⁺ is highest in children with '*high*' PRC (g). There is no difference in cSBP or cDBP between groups when stratified by local PRC reference range (h and i). However, MC_{BSA} was lowest in those children with a '*normal*' PRC (j). (*p<0.05, **p<0.01, ***p<0.001).

Figure 5

Longitudinal analysis of Plasma Renin Concentration (PRC) in 112 patients with adrenal insufficiency at baseline and follow-up (median time between assessments = 433 days, range 33-2082). Variation in PRC was defined as '*increased*' (>15% rise from baseline), '*decreased*' (>15% fall from baseline) or '*no change*' (<15% deviation from baseline). Longitudinal change in absolute PRC (a) and categorization of PRC change (b) in 80 patients with unchanged MC dose from baseline, 23 patients in whom MC dose was increased (c and d) and 9 patients with an decreased MC replacement dose (e and f).

Table 1: Baseline characteristics of 243 patients with adrenal insufficiency. Data are expressed as median (range). Abbreviations: SW-CAH=salt-wasting congenital adrenal hyperplasia, AD=Addison's disease, MC=mineralocorticoid, MC_{BSA}=MC daily dose corrected for body surface area (SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial blood pressure, PRC=plasma renin concentration, BMI=body mass index, cSBP and cDBP=centile-corrected systolic and diastolic blood pressure, sBMI=SDS-corrected BMI.

	Adults only			Children only
	Whole adult cohort	SW-CAH	AD	SW-CAH
n	188	149	39	55
Assessments(n)	386	347	39	348
Age (years)	27(16-84)	25(16-67)	49(17-84)	2.3(0-15)
Male	91(48%)	72(48%)	19(49%)	26(47%)
Female	97(52%)	77(52%)	20(51%)	29(53%)
BMI (kg/m²)	29(15-50)	29(15-50)	26(17-33)	17(12-37)
Na⁺ (mmol/L)	140(126-146)	140(130-146)	138(126-143)	139(104-148)
K⁺ (mmol/L)	4.2(2.7-5.9)	4.2(2.7-5.9)	3.9(3.1-4.7)	4.3(3.5-7.9)
SBP (mmHg)	123(90-170)	123(90-169)	124(102-170)	101(62-150)
DBP (mmHg)	79(53-104)	79(57-104)	79(53-102)	62(32-88)
MAP (mmHg)	93(70-125)	93(70-125)	93(75-120)	75(42-103)
MC daily dose (µg/day)	150(25-400)	150(25-400)	100(50-300)	100(25-375)
PRC (mUI/mL)	86(0.6-3166)	87(0.6-3166)	82(4.2-2879)	47(0.1-5090)
MC_{BSA} dose (µg/day)	/	/	/	165(15-965)
cSBP (mmHg)	/	/	/	70(10-100)
cDBP (mmHg)	/	/	/	74(10-100)
sBMI (kg/m²)	/	/	/	0.8(-1.8;3.5)

Table 2. Multiple regression modelling in adult patients with adrenal insufficiency (147 complete clinical assessments from 117 patients). The dependent variables assessed were plasma renin concentration (PRC), sodium (Na⁺), potassium (K⁺), mineralocorticoid (MC) dose, and mean arterial pressure (MAP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

Model 1 (p<0.001*)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	p
MC total daily dose	0.001	-0.001	0.002	0.515
K ⁺	0.044	-0.201	0.289	0.723
Na ⁺	-0.091	-0.126	-0.055	<0.001*
MAP	0.005	-0.006	0.016	0.351
Age	-0.006	-0.013	0.001	0.072
BMI	0.004	-0.012	0.020	0.591

Model 2 (p=0.017*)		Dependent: MC total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	p
K ⁺	-17.523	-43.350	8.303	0.182
Na ⁺	1.026	-3.072	5.123	0.621
PRC	5.846	-11.873	23.565	0.515
MAP	0.566	-0.593	1.726	0.336
Age	0.030	-0.722	0.781	0.938
BMI	2.812	1.183	4.441	0.001*

Table 3. Multiple regression modelling in adults with salt-wasting congenital adrenal hyperplasia (114 complete assessments from 82 patients). The dependent variables were plasma renin concentration (PRC), sodium (Na⁺), potassium (K⁺), mineralocorticoid (MC) dose, and mean arterial pressure (MAP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

Model 1 (p=0.008*)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	p
MC total daily dose	0.000	-0.002	0.002	0.885
K ⁺	-0.064	-0.361	0.233	0.670
Na ⁺	-0.097	-0.143	-0.051	<0.001*
MAP	0.007	-0.007	0.021	0.343
Age	0.001	-0.010	0.012	0.850
BMI	0.002	-0.016	0.020	0.832

Model 2 (p<0.001*)		Dependent: MC total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	p
K ⁺	-41.180	-71.069	-11.290	0.007*
Na ⁺	-1.393	-6.561	3.776	0.594
PRC	1.461	-18.479	21.402	0.885
MAP	1.621	0.167	3.075	0.029
Age	1.143	0.036	2.251	0.043
BMI	2.362	0.577	4.147	0.010

Table 4. Multiple regression modelling in children with salt-wasting congenital adrenal hyperplasia (55 complete assessments from 11 patients). The dependent variables were plasma renin concentration (PRC), sodium (Na⁺), potassium (K⁺), SDS-corrected body mass index (sBMI), mineralocorticoid dose adjusted for body surface area, (MC_{BSA}), and centile-corrected systolic and diastolic blood pressure (cSBP and cDBP). *p* value should be interpreted with Bonferroni correction, when significant, they are highlighted in bold and with asterisk.

Model 1 (p=0.008*)		Dependent: PRC		
Independent	B	95% CI lower bound	95% CI upper bound	p
MC _{BSA} dose	0.002	-0.003	0.006	0.493
K ⁺	-0.694	-1.151	-0.237	0.004*
Na ⁺	-0.143	-0.242	-0.045	0.005*
cSBP	0.007	-0.003	0.017	0.178
cDBP	-0.008	-0.019	0.004	0.192
Age	-0.013	-0.073	0.047	0.662
sBMI	0.027	-0.203	0.257	0.814

Model 2 (p<0.001*)		Dependent: MC _{BSA} total daily dose		
Independent	B	95% CI lower bound	95% CI upper bound	p
K ⁺	-10.671	-43.913	22.570	0.521
Na ⁺	5.602	-1.347	12.550	0.111
cSBP	0.840	0.206	1.474	0.011
cDBP	-0.446	-1.214	0.322	0.248
Age	-7.275	-10.634	-3.917	<0.001*
sBMI	-11.440	-26.370	3.490	0.130
PRC	6.814	-13.039	26.667	0.493

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

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Real-world impact of glucocorticoid replacement therapy on bone mineral density: retrospective experience of a large single-center CAH cohort spanning 24 years

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Abstract

Summary The congenital adrenal hyperplasia population seems to have an intrinsic tendency to a high frequency of low bone mass. However in this single-center and long-term evaluated cohort, the simplified corticoid regimen, with exclusive dexamethasone single dose reposition during adulthood, did not represent a risk factor for decrease in bone health.

Introduction The impact of long-term and supposedly physiological doses of gluco and mineralocorticoid (GC/MC) on bone mineral density (BMD) in congenital adrenal hyperplasia (CAH) remains discordant among studies, which contain different clinical forms and corticoid regimens. Our aim was to evaluate the BMD in CAH adults receiving similar GC regimen since childhood and to correlate it with GC/MC cumulative doses.

Methods Only patients with good compliance, who used cortisone acetate (CA) during childhood and dexamethasone after the final height achievement. Cumulative GC/MC doses were calculated from diagnosis until last evaluation. BMD was analyzed by the first and last energy X-ray absorptiometry (DXA) scans performed.

Results Twenty simple virilizing (SV) and 14 salt wasting (WS) whose mean age was 26 ± 6 years, mean CA, dexamethasone, and fludrocortisone cumulative doses were $63,813 \pm 32,767$, 812 ± 558 , and 319 ± 325 mg/m², respectively. Based on the last DXA, low BMD was observed in 11% of patients, total hip Z-score was lower in the SW than SV form ($p = 0.04$). Cumulative CA dose had an inverse correlation with femoral neck Z-score ($p < 0.01$). Total cumulative GC and MC doses had an inverse correlation with total hip Z-score ($p < 0.01$). In the analysis of sequential BMD during dexamethasone therapy, no association was observed among cumulative GC/MC doses, clinical forms, sex, and lumbar Z-score delta.

Conclusions Even though a low CA regimen during growth periods in addition to MC replacement appears to have an influence on BMD at femoral sites, interestingly a low dexamethasone one does not seem to be deleterious for bone health in adulthood.

