Universidade Federal do Rio Grande do Sul

Faculdade de Medicina

Programa de Pós-Graduação em Ciências Médicas: Endocrinologia,

Metabolismo e Nutrição

Dissertação de Mestrado

Avaliação e Seguimento da Atividade da Doença de Cushing no Pós-Operatório da Cirurgia Transesfenoidal

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Porto Alegre, 2011

Avaliação e Seguimento da Atividade da Doença de Cushing no Pós-Operatório da Cirurgia Transesfenoidal

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

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Porto Alegre, 2011

AGRADECIMENTOS

● A Deus, por estar sempre presente em minha vida, iluminando e protegendo.

● Aos meus pais: Tranqüilo, pelo exemplo de ética e entusiasmo profissional e pelos valores humanísticos aos quais me ensinou a respeitar e Jandira, pela força e ouvido amigo de tantas vezes.

● Ao professor Dr. Mauro Antônio Czepielewski, meu orientador, pelos ensinamentos diários, pelo grande professor que foi para mim ao longo destes anos. Exemplo de ética, comprometimento com a profissão médica e respeito constante ao ser humano. Um verdadeiro mestre a ser seguido.

● A professora Drª Ticiana da Costa Rodrigues, minha orientadora, por ter me mostrado o caminho correto a ser percorrido em inúmeras vezes e pela paciência. Pulso firme que me proporcionou grande crescimento na área da pesquisa clínica. Exemplo de garra, determinação e brilhantismo que me serviram de inspiração ao longo desta caminhada. Pessoa maravilhosa e orientadora exemplar.

● Ao meu amor: Samuel, pelo apoio, paciência, amor e companheirismo diário e por nunca duvidar da minha capacidade de atingir os meus objetivos.

● Aos meus irmãos: Denise e Alysson por compreenderem a minha ausência em tantos momentos importantes, pelo apoio e amizade que sempre me dedicaram.

● Aos meus queridos amigos: que Deus me permita a glória de ficar próxima a vocês para retribuir o amor e carinho que vocês me proporcionam.

● Ao Dr. Guilherme Soares Flôres Rollin: por ter dado início a esta coorte de pacientes com tanta dedicação e competência e por ter me oportunizado dar seguimento ao seu trabalho inicial.

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FORMATO DA DISSERTAÇÃO DE MESTRADO

Esta dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Metabolismo e Nutrição da Faculdade de Medicina da UFRGS, sendo apresentada na forma de texto com revisão minuciosa e um artigo original a ser submetido para publicação em periódicos Qualis A Internacional na Classificação da Coordenação de Aperfeiçoamento de Pessoal de Pessoal de Nível Superior- (CAPES).

LISTA DE ABREVIATURAS

Capítulo I

ACTH: hormônio adrenocorticotrófico

CLU: cortisol livre urinário

CRH: hormônio liberador de corticotrofina

IC: Intervalo de Confiança

CTE: cirurgia transesfenoidal

DC: Doença de Cushing

DDAVP: desmopressina

E: especificidade

GABA: ácido aminogamabutírico

HHA: hipotálamo-hipófise-adrenal

PO: pós-operatório

POMC: pro-opiomelanocortina

RC: radiocirurgia estereotáxica

RM: ressonância magnética

S: sensibilidade

SC: Síndrome de Cushing

TC: tomografia computadorizada

LISTA DE ABREVIATURAS

Capítulo II

ACTH: adrenocorticotrope hormone

BMI: body mass index

CI: confidence interval

CD: Cushing Disease

CRH: corticotropin releasing hormone

DDAVP: desmopressin

E: specificity

IPSS: inferior petrosal sinus sampling

OP: operative

PO: postoperative

Pre-OP: preoperative

TSS: transphenoidal pituitary surgery

S: sensitivity

SD: standard deviation

UFC: urinary free cortisol

ULN: upper limit of normality

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Capítulo I

Avaliação do eixo hipotálamo-hipófise adrenal no diagnóstico e na remissão da Doença de Cushing

RESUMO

A doença de Cushing (DC) permanece um desafio médico, com muitas questões ainda não respondidas. O sucesso terapêutico dos pacientes com DC está intimamente ligado à correta investigação diagnóstica sindrômica e etiológica e à escolha da melhor opção terapêutica. Várias são as alternativas terapêuticas, entre elas, as medicações anti-esteroidogênicas e as supressoras dos corticotrofos, a radioterapia, a adrenalectomia e a cirurgia hipofisária. No entanto, a adenomectomia hipofisária transesfenoidal constitui-se no tratamento de escolha para a DC. A avaliação da remissão da doença no pós-operatório e da recorrência em longo prazo constitui um desafio ainda maior, com grande divergência entre os centros médicos especializados sobre qual a melhor maneira de avaliar a atividade da doença e quais são os parâmetros indicadores de remissão e de recidiva ao longo do seguimento. Neste sentido, especial destaque deve ser dado para o papel do cortisol sérico no pós-operatório como um marcador de remissão da DC em longo prazo. Adicionalmente, a utilização de glicorticoide exógeno no pós-operatório apenas em situações de insuficiência adrenal tem sido sugerida por alguns autores, como prática fundamental para permitir a utilização do cortisol sérico neste cenário. Neste artigo, revisamos as formas de avaliação da atividade da DC e os marcadores de remissão e recidiva em longo prazo, após a realização da cirurgia transesfenoidal.

