UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

Tese de Doutorado

Estudo do Eixo Hipotálamo Hipófise Adrenal no Cushing Leve através do Cortisol Sérico no Pós-operatório de Pacientes com Doença de Cushing e Avaliação da Monitorização Ambulatorial da Pressão Arterial de 24 horas nos Pacientes com Acromegalia

Fabíola Costenaro

Porto Alegre, abril 2016.

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Fabíola Costenaro

Tese apresentada como requisito parcial para a obtenção do título de Doutor em Endocrinologia, à Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

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SUMÁRIO:

Capítulo II

Artigo original em inglês: "*The role of ambulatory blood pressure monitoring in patients with acromegaly"*

LISTA DE ABREVIATURAS E SIGLAS:

Em português

(ACTH) Hormônio Adrenocorticotrófico

(AIP) do inglês *Aryl hydrocarbon receptor interacting protein*

(CTE) Cirurgia hipofisária transesfenoidal

(CV) Cardiovascular

(DM) Diabetes melito

(DC) Doença de Cushing

(GH) do inglês *Growth hormone*

(GHRH) do inglês *Growth hormone releasing hormone*

(HCPA) Hospital de Clínicas de Porto Alegre

(HAS) Hipertensão arterial sistêmica

(h) horas

(IGF1) do inglês *Insulin-like growth factor type 1*

(MAPA) Medida da pressão arterial ambulatorial de 24 horas

(PA) Pressão arterial

(SC) Síndrome de Cushing

(UFC) Cortisol livre urinário de 24 horas

LISTA DE ABREVIATURAS E SIGLAS:

Em Inglês

(ABPM) 24-hours ambulatory blood pressure monitoring

(ACTH) Adrenocorticotrophic hormone

(AM) Antemeridian

(BMI) Body Mass Index

(BP) Blood pressure

(CD) Cushing's disease

(CS) Cushing's syndrome

(CT) Computer tomography

(CV) Cardiovascular

(DBP) Diastolic Blood Pressure

(DHEAS) Dehydroepiandrosterone sulfate

(DM) Diabetes melito

(ECLIA) Electrochemiluminescence Immunoassay

(EF) Ejection fraction

(GH) Growth hormone

(HDL) High density cholesterol

(h) Hour

(IGF1) Insulin-like growth factor type 1

(LDL) Low density cholesterol

(LVT) Left ventricular thickness

(LVM) Left ventricular mass

(MRI) Magnetic resonance image

(n) Number of patients

(OGTT) Oral glucose tolerance test

(OSAHS) Obstructive sleep apnea hypopnea syndrome

(PO) Postoperatively

(PM) Postmeridian

(RIA) Radioimmunoassay

(RLVH) Relative left ventricular hypertrophy

(RLVT) Relative left ventricular thickness

(ROC) Receiver Operating Curve

(SBP) Systolic Blood Pressure

(TCh) Total cholesterol

(TSS) Transsphenoidal surgery

(ULN) Upper limit of normality

(UFC) 24hours urinary free cortisol /

(1mg-DST) 1-mg overnight dexamethasone

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Capítulo II

APRESENTAÇÃO:

Esta Tese de Doutorado **"Estudo do Eixo Hipotálamo Hipófise Adrenal no Cushing Leve através do Cortisol Sérico no Pós-operatório de Pacientes com Doença de Cushing e Avaliação da Monitorização Ambulatorial da Pressão Arterial de 24 horas nos Pacientes com Acromegalia"**segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Metabolismo e Nutrição da Faculdade de Medicina da UFRGS, na forma de dois artigos originais, sendo apresentada da seguinte maneira:

1. Introdução
2. Desenvolvimento: dois capítulos

Capítulo I- artigo original: ***"Serum cortisol dynamics after transsphenoidal surgery in mild Cushing's disease:"***

Capítulo II- artigo original: ***"The role of ambulatory blood pressure monitoring in patients with acromegaly".***

3. Conclusões

Este trabalho foi realizado com o apoio das seguinte instituições:

- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES): através de bolsa de Doutorado.

- Fundo de Incentivo a Pesquisa do Hospital de Clínicas (FIPE).

RESUMO:

No capitulo I desta tese estudamos pacientes em remissão cirúrgica da doença de Cushing (DC) e seu comportamento em relação aos níveis de cortisol no período pós-operatório. Níveis baixos de cortisol no período pós-operatório estão claramente relacionados com a remissão cirúrgica da DC. Entretanto, alguns pacientes em remissão apresentam elevado pico de cortisol sérico durante as primeiras 48 horas (h) da avaliação após a cirurgia transesfenoidal e muitas vezes apresentam queda mais tardia do cortisol no período pós-operatório. O objetivo principal deste estudo foi caracterizar este grupo de pacientes para melhor compreensão do comportamento do eixo hipotálamo-hipófise-adrenal destes casos. Pela possibilidade de pacientes com menor gravidade da DC apresentarem menor supressão dos corticotrofos peritumorais, foram avaliados os critérios clínicos e laboratoriais relacionados ao hipercortisolismo no momento do diagnóstico da doença objetivando confirmar a hipótese de que os pacientes com uma forma mais leve da DC teriam maiores picos de cortisol nas primeiras 48 h da avaliação pós-operatória. Foram avaliados 70 pacientes em remissão cirúrgica da DC, acompanhados em média por 6 anos, procedentes de uma coorte em acompanhamento regular no ambulatório de Neuroendocrinologia do Hospital de Clínicas de Porto Alegre. Foi observado que menores níveis de cortisol livre urinário de 24 h no período pré-operatório foram associados a maiores picos do cortisol sérico no período pós-operatório. O ponto de corte definido para Cushing leve foi o cortisol livre urinário de 24 h abaixo de três vezes o limite superior da normalidade para o método utilizado (n=39). Outros critérios bioquímicos envolvidos na avaliação do comprometimento do eixo hipotálamo hipófise adrenal no momento do diagnóstico da

síndrome de Cushing e da DC foram igualmente avaliados, entre eles: a intensidade de supressão do cortisol sérico após 1 mg de dexametasona *overnight* ou após 8mg de dexametasona, níveis de cortisol sérico à meia-noite, de ACTH plasmático e de sulfato de dehidroepiandrosterona, entretanto nenhum deles foi capaz de identificar diferentes picos de cortisol sérico durante as primeiras 24-48 h do período pós-operatório. Estes 39 pacientes, também possuíam menor comprometimento da função sexual e menor prevalência de fragilidade capilar no momento da investigação da DC. Em 10-12 dias ou mesmo em 30 dias após a cirurgia transesfenoidal os níveis de cortisol sérico foram semelhantes entre pacientes com ou sem DC leve e foram compatíveis com remissão da DC.

No capitulo II foi estudado o comportamento da pressão arterial (PA) sistêmica de pacientes com acromegalia. O método utilizado foi a monitorização ambulatorial de 24h (MAPA). O artigo retrata 37 pacientes que fazem parte da coorte de pacientes com acromegalia em acompanhamento no ambulatório de Neuroendocrinologia do HCPA. O objetivo foi determinar a homeostase pressórica destes pacientes, sua associação com critérios bioquímicos de atividade da acromegalia e parâmetros ecocardiográficos. Os pacientes foram estratificados em doença em remissão, controlada por medicamentos ou ativa. Foi realizada a aferição da PA sistêmica durante as consultas ambulatoriais, a MAPA, e a avaliação de parâmetros metabólicos (perfil lipídico, níveis glicêmicos, aferição do peso e altura corporal e a medida do índice de massa corporal). Devido à elevada prevalência de síndrome de apneia do sono ou hipopneia em pacientes com acromegalia e sua possibilidade de interferência nos níveis pressóricos, esta alteração também foi investigada. Foi observado que a medida da PA sistêmica de consultório

superestimou a prevalência de hipertensão nesta coorte. A PA aferida pela MAPA classificou 23% dos indivíduos analisados como hipertensos, enquanto a pressão de consultório classificou 32% deles como portadores de hipertensão. Além disto, as medidas de pressão realizadas pela MAPA foram associadas com os parâmetros de atividade da acromegalia, seja com os níveis de fator de crescimento semelhante à insulina tipo 1 (IGF1, do inglês *Insulin-like growth factor type 1)* seja com os níveis de hormônio de crescimento humano (GH, do inglês *Growth hormone*). Os níveis pressóricos aferidos pela MAPA, especialmente PA sistólica noturna, foram associados a parâmetros ecocardiográficos de cardiomiopatia. Além disto, a ausência de descenso noturno da PA sistólica foi associada positivamente a maiores níveis de GH e à presença de hipertrofia ventricular esquerda.

Conclusões: Os dados destes dois estudos acrescentam importantes contribuições para o entendimento destas patologias. No estudo 1, pacientes com níveis de cortisolúria de 24 h < 3 x acima do limite superior da normalidade do método utilizado para esta medida, apresentaram maior pico de cortisol nas primeiras 24 h de pós-operatório, demonstrando maior resposta ao estresse cirúrgico dos corticotrofos peri-tumorais. Estes pacientes com cortisolúria de 24 h < 3 x o limite superior da normalidade foram classificados como portadores de DC leve. Estes pacientes podem necessitar aguardar entre 10 dias a 1 mês para a completa definição da remissão da DC após o procedimento cirúrgico. No estudo 2, demonstrou-se que a utilização da MAPA em pacientes com acromegalia é uma ferramenta útil para o entendimento da homeostase pressórica, e ainda observou-se associação de hipertrofia relativa do ventrículo

esquerdo a níveis de PA sistólica noturna, mesmo após o ajuste para fatores de risco classicamente associados com hipertrofia ventricular esquerda.

INTRODUÇÃO:

Doença de Cushing

A Síndrome de Cushing (SC) é causada pelo excesso de produção de glicocorticoides adrenais, especialmente de cortisol. O hormônio adrenocorticotrófico (ACTH, do inglês *adrenocorticotrophic hormone)* é o hormônio estimulador da síntese adrenal destes glicocorticoides (1,2).

Os pacientes com SC caracterizam-se por manifestações clínicas decorrentes da exposição excessiva aos glicocorticoides, que incluem: ganho de peso com obesidade centrípeta, hipertensão arterial sistêmica, intolerância a glicose/diabetes melito, transtornos psiquiátricos, hipogonadismo/ irregularidade menstrual, entretanto, estas manifestações podem estar presentes em várias outras condições clínicas. Entretanto, outras manifestações clínicas são mais específicas de pacientes expostos a glicocorticoides em excesso, entre elas: pletora e rubicundez facial, fragilidade capilar com estrias violáceas na pele, fraqueza muscular proximal e osteopenia/osteoporose (1-5).

Para o diagnóstico sindrômico, além das manifestações clínicas, os pacientes necessitam de exames laboratoriais para a confirmação da SC. Deve-se sempre ser descartado o uso de medicações que contenham glicocorticoide em sua composição (etiologia mais comum da SC em geral) antes de iniciar a investigação do quadro. Uma vez descartado o uso de glicocorticoide exógeno, devem ser realizados os exames de triagem para o hipercortisolismo endógeno. Os exames que caracterizam a SC são o

aumento do cortisol livre urinário, a ausência de supressão do cortisol após 1mg de dexametasona *overnight,* a supressão do cortisol sérico após 2mg de dexametasona, e a elevação do cortisol a meia-noite seja pela dosagem sérica, seja pela salivar. (1,5). Considera-se quadro compatível com SC a alteração de no mínimo dois exames de triagem. Após o diagnóstico da SC, deve-se dosar o ACTH para definir a síndrome como dependente ou independente de ACTH (1,5).

Descartado o uso de medicações que contenham glicocorticoide em sua composição (etiologia mais comum da SC em geral), a origem da SC endógena pode ser dependente ou independente de ACTH. Quando ACTH dependente, pode ser originada de tumor hipofisário secretor de ACTH ou mais raramente de tumor ectópico produtor de ACTH. As etiologias ACTH independente são compostas pelas lesões adrenais: adenoma, câncer ou hiperplasia adrenal (1,5,6).

A doença de Cushing (DC) , é a principal etiologia da SC endógena, caracteriza-se pela presença de um tumor hipofisário produtor de ACTH, em 90% dos casos constituído de um microadenoma (1,5). Em raros casos, a etiologia tumoral está associada a mutações da linhagem germinativa, como na neoplasia endócrina múltipla tipo 1, na mutação da proteína que interage com o receptor de aryl-hidrocarboneto (do inglês *AIP*), entre outras (2). Seu manejo ainda permanece um desafio para os endocrinologistas até os dias atuais e muitas lacunas no seu entendimento ainda precisam ser resolvidas.

Uma vez diagnosticada a DC, o tratamento de escolha é a cirurgia hipofisária transesfenoidal (CTE). A taxa de remissão em bons centros atinge 60-94%, no entanto a recidiva da doença atinge 5-30% dos casos em cinco anos (7-11).

Apesar de ser uma patologia relativamente rara, com incidência estimada em 0,7 a 2,4 casos/milhão de habitantes/ano (1, 2, 12,13), ocorre em pacientes adultos jovens, na faixa etária de 30-40 anos, com predomínio do sexo feminino 3-5:1 e está associada à elevadas taxas de morbidade e mortalidade, em torno de 2-5 vezes acima da população normal (2,12-14). A remissão do hipercortisolismo está associada `a melhora, ao menos parcial, deste prognóstico.