Keywords Bone mineral density · Classical forms · Congenital adrenal hyperplasia · Cumulative glucocorticoid dose · Long-term dexamethasone therapy · Low glucocorticoid doses

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Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a common genetic disorder caused by *CYP21A2* mutations, affecting approximately 1 in 10,000 to 20,000 live births [1]. The impairment of 21-hydroxylase activity results in decreased cortisol and/or aldosterone synthesis and leads to a spectrum of clinical manifestations, so the phenotype varies according to the degree of the enzymatic deficiency. Traditionally, clinical manifestations are grouped into two forms: classical, which includes salt wasting (SW) and simple virilizing (SV), and nonclassical (NC). The SV form is marked by the prenatal external genitalia virilization in females and by precocious pseudopuberty in males; in the SW

form, beyond the previous virilization signs, patients also present hyponatremic dehydration in the first weeks of life. The NC form is characterized by late onset hyperandrogenic manifestations [2].

The CAH therapy is based on glucocorticoid (GC) and/or mineralocorticoid (MC) replacements. During growth periods, preference is given to the use of short half-life glucocorticoids that avoid growth suppression. During adulthood several GC regimens could be used, including those with short half-life or long-acting GC. The introduction of GC allowed an increase in life expectancy of these patients; however, the long-term GC exposure has been associated with some adverse metabolic profiles, such as increased prevalence of obesity, insulin resistance, and hypertension [3, 4]. Regarding the effects of long-term GC exposure on bone mineral density (BMD), there are only a few studies with discordant results.

Low bone mass was observed in all sites surveyed in 61 Swedish CAH women (27 SW, 28 SV, 6 NC) with a mean age of 30 years (18–63 years); however, the cumulative GC dose and the degree of hormone suppression, estimated by androgens or 17-hydroxyprogesterone (17-OHP) levels, were not evaluated [5]. On the other hand, BMD was normal in 30 Dutch patients (24 SW, 6 SV) with a mean age of 21 ± 2.5 years, including both sexes. No correlation was observed between BMD and the cumulative GC dose from the last 5 years [6]. Abnormal BMD (T-score < -1) was identified in 52% of 80 American patients (47 classical and 33 NC) [7]. Interestingly, these results were associated with suppressed DHEAS values, independent of the current GC dose, although the cumulative dose was not evaluated. It is worth mentioning that this cohort's age ranged from 20 to 69 years and that a significant proportion of patients presented with the NC form.

In general, the cohorts of the available studies included heterogeneous samples regarding age, clinical forms, time of follow-up, and GC regimens, especially in adult life. Thus, it is difficult to analyze the real impact of chronic GC exposure, at supposedly physiological doses, in these patients. In addition, the impact of long-term MC therapy on BMD of this population has rarely been analyzed in the past [8].

Our aim was to evaluate the impact of cumulative corticotherapy, considering GC and MC, from diagnosis until the present moment, on BMD of a CAH cohort comprising exclusively: young adult patients with classical forms, followed in the same center throughout life and having received cortisone acetate during childhood and dexamethasone during adulthood. For this, BMD was correlated with the average daily and total cumulative doses of both GC and MC, calculated since the diagnosis.

Materials and methods

The study was approved by the Ethics Committee from Hospital das Clínicas, Faculty of Medicine of University of São Paulo. The inclusion criteria were patients with classical forms of CAH, tracked in our center since diagnosis with good compliance, who only used short half-life GC during childhood (cortisone acetate) and dexamethasone as GC after reaching final height. Compliance has been verified by achievement of normal serum levels of testosterone and androstenedione according to sex and age in quarterly evaluations during growth phases and every semester in adult life. No patient had suppressed values of plasma renin activity or 17-OHP levels < 5 ng/mL.

From a cohort of 149 CAH classical form patients, we selected 34 that fit the inclusion criteria. All patients were diagnosed by suggestive clinical signs and high basal serum 17-OHP levels. The hormonal diagnosis was confirmed by the molecular analysis of *CYP21A2* gene [9].

Clinical, laboratory, and radiologic data

Cumulative doses of GC and MC were calculated by summing partial cumulative doses for each period in which the dose remained stable, always corrected by the respective body surface. We also calculated the average daily doses, from the cumulative dose divided by the treatment time. The total cumulative dose covers the entire period from the first day of treatment to the date of the latest DXA measurement. We considered the following GC equivalence for its calculation: 1 mg dexamethasone = 16 mg prednisone = 80 mg cortisone acetate [8, 10, 11].

Questionnaires were used to estimate physical activity levels, history of clinical fractures, and daily calcium intake. Regular physical activity was considered to be at least 150 moderate-intensity minutes/week [12]. Accounting for the age groups of our sample, an adequate daily calcium intake was considered equal or greater than 1000 mg [13]. The use of cyproterone acetate and/or growth hormone (GH) during childhood (to optimize final height), as well as the use of oral contraceptive pills, age of onset of short half-life and long-acting GC and respective times of treatment with each drug were collected from records.

Patients, during their long-term follow-up, performed regular bone evaluation. BMD in our service is routinely measured by DEXA technique (dual energy X-ray absorptiometry) (DXA) with Hologic equipment. Due to the variability between individuals, we opted to raise retrospective data from the two most distant DXA measurements performed: the first DXA in life and the last (most recent) one. The age at both DXA measurements varied within the cohort, and for some individuals, the initial assessment was performed before the completion of linear growth. Consequently, in the first

evaluation, Z-score was raised only from one site: the lumbar spine. According to International Society for Clinical Densitometry's (ISCD) historical position, femoral sites are unreliable in individuals under the age of 20 [14, 15]. Therefore, as 26 out of 34 individuals of this cohort were under 20 years old in their first DXA assessment, only lumbar spine Z-scores were considered for later evolutionary analysis.

In the last DXA, as all patients had completed linear growth, Z-scores were evaluated in the three usual sites: lumbar spine, femoral neck, and total hip. In addition, T-score was evaluated in order to enable comparison of our cohort with other series, which previously used T-score < -1 as a parameter for abnormal BMD. Patients with Z-score < -2 in at least one of the sites in the last DXA were considered to have low bone mass in the present moment. Serum 25OH vitamin D and PTH measurements raised were performed at a mean interval of 2 months before the date of this second DXA measurement.

Statistical analysis

We established the evolutionary bone mass as the variation of lumbar spine Z-score between first and last exam. This variation was correlated to clinical data and only dexamethasone cumulative and average daily dose, as this was the therapy used during the covered period. As femoral neck and total hip Z-scores were not considered in the first DXA measurement, no evolutionary evaluation was made based on these sites.

Current bone mass was represented by the Z-scores in the three sites of the last DXA. It was correlated with cortisone acetate, dexamethasone, and total cumulative and average daily corticoid therapy, as well as with clinical and recent laboratory data.

The entire analysis was performed in the R Commander program. The linear regression test was used to correlate evolutionary and current BMD with laboratory and clinical data that were quantitative variables, such as corticoid doses. For the clinical data that were nominal qualitative variables, such as calcium intake and physical activity, the *t* test for independent samples was used. In this situation, the patients were compared with their peers. We used the Mann-Whitney *U* test to analyze the Z-score variation groups for quantitative clinical data in cases where the sample was not normally distributed. The significance level adopted for the statistical tests was 5% ($p < 0.05$).

Results

The cohort included 34 patients with classical forms of CAH: 13 males (M) and 21 females (F), 20 with the SV and 14 with the SW form; the mean age of women was 26 ± 6 and of men

was 25.8 ± 6.3 years. The diagnosis was performed at birth in 14 patients (12 SW and 2 SV), in the first 3 years in 9, and between 4 and 6 years in 11 individuals, these latter patients presented the SV form. Patients with the SW form were diagnosed and began regular follow-ups in our service with an average of 1 ± 2 months of life, and those with the SV form with a mean age of 3.6 ± 2.3 years ($p < 0.05$). The mean final height did not show a significant difference between clinical forms within the same sex.

The SV patients accumulated 23.8 ± 6.1 years of total treatment, 10.2 ± 4.3 years under cortisone acetate therapy and 13.5 ± 5.4 years under dexamethasone. The SW patients accumulated 24.2 ± 5.1 years of total treatment, 14.8 ± 4.2 years under cortisone acetate therapy and 9.3 ± 5.6 years under dexamethasone. Time under cortisone acetate therapy was higher in SW than SV patients ($p < 0.05$). Table 1 shows clinical descriptive data including height, chronological age, age at diagnosis, time of treatment, and average daily and cumulative doses of each medication, by sex and clinical form.

Last evaluation—current bone mass

Considering the last DXA, only 4 patients had low bone mass, representing 11% of the entire sample. Among these patients, there were 3 women (2 SW and 1 SV) and one SW man with vitamin D insufficiency. All four patients had low calcium intake and did not practice physical activity regularly. T-score < -1 in at least one of the sites was observed in 58% of the patients.

Table 2 shows the descriptive data of Z-scores of each evaluated bone site in the latest DXA measurement, by sex and clinical form. There was no significant difference in lumbar spine and femoral neck Z-score between sexes and clinical forms ($p > 0.05$); however, the total hip Z-score was lower in SW patients (-0.97 ± 1) than in SV (-0.21 ± 1) ($p = 0.014$) (Fig. 1).

The lumbar spine Z-score at the last DXA measurement had no correlation with none of the other qualitative or quantitative variables: corticoid doses and regimen, calcium intake, physical activity, or last measurements of serum PTH or 25OH vitamin D.