1. REGULAÇÃO DO EIXO HIPOTÁLAMO-HIPÓFISE-ADRENAL (HHA)

O eixo HHA constitui um importante sistema regulatório para a sobrevivência do ser humano. Este eixo é composto basicamente pelo hormônio liberador de corticotrofina (CRH), pelo hormônio adrenocorticotrófico (ACTH) e pelos esteroides adrenais. O CRH é produzido/liberado pelo hipotálamo através de vários estímulos reguladores. Os fatores estimulatórios do CRH são as catecolaminas, a serotonina, a acetilcolina, as interleucinas 1 e 6, entre outros. Desta maneira, o CRH é liberado em situações estressoras como hipoglicemia, hipotensão, frio, febre, trauma, exercício físico e cirurgia. Os fatores inibitórios do CRH são principalmente o ácido aminogamabutírico (GABA) e os glicocorticoides (1)

O CRH estimula o ACTH, que é o hormônio hipofisário estimulador da síntese adrenal de glicocorticoides, com destaque para o cortisol. O ACTH é sintetizado juntamente com outros peptídeos, a partir da clivagem de seu precursor a pro-opiomelanocortina (POMC) (1). A vasopressina hipotalâmica também é estimulatória da síntese de ACTH, atuando diretamente e ou potencializando o estímulo do CRH.

Em condições fisiológicas o ACTH é secretado obedecendo ao ritmo circadiano, e consequentemente, o cortisol também obedece este ritmo, apresentando seu pico de secreção em torno das seis horas da manhã, com uma redução gradual ao longo do dia e seu nadir entre a meia-noite e três horas da manhã (1). Além disto, em indivíduos saudáveis, o cortisol regula a secreção do ACTH através do "*feedback*" negativo sobre a hipófise e o hipotálamo (2).

2. SÍNDROME DE CUSHING

A síndrome de Cushing (SC) endógena, descrita inicialmente em 1932 por Harvey Cushing, é uma condição patológica causada pelo excesso de produção de esteroides adrenais, especialmente de cortisol. Desta maneira, ocorre perda da regulação fisiológica do cortisol sobre a hipófise, comprometendo a inibição da secreção do ACTH e ocasionando a perda do

ritmo circadiano de secreção do cortisol. Na SC, o diagnóstico sindrômico é realizado através da presença de manifestações clínicas sugestivas de hipercortisolismo associadas a exames laboratoriais confirmatórios da hipersecreção de cortisol.

As manifestações clínicas mais específicas da SC incluem: pletora e rubicundez facial, fragilidade capilar e estrias violáceas na pele, fraqueza muscular proximal, osteopenia/osteoporose e ganho de peso. Em crianças, a característica típica é o ganho de peso com redução da velocidade de crescimento. Entre as manifestações menos específicas incluem-se a obesidade centrípeta, a hipertensão arterial sistêmica, o diabetes melito e os diferentes estágios de hiperglicemia, os transtornos psiquiátricos e o hipogonadismo/irregularidade menstrual (3-5). Aproximadamente 20 a 47% dos pacientes com SC possuem intolerância à glicose e/ou diabetes e mais de 70% deles são hipertensos (6). Considerando-se que a prevalência de diabetes melito na população brasileira em geral é de 9,7% [Intervalo de confiança (IC) 95%: 9,0-10,3%] (7), e a prevalência de hipertensão arterial em adultos brasileiros é de 23,9% (IC 95%: 23-24,7%) (8), percebe-se a baixa especificidade destes caracteres clínicos para a presença de SC.

Uma situação clínica que deve sempre exigir atenção médica é a utilização de glicorticoides exógenos, que podem corresponder a 99% dos casos de SC (9). Uma vez que esta situação tenha sido descartada, o hipercortisolismo endógeno poderá ser investigado.

Entre os exames confirmatórios do hipercortisolismo estão: a ausência de supressão do cortisol após 1 mg de dexametasona *"overnight"*: cortisol sérico matinal ≥ 1,8-5 µg/dl {[≥ 1,8 µg/dl, sensibilidade (S): 95%, especificidade (E): 80%] e (≥ 5 µg/dl S: 85%, E: 95%)} (3, 10-11), o excesso de excreção do cortisol livre urinário de 24h (S: 90-100%, E: 50-98%) (3,12) e a presença de um cortisol elevado à meia-noite: salivar > 145 ng/dl (S: 95-100%, E: 93-100% (13) ou sérico com indivíduo dormindo > 1,8 µg/dl (S: 100%, E: 20%) (14) ou indivíduo acordado > 7,5 µg/dl (S: 96 % E: 83-96%) (15) e ainda cortisol sérico > 1,8-5 µg/dl após 2 mg/dia de dexametasona em 48h (0,5 mg 6/6h- teste de Liddle) com uma S: 67-95%, E: 70-100% (3).