Neste cenário, critérios de cura fidedignos são essenciais para definir a cura/remissão e se possível predizer recidiva da doença, mas várias questões ainda estão em aberto e precisam ser respondidas com evidências mais sólidas (15-17).

O paciente é considerado em remissão da DC quando preenche os seguintes critérios: duas ou três coletas de cortisol livre urinário de 24h (do inglês *UFC*) dentro do limite da normalidade (7,10,15,16),cortisol pós 1mg de dexametasona *overnight* <3 ug/dl (16), ou < 1,8 ug/dl (1) ou insuficiência adrenal/dependência do uso de corticoide exógeno (8,16-18), cortisol basal normal, ou ainda quando restabelece completamente o ritmo circadiano de secreção do cortisol (17).

A avaliação do eixo hipotálamo-hipófise adrenal no pós-operatório imediato através das dosagens de cortisol sérico seriado é de extrema importância, a curto e longo prazo, na avaliação da remissão da doença e, em algumas casuísticas, foi capaz de prever o risco de recidiva tumoral (16,21-23). Para melhor avaliação do comportamento do cortisol no pós-operatório da CTE, é fundamental que não seja suplementado glicococorticoide exógeno como rotina a todos os pacientes submetidos à CTE, e sim apenas nos pacientes que apresentarem evidência clínica ou laboratorial (cortisol sérico abaixo de 5 ug/dl) de insuficiência adrenal(16,21-25).

Há alguns anos, havia um grande temor de ocorrência de crise adrenal nos pacientes com DC que não fizessem reposição de hidrocortisona como rotina no período peri-operatório, devido ao risco de crise adrenal como consequência da supressão (“estonteamento”) dos corticotrofos peritumorais destes pacientes. Posteriormente, alguns pesquisadores evidenciaram que a prática de reposição apenas em vigência de insuficiência adrenal era segura e permitiria a avaliação do comportamento dos corticotrofos peritumorais ao estresse cirúrgico e anestésico (16,21-24). Mais recentemente foi demonstrado picos significativos de secreção de ACTH neste período nos pacientes que não fizeram uso de hidrocortisona peri-operatória (24).

Nosso grupo de pesquisa vem analisando ao longo dos anos, o comportamento dos níveis séricos do cortisol no pós-operatório imediato nos pacientes com DC, através da aferição do cortisol nos tempos 0, 6, 12, 18, 24, 36 e 48 h a contar do início da CTE e 7-12 dias e 30 dias após a realização da mesma. No estudo de Rollin e cols (16) foi observado que um nadir do cortisol em alguma destas aferições até os primeiros 10-12 dias após a CTE < 7,5 ug/dl, seria um excelente marcador de remissão da doença, com sensibilidade e especificidade de 100%. Entretanto, for observada extrema heterogeneidade na curva do cortisol destes pacientes e alguns destes indivíduos fizeram uso de hidrocortisona no período perioperatório como rotina.

Posteriormente, estudamos a coorte com um grupo maior de indivíduos com DC submetidos à CTE (23), com a orientação de que o corticoide exógeno fosse utilizado apenas sob demanda, em casos definidos de insuficiência adrenal. A presença de um nadir do cortisol sérico <3,5 ug/dl nas primeiras 48h ou < 5,7 ug/dl até os primeiros 10-12 dias após a cirurgia foi excelente marcador de remissão cirúrgica da doença, com

valor preditivo positivo de 100% para remissão cirúrgica. Entretanto, novamente foi observada intensa heterogeneidade da curva do cortisol. Alguns pacientes que entrariam em critério de remissão pelo nadir do cortisol, apresentaram um pico bastante elevado de secreção de cortisol, em comparação com os demais pacientes, durante o pós-operatório precoce, com posterior queda dos seus valores. A queda destes valores, e o menor valor encontrado (nadir) ocorreu nas primeiras 48h ou mesmo 10-12 dias e em até 30 dias após a intervenção cirúrgica.

Este comportamento de picos de secreção do cortisol no pós-operatório dos pacientes com DC e que mesmo assim atingiram a remissão cirúrgica motivou o atual estudo para melhor entendimento deste padrão de resposta e de uma possível caracterização destes pacientes.

A hipótese inicial seria de que estes pacientes tivessem uma menor supressão dos corticotrofos peritumorais e pudessem responder de forma mais intensa aos estímulos corticotróficos do período transoperatório. Entretanto, a definição de DC leve não está bem estabelecida na literatura. Apesar das críticas atuais com relação a acurácia do UFC para o diagnóstico da SC (25,26,), o critério mais aceito pelos estudos para definição de DC leve baseia-se nos pontos de corte do UFC. Entretanto, estes pontos de corte também são bastante variáveis entre o limite superior da normalidade até 3 vezes o limite superior do método utilizado para diferenciar estes pacientes (27-29).

A maior parte dos estudos sobre SC leve ou subclínica está relacionado a adenomas adrenais (29) e mais recentemente ao incidentaloma hipofisário (30). De

modo recente, foi tentado determinar um escore de gravidade da DC que envolvesse dados clínicos e laboratoriais, porém sem sucesso (31).

A avaliação da dinâmica do cortisol no pós-operatório precoce é uma maneira interessante de entender o comportamento dos corticotrofos peri-tumorais aos estímulos perioperatórios. A intensidade de resposta do eixo HPA neste período pode estar associada à intensidade de exposição prévia ao cortisol pelos corticotrofos peri-tumorais e consequentemente, à gravidade da DC.

Acromegalia

A acromegalia é ocasionada em sua grande maioria por um adenoma hipofisário secretor de GH (32,33). Apesar de ser uma doença rara, com uma incidência de 3 a 4 casos por milhão de habitantes/ano e uma prevalência de 50 a 70 casos por milhão de habitantes, acredita-se que pelo caráter insidioso da doença e pelo diagnóstico tardio, essa estimativa possa estar subestimada. A doença afeta igualmente homens e mulheres, com uma prevalência maior entre os 40-50 anos de idade (32, 34-36).

Vários fatores genéticos e mediadores que controlam o ciclo celular contribuem para a patogênese destes adenomas somatotroficos monoclonais (37) Algumas condições hereditárias, como a neoplasia endócrina múltipla tipo 1, o complexo de Carney, a síndrome de McCune-Albright, e bem raramente a síndrome de Von Hippel-Lindau, podem estar associadas ao adenoma secretor de GH (37, 38). Além disto,

mutações germinativas do gene de proteínas que interagem com o receptor do AIP têm sido descritos em casos de acromegalia familiar (37).

O GH é secretado pelas células somatotróficas na hipófise anterior e encontra-se sob controle de hormônios hipotalâmicos e mediadores da secreção do GH, que determinam a sua supressão como somatostatina e IGF1 ou liberação e síntese como *growth-hormone releasing hormone* (GHRH) e a grelina (39). O IGFI, é um peptídeo produzido principalmente pelo fígado e alvo da secreção do GH, ele é responsável por muitos dos efeitos do GH, que incluem síntese de proteínas, transporte de aminoácidos, crescimento de músculos, cartilagem e ossos, além de estimular a síntese de DNA e RNA e, comisso, a proliferação celular (40).

A secreção do GH em indivíduos com acromegalia ocorre de forma episódica, com maior duração dos pulsos e menor nadir de secreção do mesmo, mantendo o padrão circadiano, com predomínio noturno da secreção do GH (41). Este aumento de secreção de GH ocasiona níveis séricos permanentemente elevados de IGF-1.

As modificações decorrentes da acromegalia ocorrem de forma insidiosa, o que de certo modo justifica o seu diagnóstico tardio, que varia entre 7 a 10 anos após o surgimento dos primeiros sintomas (32). Podem decorrer das consequências diretas do GH ou do IGF1 ou dos efeitos secundários do crescimento da massa tumoral, já que a maioria (60%) deles se apresenta como macroadenomas (tumores maiores ou iguais a 1 cm) no momento do diagnóstico. Os sintomas são muito variáveis e sofrem influência de fatores tais como idade, duração da doença antes do diagnóstico, susceptibilidade genética do paciente para desenvolver diabetes melito ou hipertensão arterial sistêmica, tamanho e invasividade tumoral. A maioria dos pacientes é diagnosticada por acaso,

quando procura atendimento médico por cefaleia, distúrbios visuais, alterações em arcada dentária, distúrbios menstruais, artralgias ou síndrome da apneia do sono e hipopneia (33).

A atividade da acromegalia é definida pelos níveis de GH e IGF1 séricos. Conforme os critérios do consenso de 2014 e diretrizes da Endocrine Society de 2014 (37,42). Doença ativa é definida por um GH randômico ⩾1 ug/l; um nadir do GH após teste de tolerância oral com 75 gramas de glicose (TOTG) ⩾ 1 ug/l e níveis elevados de IGF-1 conforme parâmetros para sexo e idade. Doença em remissão é caracterizada por níveis de GH randômico <1 ug/l e níveis de IGF1 normais para sexo e idade. Pacientes com IGF1 normal e GH randômico detectável, a medida do GH < 1ug/l após o TOTG pode auxiliar na definição de ausência de atividade da acromegalia (37).

Pacientes que atingem critérios de remissão da doença, ou seja, GH randômico < 1 ug/l e IGF1 normal mediante o uso de medicamentos para o tratamento da acromegalia são considerados como portadores de acromegalia controlada (37).

A CTE é o tratamento de escolha para microadenomas (adenomas menores de 1 cm), macroadenomas restritos a sela túrcica ou macroadenomas que comprimem o quiasma/nervo ótico (37,42,). A cirurgia deve ainda ser considerada em macroadenomas não inteiramente ressecáveis, com o objetivo de reduzir o volume tumoral na tentativa de aumentar a eficácia do tratamento medicamentoso (39). Dependendo da experiência do cirurgião, os índices de remissão com a cirurgia em microadenomas podem chegar a 100%. Já em macroadenomas, a cura nas melhores séries não ultrapassa 50% (37,43). A mortalidade perioperatória é baixa,

correspondendo a menos que 1% em pacientes com macroadenomas invasivos. Hipopituitarismo é reportado de 10 a 30% em pacientes com macroadenomas (37, 42).

A radioterapia resulta na normalização dos níveis de GH e IGF1 em aproximadamente 50% dos pacientes em 2-10 anos, na dependência da técnica utilizada. Os efeitos colaterais principais são o hipopituitarismo, que ocorre em 100% dos pacientes em 10 anos, lesão do nervo óptico ou oftalmoplegia (mais comum na radiocirurgia), alterações cognitivas, aumento do risco de acidentes cerebrovasculares e a possibilidade de ocorrência de um segundo tumor cerebral (1 a 2%) (37, 42).

Para pacientes não curados pela cirurgia/radioterapia existem medicações disponíveis no nosso meio para tratamento clínico da acromegalia, que incluem os análogos da somatostatina os quais inibem a secreção de GH e a proliferação celular (octreotide *long acting release* e lanreotide depot/autogel), o inibidor do receptor do GH (pegvisomanto), além de agonistas dopaminérgicos (cabergolina) (32,37,42). Estas medicações podem ser usadas isoladamente ou em combinação (37). Recente metanálise de quatro estudos evidenciou benefício do uso de análogo da somatostatina antes do tratamento cirúrgico, aumentando as chances de controle da acromegalia no terceiro mês após a cirurgia (44).

A taxa de mortalidade de pacientes com acromegalia é 2 a 4 vezes maior que da população em geral, entretanto, com a normalização dos níveis de GH e IGF1, a sobrevida pode igualar-se a da população de mesma idade (37,42). O aumento da mortalidade se deve principalmente às doenças cardiovascular (24%), cerebrovascular (15%) e respiratórias (15,5%) e aos tumores malignos (15,5%) (45).

A doença cardiovascular na acromegalia é representada especialmente pela hipertensão arterial sistêmica (HAS) e pela cardiomiopatia acromegálica, entretanto arritmia cadíaca, aterosclerose e doença valvar cardíaca também fazem parte das complicações da acromegalia (46). A cardiomiopatia acromegálica se caracteriza essencialmente pela hipertrofia ventricular esquerda, sendo encontrada em até 90% dos estudos de ecografia nestes pacientes (46,47). A cardiomiopatia hipertrófica destes pacientes pode ser adequadamente avaliada pelo ecocardiograma (48).

A HAS ocorre em torno de 30% dos pacientes, entretanto, as taxas de prevalência são bastante variáveis entre os diferentes estudos, devido em parte às diferentes populações/perfis de pacientes com acromegalia, variando de 13-60% (37,46,49,50).

A HAS nos acromegálicos é predominantemente diastólica, e sua etiologia parece ser multifatorial (37). Contribuem para a fisiopatologia da hipertensão nestes pacientes a retenção hidrosalina, a disfunção endotelial, o hiperinsulinismo,o aumento da resistência vascular periférica e a diminuição do peptideo natriurético atrial (46,50,52). Neste sentido, pacientes com diabetes melito e acromegalia, tendem a ter maiores níveis pressóricos que os sem diabetes (51). A HAS tende a ser mais prevalente como aumento da idade e do tempo de acromegalia (37).