The femoral neck Z-score from the latest DXA measurement presented an inverse correlation with cortisone acetate treatment time, even with the outlier exclusion ($p < 0.01$), with cortisone acetate cumulative dose ($p < 0.01$) (Fig. 2) and with the MC cumulative dose ($p < 0.01$). No correlation was observed between femoral neck Z-score and dexamethasone average daily or cumulative doses.

Total hip Z-score from the latest DXA measurement presented an inverse correlation with cortisone acetate treatment time ($p = 0.018$), with total GC cumulative dose ($p = 0.04$) and with the MC cumulative dose ($p < 0.01$). Considering only the 14 SW patients, which

Table 1 Description of age, height, age at diagnosis, time of treatment, and average daily and cumulative doses of each medication, by sex and CAH clinical form

	SW (<i>n</i> = 14)	SV (<i>n</i> = 20)	SW/F (<i>n</i> = 10)	SV/F (<i>n</i> = 11)	SW/M (<i>n</i> = 4)	SV/M (<i>n</i> = 9)	All (<i>n</i> = 34)
Age (years)	24.2 (± 6)	27.3 (± 6)	23.2 (± 6)	29.0 (± 5.9)	27 (± 6.6)	25.2 (± 6.4)	23.9 (± 6)
Height (cm)	155.7 (± 6)	160 (± 10)	150.8 (± 9.8)	156.0 (± 10)	168.1 (± 9.3)	165.9 (± 9.3)	158.5 (± 9.8)
Age at diagnosis (years)	0.07 (± 2.3)	3.6 (± 2.2)	0 (± 2.3)	3.0 (± 2.2)	0.165 (± 2.1)	4.2 (± 2.1)	2.1 (± 2.2)
Time with CA (years)	14.8 (± 4.2)	10.2 (± 4.3)	14.3 (± 4.2)	11.2 (± 4.3)	16 (± 4.3)	8.9 (± 4.5)	12.13 (± 4.1)
Average daily CA dose (mg/m ² /day)	15 (± 3.2)	16.3 (± 3.4)	14.7 (± 3.2)	18.4 (± 3.4)	15.7 (± 3.2)	13.8 (± 3.3)	15.83 (± 3.3)
Cumulative CA dose (mg/m ²)	80,521 (± 33,264)	52,117 (± 34,025)	76,858 (± 33,264)	63,269 (± 34,025)	89,680 (± 35,562)	38,487 (± 36,634)	63,813 (± 32,767)
Dexamethasone onset age (years)	14.9 (± 2.8)	13.8 (± 2.9)	14.4 (± 2.8)	14.3 (± 2.9)	16.2 (± 3)	13.2 (± 3.2)	14.3 (± 2.8)
Time with dexamethasone (years)	9.3 (± 5.6)	13.5 (± 5.4)	8.8 (± 5.6)	14.7 (± 5.4)	10.7 (± 6.1)	12 (± 6)	11.7 (± 5.6)
Average daily dexamethasone dose (mg/m ² /day)	0.17 (± 0.07)	0.2 (± 0.08)	0.2 (± 0)	0.2 (± 0)	0.1 (± 0)	0.18 (± 0.08)	0.19 (± 0.07)
Cumulative dexamethasone dose (mg/m ²)*	523.7 (± 562)	1014.7 (± 565)	635.8 (± 562)	1189.7 (± 565)	243.3 (± 615)	801.64 (± 612)	812.56 (± 558)
Total average daily GC dose (mg/m ²)	17.3 (± 5)	16 (± 4.8)	15.3 (± 5)	17.5 (± 4.9)	20 (± 5.1)	14.3 (± 5.2)	812.56 (± 558)
Total cumulative GC dose (mg/m ²)	150,796 (± 73,611)	141,594 (± 74,347)	127,726 (± 73,611)	168,213 (± 74,347)	208,470 (± 81,001)	109,060 (± 81,930)	145,383 (± 72,520)
Total cumulative MC dose (mg/m ²)*	647,114 (± 328,875)	90,090 (± 335,568)	651,457 (± 328,875)	155,338 (± 339,336)	636,258 (± 341,635)	26,602 (± 350,501)	319,453 (± 325,443)

CA, cortisone acetate; GC, glucocorticoid; MC, mineralocorticoid; SW, salt wasting; SV, simple virilizing; F, female; M, male. *Significant difference between SW and all SV ($p < 0.05$)

were the ones using MC, total hip Z-score kept significant correlation with MC cumulative dose ($p < 0.01$). No correlation was observed between total hip Z-score and dexamethasone average daily or cumulative doses.

Only 50% of the patients had an adequate calcium intake, 42% practiced regular physical activity, and none had a history of clinical fracture. The mean serum 25OH vitamin D was 26 ± 9 ng/ml and PTH 36 ± 13 pg/ml. Twenty-one patients used cyproterone acetate ($75 \text{ mg/m}^2/\text{d}$) in childhood for 4.4 ± 2 years, 4 used GH (0.15 UI/kg/day), and 8 women used oral contraceptive pills. Testosterone levels in all females and androstenedione levels in both sexes were normal within sex and age reference values. None of these factors had significant correlation with Z-scores of lumbar spine and femoral sites evaluated in the last DXA measurement ($p > 0.05$).

Evolutionary evaluation

Thirty patients (4 SW/M, 7 SV/M, 8 SW/F, 11 SV/F) performed at least two DXA measurements in their follow-up, making possible an evolutionary analysis based on the lumbar spine Z-score only. The femoral sites were not used for comparison. The mean age at first DXA measurement was 16.8 ± 6.4 years and 27.1 ± 6.1 years at the last one, reaching an interval between the two exams of 10.3 ± 5.9 years. In the first DXA, all patients had already started the exclusive use of dexamethasone, allowing evolutionary analysis of the bone condition under dexamethasone therapy.

Lumbar spine site was exclusively used for this comparison. Table 2 shows the Z-score variation between the first and second DXA measurement. The mean Z-score in the first assessment was -0.71 ± 1.02 and in the last assessment was -0.77 ± 0.85 , representing a delta Z-score of -0.06 ± 0.75 ($p > 0.05$). No significant BMD variation was found according to sex (-0.06 in M/ -0.04 in F) or clinical form ($+0.16$ in SW/ -0.22 in SV) (Fig. 3).

Lumbar spine Z-score variation between the first and last densitometry was independent from the therapy associated varieties: delta age, time of treatment with dexamethasone, or average daily and cumulative doses of dexamethasone. In addition, no correlation was found between lumbar spine Z-score variation and clinical varieties such as calcium intake, practice of physical activity, previous use of cyproterona acetate, GH, or contraceptive pills. No statistical analysis was made between lumbar spine Z-score variation and cortisone acetate average daily or cumulative doses, as the covered period did not comprehend directly this short half-life corticoid therapy.

Discussion

Literature is still unclear about the impact of GC and MC replacement therapy on the BMD of CAH patients. Previous

Table 2 Z-scores of each evaluated bone site by sex and clinical form

	Site	SW (<i>n</i> = 14)	SV (<i>n</i> = 20)	SW/F (<i>n</i> = 10)	SV/F (<i>n</i> = 11)	SW/M (<i>n</i> = 4)	SV/M (<i>n</i> = 9)
Z-score L1-L4	Lumbar spine	−0.87 (± 0.8)	−0.76 (± 0.82)	−0.95 (± 0.8)	−0.96 (± 0.8)	−0.67 (± 0.8)	−0.52 (± 0.86)
Z-score femoral neck	Femoral neck	−0.85 (± 0.85)	−0.44 (± 0.86)	−0.87 (± 0.85)	−0.58 (± 0.83)	−0.82 (± 0.79)	−0.27 (± 0.87)
Z-score total hip	Total hip*	−0.97 (± 1)	−0.21 (± 1)	−1.02 (± 1)	−0.44 (± 1.02)	−0.85 (± 0.83)	−0.05 (± 1.08)

SW, salt wasting; SV, simple virilizing; F, female; m, male. *Significant difference between SW and all SV ($p < 0.05$)

studies have found variable frequencies of abnormal BMD (T-score < -1); although, all these studies evaluated young adults. In 47 American patients with classical form (mean age 35 years), T-score < -1 was identified in 65% of the series [7]. One French cohort ($n = 104$; 34 NC; mean age 28 years), identified abnormal BMD in 60% of the patients [8]. Another French study ($n = 38$; 9 NC; mean age 25 years) found 45% [10]. Among 61 women (age range 18–63 years), a Swedish study found 62% of abnormal BMD [5], and a multicentric British study ($n = 203$; 37 NC; age range 18–69 years) found 47% [4]. In our cohort of young adults, low BMD based on Z-score < -2 was found in 11% of patients; we also evaluated T-score in order to compare our data with previous studies and similarly, T-score < -1 was found in 58%. All of those results were higher than the 16% of abnormal BMD expected among general population according to its Gaussian distribution.