Importante ressaltar que é necessária a presença de dois ou mais testes alterados para realização do diagnóstico sindrômico (3). Com a validação do

cortisol salivar à meia-noite, tornou-se possível a avaliação ambulatorial do ritmo circadiano dos pacientes, tendo em vista que o cortisol plasmático à meia-noite necessita de internação hospitalar para sua realização. Para a exclusão do diagnóstico da SC, é necessária a realização de no mínimo dois testes distintos e em momentos diferentes (3,16), tendo em vista a elevada prevalência de um dos testes normais nos casos de SC leve ou cíclica: 54% para o cortisol pós 1 mg de dexametasona "*overnight*" e 92% para cortisol salivar.(16)

Uma vez diagnosticada a SC, é necessário estabelecer seu diagnóstico etiológico. A SC pode ser originada de um tumor hipofisário secretor de ACTH (70-80% dos casos), de um tumor adrenal, de hiperplasia nodular adrenal, de um tumor ectópico produtor de ACTH ou de CRH, ou mais raramente, pela síndrome de McCune-Albright (3,17).

3. DOENÇA DE CUSHING

3.1. Diagnóstico e aspectos demográficos da Doença de Cushing

A doença de Cushing (DC) caracteriza-se pela presença de um tumor hipofisário produtor de ACTH, em sua grande maioria um microadenoma (90% dos casos). Ocorre predominantemente em mulheres (relação 3-4:1), com pico de incidência entre a terceira e quarta décadas de vida (17). A incidência estimada é de 0,7 a 2,4 casos/milhão/ano (3,18-20), com prevalência de 39,1 casos por milhão de habitantes (21).

O risco relativo de morbidade e mortalidade na DC varia de 2 a 6 vezes acima da população em geral (18-20), podendo equiparar-se ao risco da população em geral durante a remissão da doença (19). Neste cenário, a DC exige diagnóstico preciso para idealmente obter-se tratamento eficaz, com resultados rápidos e remissão em longo prazo.

O diagnóstico etiológico de DC nos pacientes com SC é estabelecido através de: 1) ACTH sérico normal ou elevado, 2) supressão do cortisol sérico ou urinário > 50% em relação ao basal, após a administração de 8 mg de dexametasona "overnight" ou 2 mg 6/6h por 48h (S: 65-100%, E: 50-100%) (17,22), 3) presença de adenoma em imagem hipofisária: tomografia

computadorizada (TC) (S: 20-50%) ou ressonância magnética (RM) (S: 60-70%) (10,23), 4) nos pacientes com imagem hipofisária negativa é necessária a presença de gradiente do ACTH durante cateterismo bilateral simultâneo do seio petroso inferior: ≥ 2 em relação ao ACTH periférico ou ≥ 3 após estímulo com CRH (S: 96%, E: 93%-100) ou com Desmopressina (DDAVP) (S: 84-95%, E: 62-100%) (17,24,25). Adicionalmente, pode ser utilizado o estímulo periférico do ACTH ao CRH como ferramenta auxiliar no diagnóstico da DC: elevação > 50% do ACTH sérico em relação ao seu basal (S: 86%, E: 94%) ou ainda a resposta ao DDAVP: elevação >50% do ACTH em relação ao seu basal sérico (S: 82-87%, E: 85-91%) (26,27).

Com relação ao teste do DDAVP, é relevante o fato de alguns tumores produtores de ACTH ectópico, especialmente o carcinoide brônquico, responderem ao DDAVP (28), comprometendo a acurácia deste teste na diferenciação da DC dos tumores ectópicos produtores de ACTH. Por outro lado, este teste tem se mostrado acurado na diferenciação da DC dos casos que mimetizam a doença (Pseudo-Cushing). Neste cenário atingiu acurácia de 85-94% para um pico de ACTH ≥ 27 pg/dl após DDAVP (29,30). Tirabassi e cols (31) também encontraram boa acurácia (90%) para o incremento do ACTH > 18 pg/dl após DDAVP associado ao cortisol sérico basal > 12 ng/dl na diferenciação da DC com estados de Pseudo-Cushing.

Devido à relativa raridade da DC, poucos centros médicos têm experiência suficiente para estabelecer protocolos diagnósticos e terapêuticos baseados em suas casuísticas.

3.2. Terapêutica da Doença de Cushing

O tratamento ideal para a DC inclui a resolução das alterações clínicas e bioquímicas da DC, com mínima morbidade, além de controle da doença em longo prazo sem recidiva (32). Atualmente, a cirurgia hipofisária transesfenoidal (CTE) constitui-se no tratamento de escolha para a maioria dos pacientes com DC.