Pelo fato de a doença cardiovascular ser uma das principais causas de mortalidade na acromegalia, a aquisição de hábitos de vida saudáveis como suspensão do tabagismo, o adequado controle de fatores de risco como hipertensão, hiperglicemia e dislipidemia devem ser agressivamente perseguidos nestes pacientes (37).

Na população em geral, o papel da avaliação da PA através da MAPA está bem estabelecido, com aumento de complicações e da mortalidade associados a níveis mais elevados da PA. Especial destaque é dado à hipertensão noturna e à ausência do descenso noturno significativo, definido por redução mínima > de 10% entre a média da pressão noturna sistólica ou diastólica em relação à média da pressão diurna sistólica e diastólica, respectivamente (53).

Contudo, poucos estudos avaliaram a importância da MAPA em pacientes com acromegalia (51, 54-56). Devido à raridade da acromegalia, os estudos são pequenos, controversos e nem sempre avaliam a repercussão cardiovascular dos diferentes níveis de PA aferidos pela MAPA. Por estas razões, as conclusões neste cenário ainda não são definitivas. Entretanto, dois estudos evidenciaram maior prevalência do perfil *non-dipping* (sem o descenso noturna da PA) em pacientes com acromegalia em relação à população normal (54,55), reforçando a relevância deste exame nestes pacientes.

Além disto, os níveis de PA estão associados aos níveis séricos de GH e IGF1, e ao controle da acromegalia (46, 55). Entretanto, a associação dos níveis de IGF1 com os níveis pressóricos é menos compreendida, com alguns estudos mostrando resultados divergentes (57). A aferição da PA apenas pelas medidas de consultório tende a superestimar a prevalência de hipertensão neste grupo de pacientes (56), possivelmente associado ao fenômeno do jaleco branco.

Em indivíduos sem acromegalia, a hipertensão do jaleco branco pode estar associada ao aumento de mortalidade por todas as causas (58). Entretanto, até o presente momento, dados de prognóstico envolvendo as medidas de PA do consultório e da MAPA em pacientes com acromegalia não estão disponíveis na literatura. Estudos

envolvendo estas ferramentas poderão modificar a abordagem terapêutica dos pacientes acromegálicos.

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Capítulo I

ARTIGO ORIGINAL

“Serum cortisol dynamics after transsphenoidal surgery in mild Cushing’s disease”

Artigo a ser submetido para publicação.

“Serum cortisol dynamics after transsphenoidal surgery in mild Cushing’s disease”

Running Title: Mild Cushing’s disease

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Abstract:

Objective: To describe the corticotrophs behavior based on cortisol levels after transsphenoidal surgery (TSS) of patients with higher answer of hypothalamic pituitary adrenal axis (HPA) to perioperative stress. To describe the clinical and biochemical characteristic of this patients in terms of Cushing's Disease (CD) severity.

Methods: A cohort of 70 CD remission patients with 39 of them classified as mild CD based on 24h-Urinary free cortisol (UFC) less than 3 times the upper limit of normality (ULN). Exogenous glucocorticoid was not used preoperatively and during surgery, as routine. Serum cortisol level was measured on the morning of the surgery and every 6h for 48h after TSS. Clinical and biochemical data on CD diagnosis were analyzed to identify patients with a higher peak of serum cortisol in the first 48h after TSS.

Results: Patients with UFC < 3 times ULN presented higher serum cortisol peak 24h after TSS: 28.7 ± 15.9ug/dl (791.8 ±438.7 nmol/l) versus 19.6 ± 18.7 ug/dl (540.7 ± 515.9 nmol/l) than those with UFC 3 times above the ULN (p=0.03). Patients with UFC < 3 times ULN were considered mild CD. On CD diagnosis, mild CD patients presented less impaired sexual function and less bruising.

Conclusions: Based on the serum cortisol levels soon after TSS, patients with UFC less than 3 times ULN before surgery presented higher serum cortisol peak after TSS. This higher serum cortisol peak soon after TSS can be attributed to lower peritumoral corticotrophs suppression and higher answer of HPA axis of these patients to surgical stress, possible patients with a milder CD.

INTRODUCTION

Low levels of serum cortisol early (48h) after transsphenoidal surgery (TSS) has been proposed as an important tool in defining surgical remission of Cushing's Disease (CD) in patients without routine perioperative glucocorticoid administration (1-5). However, serum cortisol secretion presents an important heterogeneity in patients with surgical remission (3,5). It has been suggested that the surgical and anesthetic stress may awake the stunned corticotrophs around the adenoma (6). Therefore, some patients could present a higher serum cortisol peak soon after TSS, probably because these corticotrophs would have a preserved response to acute stress.

In this scenario, even patients with surgical remission could present a delay of a week or even a month to achieve their serum cortisol nadir after surgery (3,5). A possible group that could present this behavior during the early pos-operative (PO) cortisol evaluation would be the patients with mild CD.

The criterion of "mild" CD is not well established. The most widely accepted definitions are based on 24h urinary free cortisol (UFC) less than two or three times the upper limit of normality (ULN) (7-9) or at upper limit of normality (10). The definitions of mild and subclinical Cushing are very often intermingled.

Our objective was to study the behavior of peritumoral corticotrophs during perioperative time based on serum cortisol dynamics. We aimed to investigate if patients with preoperatively mild CD were those with a higher response of peritumoral corticotroph to surgical and anaesthetic stress and in consequence presenting a higher peak of serum cortisol in the first 24-48h after surgery.

PATIENTS AND METHODS

Patient Cohort

Seventy CD patients followed in the Outpatients Division of Neuroendocrinology at Hospital de Clínicas de Porto Alegre (HCPA) were included. They were in TSS remission and they had their serum cortisol dynamic evaluated at PO period. These patients were analyzed for clinical and biochemical characteristics of CD severity. The study protocol was approved by the local Research Ethics Committee and all patients provided written informed consent.

Clinical evaluation and comorbidities:

The clinical aspects of CS (obesity, central obesity, easy bruising, purple striae, supraclavicular pad, buffalo hump, skin pigmentation, plethora, proximal myopathy, acne, hirsutism, alopecia, menstrual irregularity and, sexual disturbance), comorbidities (diabetes, hypertension, psychiatric and bone disease) and the laboratory parameters for CS and CD [adrenocorticotrophic hormone (ACTH) level, Dehydroepiandrosterone sulfate (DHEAS) level, serum and urinary free cortisol in 24h (UFC)] were evaluated in relation to the serum cortisol PO behavior. The clinical information was collected retrospectively from medical records. Sexual disturbance was considered positive if the patient mentioned any sexual problem during clinical interview. Easy bruising was considered as spontaneous hematomas or telengiectasias on physical examination. The patients were considered hypertensive if systolic blood pressure was ≥ 140 mmHg or diastolic blood pressure was ≥ 90 mmHg in the two consecutive measurements or if there was a previous history of anti-hypertensive medication (19).

Biochemical diagnosis of Cushing's disease and perioperative approach

CS and CD were diagnosed after admission as previously described (3,5) and in accordance with 2008 Endocrine Society Guidelines (16). All of the patients had at least two samples of UFC on diagnosis and most of them three UFC samples. Patients without a visible adenoma on pituitary Computed Tomography Scan and Magnetic Resonance Imaging underwent bilateral inferior petrosal sinus sampling with a positive central/peripheral gradient (16,20). All the patients were submitted to TSS without routine hydrocortisone use during the preoperative period. These subjects received glucocorticoids only in case of clinically diagnosed adrenal insufficiency and/or laboratory evidence of adrenal insufficiency (serum cortisol < 5 µg/dl [138 nmol/l]). Serum cortisol was measured on the morning of the surgery, every 6h for 48h after PO, at 10-12 days and a month after surgery, until glucocorticoid therapy was initiated, as necessary. Until seven days after surgery the patient was kept in hospital. These patients under remission without glucocorticoid use have adrenal insufficiency symptoms actively investigated and have serum cortisol determination, until glucocorticoid replacement be initiated, as necessary. This protocol and data from 61 of these 70 patients were previously described in other papers (3, 5).

Follow up

This cohort was followed for 71.9 ± 55.5 months. Patients with low or undetectable serum cortisol levels, regardless of protocol, were kept on glucocorticoid therapy until recovery of the pituitary-adrenal axis. Ten to fourteen days after surgery the

patients were reviewed and then followed at least once a year. Patients underwent subsequent oral 1-mg overnight dexamethasone suppression testing (1mg-DST) and/or two urinary free cortisol (UFC) tests at least once a year. For all glucocorticoid-dependent patients, attempts at weaning were made at every visit and a Synacthen® test (tetracosactide 1 µg, cutoff for pass 18 µg/dL [497 nmol/L]) was performed to confirm the need for glucocorticoids if clinical judgment of adrenal insufficiency showed the need (3,5).

Remission and recurrence criteria

Remission was defined as serum cortisol less than 3ug/dl after 1mg-DST or normal UFC at the time of the last follow up visit (3,5). Recurrence of CD was defined as the loss of remission criteria at least 1 year after TSS. Patients who ceased to meet the remission criteria within 1 year of TSS were classified as surgical failure rather than recurrence (3,5).

Biochemical methods

Cortisol was measured until April 2004 by a commercially available RIA kit (Diagnostic Systems Laboratories, Webster, TX) with intra- and interassay coefficients of variation of 8.3% and 9.8%, respectively, and a lower limit of detection of 0.15 µg/dl (4.14 nmol/l). The normal range for UFC was 20–90 µg (55-248 nmol) and for serum cortisol, 7-24 µg/dl (193-662 nmol/L). From 2004 to March 2010, the cortisol method was changed to an electrochemiluminescence immunoassay **(**ECLIA) kit **(**Modular Analytics E 170, Roche), intrassay coefficients of variation: 1.7%, interassay coefficients

of variation: 2.8% and a lower limit of detection: 0.15 μg/dl (4.14 nmol/l). The normal range was 36–137 μg (99-102 nmol) for UFC and 6.2-19.4 μg/dl (171-535 nmol/l) for serum cortisol. From March 2010 through the end of the study, cortisol was measured by chemiluminescence immunoassay (ADVIA Centaur XP Immunoassay System), intra-assay coefficients of variation: 4.8% and interassay coefficients of variation: 5.5% and lower limit of detection: 0.38 μg/dl (10 nmol/l). The normal range for UFC was 55.5-286 μg (153-789 nmol) and for serum cortisol, 4.3-22.40 μg/dl (118-618 nmol/l). Since the UFC was considered in relation to the reference range, UFC levels changed during the follow-up period and were thus adjusted by percentage over the upper limit of normal range (ULN) (21,22). DHEAS until April 2004 and before November 2010 was Chemiluminescence Immulite DPC. Siemens Healthcare Diagnostics Products Ltd. United Kingdom. Normal range: females 35 – 430 μg/ml (95-1167 nmol/l), males 80 – 560 μg/mL (217.1- 1519.8 nmol/l), lower limit of detection: 3 μg/mL, intra-assay coefficients of variation: 9.8%, interassay coefficients of variation: 13%. From April 2004 to November 2010 DHEAS was measured by Electrochemiluminescence Roche. Modular Analytics E170. Roche Diagnostics GmbH, D-68298 Mannheim. Lower limit of detection: 0.100 μg/dl, intra-assay coefficients of variation: 3.2%; interassay coefficients of variation: 2.7%. Normal DHEAS ranges for Electrochemiluminescence are found in in Supplement 1 (online appendix). Since the DHEAS was considered in relation to the reference range, similar to UFC levels that changed during the follow-up period, the DHEAS levels were adjusted by percentage over ULN.

Statistical analysis

Statistical evaluation was carried out by Student's t-test and the Mann-Whitney *U* test for unpaired data. The chi-square test was used to compare categorical variables. Logistic regression was used to show the variables associated with the presence of mild CD, and a Receiver Operating curve (ROC) was performed to determine the best peak of cortisol to discriminate mild CD, using as gold standard < 3x ULN for mild CD diagnosis. A *p* value of < 0.05 was considered significant. All analyses were performed using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 70 CD patients of the study cohort, 75.7% were female. Median age at CD diagnosis was 38 ± 1.6 yrs (range: 12-64 yrs), 90% were white. Preoperative laboratory characteristics were: median of 8h serum cortisol 25.5 ± 1.3 µg/dl (range 11.3-71.8 µg/dl) 703.5 ± 35.9 nmol/l (range 311.8 ± 1980.1 nmol/l); median UFC 2.52 ± 0.5 times ULN (range 0.5-32.0); serum cortisol after 1 mg-DST was 13.0 ± 1.6 µg/dl (range 4.2-48.4 µg/dL) 358.7 ± 44.1 nmol/l (range 115.9- 1216.7 nmol/l); median midnight serum cortisol 19.9 ± 1.1 µg/dl (range 9.4-46.2) 549.0 ± 1274.6 nmol/l (range 259.3 ±1274.6 nmol/l). Median ACTH level 59.2 ± 5.5 pg/ml (13.0 pmol/l ± 1.21 pmol/l), median DHEAS 205.0 ± 24.5 µg/dl (556.4 ± 66.5 nmol/l). After 8mg (2mg/6/6h for 48h) dexamethasone test 78.1% had suppressed serum cortisol > 50% basal value and 64% have suppressed 24h UFC < 200 µg/24h (5518 nmol/24h).