We observed that the average daily doses of corticosteroids used in our center (13–18 mg cortisone acetate/m²/day) are lower than the average doses used in other centers (20–25 mg cortisone acetate/m²/day) [4, 10]. The dose adjustment in our series was based on the achievement of normal serum androstenedione and testosterone levels according to age and sex, and not on suppression of 17-OHP values [16]. In theory, this measure would protect bone health, which is known to be damaged by corticoid excess and hypoandrogenism.

Fig. 1 Total hip Z-score is lower in SW than in SV patients

Our methods and results are similar to those found by Chakhtoura et al. [10], one of the few studies that calculated in detail the GC cumulative dose over the patients' lives. The authors observed a negative correlation of the total cumulative and average daily GC doses (184.132 ± 71.175 mg/m² and 20.6 ± 5.6 mg/m²/day) with the T-score at lumbar spine and femoral neck in 38 (9 NC) patients with average treatment time of $24.2 (\pm 4.5)$ years. In our sample, we observed a negative correlation of the cumulative dose only of cortisone acetate with femoral neck and total hip Z-score, indicating the importance of avoiding over-treatment during growth periods. The total cumulative (cortisone acetate and dexamethasone = 145.383 ± 72.520 mg/m²) and the average daily dose of GC (16.2 ± 4.7 mg/m²/day) of our patients, although lower than in the French cohort, had no influence on the Z-score at any site. However, we cannot rule out a sample size effect.

Interestingly, in spite of the known preferential action of GCs on trabecular bone, similar results are described in CAH patients regarding the greater impact of corticotherapy on sites with a higher proportion of cortical bone. Ceccato et al. found that BMD in 38 patients was lower than controls only at the femoral neck site, as well as in a 10-year evolution analysis with 15/38 patients: femoral neck BMD was reduced while lumbar spine increased. In this latter study, the average daily GC dose was evaluated in the last 3 years (10 ± 5 mg/m²/day) [17]. El-Maouche et al. found a significantly lower BMD in 47 classic patients in comparison with 33 NC patients, the major difference being in the forearm, which has a high representation of cortical bone. This finding was related to lower serum DHEAS levels among classical patients, perhaps suggesting a higher suppression [7].

Another point that deserves attention is the impact of the cumulative MC dose on BMD, whose effect on bone is unclear. The only study that previously addressed this analysis [8] did not find an influence of MC on BMD. It is noteworthy that this study included patients with classical and NC forms, in which individuals from both groups used MC with extremely varied cumulative doses, being 22 to 24 mg/m² among the 70 classical patients and 0.2 to 0.6 mg/m² among the NC.

In our sample, we decided to analyze the SW patients separately, since they presented a continuous and regular use of MC. Despite the small sample size, we identified the cumulative MC dose impact, which suggested a possible negative effect on total hip BMD. It is known that physiologically, in

Fig. 2 Femoral neck Z-score presented an inverse correlation with cortisone acetate cumulative dose

animal models, MC receptor inhibition increases bone mass [18]. In addition, MC also increases calciuria and reduces intestinal calcium absorption [19], which could lead to relative secondary hyperparathyroidism [20]. We do not have enough evidence to support our findings in this theoretical model, because our patients who used MC did not perform serial serum PTH dosages throughout life, and the last PTH value did not show a significant correlation with the cumulative MC dose. Moreover, high MC doses could present a synergistic hypocalcemic effect as well as observed with GC. We recognize the limitation of our study concerning PTH analysis, as we do not have serial measurements during treatment.

The comparison between the two DXA performed in our patients with a significant time interval showed no significant variation of BMD during continuous use of dexamethasone. Frequently avoided in clinical practice [21] because of its high potency, dexamethasone was not detrimental to the bone health in our series, since it was used at low doses and introduced only after the achievement of final height. Thus, our preliminary data suggest the usefulness of dexamethasone in adults as a way of facilitating the treatment compliance, since it is used as single daily dose. Previously, only the study of

Ceccato et al. [19] performed sequential DXA analysis in CAH patients; it identified a progressive increase in lumbar BMD and a decline in femur BMD without association with corticotherapy in the last 3 years.

In spite of the absence of correlation between calcium intake and BMD in this young adult cohort, it is relevant to point out the high frequency of insufficient calcium intake noticed: 50% of this cohort. Besides the continuous recommendation for an adequate intake and sun exposure, unfortunately due to social, financial, or cultural reasons several patients do not adhere. In fact, the average calcium intake of the Brazilian population is 40% of the daily adequate intake [22, 23].

Strengths of our study are (1) a sample comprising exclusively classical patients, followed in the same center throughout life and submitted to a similar regimen of corticosteroids, (2) the analysis of the long-term corticosteroid therapy since diagnosis, and (3) the evolutionary analysis of the BMD during treatment with GC.

Weaknesses are (1) a retrospective study with limited analysis of bone metabolism markers. Despite androstenedione and testosterone periodical measurements, and constantly normal levels within sex and age in these 34 well controlled

Fig. 3 Lumbar spine Z-score evolution by clinical form between first and second evaluation in 30 patients (19SV/11SW) during the use of dexamethasone

patients, PTH and vitamin D were not routinely measured in the past and were recently introduced in patients follow-up. Considering this retrospective analysis spanning 24 years, data of these parameters were heterogeneous among patients, and we opted not to include in the evolutionary analysis. (2) Limited sample size with predominantly young adults. Thus, we emphasize the need to re-address this issue in older patients, in which the long-term effects of therapy may be more clearly shown. (3) For this retrospective analysis, we did not have the opportunity to include a control group, and, thus, we have opted to analyze Z-score evolution rather than BMD evolution. However, Z-scores were calculated by the DXA machine based on information of thousands of individuals in the similar age and sex group. Therefore, the fact that our cohort did not have significant Z-score changes in the studied interval can be seen as a positive outcome, meaning that, similar to a vast control population, they gained BMD during these critical years and therefore maintained their Z-score.

In conclusion, in this CAH cohort, we observed a negative effect of cumulative cortisone acetate dose used during growth periods and of cumulative MC dose on bone mass in femoral neck and total hip. In view of this result, we emphasize the recommendation of preventive habits to young CAH patients, particularly for SW patients. In addition, we observed a similar frequency of abnormal BMD in this CAH cohort compared with others. Concerning long-term follow-up during adulthood, the use of a single daily low dexamethasone dose seems to be a valid strategy to improve the adherence without increasing risk for bone deterioration.

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Compliance with ethical standards

Conflicts of interest None.

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Guia Prático de Atualização

Departamento Científico
de Endocrinologia (2019-2021)

Hiperplasia adrenal congênita: triagem neonatal

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Introdução

A hiperplasia adrenal congênita (HAC) é uma doença com significativa morbimortalidade, frequentemente sub-diagnosticada no período neonatal, principalmente em crianças do sexo masculino, antes de sua inclusão nos testes de triagem neonatal. O diagnóstico e tratamento precoces são disponíveis e capazes de prevenir as complicações pós-natais. Estas características, associadas a um teste de triagem de execução fácil, fizeram com que a HAC fosse incluída nos programas públicos de triagem neonatal.¹

Tendo em vista a importância desse tema, a Sociedade Brasileira de Pediatria e seu Departamento Científico de Endocrinologia, decidiram redigir esse Documento para atualizar os Pediatras sobre a triagem neonatal no diagnóstico e tratamento da HAC.

O que é hiperplasia adrenal congênita e quais são suas principais causas?

Hiperplasia adrenal congênita (HAC) é o termo utilizado para caracterizar um conjunto de doenças, de herança autossômica recessiva, potencialmente letais, resultantes da deficiência de uma das enzimas responsáveis pela síntese de cortisol nas glândulas adrenais (Figura 1).

Em populações caucasianas, 90 a 95% dos casos de HAC ocorrem por redução da atividade da enzima 21-hidroxilase (21OH).²⁻⁴ A HAC resultante da deficiência da enzima 21-hidroxilase (HAC-21OH) é causada por mutações no gene *CYP21A2*, que codifica a enzima *CYP21A2*, e resultam em diferentes comprometimentos da atividade enzimática e amplo espectro de formas clínicas.

Na maioria dos grupos étnicos, a deficiência de 11 β -hidroxilase corresponde a segunda causa mais comum (5% dos casos). Mas, no Brasil essa deficiência é muito rara e a da 17 α -hidroxilase é a segunda causa de HAC.⁵

Figura 1. Esteroidogênese adrenal. A enzima 21-hidroxilase (P450c21) catalisa a conversão da 17-OH-progesterona em 11-desoxicortisol e da progesterona em desoxicorticosterona. A diminuição de sua atividade causa prejuízo na síntese de cortisol, com consequente aumento compensatório na produção do ACTH (hormônio adrenocorticotrófico), hiperplasia do córtex adrenal e estímulo da produção excessiva dos precursores do cortisol. Estes precursores são desviados para a síntese de andrógenos adrenais.

A incidência da HAC-21OH varia de acordo com a população estudada. Em 1993, Pang *et al.* coletaram dados de 29 programas de triagem neonatal existentes em 13 países e estimaram que a incidência mundial para a forma clássica, a partir dos dados de 6,5 milhões de recém-nascidos, foi de 1:15.000 nascidos vivos, variando de 1:10.000 a 1:14.000 na maioria das populações caucasianas.⁶ Estudos recentes realizados na população brasileira demonstraram que a HAC também possui frequência elevada em

nosso meio, variando de 1:10.000 a 1:18.000 nascidos vivos.⁷⁻¹⁰

Quais são as manifestações clínicas da hiperplasia adrenal congênita?