Os tratamentos alternativos encontram seu papel na terapêutica da DC em algumas circunstâncias como, por exemplo, na falência terapêutica da CTE, na recidiva após CTE ou em pacientes muito enfermos, no sentido de permitir

uma posterior realização da CTE. Os tratamentos de segunda linha constituem-se na radioterapia, na adrenalectomia bilateral e no uso de medicamentos que inibem a síntese de esteroides adrenais ou antagonizem os seus efeitos (33,34) ou ainda nas medicações que inibem a síntese de ACTH.

A adrenalectomia bilateral está associada à taxa de cura de aproximadamente 100% dos casos de DC, com rápida resolução do hipercortisolismo. No entanto, implica em uso de reposição de glicocorticoide e mineralocorticoide ao longo de toda a vida do indivíduo, além do risco de 8-45% dos pacientes desenvolverem Síndrome de Nelson (18). A Síndrome de Nelson caracteriza-se pelo crescimento, muitas vezes agressivo, do adenoma hipofisário secretor de ACTH, associado à hiperpigmentação cutânea e sinais e sintomas de tumor em sela túrcica, como cefaleia e alterações de campo visual. A Síndrome de Nelson ocorre devido à suspensão da supressão hipofisária pelo cortisol adrenal em decorrência da adrenalectomia (35). Em estudos antigos, o risco de mortalidade perioperatória da adrenalectomia chegava a ser 5-7 vezes superior ao da CTE (36,37). Porém mais recentemente, em estudo com pacientes operados por videolaparoscopia, observou-se uma mortalidade PO de 1,1% em 90 dias (38). Poucos estudos avaliaram a sobrevida dos pacientes com DC após adrenalectomia como tratamento isolado e os dados variam desde um prognóstico pior ao da população em geral (39), até um prognóstico semelhante ao da população em geral para os pacientes que sobrevivem ao PO precoce (36).

A radioterapia convencional, neste contexto, é geralmente um tratamento complementar aos pacientes não curados com a CTE, chegando a atingir remissão em até 83% dos casos (40). A remissão pode ocorrer, porém, até 10 anos após o tratamento radioterápico. A complicação mais comum da radioterapia é o hipopituitarismo, ocorrendo em até 76% dos pacientes tratados (40). O risco de uma segunda neoplasia após radioterapia é de 2% após 10 anos e 2,4% após 20 anos de seguimento (41). As complicações cerebrovasculares também são temíveis, sendo identificado risco relativo de mortalidade cerebrovascular ao redor de 5,23 após radioterapia, em coorte de pacientes portadores de adenoma hipofisário secretor e já submetidos à cirurgia hipofisária, no Reino Unido (42). A prevenção da Síndrome de Nelson

nos pacientes adrenalectomizados, também constitui indicação de radioterapia, pois reduz de 50 para 25% o risco do seu desenvolvimento (43).

A radiocirurgia estereotáxica (RC) tem sido utilizada como alternativa à radioterapia convencional no tratamento da DC. A RC consiste na emissão de uma alta dose de radiação direcionada ao adenoma hipofisário, geralmente realizada em uma única sessão, podendo ser utilizado sistema de imagem cerebral para auxiliar na determinação do alvo (44-46). Em uma recente revisão (45) foi observado um tempo de remissão da DC mais frequentemente entre 12 e 24 meses após RC e as taxas de remissão, incluindo estudos com mais de 10 pacientes, variaram entre 54 a 83% (45,47-49). Hipopituitarismo e recidiva da DC ocorreram na mesma proporção, em torno de 20% dos casos (47,49). Desta maneira, devido à menor latência de tempo para a remissão da doença, menor risco de hipopituitarismo e menor radiação às estruturas adjacentes sugere-se que a RC seja superior à radioterapia convencional no tratamento da DC (44,49).

As medicações disponíveis para o tratamento da DC constituem-se até o momento em medida adjuvante. Os fármacos mais utilizados são os inibidores da esteroidogênese adrenal, destacando-se no Brasil o mitotano e o cetoconazol. O cetoconazol é a droga mais comumente utilizada em nosso meio. Em meta-análise de oito estudos com o uso do cetoconazol, foi identificada remissão da DC em 70% (IC 95%: 25-93%) dos pacientes (50). Os paraefeitos mais comuns do cetoconazol são gastrointestinais e rash cutâneo, no entanto, o maior temor é a disfunção hepática, com risco de 10% de alteração de transaminases hepáticas e raros casos de insuficiência hepática fulminante (51-53). O mitotano apresenta maior frequência de eventos adversos, especialmente gastrointestinais, neurológicos e insuficiência adrenal. Destaca-se o potencial teratogênico do mitotano mantido por até cinco anos após a suspensão da medicação (51).

Recentemente, outras classes de medicação têm adquirido destaque no tratamento da DC: os agonistas dopaminérgicos e os análogos da somatostatina direcionados para o corticotrofinoma. Estudo recente avaliou a utilização da cabergolina em 20 pacientes com DC e evidenciou a persistência da normalização do cortisol livre urinário (CLU) em 8/20 (40%) pacientes após dois anos de seguimento (54). Estudos com octreotide não mostraram

respostas consistentes na DC (55,56), no entanto, outro análogo da somatostatina: o pasireotide demonstrou, em estudos de fase II, a normalização do CLU em 17% dos 29 pacientes avaliados (57).