The diagnosis of micro- or macroadenoma was based on pituitary imaging findings. However, adenomas were identified in all patients during TSS. Of all pituitary images analyzed, 57 patients (81.4%) had an adenoma, 17 of these 57 patients (24.3%)

had a macroadenoma and 13 patients (18.6%) have not adenoma visible on pituitary CT or MRI. In the entire cohort, 75.7% had adenoma on pituitary histopathology and three had negative ACTH immunohistochemistry. In these three patients, the tumor was aspirated by endoscope during surgery, and then they presented complete resolution of CS after TSS.

Clinical aspects and comorbidities

On CD diagnosis, the body mass index (BMI) was 31.6 ± 7.1 kg/m^2. Obesity in 37 (52.9%), 59 (84.3%) had abdominal obesity, 48 (67.1%) patients had buffalo hump, 48 (68.6%) supraclavicular pad, 54 (68.6%) plethora, 50 (72.4%) moon face, 30 (42.9%) purplish striae, 33 (47.1%) had easy bruising, hyperpigmentation 12 (17.1%), 36 (51.4%) hirsutism, 8 (11.4)% alopecia, 23 (32.9%) acne, 22 (31.4%) had edema of the lower limbs 12 (15.8%) had skin pigmentation, proximal myopathy 47 (67.1%), menstrual irregularity 29 (41.4%), sexual dysfunction 8 (11.4%). Seven (10%) of the patients had an infection on CD diagnosis.

Twenty-four (34.3%) had DM, 53 (75.7%) patients had hypertension, 31 (44.3%) had dyslipidemia 43 (56.6%) had osteoporosis/osteopenia of the spine and 31 (40.8%) of the hip, 8 (11.4%) had bone fracture and 9 (12.9%) had nephrolithiasis. Twelve (17.2%) had major depression, 2 (2.9%) had psychosis and 17 (24.3%) other psychiatric disease.

The patients have no significant clinical or surgical complications due to adrenal insufficiency in perioperative time. Exogenous glucocorticoid was administrated to the patient as soon as adrenal insufficiency was identified.

Analysis based on cortisol levels

Firstly, we analyzed the group of patients with a higher peak of serum cortisol response during the first 24-48h after TSS in relation to clinical and biochemical parameters of Cushing investigation and to CD comorbities aiming to differentiate this group of patients with a higher peak of serum cortisol after TSS from the rest of the cohort.

The criterion of <3 times ULN for UFC was the only marker that discriminated these patients with a higher peak of serum cortisol soon after surgery and we then considered these patients to have mild CD. Lower levels of UFC (1,1.5, 2,2.5 times ULN) were also tested but none of them was able to identify the different behavior of serum cortisol during the first 24-48h after TSS.

Moreover, the achievement of a peak higher than 34 ug/dl (938.4 nmol/l) (defined as adrenal sufficiency during critical stress) was more frequent between patients with UFC < 3ULN than those to UFC > 3 ULN [17/39 versus 6/31 patients (p=0.028)].

Other tests like midnight serum cortisol levels SDHEA levels, ACTH levels and different degrees of serum cortisol suppression after 1mg overnight dexamethasone test or 8 mg dexamethasone test were also tested to find a discriminative point that could conceive a different serum cortisol dynamic after TSS. However, there was not found any difference in the serum cortisol peak after TSS between patients with these tests more intensively changed to those with these tests only slightly compromised.

During the first 12h after TSS to 24 h after TSS the serum cortisol behavior was different between patients with mild disease from those with more severe CD. Patients with mild CD presented higher serum cortisol peak and nadir during the first 24 PO than those without mild CD. However, during 10-12 days and 30 days it was similar between patients with mild or not mild CD, both groups achieving remission serum cortisol levels. Table 1 and Figure 1.

Mild Cushing Disease

Thirty-nine out of seventy patients were classified as mild CD based on UFC < 3 times ULN. Besides UFC, mild CD patients presented the similar hypothalamic-pituitary-adrenal axis dysfunction during CD investigation, pituitary images and anatomo-pathological characteristics as patients without mild CD, as shown in Table2.

Table 3 shows clinical aspects and comorbidities in patients with and without mild CD. On diagnosis, mild CD patients presented less impaired sexual function than those with more severe CD (1/40 vs. 7/30 patients, p=0.012) and easier bruising (13/40 vs. 20/30 p=0.008).

We analyzed the 24h and 48h serum cortisol peak excluding patients with definite adrenal insufficiency during the last follow-up (n=47). The serum peak during the first 24h after surgery was higher between patients with UFC < 3 times ULN than those with UFC> 3 times ULN (p=0.015). However, the median peak during 48h and the nadir during 24h and 48h were similar between the two groups of patients (p=0.068, p=0.223, p=0.168) Figure 2.

Additionally, we have tested the other cut-off of 1.1, 1.5, 2, 2.5 times ULN of the UFC, but no differences in cortisol dynamics or in other parameters were observed using that criterion for mild CD. Other parameters of HPA damage were also tested, without statistically significance with a different serum cortisol behavior in the first 48h after TSS. (Data not shown).

ROC Curve shows that serum cortisol peak 24h after surgery presented an area under the curve of 0.676 (0.54-0.80) p=0.012 to differentiate patients with and without mild CD. A value ≥ 34.1 µg/dl (940.8 nmol/l) presented a specificity of 83.3% and sensitivity of 42.5% to differentiate patients with mild CD from those without. However, to find a specificity of 100%, the peak of serum cortisol 24h PO ≥ 68.8 µg/dl (1898.2 nmol/l) presented a sensitivity of 0% for mild disease.

Clinical, biochemical and after surgery serum cortisol behavior

Table 4 presents the adjusted regression for 24h cortisol peak, easy bruising and sexual dysfunction related to mild CD determination. Data shows that 24h cortisol peak is a good tool to discriminate patients with mild disease from those without. Preoperatively, sexual dysfunction and/or easy bruising default could predict a different 24h performance of serum cortisol compatible with mild CD.

Recurrence of CD

Seven patients of the cohort presented recurrence during the follow up of 37.6± 15.1 months. It was identified that patients with recurrence presented higher levels of serum cortisol at 24h and 48h after TSS than those without recurrence (41.5 ± 13.9

µg/dl versus 21.3 ±17.0 µg/dl, P=0.004). However it was not associated with the time to recurrence P=0.224. One of these seven patients was classified as mild CD. Mild CD was not associated with recurrence P=0.136. The patients with recurrence presented more acne (5/7 versus 16/61 patients) and higher body mass index (37.5 ±8.6 Kg/ m2 versus 30.6 ± 6.7 Kg/ m2) preoperatively than those without recurrence.

DISCUSSION

Patients with mild forms of CDpresented a different behavior in their cortisol dynamics early after TSS, probably because a lower suppression of peritumor corticotrophs. Another interesting finding was that three times ULN of the UFC was able to identify these individuals. Clinical characteristics of easier bruising and sexual dysfunction were also predictors of these higher levels of serum cortisol dynamics 48h after surgery.

The best understanding of serum cortisol behavior soon after TSS is important to our knowledge of the peritumoral corticotrophs performance during perioperative stress in patients with a milder form of CD. Additionally, we observed that a different perioperative behavior of serum cortisol could be predicted by UFC levels and also by the presence of easy bruising and sexual dysfunction, all presented at CS diagnosis.

These patients with mild CD presented similar levels of cortisol nadir during 10-12 days and 30 days as those without mild CD, compatible with surgical remission. This could suggest postpone the definition of surgical remission in these mild CD patients.

Recent evidence suggesting special concern for subclinical CS consequences of not surgically treated adrenal adenoma (11,12) and the increasing frequency of patients

with mild forms of CD in clinical practice (7, 8,23) support the importance of a better understanding of the special behavior of the hypothalamic-pituitary-adrenal axis in these patients especially in the perioperative period. In pituitary incidentaloma, Toini et al (24) found a 7.3% prevalence of pituitary hypercortisolism diagnosed by biochemical criteria in patients without overt hypercortisolism.

The challenges in the diagnosis of mild forms of CD fell into the pitfalls of laboratory criteria (7,8 10,17,25) and in the lower sensitivity of more specific clinical aspects of CD in these patients (8,17). Moreover most of studies with the evolution of patients with mild/subclinical Cushing are based on adrenal incidentaloma (11,12,14), and not on patients with CD. Nowadays, with the public accessibility to health information and with the widespread use of laboratory investigation, progressively less severe cases will be diagnosed. This study is an opportunity to better understand the HPA axis dysfunction of these mild CD patients, since the cohort is composed by CD patients whose diagnosis was confirmed by transoperative adenoma identification, histopathology confirmation and whose remission was validated by long term follow up.

Recently, Elias et al (18) analyzing patients with different etiologies of CS did not find a correlation between clinical and laboratory aspects of Cushing, to determine the severity of disease. On the other hand, we only studied individuals with CD with a subset of mild CD.

Petersenn et al (26) evaluated the UFC of patients with a persistent/recurrent or *de novo* CD, most of them submitted to previous surgical procedure (79.6%). The majority of those patients had moderate-to-severe hypercortisolism (83%) and they could not find a linear correlation between mean UFC ULN and clinical parameters of

glycemic profile, BMI or systolic/diastolic blood pressure. Physical aspects of CS were not evaluated and mild CD patients were not included.

Patients with definite adrenal insufficiency after surgery could not recover the pituitary adrenal axis by surgical stress. When these patients were excluded from analyses the serum cortisol peak during 24h kept higher in patients with < three times ULN for UFC than those with higher UFC levels. The exclusion of patients with definite adrenal insufficiency from the analysis was performed to clarify the perioperative serum cortisol behavior of those patients that still have peritumoral corticotrophs residual with an HPA axis that could recover at some time after surgery. However, definite adrenal insufficiency is not predictable before surgery and to enhance the clinical applicability of our results all the other analyses included these patients.

We have no late night salivary cortisol available for this cohort of patients, but we analyzed the serum midnight cortisol on CS diagnosis and it was not different between patients with and without mild CD. Nonetheless, in the literature even late night salivary cortisol presented limitations for mild CD diagnosis (8,27).

We do not minimize the accuracy limitations of UFC on CS diagnosis (7,8,16,18,28). However, this study showed that UFC was the only biochemical marker able to predict a different performance of serum cortisol dynamics after TSS in this cohort of CD patients.

We highlighted that all of the patients had at least two samples of UFC on diagnosis and most of them three UFC samples. The methodology used at our center for UFC analysis changed during the years of CS investigation. To overcome this limitation, we standardized the mean of the UFC value over ULN to enable a

comparison between different patients over the years. We suggest that this standardization can enhance the applicability of our results to other centers, apart from the UFC methodology used during Cushing's investigation, similar to what is done in other centers (21,22).

Patients with recurrence presented higher levels of serum cortisol in first 24-48h, and only one of the seven patients was classified as mild CD. Mild CD was not associated with recurrence. However, because only seven patients had a recurrence it is difficult to come to any conclusion about recurrence based on serum cortisol levels.

Another possible limitation of this study was that absence of the dynamic of ACTH levels of perioperative time to demonstrate the complete HPA answer of perioperative stimulus. Moreover, the clinical characteristics were evaluated by medical records. However the same staff at our center evaluated all the patients during the years of CD diagnosis and follow-up, validating the presence or not of the clinical aspects of CD.

CONCLUSION

Patients with mild CD based on UFC < 3 times ULN, in surgical remission, presented a higher peak of serum cortisol in the first 24h after surgery, probably because of a lower suppression of normal corticotrophs near the adenoma and a preserved answer of HPA axis to surgical and anesthetic stress stimulus. These patients with mild CD will have a nadir of PO serum cortisol compatible with surgical remission; however it could happen after a week or a month after TSS.