As manifestações clínicas da deficiência da 21OH estão correlacionadas com o grau de deficiência enzimática. Os sinais e sintomas podem

ser decorrentes da deficiência de glicocorticoide, associada ou não à deficiência de mineralocorticoide, e do excesso de andrógenos nos períodos pré- e pós-natal.

As formas clínicas da HAC-21OH são tradicionalmente divididas em dois grandes grupos: *Forma clássica*, com manifestações ao nascimento e subdividida em forma perdedora de sal (PS) e virilizante simples (VS)^{1,2} e *Forma não clássica*, cujos sintomas se iniciam tardiamente, podendo ser na infância, puberdade ou vida adulta, mas com intensidades e complicações muito menos graves que a forma clássica.

Na forma clássica VS, os pacientes são portadores de mutações que resultam em 3-7% de atividade residual da 21OH causando total comprometimento da atividade enzimática.¹ Caracteriza-se, no sexo feminino, por virilização pré-natal da genitália externa, que varia desde clitoromegalia isolada até a aparência semelhante a de uma genitália masculina, porém sem a presença de gônadas palpáveis.¹¹ Esta forma clínica também causa virilização pós-natal em ambos os sexos, com aumento progressivo do clitóris ou pênis, surgimento precoce dos pelos pubianos, aumento da massa muscular, acne e engrossamento da voz.¹² Ocorre também, incremento da velocidade de crescimento e avanço da idade óssea resultando em fechamento epifisário precoce e baixa estatura final.¹⁻³ Todos os recém-nascidos com atipia genital (AG) e sem gônadas palpáveis no períneo ou na região inguinal, devem ser investigados para HAC, antes mesmo da alta do berçário e do resultado do cariótipo, por esta ser a principal causa de AG e pelo fato da forma perdedora de sal ser potencialmente letal.^{13,14}

A forma clássica PS, corresponde a 70-75% das formas clássicas.¹ Estes pacientes são portadores de mutações que resultam em comprometimento total ou quase total da atividade enzimática (< 2%). O comprometimento na síntese de aldosterona resulta em perda de peso seguida de desidratação hiponatrêmica nas primeiras semanas de vida, com potencial evolução para choque hipovolêmico e óbito, se o diagnóstico não for precoce. Essa manifestação, conhecida como crise de perda de sal, surge, em geral, entre

o 8º e 15º dias de vida. As manifestações hiperandrogênicas de virilização também ocorrem na forma perdedora de sal.^{2,3}

Como fazer a suspeita diagnóstica da hiperplasia adrenal congênita?

A virilização da genitália externa no recém-nascido do sexo feminino com forma clássica da HAC-21OH, é o principal sinal clínico da doença. Nos recém-nascidos do sexo masculino, os sinais clínicos estão ausentes ou pouco evidentes ao nascimento; neles o diagnóstico só é realizado com a crise de desidratação hiponatrêmica e hipercalêmica nos primeiros dias de vida nos afetados pela forma clássica perdedora de sal, ou na infância pela investigação de sinais de antecipação puberal nos pacientes com a forma virilizante simples.

A deficiência enzimática da HAC determina o acúmulo de metabólitos precursores da síntese de cortisol e da aldosterona, principalmente a 17OH-progesterona (17OHP), que está geralmente muito elevada nos pacientes sintomáticos com as formas clássicas. O diagnóstico laboratorial é confirmado por sua dosagem, geralmente > 50 ng/mL, porém em situações de uso materno de corticosteroide no final da gestação os valores de 17OHP na triagem neonatal podem ser menores¹⁵. Os andrógenos como androstenediona e testosterona também estão elevados nesses pacientes.¹⁻³ Ressalta-se que, mesmo em meninos não afetados, até os 6 meses de idade, os valores séricos de testosterona encontram-se elevados em decorrência da mini puberdade fisiológica; nesta situação deve-se valorizar os valores de androstenediona e 17OH-progesterona.

Qual é o objetivo da triagem neonatal para a hiperplasia adrenal congênita?

Os programas de triagem neonatal focam na investigação das formas clássicas da HAC.

Isso porque, quando não diagnosticadas precocemente, elas podem evoluir com complicações potencialmente graves, tais como perda de sal com desidratação hiponatrêmica em ambos os sexos, erros de determinação do sexo feminino ao nascimento e pseudo-puberdade precoce, especialmente no sexo masculino, com comprometimento da altura final.¹

A triagem possibilita o diagnóstico precoce da doença, previne e/ou atenua a gravidade da crise perdedora de sal, além de evitar complicações em longo prazo, principalmente as relacionadas à hiponatremia neonatal, tais como retardo mental e/ou déficits cognitivos^{1,9,10}. Além disso, a triagem possui o benefício do diagnóstico neonatal da forma clássica virilizante simples, o que permite a imediata correção de erros de registro de sexo social em meninas com virilização genital importante e a instituição da terapia de reposição hormonal^{9,10,15}, prevenindo o desenvolvimento de pseudo-puberdade precoce e comprometimento da estatura final em ambos sexos.

Quando os programas de triagem neonatal para hiperplasia adrenal congênita tiveram início?

Nos Estados Unidos, o Alasca, no final da década de 1970, foi o primeiro estado a incluir a HAC no programa de triagem neonatal, pois a incidência desta doença é muito elevada entre os esquimós *Yupik* (1:282).¹⁶ Desde 2009, todos os 50 estados dos Estados Unidos e mais 12 outros países, incluindo França, Suécia, Suíça, Japão e Holanda, adotaram a triagem neonatal para HAC.³

No Brasil, o Estado de Goiás foi o primeiro a adotar o programa de triagem da HAC, com verbas do próprio Estado, sendo seguido pelo Estado de Santa Catarina. Alguns Estados, como Bahia, Minas Gerais, Rio Grande do Sul e São Paulo implementaram projetos pilotos e em dezembro de 2012, o Ministério da Saúde anunciou a inclusão da HAC no Programa Nacional de Triagem Neonatal (PNTN), inicialmente em 9 estados.^{10,17} (Figura 2).

Figura 2. Mapa da incidência da hiperplasia adrenal congênita no Brasil¹⁰.

Por que a triagem neonatal para hiperplasia adrenal congênita é indicada?

Apesar da sua eficácia, sobretudo no diagnóstico da forma clássica perdedora de sal, um dos grandes problemas da triagem da HAC é a elevada frequência de resultados falso-positivos (FP).^{1,10,18} Considerando que em países desenvolvidos, de forma geral, a HAC é adequadamente diagnosticada sem a triagem neonatal, diversos estudos não consideraram esta triagem custo-efetiva.¹⁹

Entretanto, dados de aproximadamente 210 pacientes do Hospital das Clínicas de São Paulo (HCFMUSP), diagnosticados clinicamente, antes da Triagem Neonatal, mostrou uma proporção menor de pacientes do sexo masculino com a forma perdedora de sal, sugerindo perda do diagnóstico por óbito no período neonatal. Além disso, uma parcela significativa dos pacientes possuía diagnóstico tardio, com presença de comorbidades, como a pseudo-puberdade precoce, muitas vezes necessitando da adição de medicações de custo elevado para bloquear a puberdade e recuperar o potencial de altura final. Resultados semelhantes foram observados em outros

serviços universitários brasileiros, a exemplo da Universidade Federal da Bahia.

Tem-se debatido sobre a necessidade da triagem para HAC em meninas, considerando-se que a atipia genital é uma característica evidente ao nascimento, o que facilitaria o diagnóstico precoce. No Texas, onde a HAC está incluída no programa de triagem, observou-se que o diagnóstico foi possibilitado apenas pelo resultado do teste neonatal em 8/15 crianças com as formas clássicas sendo que a atipia genital não foi identificada ao nascimento em 3/8 meninas.²⁰ Ainda, no Brasil, dados do Programa de Triagem Neonatal do Rio Grande do Sul demonstraram erros de registro civil em 2/9 meninas com ambiguidade genital, sendo possível o ajuste após o adequado diagnóstico da doença¹⁵. Dados não publicados do HCFMUSP, sugerem realidade ainda pior, a atipia genital não foi identificada ao nascimento em 40% das meninas, sendo que 16% delas foram registradas no sexo social masculino e metade destas últimas, por terem tido diagnóstico muito tardio, cresceram no sexo social masculino. No Estado de Goiás, que tem realizado a triagem da HAC, 28% das pacientes com atipia genital só tiveram sua identificação após o teste da triagem.²¹

Esses dados reforçam a importância da Triagem Neonatal para HAC para ambos os sexos em todo o Brasil.

Como é feita a triagem neonatal para hiperplasia adrenal congênita no Brasil?