Uma nova alternativa no uso das medicações na terapêutica da DC tem sido a associação de diferentes classes medicamentosas. Neste sentido, Vilar e cols avaliaram em uma coorte de 12 pacientes com DC não curados com a CTE, o cetoconazol foi associado à cabergolina nos indivíduos em que esta não normalizava o CLU e foi observada completa normalização da excreção do CLU em dois terços dos pacientes (58). Feelders e cols associaram cetoconazol, pasireotide e cabergolina de forma escalonada em 17 pacientes e as respostas encontradas foram às seguintes: a monoterapia com pasireotide normalizou o CLU em 5/17 (29%) pacientes, o acréscimo de cabergolina normalizou o CLU em mais 4/17 (24%) pacientes e o acréscimo de cetoconazol às duas drogas controlou a doença em 6/8 pacientes que ainda apresentavam elevação do CLU, alcançando-se 88% de normalização do CLU com o protocolo utilizado (59). Kamenický e col (60) avaliaram o uso da combinação simultânea do mitotano, metirapona e cetoconazol em 11 pacientes com SC grave dependente de ACTH e identificou normalização do CLU em todos os pacientes, em 24-48h após introdução da terapêutica.

A CTE com adenomectomia seletiva constitui-se no tratamento de escolha para a maioria dos pacientes com DC (61). A mortalidade perioperatória após CTE para DC é mínima (1-4%) em centros com experiência em cirurgia hipofisária, e na maioria das vezes, ocorre por infarto agudo do miocárdio ou tromboembolismo pulmonar (62-64). O risco de complicações perioperatórias maiores (fístulas, hiponatremia severa, diabetes insipidus permanente) está em torno de 3-15% (63-65) e o risco de hipopituitarismo fica em torno de 30-50%, quando incluída a deficiência de hormônio de crescimento (64, 66-68).

Há evidências de que pacientes com DC curados após CTE apresentam mortalidade em longo prazo semelhante à da população em geral, controlada para sexo e idade (20,69). A taxa de remissão após CTE observada em bons centros varia entre 53-96% (10,63,64,70-73), chegando a 96% nos microadenomas (64) e tão baixas quanto 53% nos macroadenomas (72). Além disso, as taxas de cura na segunda CTE são inferiores as da primeira CTE e

variam em torno de 28-67% (10,73-75,76), já as chances de hipopituitarismo aumentam nas reintervenções, alcançando taxas de 46-100% após a segunda CTE (73-76).

A possibilidade de reintervenção cirúrgica precoce (menos de 15 dias de intervalo entre as CTE), objetivando-se encontrar menor reação local cicatricial com menor mudança da anatomia da sela, foi proposta por alguns autores (68,76). Na casuística de Locatelli e cols (76) apesar do sucesso em 67% dos 12 casos, 100% dos indivíduos desenvolveram algum grau de hipopituitarismo após a segunda intervenção cirúrgica e em 83% dos casos houve alguma complicação leve no pós-operatório (PO). Na publicação de Rollin e cols (73) a taxa de remissão foi de 0% dos cinco pacientes com doença persistente submetidos precocemente à segunda CTE.

A recidiva da DC ocorre em 5 a 64%% das vezes em até cinco anos após a CTE (10,73,74,77). Estas discrepâncias entre os diferentes centros devem-se aos diferentes critérios de remissão adotados e à duração do seguimento dos pacientes.

4. AVALIAÇÃO DA REMISSÃO E DA RECIDIVA DA DOENÇA DE CUSHING NO PÓS-OPERATÓRIO

Devido à elevada morbimortalidade dos pacientes com DC ativa, é de fundamental importância que pacientes não curados pela CTE ou com risco de recidiva da doença sejam identificados precocemente, para que se possa introduzir um segundo tratamento que seja efetivo assim que a falência terapêutica tenha sido identificada. Neste cenário, critérios de cura fidedignos são essenciais para a definição de remissão da DC e a identificação de marcadores capazes de predizer a recidiva da doença é fundamental para o seguimento destes pacientes (78).

Os critérios mais aceitos para remissão da DC são a resolução clínica do hipercortisolismo associada a algum dos seguintes critérios: cortisol suprimido após a administração de 1mg de dexametasona overnight (57,79,80), duas ou três cortisolúrias de 24h dentro do limite da normalidade (10,65,71,78,81,82), dependência ao uso de glicocorticoide exógeno por insuficiência adrenal (68,74) ou mesmo algum destes critérios isolados ou em associação. No

entanto, a melhor maneira de avaliar a remissão e o risco de recidiva no PO ainda não está definida.