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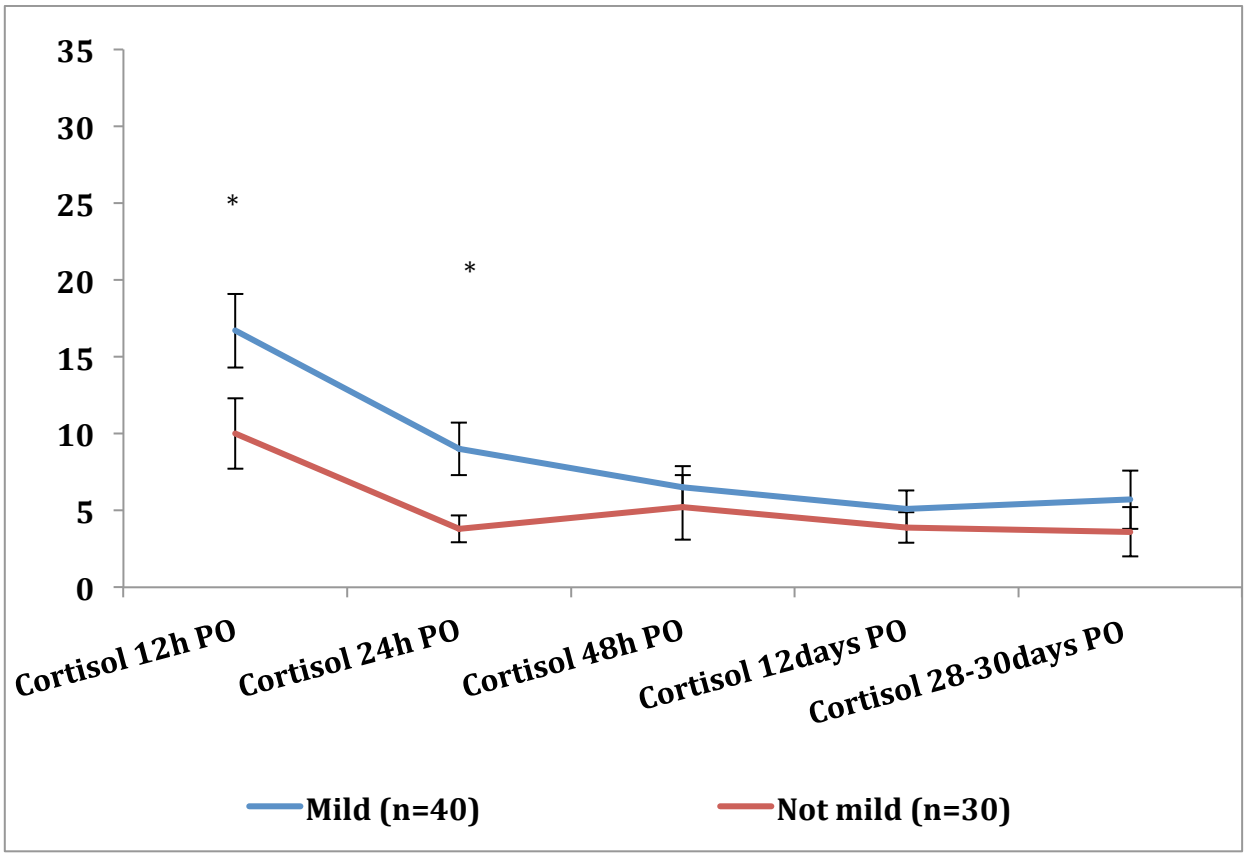

Figure 1. Serum cortisol dynamics of patients with and witout mild CD. * p<0.05

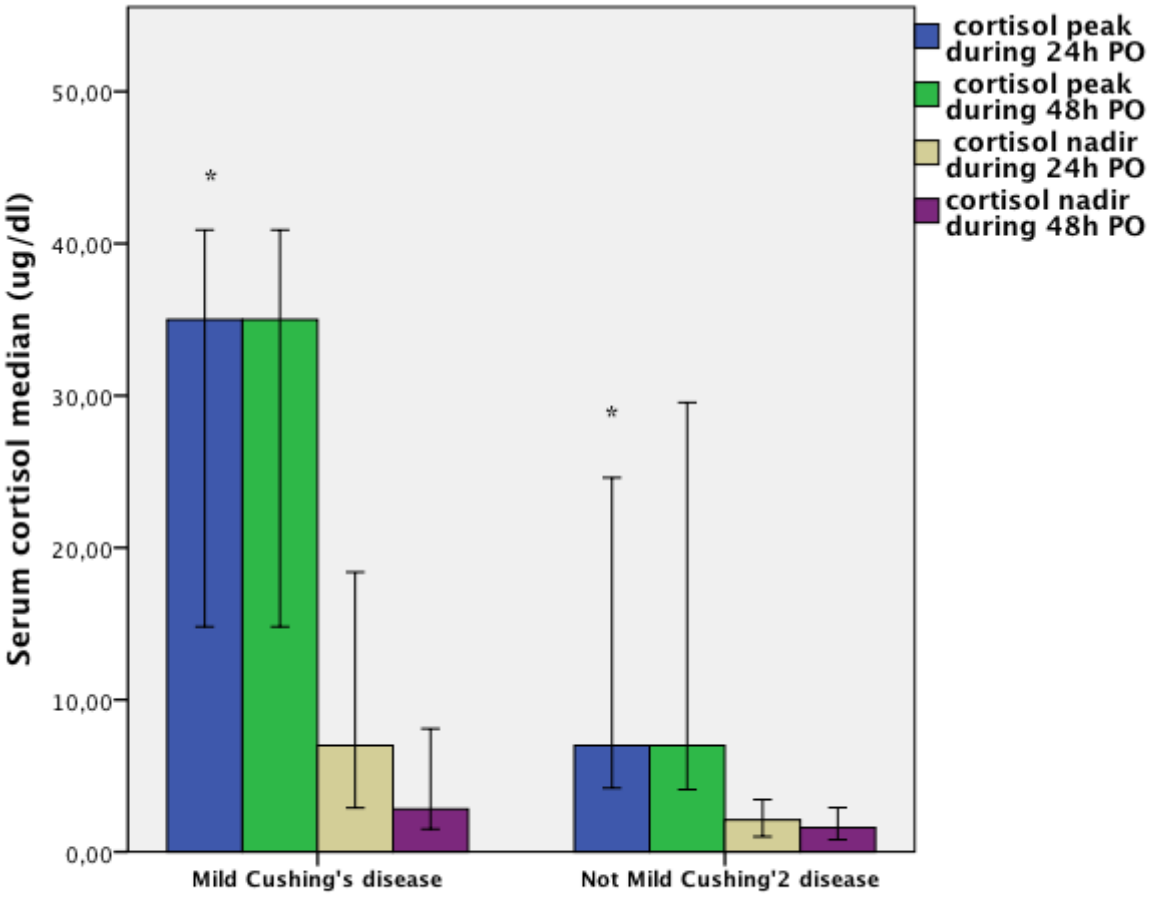

Figure 2. Serum cortisol peak and nadir during 24 and 48h after surgery in patients with or without mild CD, excluding those with definitive adrenal insufficiency. Cortisol in median and standard error. * p<0.05.

Table 1: Differentiation of serum cortisol levels, peak and nadir at different times after surgery in patients with or without mild CD.

Serum cortisol (ug/dl)	Mild (n=39)	Not mild (n=31)	p
Cortisol at 12h PO	**16.7 ± 15.3**	**9.7 ± 12.3**	**0.047**
Cortisol at 24h PO	**8.7± 10.6**	**3.8 ± 4.8**	**0.013**
Cortisol nadir during 24h PO	**4.5 ± 1.6**	**2.3 ± 0.84**	**0.630**
Cortisol peak during 24h PO	**28 ± 2.6**	**14.3 ± 3.3**	**0.016**
Cortisol at 48h PO	**6.5 ± 7.5**	**5.2 ± 10**	**0.673**
Cortisol nadir during 48h PO	**2.2 ± 1**	**1.6 ± 0.37**	**0.792**
Cortisol peak during 48h PO	**28 ± 2.6**	**14.3 ± 3.4**	**0.054**
Cortisol at 12 d PO	**4.9 ± 6.3**	**3.9 ± 4.1**	**0.572**
Cortisol nadir during 12d PO	**2.3 ± 2.3**	**1.9 ± 2.2**	**0.604**
Cortisol at 30d PO	**4.7 ± 7.5**	**3.3 ± 4.8**	**0.585**
Cortisol nadir during 30d PO	**1.8 ± 2.3**	**1.3 ± 1.4**	**0.509**

Data were displayed as mean ± Standard Deviation or median ± Standard error. n= number of patients in each group, d= days, h= hours. PO: postoperative. Cortisol 12h, 24h PO or 48h PO: the median and SD of serum cortisol at 12h, 24h or 48h after surgery, respectively; cortisol 24h PO nadir and 48h PO nadir: the lowest level of serum cortisol during 24h or 48h after surgery, respectively; cortisol 24h PO peak and 48h peak: the highest level of serum cortisol during 24h and 48h after surgery, respectively. To convert serum cortisol µg/dl to nmol/l multiply by 27.6.

Table 2: Biochemical and image data of Cushing´s disease (CD) patients in surgical remission with or without mild CD.

	Mild	Not mild	p
Cortisol 8h µg/ dl*	**27.1±10.7**	**25.4±9.6**	**0.52**
24h-UFC ULN	**1.63 (0.52-2.9)**	**4.6 (3.1-11)**	**0.00**
1mg -DSTcortisol (µg/ dl)	**12.9 (4.2-48.4)**	**13.8 (4.8-46.6)**	**0.99**
Midnight cortisol (µg/dl)	**19.8 (11.1-46.2)**	**21.1 (9.4-39.8)**	**0.74**
8 mg-DST cortisol < 50%	**17 (89.5)**	**8 (61.5)**	**0.091**
8 mg-DST UFC < 20 µg/ 24h	**11 (73.3)**	**5 (50)**	**0.4**
Plasma ACTH (pg/ml)	**61.8 (22.6-199)**	**54.9 (11.5-175)**	**0.8**
Serum DHEAS	**204 (8-761)**	**231.5 (27.1-914)**	**0.37**
Microadenoma	**22/35 (62.8)**	**17/21 (80.9)**	**0.23**
Normal pituitary image	**5/ 40 (12.5)**	**7/29 (24.1)**	**0.33**
Adenoma in histopathology	**32/37 (86.5)**	**21/22 (95.4)**	**0.39**
ACTH in immunohistochemistry	**16/19 (84.2)**	**7/7 (100)**	**0.54**
Isolated adrenal insufficiency ***	**12/ 38 (31.6)**	**6/24 (25)**	**0.77**
Hypopituitarism ***	**14/38 (36.8)**	**10/25 (40)**	**1**
Panhypopituitarism ***	**6/ 38 (15.8)**	**5/20 (25)**	**0.74**

Data were displayed as mean ± Standard Deviation, median (range) or positive /total number of cases and (%). UFC: urinary free cortisol; *****Cortisol 8h: morning cortisol during Cushing diagnosis. ULN: times upper limit of reference; 1-mg DST: overnight 1mg dexamethasone suppression testing; 8mg-DST (2mg dexamethasone 6/6 hours for 48h): cortisol < 50%: serum cortisol <50% of baseline; 8 mg-DST (2mg dexamethasone 6/6 hours for 48h): UFC < 20 µg/24h: 24h UFC during 8mg-DST test lower than 20 ug/24h;DHEAS: dehydroepiandrosterone sulfate; *** After transsphenoidal surgery. (To convert serum cortisol µg/dl to nmol/l multiply by 27.6 and plasma ACTH pg/ml to pmol/l multiply by 0.22).

Table 3.Clinical aspects and comorbidities associated with Cushing in patients in surgical remission with or without mild CD.

	Mild	Not mild	p
Female	**30 (75)**	**23 (76.6)**	**1**
Age (years)	**41(12-64)**	**33.5 (19-61)**	**0.28**
Obesity	**19 (54.2)**	**18/27(66.7)**	**0.58**
Abdominal Obesity	**35 (94.6)**	**24 (96)**	**0.48**
Pre-operative BMI (kg/m²)	**31.8 ± 7**	**31.2 ± 7.3**	**0.77**
Hirsutism/Acne/Alopecia	**24 (64.8)**	**18 (69.2)**	**0.67**
Plethora/moon face	**31 (83.7)**	**26 (96.3)**	**0.27**
Sexual dysfunction *	**1 (2.8)**	**7 (26.9)**	**0.012**
Buffalo/supraclavicular pad	**32 (86.4)**	**21 (80.8)**	**0.6**
Myopathy	**30 (81)**	**17 (65.4)**	**0.27**
Capillary fragility*	**13 (35.1)**	**20 (74.1)**	**0.008**
History of coagulopathy	**6 (15.8)**	**2 (8.3)**	**0.10**
Depression/ psychosis	**8 (22.2)**	**5 (19.3)**	**0.87**
Osteopenia/ Osteoporosis hip	**14 (53.8)**	**16 (72.7)**	**0.30**
Osteopenia/ Osteoporosis spine	**22 (84.6)**	**20 (90.9)**	**0.67**
Nephrolithiasis	**5 (14.3)**	**4 (15.4)**	**0.98**
Bone fracture	**3 (8.6)**	**5 (18.5)**	**0.48**
Hypertension	**32 (88.9)**	**21 (77.8)**	**0.54**
Dyslipidemia	**21 (53.8)**	**10 (40)**	**0.094**
Diabetes	**16 (45.7)**	**8 (26.7)**	**0.41**
Infection on diagnosis	**4 (12.1)**	**3 (13)**	**1**

Data were displayed as mean ± Standard Deviation, median (range) or absolute (%).

BMI: body mass index. *p< 0.05.

Table 4. Odds ratio to not mild Cushing disease diagnosis in different adjusted models.

	OR	CI (95%)	P
Model 1			
Peak 24 hrs	**0.96**	**0.92-0.99**	**0.024**
Sexual Dysfunction	**18.4**	**1.67-201.8**	**0.017**
Model 2			
Peak 24 hrs	**0.96**	**0.93-0.99**	**0.025**
Easy Bruising	**5.7**	**1.82-18.1**	**0.003**
Model 3			
Peak 24 hrs	**0.96**	**0.92-0.99**	**0.023**
Easy Bruising	**5.5**	**1.53-19.7**	**0.009**
Sexual Dysfunction	**10**	**0.94-106.4**	**0.056**
Model 4			
Peak 24 hrs	**0.96**	**0.93-0.99**	**0.022**
Sexual Dysfunction plus Bruising	**13.9**	**1.28-151.2**	**0.031**

Model 1:adjustment for 24h cortisol peak and sexual dysfunction.