O processo de triagem consiste na coleta do sangue por meio de uma punção no calcanhar em cartões de papel-filtro, idealmente dois a cinco dias após o nascimento.²² A dosagem da 17OHP é realizada nas amostras de todos os recém-nascidos. Em seguida, são determinados os limites de convocação para nova coleta em papel de filtro (resultados moderadamente alterados) e de emergência (resultados muito elevados) para imediata consulta médica e coleta de exames confirmatórios séricos.^{10,23}

Na prática, a idade na coleta de amostras varia no Brasil. A maioria dos estados realiza entre o 4º e o 7º dia de vida nas Unidades Básicas de Saúde, enquanto que o estado de São Paulo as realiza 48-72 horas após o parto nas maternidades privadas ou nos hospitais públicos da Grande São Paulo. Todas as amostras são então enviadas para os laboratórios de referência de triagem neonatal.

As técnicas disponíveis para a dosagem da 17OHP neonatal incluem radioimunoensaio (RIA), testes imunoenzimáticos -*enzyme-linked immuno absorbent assay* (ELISA) e testes imunofluorimétricos (FIA). O método imunofluorimétrico *dissociation-enhanced, lanthanide fluorescence immunoassay* (DELFI[®]) tem sido o mais utilizado para a triagem inicial.²⁴

Existe diferença na triagem neonatal do recém-nascido a termo *versus* o recém-nascido pré-termo ou em situações especiais?

Sim. Aproximadamente 70% dos resultados falso-positivos na triagem da HAC são devidos à prematuridade e/ou baixo peso ao nascer.^{1,6,10,25-29}

Quais são as causas de resultados falso-positivos e falso-negativos na triagem neonatal para hiperplasia adrenal congênita?

Prematuridade e baixo peso ao nascer são as principais causas de resultados falso-positivos na triagem neonatal para HAC.^{10,27-29} Considerando, que no Brasil a ultrassonografia obstétrica não é realizada rotineiramente para se aferir a idade gestacional, tem-se adotado no país a padronização dos valores de normalidade da 17OHP de acordo com a faixa peso do recém-nascido (<1500g, 1500-2000g, 2001-2500g e >2500g).^{8,10}

A coleta de amostras antes de 24-48 horas de vida pode prejudicar a precisão diagnóstica da triagem neonatal, contribuindo para a ocorrência de resultados falso-negativos, com alguns dos recém-nascidos gravemente afetados apresentan-

do valores de 17OHP neonatal apenas moderadamente aumentados, quando submetidos à coleta precoce de amostras (< 48 horas de vida).^{8,30,31}

A administração de glicocorticoides à mãe no periparto, ou ao recém-nascido previamente à coleta da amostra neonatal, são fatores que contribuem para resultados falso-negativos. Nestes casos, a triagem da HAC é ainda recomendada, mas o uso da medicação deve ser registrado no formulário de pedido.³² A realização de uma se-

gunda amostra de controle pode revelar valores mais elevados preditores da doença.¹⁵

A transfusão sanguínea é outro fator relacionado a resultados falso-negativos. Se um bebê necessitar de uma transfusão, deve ser obtida uma amostra de sangue antes da transfusão ou, no mínimo, 5 dias após.³¹

O Quadro 1 sumariza as principais causas de resultados falso-positivos e falso-negativos da 17OHP na triagem neonatal da HAC.

Quadro 1. Causas de resultados falso-positivos e falso-negativos da 17OHP na triagem neonatal da hiperplasia adrenal congênita.

Resultados falso-positivos	Resultados falso-negativos
<ul style="list-style-type: none"> • Recém-nascidos: <ul style="list-style-type: none"> – Prematuros, – Baixo peso, – Desidratados, – Ictéricos, – Submetidos a estresse neonatal. • Metodologia utilizada (reação cruzada com outros esteroides) 	<ul style="list-style-type: none"> • Recém-nascidos: <ul style="list-style-type: none"> – Tratados com corticosteroide, ou cujas mães foram tratadas com corticosteroide durante a gestação, – Submetidos a transfusão sanguínea. • Coleta do exame do teste do pezinho antes do 2º dia de vida.

Como interpretar o resultado da 17OHP na triagem neonatal para hiperplasia adrenal congênita?

O resultado da 17OHP no Teste de Triagem Neonatal deve levar em consideração o peso ao nascimento e a idade da coleta (Quadro 2), além das causas de resultados falsos-positivos e falsos-negativos discutidas anteriormente

Na maior parte dos Estados brasileiros, seguindo orientações do Ministério da Saúde, é utilizado o percentil 99,0 como ponto de corte para a 17OHP.⁸ Estudo realizado pelo HCFMUSP em parceria com Serviço de Referência em Triagem Neonatal do Estado São Paulo (APAE-SP) mostrou que a utilização do percentil 99,8 foi capaz de aumentar o valor preditivo do teste, sem incorrer na presença de resultados falso negativos.³³

Os valores abaixo do percentil de corte são considerados normais. Aqueles com valores acima de 2 vezes o percentil de corte são considera-

dos altamente suspeitos e receberão convocação de emergência para consulta médica e realização de exames confirmatórios séricos. Valores moderadamente elevados (acima do percentil de corte, mas menor que seu dobro) terão a coleta no papel-filtro repetida e os critérios de avaliação serão os mesmos para a primeira amostra. Se a nova amostra neonatal não apresentar valores normais, o recém-nascido deverá ser encaminhado para consulta médica e mantido em acompanhamento até que haja uma definição diagnóstica ou a normalização da 17OHP sérica.³³⁻³⁵

Os recém-nascidos, com resultados positivos serão encaminhados para a consulta médica e deverão receber atenção especial para a avaliação de presença de atipia genital nas meninas ou presença de “hipospádia” em bebês de sexo social masculino sem a presença de testículos palpáveis no períneo ou região inguinal. Aqueles com resultados bastante elevados devem ser examinados com urgência a fim de evitar ou minimizar a crise de perda de sal.

Qual a conduta a ser tomada quando do recebimento de resultado alterado na triagem neonatal para hiperplasia adrenal congênita?

Dependendo da magnitude da elevação dos valores alterados em papel de filtro, a segunda coleta poderá ser realizada em papel filtro ou será necessária a realização de testes confirmatórios no soro e consulta médica imediata.

O Quadro 2, mostra a recomendação do Ministério da Saúde, usando como variáveis: o peso ao nascimento (<1499g, 1500-1999g, 2000-2499g e >2500g), tempo de vida no momento da coleta (3^o-5^o dia) e definindo como ponto de corte para a 17OHP, o P. 99%. Recém-nascidos

com valores de 17OHP $\leq$ P. 99% serão considerados como normais. Valores de 17OHP > P.99% e < 2 vezes o P.99%, será considerado como aumento moderado, devendo repetir segunda dosagem em papel de filtro. Valores de 17-OHP > P. 99% e > 2 vezes o P. 99%, serão considerados altamente suspeitos e as crianças serão convocadas para avaliação médica e testes confirmatórios.^{33,35}

O Quadro 3 mostra a recomendação da Universidade de São Paulo, usando como variáveis para triagem neonatal da 17-OHP: peso ao nascimento (<1500g, 1500-2000g, 2001-2500g e >2500 g), tempo de vida no momento da coleta (<72 h ou > 72 horas de vida) e o ponto de corte para 17OHP situado no P.99.8%.^{33,35}

Quadro 2. Conduta a ser tomada quando do recebimento dos resultados da triagem neonatal da 17OHP no papel de filtro, de acordo com peso ao nascimento, tempo de vida na coleta (3^o-5^o dia), utilizando o P.99% recomendado pelo Ministério da Saúde.

Peso ao nascimento (g)	17OHP (ng/mL) Entre 3 ^o -5 ^o dia de vida	17OHP (ng/mL) Entre 3 ^o -5 ^o dia de vida	17OHP (ng/mL) Entre 3 ^o -5 ^o dia de vida
< 1500	<110	≥ 110 e < 220	≥ 220
$\geq 1501-1999$	< 43	≥ 43 e < 86	≥ 86
$\geq 2000-2499$	<28	≥ 28 e < 56	≥ 56
≥ 2501	<15	≥ 15 e < 30	≥ 30
Interpretação	17OHP normal	17OHP elevada	17OHP muito elevada
Conduta	Desnecessário novas investigações	Repetir dosagem em papel-filtro	Convocação de emergência para teste confirmatório sérico

Quadro 3. Conduta a ser tomada quando do recebimento dos resultados da triagem neonatal da 17OHP no papel de filtro (ng/mL), de acordo com o peso ao nascimento tempo de vida na coleta (< 72 e > 72 horas) para a convocação de segunda coleta em papel de filtro e para convocação de emergência com coleta de soro, utilizando o percentil 99,8, recomendado pela USP-SP.

Peso ao nascimento (g)	Resultado da 17OHP (ng/mL) de acordo com a idade da coleta da triagem neonatal e o peso ao nascimento					
	<72 h	>72 h	<72h	>72h	<72h	>72h
<1500	<120	<173	120-240	173-346	>240	>346
1500-2000	<71	<90	71-142	90-180	>142	>180
2001-2500	<39	<66	39-78	66-132	>78	>132
>2500	<20	<25	20-40	25-50	>40	>50
Interpretação	17-OHP normal		17-OHP elevada		17-OHP muito elevada	
Conduta	Desnecessário novas investigações		Repetir dosagem em papel-filtro		Convocação de emergência para teste confirmatório sérico	

Como confirmar laboratorialmente a suspeita diagnóstica da hiperplasia adrenal congênita?