A avaliação de cura da DC inicia antes mesmo da realização da CTE, pois pacientes com doença mais severa, com tumores maiores (macroadenomas) e com extensão além da sela túrcica geralmente possuem menor chance de cura (20,65,71-74,78,79). Apesar disso, um grupo não identificou a influência do tamanho tumoral, invasão tumoral ou crescimento extra-selar como marcadores de desfechos cirúrgicos (63). Os pacientes com presença de lesão bem visualizada no exame de imagem hipofisária (TC ou RM) são considerados como tendo maior chance de cura (63,83). A experiência e habilidade do neurocirurgião também são fatores de grande impacto para o sucesso da CTE (84-86). A presença de um adenoma produtor de ACTH no anatomopatológico do PO também é considerada um dos fatores associados à maior remissão da DC (63,71,72,81). Entretanto, Chee e cols observaram que o anatomopatológico com presença de adenoma foi um bom marcador de remissão da DC apenas quando em associação ao achado radiológico positivo (75). O tempo de utilização de glicocorticoide pelos pacientes no PO também é sugerido como inversamente relacionado com o risco de recorrência da DC (63). Entretanto, um grupo de pesquisadores (74), não encontrou correlação entre a duração da insuficiência adrenal com tempo de remissão da DC nos pacientes com macroadenomas.

4.1. AVALIAÇÃO DO EIXO HIPOTÁLAMO-HIPÓFISE-ADRENAL NO PÓS-OPERATÓRIO

4.11. Níveis de Hormônio Adrenocorticotrófico no pós-operatório

A queda dos níveis de ACTH no PO tem sido sugerida como marcador de remissão da DC em alguns estudos. Grahan e cols (87) identificaram queda de 40% nos níveis de ACTH uma hora após a CTE em 82% dos indivíduos considerados em remissão para DC e uma queda menor que 40% nos níveis

de ACTH em 71% dos indivíduos não curados. Entretanto, a acurácia do teste foi de apenas 78%, sugerindo-se que o declínio do ACTH no PO não seja uma medida acurada para predizer a completa ressecção tumoral na DC (87). No estudo de Pereira e cols (88) um valor mais baixo do ACTH no PO foi marcador de remissão e não de recidiva da DC. Acebes e cols (21) encontraram o ponto de corte de ACTH no PO de 34 pg/dl com S: de 80% e E: 97,5% para identificar pacientes com remissão da DC. Para Invitti e cols (10) houve associação entre risco de recorrência e os valores de ACTH mais elevados no PO.

4.12. Teste de supressão com dexametasona

O uso do cortisol após supressão com dexametasona "*overnight*" foi avaliado por Bochichio e cols em 1995 (63), dos 510 pacientes com teste de supressão normal, 65 (12,7%) sofreram recorrência da DC. Na publicação de Chen e cols (80) 93% dos pacientes com supressão do cortisol matinal das 8h < 3 µg/dl, avaliado no terceiro dia de PO, permaneceram em remissão da DC durante cinco anos de seguimento.

4.13. Teste estímulo com Hormônio Liberador de Corticotrofina (CRH) ou com Desmopressina (DDAVP) no pós-operatório

A resposta do cortisol/ACTH ao CRH exógeno logo após a cirurgia é sugerido como sendo um bom marcador de persistência do tumor hipofisário (10,89,90). Porém, devido à dificuldade de importação do CRH humano e ao seu alto custo, o uso do teste do DDAVP (Desmopressina) no PO vem sendo sugerido como alternativa como marcador de persistência e recidiva da DC (91-94), com raros e leves paraefeitos após a sua aplicação como náuseas e rubor facial. O DDAVP é um análogo sintético da vasopressina, sem efeito vasopressor, que atua especificamente nos receptores V2 renais e nos receptores V3 que estão "*up-regulated*" nos adenomas corticotróficos. Desta maneira induz uma significativa elevação do cortisol e do ACTH na maioria dos pacientes com DC (95,96). Os indivíduos saudáveis não costumam responder ao DDAVP (97). Sendo assim, para os pacientes com DC responsivos ao

DDAVP na avaliação pré-operatória, o desaparecimento da resposta do cortisol e/ou do ACTH ao DDAVP no PO foi sugerido como marcador de remissão da DC (92) e o retorno dessa resposta como sendo um indicador de recidiva (84,95,97,98).

Estudos recentes avaliaram o uso do estímulo com CRH ou DDAVP após supressão com dexametasona para predizer remissão em longo prazo no PO da CTE dos pacientes com DC. O objetivo da dexametasona seria a supressão dos corticotrofos normais, permitindo que apenas as células tumorais respondessem ao CRH ou ao DDAVP. O teste com CRH não atingiu uma boa acurácia (32-47%) para avaliar recorrência da DC (99). Entretanto, com relação ao teste com DDAVP Castinetti e cols (100) acompanharam 38 pacientes submetidos à CTE por aproximadamente 60 meses e o teste alcançou S: 100, E: 89% como marcador de recorrência da DC, sinalizando a recidiva da DC com 6-60 meses de antecedência em relação aos marcadores clássicos do hipercortisolismo.