Model 2: adjustment for 24h cortisol peak and easy bruising.

Model 3: adjustment for 24h cortisol peak, sexual dysfunction and easy bruising.

Model 4: adjustment for 24h cortisol peak and both sexual dysfunction plus easy bruising in the same patient.

Peak 24 hrs: serum cortisol peak first 24h after transsphenoidal surgery. OR: odds ratio; CI: Confidence Interval.

Supplement 1. Normal dehydroepiandroesterone sulfate (DHEAS) value for age and sex

Age (yrs)	Female normal range (µg/dL)	Male normal range (µg/dL)
5-10	2.8-85.2	2.8-85.2
10-14	33.9-280	24.4-247
15-19	65.1-368	70.2-492
20-24	148-407	211-492
25-34	98.8-340	160-449
35-44	60.9-337	88.9-427
45-54	35.4-256	44.3-331
55-64	18.9-205	51.7-295
65-74	9.40-246	33.6-249
>75	12.0-154	16.2-123

To convert serum DHEAS µg/dl to µmol/l divide by 36.923

Capítulo II

ARTIGO ORIGINAL

“The role of ambulatory blood pressure monitoring in patients with acromegaly”

Artigo aceito para publicação no periódico Journal of Hypertension

The role of ambulatory blood pressure monitoring in patients with acromegaly.

Short title: blood pressure in acromegaly.

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Abstract:

Background: Hypertension is associated with increased cardiovascular deaths in patients with acromegaly.

Objective: To evaluate the accuracy of blood pressure (BP) by 24-h ambulatory blood pressure monitoring (ABPM) and office BP measurements to represent the real BP status in acromegalic patients and its relationship with acromegalic activity and echocardiogram parameters.

Patients and Methods: cohort of 37 patients with acromegaly in a tertiary endocrine outpatient service.

Results: Twenty three percent of the patients were considered hypertensive by ABPM versus 32% by office BP measurements (p=0.006). BP obtained from the ABPM was associated with GH, IGF1 levels and echocardiogram parameters of acromegalic myocardiopathy. Patients with non-dipper systolic BP and diastolic BP behavior presented higher GH levels (p=0.002 and p=0.014) than dipper patients, respectively. Nighttime systolic BP remained positively associated with relative left ventricular thickness after control for other variable associated with myocardial hypertrophy (p=0.017).

Conclusions: BP levels assessed by ABPM were associated with acromegalic activity and echocardiogram parameters. ABPM can correctly identify BP levels and their repercussion on acromegalic patients.

Key words: acromegaly, ambulatory blood pressure, hypertension

Introduction

One of the main causes of increased mortality rate in patients with acromegaly is cardiovascular disease (1). Cardiovascular complications include acromegalic cardiomyopathy, valve disease, atherosclerosis, arrhythmia and hypertension. Acromegalic cardiomyopathy is characterized primarily by left-ventricular hypertrophy, and it is the most prevalent cardiovascular abnormality, found in up to 90% of the echocardiographic studies performed in acromegalic patients (1,2). Hypertension affects approximately one third of patients with acromegaly, and its prevalence has a significant variation from 13% to 60% (3,4). However, few studies have measured the prevalence of hypertension using 24-h ambulatory blood pressure monitoring (ABPM) (4-7). Moreover, when the clinical measurements were compared to ABPM, it has been suggested that office measurements overestimate the prevalence of hypertension in patients with acromegaly (5). ABPM also showed a higher prevalence of non-dippers (48%) in acromegalic patients when compared to subjects without acromegaly (5,6).

The pathological mechanisms that lead to hypertension in these patients appear to be multifactorial. Possible explanations are differences in sodium and water regulation that lead to increased plasma volume; insulin resistance; peripheral vascular resistance and decreased secretion of type B atrial natriuretic peptide (3,8,9); polymorphism involving aldosterone synthetase transcription (10). In this sense, acromegalic patients with diabetes have higher blood pressure (BP) levels than those without diabetes (9).

It is also known that hypertension in acromegaly is related to growth hormone (GH) hypersecretion, as well as the duration of this hypersecretion (6) and IGF1 levels

(8). Minniti G.et al evaluated BP after transsphenoidal surgery and detected a decrease in systolic BP and a normalization of the circadian BP rhythm in patients who achieved normal GH levels after surgery, but no reduction in diastolic BP levels was found (11). However, the relationship between BP levels and insulin-like growth factor type 1 (IGF-1) is more obscure than with GH levels. Recently, this association was reported as positive when IGF-1 levels were higher than the cut-off values, and there was an inverse association when IGF-1 levels were in the normal values, suggesting that the relationship between BP and IGF-1 depends on or is related to IGF-1 concentrations (12).

In the present study we evaluated the accuracy of office and ABPM BP measurements to represent the homeostasis of BP in a cohort of patients under surgical and/ or pharmacological treatment for acromegaly; we also analyzed the impact of a previous diagnosis of hypertension on BP levels by office and ABPM measurements and its relationship to acromegalic parameters.

Patients and methods

A prospective cohort study was performed with 37 patients previously diagnosed with acromegaly followed in the Outpatients Division of Neuroendocrinology at Hospital de Clínicas de Porto Alegre (HCPA). All patients signed a consent form before any participation in the study. The protocol was approved by the Local Ethics Committee of our institution.

- **Classification Criteria for Acromegalic activity:**

Thirty-seven patients were separated into three groups based on defining criteria from the Acromegaly Consensus of 2014 and on the presence of current medication for acromegaly (13,14). Active disease was defined as a random GH ≥1 ug/l; a nadir GH after oral glucose tolerance test (OGTT) ≥ 1 ug/l and elevated IGF-1 matched for age and sex. Disease in remission was defined as a random GH <1 ug/l and IGF-1 levels normalized for age and sex. Patients with normal IGF1, and a detectable random GH underwent an OGTT and a nadir < 1ug/l was needed to define disease remission (13,14). Patients with GH < 1 ug/l and IGF-1 normal for age and sex, but on any medication for acromegaly treatment were considered as controlled. In patients under medication for acromegaly treatment with discordant GH and IGF-1 levels, IGF1 levels were preferred to define the activity of the disease.

- **Hormone assay:**

IGF-1 dosage was performed by immunoradiometric assay (commercial kit of Diagnostic Systems Laboratories, Inc, Webster, TX, USA). Interassay coefficient of variation, CV: 8.2%, 1.5% and 3.7%; intrassay CV: 3.4%, 3.0% and 1.5%, for low, medium and high points of the standard curve, respectively). Normal values were 109-284 ug/l for patients 36-40 years, 101-267 ug/l for patients between 41-45 years old, 94-252 ug/l for 46-50 years old, 87-238 ug/l for 51-55 years, 81-225 ug/l for 56-60 years old and 75-212 ug/l for 61-65 years old, 69-200 ug/l for patients between 66-70 years old, 64-188 ug/l for patients between 71-75 years, 59-177 ug/l for 76-80 years old. GH was

determined by chemiluminescence (commercial kit of Diagnostic Products Corporation (DPC), Los Angeles, CA, with IMMULITE® analyzer 1000). Method sensitivity was 0.01 ug/L, inter and intra-assay CV were, respectively, 6.2% and 6.5%.

- **Clinical and laboratory evaluation:**

Clinical assessment consisted of a medical history focusing on acromegaly and comorbidities and physical examination with weight and height measurements for body Mass Index (BMI). Office BP levels were assessed by an aneroid sphygmomanometer during the physical examination: two BP measurements, 2 minutes apart, with the patient seated for at least 5 minutes, on the right arm supported at the level of the heart, with a cuff appropriate to the size of the arm. We used the average of both measurements (15). The patient was considered hypertensive at the office if systolic blood pressure (SBP) was ≥ 140mmHg or diastolic blood pressure (DBP) was ≥ 90 mmHg in the two consecutive measurements or if there was a previous history of anti-hypertensive medication.

Laboratory analyses included: total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, creatinine, and glucose parameters. All laboratory values were assessed on the day of the current interview. Serum creatinine was measured using the Jaffe method, and the lipid profile was determined by an enzymatic colorimetric method. LDL cholesterol was calculated using the Friedewald equation.

Diabetes mellitus (DM) was diagnosed using American Diabetes Association criteria (16). The diagnosis was made if HbA1 ≥6.5%; or fasting plasma glucose ≥7 mmol/l or two-hour plasma glucose ≥11.1 mmol/l during the oral tolerance glucose test after 75g of

glucose on at least two different days; or by current use of oral anti hyperglycemic medications and/or insulin. Impaired glucose tolerance was diagnosed if the 2-hour blood glucose was between 7.7 and 11 mmol/l or if the fasting plasma glucose level was between 5.5 and 6.9 mg/dl (16). HbA1c was measured by high performance liquid chromatography, on a Merck-Hitachi 9100 chromatographer using a cation-exchange column. Glucose was measured by the UV kinetic method with hexokinase, in a Dimension RXL Dade Behring analyzer. Method sensitivity was 0.04 mmol/l, and inter and intra-assay CV were equal to 1.4% and 2.82%, respectively; and RV was 3.8 – 5.5 mmol/l.

Obstructive sleep apnea hypopnea syndrome (OSAHS) was defined by previous diagnosis by the patients and risk for OSAHS was defined by STOP-bang questionnaire (17). High risk for OSAHS was defined as a score of three or more positive answers and low risk < 3 positive answers.

- **24-hour Ambulatory Blood Pressure Monitoring (ABPM):**

All 37 patients had their blood pressure analyzed by oscillometry (Spacelabs 90207, ser. nos. 207/024751 and 207/038016, with calibration certification) as previously reported at our center (18). Briefly, BP was recorded every 15 minutes during daytime and every 30 minutes during the night. Sleep time was recorded as the period between the time when the patient went to bed and the time when the patient woke up the next morning. ABPM was considered satisfactory if at least 80% of the measures were appropriate. The quality of the exam of two patients was compromised and therefore some of the test data were not available. Hypertension (or high blood pressure) was defined as mean 24h-BP $\geq$ 130 x 80 mmHg, daytime BP $\geq$ 135 x 85 mmHg, or nighttime

BP $\geq$ 120 x 70 mmHg (19). A non-dipper profile was considered when the ratio of nighttime to daytime BP was < 10% for SBP or DBP, respectively (19).

Echocardiogram:

Nineteen patients underwent an echocardiography study within the two years before or after the ABPM BP measurement. We assessed ejection fraction (EF), left ventricular thickness (LVT), left ventricular mass (LVM) and relative wall thickness (RLVT). We considered left ventricular hypertrophy (LVH) according to the recommendation of the American Society of Echocardiography and European Association of Echocardiography. LVM was calculated according to the ASE convention based on the formula validated by Devereaux et al (20) with indexation obtained by dividing LVM by body surface area. LVH was considered when women had 96cm/m² or more, and men had 116cm/m² or more of LVM (21). RLVT [(septal thickness + posterior wall thickness) / LV internal end-diastolic diameter] was calculated. The cut-off point of 0.42 was used to define RLVH (relative left ventricular hypertrophy).

Table 1 describes the clinical and laboratory characteristics of the entire cohort of patients.

- **Statistical Analysis:**

Categorical variables were described by their absolute and relative frequencies and quantitative variables as mean and standard deviation. Pearson's correlation was used for correlations between variables with normal distribution. Student's t-test for unpaired data and ANOVA for repeated measures were performed. The chi-square test was used

to compare categorical variables.Multivariable linear regression analysis was performed. Data are presented as mean ± Standard Deviation (SD) or frequency. P<0.05 was considered significant. The statistical program used for these analyses was SSPS 22.0 (Armonk, NY: IBM Corp).

Results

Clinical and laboratory characteristic of the entire cohort are described in Table 1 When the office BP measurements were evaluated, a higher diagnosis of hypertension was observed compared to ABPM 32.4% versus 22.9% (p=0.006), respectively. Nighttime hypertension was present in 40 % of the cohort (Table 2).

The BP obtained by the ABPM showed a positive correlation between BP and IGF1 levels: 24h-DBP (r=0.407, p=0.014), daytime DBP (r=0.350, p=0.039) and nighttime DBP (r=0.401, p=0.017). And the SBP night-to-day ratio showed a positive correlation with GH levels (r=0.370, p=0.031). BP assessed in the office showed no correlation between IGF-1 or GH levels.

Table 3 presents the characteristics of the patients according to acromegaly status, separated into three groups (active, inactive and controlled group). The previous diagnosis of hypertension was higher in patients in remission versus those with controlled disease (p=0.005) and higher in active versus controlled disease (p=0.021). Patients in acromegaly remission underwent more surgery (p=0.013), used less octreotide (p=0.001), and presented lower IGF1 levels than active patients (p=0.001). Patients with active acromegaly presented higher BP at some points during the ABPM measurements than patients in remission (Table 4).

There was a positive correlation between IGF1 levels and 24h SBP (r=0.657, p=0.015), daytime SBP (r=0.673, p=0.012) and nighttime SBP (r=0.590, p=0.034) in patients in remission. In patients with active acromegaly, only the positive correlations at 1:00 AM DBP with IGF1-1 levels (r=0.546, p=0.044,) and at 1:00 AM DBP with GH levels (r=0.580, p=0.038) were significant. When patients with controlled/remission disease were grouped, there was no correlation between GH or IGF1 levels and office BP or ABPM measurement. There was no correlation between any BP office and activity of acromegaly.