São encaminhados para coleta de testes confirmatórios séricos, aqueles RNs cujos valores da primeira amostra em papel de filtro são altamente suspeitos (>2 vezes, o percentil de corte) e aqueles cuja segunda amostra em papel de filtro permaneceu alterada (Quadros 2 e 3).

As amostras de soro serão utilizadas para as dosagens da 17OHP, cortisol, androstenediona, testosterona, sódio, potássio. Embora não façam parte do Protocolo Clínico de Diretrizes Terapêuticas do Ministério da Saúde, alguns Serviços também solicitam ureia, creatinina, glicemia e gasometria, uma vez que muitos recém-nascidos com atraso na realização da triagem neonatal, chegam nas consultas gravemente desidratados, acidóticos, hipovolêmicos e hipoglicêmicos.

A coleta rápida e envio imediato das amostras para o Laboratório de Triagem Neonatal visa permitir tempo hábil para realização do diagnóstico e tratamento precoce dos afetados com a forma perdedora de sal.³⁵ Em pacientes com genitália atípica, a determinação do sexo genético pode ser guiada pela realização de cariótipo, exame que demora em média 30 dias, ou da realização da reação de polimerização em cadeia para sequências do cromossomo Y, técnica de resultado mais rápido e já disponível em vários estados.

Quais são as peculiaridades da dosagem da 17OHP na confirmação diagnóstica da HAC?

Diferentes metodologias são utilizadas na dosagem sérica da 17OHP, destacando-se o radioimunoensaio (RIA), imunoensaios e a cromatografia líquida acoplada à espectrometria de massas em *tandem* (LC-MS/MS). Esta última metodologia tem a vantagem de dosar simultaneamente mais de um composto esteroide, separando-os por peso molecular e não por reações

antígeno-anticorpo, o que confere especificidade maior e interferência menor das reações cruzadas entre diferentes analitos.¹

Entretanto, 7% a 17% dos casos com resultados falso-positivos em papel de filtro ainda não apresentam normalização da 17OHP no teste confirmatório sérico. Estes recém-nascidos assintomáticos e que permanecem com resultados alterados necessitarão de seguimento clínico e laboratorial até que haja definição diagnóstica ou normalização da 17OHP sérica.^{29,35,36} Este procedimento eleva os custos para o sistema de saúde e causa ansiedade às famílias, o que evidencia a necessidade da padronização de testes confirmatórios com maior especificidade.³⁷

Tem sido proposto, para a otimização dos testes confirmatórios, que o valor preditivo positivo (VPP) da concentração sérica elevada da 17OHP pode ser melhorado se interpretada em conjunto com outros esteroides, como cortisol, androstenediona e 21-desoxicortisol. Adicionalmente, outras metodologias têm sido avaliadas, tais como a dosagem da 17OHP por LC-MS/MS, bem como a dosagem da androstenediona, cortisol, 21-desoxicortisol pelo mesmo método. Outra maneira de se melhorar significativamente o VPP dos testes confirmatórios é a de se avaliar as razões entre os esteroides, tais como 17OHP/cortisol, 17-OHP+androstenediona/cortisol e 17-OHP+21-desoxicortisol/cortisol.^{38,39}

Quando indicar exames de imagem nos pacientes com suspeita de hiperplasia adrenal congênita?

Recém-nascidos com atipia genital e elevação da 17OHP no teste de triagem neonatal, devem realizar ultrassonografia para identificação de útero e, se possível, ovários e auxílio na definição do sexo genético. No entanto, é necessário que o exame seja realizado por profissional experiente na identificação de estruturas *mullerianas* em recém-nascidos. A presença de genitália interna feminina (útero e ovários) em RN com alteração de 17OHP muito elevada sugere fortemente tratar-se de menina virilizada.

Quando indicar testes confirmatórios moleculares?

Além dos métodos hormonais para confirmação diagnóstica de um teste de triagem positivo, a pesquisa de mutações no gene da 21-hidroxilase (*CYP21A2*) tem também sido proposta como teste confirmatório, em análise de amostras de DNA do sangue periférico ou do papel de filtro.^{15,36,40,41}

Porém, a biologia molecular não é essencial na confirmação diagnóstica dos recém-nascidos sintomáticos e com testes hormonais claramente alterados; sua maior utilidade seria em descartar a doença em pacientes assintomáticos com valores persistentemente elevados, diminuindo o tempo de acompanhamento desnecessário. Um exemplo prático são os casos de recém-nascidos do sexo masculino assintomáticos com valores moderadamente elevados, nos quais a biologia molecular seria importante para diferenciá-los entre portadores da forma virilizantes simples, forma não clássica ou de resultados falso-positivos¹⁵. Em estudo prévio, observou-se que alguns dos casos falso-positivos demoraram de 6 a 12 meses para normalizarem os valores de 17OHP sérica, o que eleva o custo da triagem e causa grande estresse emocional às famílias.³⁶

O último Consenso da *Endocrine Society*⁴², recomenda testes moleculares nas seguintes situações: (1) Resultados dos hormônios adrenocorticais após teste de estímulo com ACTH forem inconclusivos; (2) Dosagem hormonal não puder ser feita com segurança (ex: uso atual de glicocorticoide); ou (3) Para aconselhamento genético.

Como efetuar o tratamento das formas clássicas de hiperplasia adrenal congênita diagnosticadas na triagem neonatal?

Os objetivos gerais do tratamento da HAC-21OH são suprir as deficiências de glicocorticoide e mineralocorticoide a fim de: prevenir a crise

de insuficiência adrenal, reduzir a hipersecreção adrenocortical dos esteroides androgênicos, promover crescimento e puberdade adequados, com preservação do potencial de altura final. Deve-se evitar o excesso de glicocorticoide no tratamento da doença.

O tratamento é indicado para todos os pacientes com a forma clássica da doença. Na infância são preconizados glicocorticoides de curta ação por via oral,¹ cujas doses de manutenção são: hidrocortisona 10 a 15 mg/m² por dia e acetato de cortisona 18 a 20 mg/m², divididos em 3 tomadas.

Vale ressaltar que o acetato de hidrocortisona/cortisona em comprimido e em suspensão não são bioequivalentes. A suspensão é inadequada para controle da HAC, pois não há uma distribuição uniforme da medicação na solução.¹ O recomendável é diluir um comprimido macerado em pequena quantidade de líquido imediatamente antes da administração. Infelizmente, o acetato de cortisona e hidrocortisona, sob a forma de comprimidos, não são comercializados no Brasil, requerendo seu preparo em farmácias de manipulação, o que pode levar a problemas de controle de qualidade e biodisponibilidade com potencial de alterar a resposta terapêutica. É necessário, com urgência, que o Ministério da Saúde a agências reguladoras como a ANVISA, busquem soluções imediatas para esse problema.

Para os portadores da forma clássica perdutora de sal, o tratamento inclui além da reposição de glicocorticoide, a reposição de mineralocorticoide:^{1,3} 9 α -fluor-hidrocortisona (acetato de fludrocortisona) na dose de 100-200 mcg/dia no período neonatal. Existe uma ampla variação da necessidade de dose de reposição entre os recém-nascidos, com dose média inicial até o sexto mês de vida de aproximadamente 200 mcg/dia. A necessidade de mineralocorticoide tende a diminuir após os primeiros meses de vida, principalmente devido à melhora da imaturidade renal presente ao nascimento. A dose deve ser ajustada de acordo com a pressão arterial e valores de renina. A dose média aos 2 anos de idade fica, geralmente, em torno de 100-150 mcg/dia e aos 4 anos, em torno de 50 mcg/dia.⁴¹

Para as crianças em aleitamento materno exclusivo ou em uso de fórmulas infantis, é essencial a oferta de cloreto de sódio (sal de cozinha) na dose de 1 a 2 g/dia, diluído em 20-30 mL de água e oferecido entre as mamadas. A quantidade de sódio no leite materno/fórmulas é insuficiente para suprir a demanda desses pacientes.³

O Quadro 4 sumariza o tratamento das formas clássicas da hiperplasia adrenal congênita⁴² para crianças e adolescentes em fase de crescimento⁴².

Os pacientes e familiares deverão ser orientados sobre a prevenção, reconhecimento dos sintomas e as modificações do tratamento durante situações de estresse. Se a ingestão oral não estiver comprometida, durante situações de estresse (ex: temperatura > 38,5°C, cirurgia, grande trauma), a dose do glicocorticoide deve-

rá ser duplicada ou triplicada enquanto durar o estresse. O último *Guideline* da *Endocrine Society* sugere, em situações de estresse, ao invés de administrar a hidrocortisona ou cortisona de 8/8 horas, como de costume, aumentá-la para uma quarta dose, administrando o medicamento de 6/6 horas⁴². Se a ingestão oral estiver comprometida, deverá ser administrado hidrocortisona conforme descrito no Quadro 5. A dose do mineralocorticoide não necessita ser aumentada em situações de estresse.