4.14. Dosagem do cortisol pós-operatório

A avaliação do cortisol no PO da CTE tem sido sugerida como a maneira mais fidedigna de predizer remissão da DC. Os protocolos de aferição variam entre os diferentes centros, seja com relação ao momento de aferição do cortisol mais precoce (desde o PO imediato até duas semanas após a cirurgia) (21,64,68,73,75,77,79,82,86,88,101-107) e/ou mais tardio (entre 1 a 6 meses de PO) (10,66,75,86,88), seja pela utilização de diferentes esquemas de corticoide exógeno como rotina desde o período perioperatório ou apenas em caso de evidência clínica ou bioquímica de insuficiência adrenal (21,79,102,107). Esta variabilidade entre os diferentes estudos e centros com relação aos protocolos de aferição do cortisol sérico no PO acarreta dificuldades na comparação dos resultados.

Na década de 90, após a publicação em 1993 de Treiner e cols (68) reforçada pelas publicações de McCance e cols (101,108) acreditava-se que valores de cortisol indetectáveis (cortisol sérico< 1,8 µg/dl) no PO eram indispensáveis para definir a remissão da DC e que níveis detectáveis de cortisol neste período seriam marcadores de falência cirúrgica. Propunha-se

inclusive, a indicação de nova intervenção terapêutica, seja cirúrgica ou radioterápica (68,109,76). A partir do final da década de 90, alguns estudos começaram a identificar casos de pacientes com níveis indetectáveis de cortisol no PO que recidivaram a DC (67,101).

O questionamento sobre a necessidade de valores indetectáveis de cortisol no PO para definir remissão da DC iniciou após a publicação de Bochicchio e cols (63) em 1995, na qual 33 (24,4%) dos 135 pacientes com níveis detectáveis de cortisol matinal no PO apresentaram recorrência, e 4 (4%) dos 94 pacientes com níveis indetectáveis de cortisol apresentaram recorrência. Yap e cols (86) observaram que 7 (11,5%) dos 61 pacientes com níveis de cortisol sérico < 1,8 µg/dl sofreram recorrência da doença em 36 meses de seguimento. Outro estudo identificou recorrência em longo prazo de até 20% nos pacientes com insuficiência adrenal no PO (110). Observou-se também que níveis detectáveis de cortisol no PO precoce poderiam lentamente declinar ao longo dos dias e estes pacientes permaneciam em remissão por longos períodos (79,88,111).

A premissa da necessidade de valores indetectáveis de cortisol no PO vem da lógica de que a supressão crônica dos corticotrofos saudáveis pelo excesso de cortisol na DC se manifestaria por níveis indetectáveis de ACTH e consequentemente de cortisol, após a remoção completa do adenoma produtor de ACTH (112). Neste sentido, a persistência de níveis de cortisol sérico detectáveis no PO poderia ser um marcador de persistência da lesão tumoral (78,111). No entanto, é possível que a adrenal hiperplásica resultante do estímulo crônico de ACTH pelo tumor hipofisário desenvolva uma semi-autonomia e persista produzindo níveis detectáveis de cortisol, mesmo na ausência do estímulo tumoral do ACTH, ocasionando queda mais tardia nos níveis séricos do cortisol no PO (88). Outra especulação para este declínio mais lento do cortisol seria a de que uma porção das células tumorais sofreria necrose após a cirurgia, com redução gradual dos níveis séricos de cortisol ao longo dos dias de PO. A menor confirmação histológica de adenoma nestes pacientes com queda mais lenta dos níveis do cortisol no PO, sugerindo necrose "in situ", reforçaria esta teoria (111). Sugere-se ainda que pacientes com DC mais leve teriam menor inibição dos corticotrofos saudáveis e a redução do cortisol PO poderia ser mais gradual (102,107). Além disso, Pereira

e cols (88) observaram que indivíduos com DC por macroadenoma que atingiram remissão com a CTE apresentaram queda mais tardia dos níveis de cortisol no PO.

A avaliação no PO imediato através das dosagens de cortisol sérico mostra-se de extrema importância a curto e longo prazo na avaliação da remissão da DC e em alguns estudos pôde prever o risco de recidiva tumoral (63,68,79,81,101). No entanto Pereira (88) e Chee (75) não encontraram relação dos níveis de cortisol no PO e risco de recidiva na DC.

Metanálise recente (114) sobre os níveis de cortisol no PO na DC, incluiu 14 estudos, totalizando 786 indivíduos com níveis subnormais de cortisol no PO e 319 indivíduos com valores normais, com um seguimento variável de 2 a 9,6 anos. Esta metanálise identificou uma taxa de recorrência cumulativa da DC de 9% (IC 95%: 6-12%) no grupo com cortisol indetectável e de 24% (IC 95%: 17-31%) no grupo com cortisol normal. O risco relativo cumulativo de recidiva foi de 0,39 nos indivíduos com cortisol subnormal (IC 95%: 0,25-0,47 p< 0, 001).