Patients with a previous diagnosis of hypertension had higher BMI (p=0.004), LVM (p=0.001), nighttime SBP (p=0.03), and lower IGF-1 levels (p=0.019) than previously normotensive patients, Table 5. Office BP measurements were not correlated with GH or IGF1 in patients with or without previous hypertension.

Echocardiogram parameters were correlated with clinical variables: LVM with BMI (r=0.820, p=<0.001), with 24h SBP (r=0.47, p=0.04), with daytime SBP (r=0.44, p=0.06) and with nighttime SBP (r=0.47, p=0.04). RLVT was correlated with BMI (r=0.845, p < 0.001), with 24h SBP (r= 0.44, p=0.05), with daytime SBP (r=0.44, p=0.06), with nighttime SBP (r=0.55, p=0.01), with non-dipper SBP (r= 0.46, p=0.05) and with non-dipper DBP (r=0.55, p=0.01). Office BP levels were not correlated with echocardiogram parameters.

According to the presence of dipping profile, non-dipper SBP patients presented higher GH levels than dipper SBP (1.63 ± 1.66 ug/l vs. 0.57 ± 0.38 ug/l, p=0.002) and non-dipper DBP had higher GH levels than dipper DBP (1.69 ± 1.93 vs. 1.18 ± 1.17 ug/l, p=0.014). Non-dipper DBP also presented higher triglyceride levels (1.8 ± 0.9 mmol/l vs.

1.3± 0.5 mmol/l, p=0.003) and higher LVT (1.0 ± 0.22 cm/m² vs. 0.84 ± 0.14 cm/m², p=0.016), than dipper DBP patients. Female patients more frequently presented a non-dipping profile for DBP and for SBP than male patients (13 from 21 vs. 3 from 14, p=0.019 and 20 from 21 versus 8 from 14, p=0.006, respectively).

Patients at high risk for OSAHS presented a positive association with previous hypertension diagnosis 13/13 versus 2/11 patients at low risk for OSAHS (p< 0.001) and also presented a lower prevalence of nondipper SBP than patients at low risk for OSAHS (9/14 versus 9/9 patients, p=0.043). No difference was found for nondipper DBP and risk for OSAHS (p= 0.65).

Independent models of multivariable regression analysis were performed with RLVT as dependent variable. Nighttime SBP and 24h SBP remained associated with the outcome, even after adjustment for BMI, GH or IGF1, OSAHS and DM. However, when age was introduced into the model, only nighttime SBP remained positively associated with RLVT (Table 6).

Discussion

In our study, office BP overestimated the frequency of individuals with high BP levels compared to ABPM. BP levels measured by ABPM and parameters of acromegaly activity measured by IGF-1 and GH were associated and showed that ABPM is a tool that identifies the mediation among BP levels, somatotrophic axis and hypertensive cardiomyopathy.

Nighttime BP has been associated with cardiovascular complications in the general population (22, 23); however, few data are available for acromegalic individuals

(5). In this study, nighttime parameters of BP were associated with IGF1 and GH and also echocardiogram parameters related to hypertensive cardiomyopathy. This emphasizes the interaction and impact of BP variability throughout 24h in patients with acromegaly.

Moreover, in this cohort nighttime SBP level was an independent predictor of RLVT, an important parameter of hypertensive myocardiopathy even after control for parameters possibly associated with hypertension and myocardial damage. This emphasizes the impact of nighttime SBP levels in cardiovascular damage of patients with acromegaly.

Terzolo et al (5) compared 16 patients with active acromegaly to controls without the disease. On the contrary of our study, there was no significant correlation between BP levels assessed by ABPM and age or hormone profile of those acromegalic patients. However, they found higher BP levels and a higher frequency of non-dipping status (62%) for both SBP and DBP in subjects with acromegaly compared to controls (12%). In our study, we found 76% of prevalence of non-dipping profile for SBP and, 43% for DBP. Additionally, we found a positive association between GH levels and non-dipper profile.

Pietrobelli et al (4) analyzed 25 acromegalic patients with ABPM. Similar to us, they found 12 non-dippers patients who presented higher GH levels, higher LVM, than dippers, but without a difference in BMI.

Minitti et al (7) studied 40 patients with active acromegaly. They also found a higher prevalence of hypertension by office measurements than ABPM and a positive

correlation between age and 24h SBP, without association between BP levels and GH or IGF-1.

Obstructive sleep apnea syndrome (OSAHS) is associated with non-dipper behavior in the general population (24). It is also known that patients with acromegaly presented a high prevalence of OSAHS (14, 25). Our cohort was comprised of 40% of individuals at high risk for OSAHS, and as expected they were more hypertensive than patients at low risk for OSAHS. However an inverse association was observed between non-dipper SBP and high risk for OSAHS. This borderline association could be accounted for by our small sample size.

Schutteet al (12) recently pooled 20 studies including 11704 subjects, and found an association between IGF1 levels and BP in the general population, not only in acromegalic patients. They also found a positive association between high levels of IGF1 and BP.

The applicability of ABPM measurements in acromegaly patients was suggested by Sardella et al (6) in a retrospective cohort of 58 patients with acromegaly. They showed that in previous normotensive patients, acromegaly control was associated with lower BP levels assessed by ABPM. In our study, acromegaly in remission presented lower levels of BP at some times when ABPM was measured than those with active disease.

We recognize that the retrospective nature of the study could be a major limitation. However, this does not minimize the impact of our data on the comprehension of hypertension in acromegalic patients and on the relevance of ABPM in the clinical practice of these patients. Moreover, BP levels by ABPM were associated with

echocardiographic parameters associated with cardiomyopathy as independent predictor even after controlling for IGF1 and GH levels and other important variables associated with acromegalic myocardiopathy.

In this scenario, the ABPM correctly recognized the truly hypertensive individuals, independently of previous diagnosis of hypertension and identified nighttime BP levels. Therefore, ABPM can be of benefit in the clinical practice dealing with acromegaly patients.

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Table 1. Clinical and laboratory characteristics of the subjects of the cohort

Characteristics	Value
Age (years)	56 ± 12
Female (%)	59
BMI (kg/m^2)	29 ± 7
Overweight (%)	44
Obesity (%)	29
Hypertension (%)	59.5
Diabetes Mellitus (%)	27
Glycemia (mmol/l)	5.59 ± 1.43
Total cholesterol (mmol/l)	5 ± 0.9 97
HDL cholesterol (mmol/l)	1.3 ± 0.4

Characteristics	Value
LDL cholesterol (mmol/l)	3.2 ±0.8
Triglycerides (mmol/l)	1.5 ± 0.77
Family CV disease (%)	10.8
Family hypertension (%)	45.9
Active smoker /Previous smoker (%)	29.7/ 13.5
OSAHS n* (%)	6 (16.2)
High risk OSAHS **n (%)	15 (40.5)
Surgical remission (%)	37.8%
Radiotherapy (n)	8.1%
Cabergoline n (%)	7 (18.9)
Octreotide n (%)	17 (43.2)
Pegvisomant n (%)	1 (2.7)
Pharmacological control n (%)	8 (21.6)
Active acromegaly n (%)	15 (40.5)
IGF-1 (ugl/l)	293 ± 237
GH (ug/l)	1.35 ± 1.5

n: number of patients, BMI: body mass index, IGF-1: insulin-like growth factor type 1, GH: growth hormone, CV: cardiovascular, HDL: high density cholesterol, LDL: low density cholesterol, OSAHS: Obstructive sleep apnea hypopnea syndrome. Values are expressed as absolute values, mean ± SD or frequency.

Table 2. Twenty-four hours ambulatory blood pressure monitoring and echocardiographic characteristics of the cohort.

Characteristics	Value
Office SBP (mmHg)	128 ± 16
Office DBP (mmHg)	82 ± 13
24h SBP (mmHg)	118.6 ± 13.9
24h DBP (mmHg)	72.9 ± 8.5
Daytime SBP (mmHg)	120.8 ± 13.1
Daytime DBP (mmHg)	75.7 ± 8.4
Nighttime SBP (mmHg)	114.8 ± 16.9
Nighttime DBP (mmHg)	66.8 ± 10.2
Hypertensive by ABPM BP n (%)	8 (22.9)
Hypertensive by office BP n (%)	12 (32.4)
Night hypertension n (%)	14 (40)
Non-dipper SBP (%)	76
Non-dipper DBP (%)	43
Ejection fraction*** (%)	66 ± 8.2
LVM*** (cm/m²)	168 ± 85.68
LVT*** (cm/m²)	1.17 ± 1.09
RLVT***	0.41 ± 0.10
LVH***(%)	93.3%
RLVH*** (%)	42.1%

BP: blood pressure, SBP: systolic BP DBP: diastolic BP, ABPM: ambulatory blood pressure measurements, LVM: left ventricular mass, LVT: Left ventricular thickness, LVH: Left ventricular hypertrophy, RLVT: Relative left ventricular thickness, RLVH: Relative left ventricular hypertrophy. *n=30, **n=24, ***: n= 19. Values are expressed as absolute values, mean ± SD or frequency.

Table 3. Clinical and laboratory characteristics of the subjects according to the acromegaly activity status.

CHARACTERISTICS	REMISSION (n=15)	CONTROLLED (n=8)	ACTIVE (n=14)	P
Age (years)	60.2 ± 8.9	52.4 ± 8.5	55.4 ± 8.5	0.327
Female (%)	42.9	75	60	0.324
Date of diagnosis (year)	2001 ± 8.2	2003 ± 8.3	2004 ± 8.7	0.401
Macroadenoma (%)	85.7	100	84.6	0.795
Primary surgery (%)	100***	50	73.3	**0.013**
Secondary surgery (%)	0	50	13.3	
Radiotherapy (%)	7.1	12.5	6.7	0.875
Cabergoline (%)	0	25	33.3	0.065
Octreotide (%)	0***	87.5	60*	**0.001**
Pegvisomant (%)	0	0	6.7	0.471
BMI (kg/m^2)	30.8 ± 7.4	25.4 ± 4.6	29.9 ± 6.4	0.133
Hypertension (%)	78.6***	12.5**	66.7	**0.008**
Family CV disease (%)	7.7	16.7	18.2	0.635
Family hypertension (%)	61.5	50	54.5	0.881
Active or previous smoker (n)	5	3	8	0.349
OSAHS (%)	15.4	16.7	27.3	0.749
High risk OSAHS (%)	81.8	20	62.5	0.061
Diabetes Mellitus (%)	21.4	12.5	40	0.308
Previous IGF-1 (ug/l)	842.1 ± 402.4	936.9 ± 582.8	859.3 ± 475.2	0.925

Previous GH (ug/l)	13.0 ±1 5.3	46.1 ± 87.2	15.3 ±17.2	0.391
IGF-1 (ugl/l)	142.7 ± 1.5	180.2 ± 70.1**	493.3 ± 259.4*	**0.001**
GH (ug/l)	0.39 ± 0.33	1.32 ± 1.14	2.34 ± 1.83*	**0.001**
Glycemia (mmol/l)	5.0 ± 0.6	5.26 ± 0.57	6.3 ± 1.9	0.063
TCh (mmol/l)	5 ± 1	4.5 ± 0.45	5.5 ± 0.95	0.075
HDL cholesterol (mmol/l)	1.2 ± 0.2	1.4 ± 0.8	1.3 ± 0.3	0.713
LDL cholesterol (mmol/l)	3.1± 0.8	2.6 ± 0.8	3.4 ± 0.8	0.100
Triglycerides (mmol/l)	1.5±0.9	1.2 ± 0.6	1.6 ± 0.7	0.516

n: number of patients , IGF-1: insulin-like growth factor type 1, GH: growth hormone, CV: cardiovascular, TCh: Total cholesterol HDL: high density cholesterol, LDL: low density cholesterol, OSAHS: Obstructive sleep apnea hypopnea syndrome *: remission versus active p< 0.05, **: controlled versus active p<0.05, ***: remission versus controlled< p=0.05. Values are expressed as absolute values as mean ± SD or frequency.

Table 4. Twenty-four hours ambulatory blood pressure monitoring and echocardiographic characteristics of the subjects according to the acromegaly activity status.