Adicionalmente, os pacientes e familiares deverão ser instruídos sobre o uso de um cartão, pulseira ou colar de identificação descrevendo que eles fazem uso crônico de corticoterapia devido à hiperplasia adrenal congênita.

O Quadro 6 sumariza as orientações a serem tomadas durante situações de estresse.

Quadro 4. Terapia de manutenção de pacientes com formas clássicas de hiperplasia adrenal congênita, que se encontram em fase de crescimento⁴¹.

Medicamento	Dose diária	Distribuição das doses
Hidrocortisona (comprimidos)	10-15 mg/m ² /dia	3 vezes ao dia
Cortisona (comprimidos)	18-20 mg/m ² /dia	3 vezes ao dia
Fludrocortisona (comprimidos)	50-200 mcg/dia	1-2 vezes ao dia
Cloreto de sódio (sal de cozinha)	1-2 gramas/dia (17-34 mEq/dia)	Dividido em várias refeições

Quadro 5. Doses de hidrocortisona durante situações de estresse.

Faixa etária	Dose inicial de hidrocortisona (IM, ou EV)
Lactentes e pré-escolares	25 mg
Escolares	50 mg
Adolescentes	100 mg

*Após a dose inicial, acima descrita, a terapia pode ser mantida, se necessário, com modificação das doses subsequentes para ½ a ¾ da dose inicial, administradas a cada 6 horas.

Quadro 6. Orientações para o tratamento de pacientes com hiperplasia adrenal congênita durante situações de estresse.

Nome: é tem Hiperplasia Adrenal Congênita

Faz uso de:

Acetato de Hidrocortisona, na dose de: ; e

Acetato de Fludrocortisona, na dose de:

Na última consulta, em / /, o peso era de Kg e a altura de cm

Em situações de estresse, agir da seguinte forma:

Estresse Mínimo

- Exemplos: Imunização, resfriado sem febre, tosse.
- Conduta: Não há necessidade de modificar a dose do glicocorticoide.

Estresse leve

- Exemplos: Vômitos e diarreia, redução da ingesta oral, infecções com febre (até 38,5°C), extração dentária simples, pequenas suturas, cirurgia sob anestesia local e traumas leves.
- Conduta: Dobrar ou triplicar a dose em uso do Acetato de Hidrocortisona, ou modificá-la de 8/8 horas, para 6/6 horas, até resolução dos sintomas, quando retornará ao corticoide em uso na sua dose habitual.

Estresse moderado

- Exemplos: Desidratação, recusa alimentar, recuso alimentar, febre acima de 38,5°C, cirurgias, extrações dentárias múltiplas.
- Conduta: Triplicar a dose em uso do acetato de hidrocortisona, por via oral. Se a ingestão oral não for possível, administrar dose de ataque de Hidrocortisona, IM/EV, de acordo com a idade (lactentes e pré-escolares: 25 mg; escolares: 50 mg; e adolescentes: 100 mg) (I); enquanto durar o stress (24-48 horas). Se o estresse persistir e a ingestão oral for impossibilitada, manter Hidrocortisona (30-50 mg/m²/dia, IM/EV), até resolução dos sintomas, quando retornará ao corticoide em uso na sua dose habitual.

Estresse grave

- Exemplos: Crise de perda de sal, choque, pneumonia grave, politrauma, queimadura grave, múltiplas fraturas, infecção sistêmica grave, cirurgia de grande porte.
- Conduta: Administrar dose de ataque de Hidrocortisona, IM/EV, de acordo com a idade (lactentes e pré-escolares: 25 mg; escolares: 50 mg; e adolescentes: 100 mg). Manter Hidrocortisona (50-100 mg/m²/dia, IM/EV), até resolução dos sintomas, quando retornará ao corticoide em uso na sua dose habitual. Em caso de crise de perda de sal: expandir e restaurar volume (EV), corrigir hiponatremia, hipercalemia e hipoglicemia

Como fazer o acompanhamento dos pacientes com formas clássicas da hiperplasia adrenal congênita?

O acompanhamento dos bebês afetados deverá ser feito por equipe multidisciplinar. Depois de iniciado o tratamento, especialmente nos perdedores de sal, recomenda-se acompanhamento semanal durante os primeiros 2 meses de

vida, com dosagens de sódio e potássio. Deve-se, a cada consulta, observar a aderência ao tratamento, o ganho pômbero-estatural, procurando-se evitar sinais de hiperandrogenismo ou de hipercortisolismo, hipo ou hipertensão, além de realizar exames complementares rotineiros (androstenediona, 17-hidroxiprogesterona e para o sexo feminino também testosterona).

Segundo as recomendações do Ministério da Saúde¹⁶, a frequência das consultas deve

ser gradualmente modificada de acordo com o tempo de vida da criança (Quadro 7). Valores de Atividade de Renina Plasmática (ARP) ou de renina na metade inferior da normalidade indicam super-tratamento, associando-se com diminuição da velocidade de crescimento e hipertensão. A radiografia do punho e mão esquerda para idade óssea deve ser realizada

anualmente, após a idade de 2 anos.¹⁷ Não se deve esperar normalização da 17OHP sérica nas formas clássicas; em pacientes com valores abaixo de 10ng/ml, deve-se observar a velocidade de crescimento, já nos casos com valores da 17OHP abaixo de 5 ng/mL, recomenda-se reduzir a dose de glicocorticoide para evitar o super-tratamento.

Quadro 7. Recomendações do Ministério da Saúde para frequência das consultas e exames dos pacientes com HAC.

Idade	Frequência das consultas	Exames sugeridos
2-6 meses	1/1 mês	Sódio, Potássio 17-OHP Androstenediona Testosterona Atividade da renina plasmática
6-12 meses	2/2 meses	
>12 meses	3/3 ou 4/4 meses	

Quando indicar a correção cirúrgica da genitália externa?

A genitoplastia feminizante deve ser realizada antes dos 2 anos de idade e tem como objetivos corrigir a virilização da genitália, assim como permitir a sua funcionalidade.¹ A cirurgia consiste na clitoroplastia, com manutenção da glândula clitoriana tópica, ressecção do tecido fálico erétil, preservação do pedículo vasculo-nervoso, abertura e ampliação do seio urogenital a fim de assegurar um orifício amplo e sem estenose, dispensando a necessidade de cirurgia ou mesmo do uso de moldes para dilatação na idade adulta.⁴³ Em pacientes com o diagnóstico tardio, a cirurgia feminizante só deve ser indicada após avaliação psicológica para definição da identidade de gênero.

Quando indicar suporte psicológico?

Como toda doença crônica, é importante o acompanhamento psicológico para auxiliar na aceitação da doença pelo paciente e seus fami-

liares, na aderência à terapia e na adequação do sexo social com a identidade de gênero.⁴⁴

Considerações finais

O principal desafio à triagem para HAC é a determinação de um ponto de corte para a 17OHP que resulte em adequado custo/benefício. A utilização de diferentes valores de referência, conforme a idade gestacional ou peso ao nascimento e idade à coleta são estratégias frequentemente adotadas para tornar viável o processo de triagem, promovendo redução da taxa de falso-positivos.

Para se atingir o objetivo da triagem neonatal todo o processo deve ser realizado de forma rápida para que o tratamento adequado seja iniciado o mais precoce possível. Para isso é necessário o trabalho conjunto do gestor municipal para o rápido envio das amostras neonatais, do Laboratório de Triagem, da rede complementar e do ambulatório especializado. O paciente com HAC deve ter seu seguimento realizado por uma equipe multiprofissional especializada, envolvendo pediatras, endocrinologistas, cirurgiões, psicólogos e enfermeiros.

O Quadro 8 faz um resumo de “*red flags*” a serem memorizadas pelos pediatras quando da avaliação de resultados alterados na triagem neonatal para HAC.

Quadro 8. Aspectos a serem lembrados pelo pediatra quando da avaliação da triagem neonatal da hiperplasia adrenal congênita (HAC).

- A triagem neonatal para HAC reduz a demora diagnóstica, que provoca risco de vida, em pacientes do sexo masculino e o erro da designação do sexo em pacientes femininas virilizadas.
- Deve-se avaliar a possibilidade de resultados falsos positivos e falsos negativos
- Os resultados devem ser interpretados de acordo com o peso ao nascimento.
- O pediatra deve estar atento para dobrar ou triplicar a dose do glicocorticoide em vigência de situações de estresse.
- O pediatra que atua em unidades de pronto-atendimento deve estar atento para administrar hidrocortisona endovenosa associado à hidratação endovenosa com soro glicofisiológico em situações de estresse agudo, principalmente se o paciente apresentar vômitos, sinais de desidratação e hipotensão.
- O pediatra deve recomendar a administração de hidrocortisona endovenosa na indução anestésica de qualquer cirurgia.
- A confirmação diagnóstica, tratamento e acompanhamento da HAC devem ser feitos por especialistas, preferencialmente em centros terciários.

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