CHARACTERISTICS	REMISSION	CONTROLLED	ACTIVE	P
Ejection fraction (%)	64.6 ± 12.3	70.3 ± 1.5	65.7 ± 5.7	0.613
LVM (cm/m²)	232.2 ± 93.8	125.1 ± 24.5	139 ± 72.5	0.068
LVT (cm/m²)	0.99 ± 0.2	0.83 ± 0.1	1.43 ± 1.6	0.629
RLVT	0.45 ± 0.2	0.36 ± 0.03	0.4 ± 0.06	0.461
Office SBP (mmHg)	129.9 ± 10.2	124.9 ± 25	129.4 ± 16.7	0.776
Office DBP (mmHg)	80.6 ± 11.1	84.6 ± 16.5	82.2 ± 13.6	0.793
24h SBP (mmHg)	119 ± 11.1	112.5 ±13.2	121 ± 16.0	0.333
24h DBP (mmHg)	70.2 ± 6.8	71.9 ± 8.0	75.9 ± 9.6	0.202
Daytime SBP (mmHg)	121.1 ±10.4	114.7 ±13.6	124.1 ± 14.7	0.279
Daytime DBP (mmHg)	72.9 ± 7.8	75.6 ± 8.5	78.2 ± 8.6	0.270
Nighttime SBP (mmHg)	113.8 ± 13.1	106.1 ± 14.1	120.8 ± 19.9	0.142
Nighttime DBP (mmHg)	63.6 ± 6.9	65 ± 8.7	70.9 ± 12.5	0.155
4:00 AM DBP (mmHg)	54 ± 20.26	65.3 ± 8.1	74.2 ± 13.6*	**0.011**
1:00 PM DBP (mmHg)	58.4 ± 22.2	75.7 ± 12.7	76.1 ± 12.1*	**0.026**
2:00 PM DBP (mmHg)	47.9 ± 31.9	68.4 ± 30.7	78.9 ± 8.2*	**0.012**
Non-dipper SBP (%)	76.9	75	85.7	0.784
Non-dipper DBP (%)	38.5	37.5	57.1	0.541

SBP: systolic blood pressure, DBP: diastolic blood pressure, ABPM: ambulatory blood pressure measurement, LVM: left ventricular mass, LVT: Left ventricular thickness, LVH: Left ventricular hypertrophy, RLVT: Relative left ventricular thickness, RLVH: Relative left ventricular hypertrophy, AM: antemeridian, PM: postmeridian *: remission versus active p< 0.05, **: controlled versus active p<0.05, ***: remission versus controlled< p=0.05. Values are expressed as absolute values as mean ± SD or frequency

Table 5. Main characteristics of the sample with and without the diagnosis of previous hypertension.

Variable	Hypertensives (n= 22)	Normotensives (n=15)	P value
Age (year)	60.8 ± 9.2	53.2± 10.3	0.92
BMI (Kg/m2)	32±6.7	25.1±4	**0.04**
Total Cholesterol (mmol/l)	5.2 ±1	4.9 ±0.8	0.43
HDL cholesterol (mmol/l)	1.2 ± 0.2	1.3± 0.5	0.07
Triglycerides (mmo/l)	1.6± 0.9	1.3 ± 0.51	0.09
LDL cholesterol (mmol/l)	3.2± 0.9	3± 0.8	0.99
Glycemia (mmol/l)	5.8 ±1.7	5.3± 0.7	0.12
DM (%)	36.4	13.3	0.12
Tabaco	44.4	66.6	0.23
High risk OSAHOS (%)*	100	18	**0.00**
OSAHOS (%)*	27.8	8.3	0.32
Family CV disease	16.6	8.3	0.51
Family Hypertension	61.1	50	0.55
IGF-1 (ug/l)	249.1± 115.9	322.8± 291.5	**0.019**
GH (ug/l)	1.1 ±1.3	1.7±1.7	0.14
Ejection Fraction (%)**	65.7 ± 9.3	66.6±6.2	0.41
LVM (cm/m²)**	204.7 ±91.1	110.3 ± 24.7	**0.00**
LVT (cm/m²) **	1.4± 1.33	0.8±0.1	0.15
RLVT**	0.45 ± 0.11	0.34 ± 0.03	0.09

Office SBP (mmHg)	132.7±17.1	122.6±14	0.74
Office DBP (mmHg)	83±14.8	80.8±10.6	0.10
24h SBP (mmHg)	122±14.9	113.9±11.1	0.15
24h DPB (mmHg)	72.6±9.5	73.4±7.0	0.53
Daytime SBP (mmHg)	124.8 ±13.5	115.7± 10.9	0.22
Daytime DBP (mmHg)	75.0± 9.3	76.5±7.3	0.56
Nighttime SBP (mmHg)	118.6 ±19.5	109.9± 11.5	**0.03**
Nighttime DBP (mmHg)	67.4±11.6	66.1±8.3	0.21
Nondipper SBP (%)	70	93.3	0.08
Nondipper DBP (%)	50	40	0.557
Hypertensive according to ABPM	5	3	**1**

BMI: body mass index, HDL cholesterol: high density cholesterol, LDL cholesterol: low density cholesterol, DM: Diabetes Mellitus, IGF-1: insulin-like growth factor type 1, GH: growth hormone, OSAHS: Obstructive sleep apnea hypopnea syndrome, CV: cardiovascular, LVM: left ventricular mass, LVT: Left ventricular thickness, RLVT: relative left ventricular thickness, BP: blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, ABPM: ambulatory blood pressure measurement, ABPM: ambulatorial blood pressure, n: number of patients. *: n= 19 patients, **: n=25 patients. Values are presented as mean ± Standard Deviation or frequency.

Table 6. Regression coefficients (beta, ß) of nighttime systolic blood pressure levels and 24 hours (24h) systolic blood pressure levels according to Independent models of multivariable regression with Relative left ventricular thickness as dependent variable.

	Unadjusted			Adjusted[1]			Adjusted[2]		
	β (SE)	P	R^2	β (SE)	P	R^2	β (SE)	P	R^2
Nighttime SBP	0.004 (0.001)	0.017	0.30	0.003 (0.001)	0.044	0.92	0.003 (0.001)	0.035	0.92
24h SBP	0.004 (0.002)	0.056	0.19	0.003 (0.001)	0.045	0.91	0.003 (0.001)	0.049	0.89

	Adjusted[3]			Adjusted[4]		
	β (SE)	P	R^2	β (SE)	P	R^2
Nighttime SBP	0.004 (0.001)	0.021	0.95	0.004 (0.001)	0.017	0.95
24h SBP	0.003 (0.001)	0.052	0.92	0.003 (0.001)	0.064	0.90

Adjusted [1]: Diabetes mellitus, body mass index, Growth hormone, Obstructive sleep apnea hypopnea syndrome.

Adjusted [2]: Diabetes mellitus, body mass index, Insulin like peptide type 1, Obstructive sleep apnea hypopnea syndrome.

Adjusted[3]: Diabetes mellitus, body mass index, Growth hormone, Obstructive sleep apnea hypopnea syndrome, age.

Adjusted[4]: Diabetes mellitus, body mass index, Insulin like peptide type 1, Obstructive sleep apnea hypopnea syndrome, age.

SBP: systolic blood pressure, β: beta, SE: standard error, R^2: entire model, p= p value.

CONCLUSÕES DA TESE:

A avaliação do comportamento do cortisol sérico no pós-operatório constitui uma importante ferramenta para o entendimento do funcionamento corticotrófico peritumoral. Alguns pacientes que atingem a remissão cirúrgica, apresentam um pico de cortisol no pós-operatório imediato maior do que outros. Alguns destes pacientes podem necessitar de um tempo maior para a redução dos níveis de cortisol no pós operatório, podendo ser necessário aguardar de 10 dias a 1 mês para que se defina com segurança o estabelecimento ou não da remissão cirúrgica da DC. O artigo original I, evidenciou que estudando as características destes pacientes, foi possível classificá-los como portadores de DC mais leve, representada por níveis mais baixos de CLU no momento do diagnóstico da SC. Acreditamos que este maior pico do cortisol durante as primeiras 24h de pós-operatório deva-se a menor supressão dos corticotrofos peritumorais secundária a uma forma mais leve de DC nestes indivíduos, com maior resposta ao estresse cirúrgico e anestésico.

Com relação aos pacientes com acromegalia, o uso da MAPA foi capaz de representar de maneira mais fiel a homeostase pressórica e o comprometimento miocárdico destes indivíduos. Os níveis pressóricos medidos pela MAPA foram associados com os marcadores de atividade da acromegalia nestes pacientes e com parâmetros ecocardiográficos marcadores de cardiomiopatia. A PA medida no consultório, além de superestimar a prevalência de HAS, não foi capaz de se associar com estes parâmetros. Tendo em vista que uma das principais causas de mortalidade na acromegalia é a doença cardiovascular, e que a miocardiopatia constitui um importante componente desta, o uso da MAPA constitui ferramenta fundamental para o

manejo destes pacientes. A aferição precisa da PA e o controle adequado desta devem fazer parte da atenção médica oferecida aos pacientes com acromegalia.

ANEXOS:

Outros trabalhos acadêmicos desenvolvidos durante o período do doutorado:

- Artigos publicados em periódicos internacionais:

***1. A successful case of Cushing's disease pregnancy treated with ketoconazole*.** **Costenaro F,** Rodrigues TC, de Lima PB, Ruszczyk J, Rollin G, Czepielewski MA. Gynecol Endocrinol. 2015 Mar;31(3):176-8.

2. Evaluation of the DDAVP Test in the Diagnosis of Cushing's Disease. Rollin GA, **Costenaro F**, Gerchman F, Rodrigues TC, Czepielewski MA. Clin Endocrinol (Oxf). 2015 Jun;82(6):793-800.

3. Low Gestational Weight Gain in Obese Women and Pregnancy Outcome. Moehlecke M, **Costenaro F**, Reichelt AA, Oppermann ML, Leitão CB. American Journal of Perinatology Reports2016 Mar;6(1):e77-82.

- Co-autoria em capítulos de livro:

1. Co-autorado capítulo: **Doença de Cushing: Tratamento Clínico,** do livro Hipófise: Glândula fundamental em Endocrinologia, Editora Atheneu, 2013.

2. Primeira autora do capítulo: **Neuropatia Diabética** do livro do Sistema de Educação continuada a distância PROENDÓCRINO, Ciclo 6- volume 2. Editora Artmed, 2014

3. Segunda autora do capítulo: **Tratamento clínico da Doença de Cushing** do livro do Sistema de Educação continuada a distância, PROENDÓCRINO, Ciclo 7- volume 2. Editora Artmed, 2015

4. Primeira autora dos seguintes capítulos: **Síndrome de Cushing, Acromegalia, Ginecomastia e Neuropatia diabética** e co-autora no capítulo **Doenças Endócrinas na gestação** do livro de Rotinas em Endocrinologia do HCPA, Editora Artmed, 2015.

5. Primeira autora do Capítulo: **Baixa Estatura** do livro de Pediatria Baseada em Evidências. Sociedade de Pediatria do Rio Grande do Sul. Editora Manolle, 2016

- Palestra e aulas proferidas:

1. Palestra "Tratamento da Doença de Cushing": apresentada no IX Simpósio Gaúcho de Neuroendocrinologia, Santa Casa de Porto Alegre, 2013.

2. Palestra "Prolactinoma e Gestação": apresentada no curso de Controvérsias em Obstetrícia: Doenças Endócrinas e Gestação do Hospital de Clínicas de Porto Alegre, 2014

3. Palestra "Pubarca Precoce": apresentada no VII Congresso Gaúcho de Atualização em Pediatria, 2015

4. Palestra "Atualização de anticoncepcionais e sua influência nas manifestações clínicas de hiperandrogenismo": apresentada na 40 Jornada Gaúcha de Dermatologia,2015

5. Palestra "Distúrbios do crescimento, baixa estatura: diagnóstico e manejo clínico" : apresentada na 1 ª Jornada de Pediatria: Pediatria em Foco, ULBRA, 2015

6. Palestra "Obesidade: O novo perfil de nossas crianças": apresentada na1ª Jornada de Pediatria: Pediatria em Foco, ULBRA, 2015

7. Palestra "Insuficiência adrenal na infância": apresentada no VIII Congresso Gaúcho de Atualização em Pediatria, 2015

8. Aula "Alterações do crescimento": Apresentada para alunos de graduação em Nutrição: UFRGS, Disciplina: Patologia da nutrição I, em 2014, 2015 e 2016.

- Participação Acadêmica em Congressos:

1. 31°Congresso Brasileiro de Endocrinologia 2014

Apresentação de Pôster: "A successful case of Cushing's disease pregnancy under ketoconazole treatment".

2. Simpósio Internacional de Endocrinologia-SINE 2014

Co-autoria do Pôster: “Correlação da pressão arterial de 24h e IGF1 na acromegalia”. Prêmio de melhor Pôster.

3. Congresso Endocrine Society, ENDO 2014

Co-autora do Pôster: “Diagnostic challenge of 8 patients with occult ACTH tumor”

4. Congresso Endocrine Society, ENDO 2015

Co-autora do Pôster: ”Mild Cushing's Disease: Definition and Serum Cortisol Dynamics after Transphenoidal Surgery”

5. XX Congresso da Sociedade Brasileira de Diabetes 2015

-Coordenadora da sessão de mini-simpósio: Diabetes na infância e adolescência

-Avaliadora de Pôsteres e temas livres orais

-Co-autora do tema livre oral: “A remissão da Síndrome de Cushing é associada à remissão do Diabetes Mellitus? Análise de 108 pacientes com Doença de Cushing”

6. Congresso Endocrine Society, ENDO 2016

Co-autora do Pôster:“ Cushing's Syndrome and Bone: An Analysis of Related Factors Leading to Increased Risk of Fractures”

PERSPECTIVAS FUTURAS

Pretendo prosseguir na carreira acadêmica dentro de duas linhas de pesquisa que me encantam profundamente, a Neuroendocrinologia e a Endocrinologia Pediátrica. Pretendo fazer pós-doutorado e assim que possível me vincular a alguma instituição de ensino na área da saúde.