É importante salientar que muitos centros médicos administram corticoide exógeno antes, durante e após a CTE, através de diferentes protocolos de emprego da medicação; tais procedimentos são justificados pelo temor da potencial insuficiência adrenal central no PO (74). Contudo, por definição, os pacientes com DC possuem um eixo HHA altamente ativado e estudos de medida intra-operatória dos níveis de cortisol e ACTH observaram que tanto o ACTH quanto o cortisol elevados declinam gradualmente após a adenomectomia hipofisária completa (87). De fato, considerando a meia vida do cortisol sérico e do ACTH, os valores de ambos ainda são detectáveis nas primeiras 12 a 14 horas de PO (34). Os glicocorticoides levam em torno de 15 minutos para inibir a transcrição do gene da POMC no corticotrofo normal (115). Além disto, mesmo em condições patológicas como na DC, aproximadamente 2% dos pacientes suprimem completamente o cortisol após teste de 2 mg de dexametasona, indicando que há relativa sensibilidade dos corticotrofos (113). Desta maneira, no período PO, a síntese e liberação do ACTH pelos corticotrofos tumorais remanescentes pode ser inibida pela administração de glicocorticoide exógeno, mesmo em baixas doses, especialmente por períodos mais prolongados (dias a semanas) (113). Além

disso, o uso rotineiro de dexametasona pode estender ainda mais o período de supressão do eixo HHA.

É importante salientarmos a pobreza de informações a respeito do uso de medicação inibidora da esteroidogênese adrenal no período pré-operatório. Tendo em vista a meia-vida prolongada destas medicações, podendo chegar a meses no caso do Mitotano, poucos grupos (10,88,102) fazem menção ao uso ou não destas medicações e o período de suspensão no pré-operatório antes da avaliação do eixo HHA.

Portanto, o protocolo ideal de avaliação no PO da CTE seria aquele que além do controle para o uso de medicações que interfiram no eixo HHA no pré-operatório, realizasse avaliação clínica e laboratorial do paciente no PO, com coleta de cortisol sérico a cada 6 horas do PO e com a administração de corticoide exógeno apenas no paciente que apresentasse sintomas de insuficiência adrenal como hipotensão arterial ou valor de cortisol sérico abaixo de 2-5 µg/dl, mesmo na ausência de sinais e sintomas clínicos de insuficiência adrenal (34,79). Poucos grupos têm analisado o cortisol sérico no PO sem a reposição de glicocorticoides. Temos conhecimento de quatro publicações destes grupos (21,79,102,107) descritos na Tabela 1

Simmons e cols. (107) foram os pioneiros na avaliação da dinâmica do cortisol no PO da CTE sem uso de corticoide exógeno como rotina. Analisaram 27 pacientes com DC através da medida do cortisol da meia-noite no pré-operatório e a cada 6h (6h, 12h, 18h e meia-noite) nos primeiros três dias de PO. Durante o primeiro dia de PO identificaram excelente acurácia para um nadir de cortisol sérico abaixo de 10 µg/dl associado a um nadir do cortisol PO abaixo do valor do cortisol medido a meia-noite do pré-operatório, com S: 95,4% e E: 100% para remissão da DC, durante seguimento de 27 meses. No entanto, avaliando isoladamente um valor do cortisol abaixo de 10µg/dl nas primeiras 24h de PO encontrou S: 72.7% para remissão da DC. A publicação evidenciou de forma pioneira a segurança do uso do corticoide exógeno neste período apenas se evidência clínica ou bioquímica de hipocortisolismo.

Rollin e cols (79) avaliaram prospectivamente 41 pacientes, a dosagem do cortisol sérico <7,5 µg/dl 10-12 dias após a CTE foi considerado um excelente marcador de remissão da doença, com S: 100% e E: 100%. Entretanto, os 15 pacientes iniciais foram analisados com o uso de corticoide

como rotina no transoperatório, tendo em vista a prática aceita na época (110). Sendo assim, a medida do cortisol nestes pacientes foi realizada apenas uma semana após a realização da CTE. Os 26 indivíduos analisados através da medida do cortisol sérico a cada 6h por 24h de PO, usando corticoide apenas se necessário (sintomas de insuficiência adrenal e ou cortisol sérico abaixo de 5 µg/dl) apresentaram em 24h um valor bem acurado para o nadir do cortisol: < 10 µg/dl para predizer a remissão da DC, com S: 90% e E: 100 %.

Esposito e cols (102) estudaram prospectivamente 40 pacientes com DC submetidos à CTE com uso de corticóide exógeno apenas se insuficiência adrenal no PO, através da medida do cortisol das oito horas da manhã no primeiro e no segundo dias de PO. Utilizaram como ponto de corte o nadir do cortisol ≤ 5 µg/dl entre estas duas medidas e identificaram 97% indivíduos em remissão durante seguimento médio de 32 meses. A média do nadir do cortisol no PO foi de 2,05 ± 1,2 µg/dl nos indivíduos em remissão da DC e 22 ± 12,8 µg/dl nos indivíduos com persistência da doença.

Acebes e cols (21) avaliaram prospectivamente 44 indivíduos com DC operados por CTE, através da dosagem do cortisol às oito horas da manhã do primeiro de PO e o ponto de corte de 21 µg/dl apresentou S: 97% e E: 100% para definir remissão da DC ao longo do seguimento médio de 49 meses.

Conforme supracitado, estes quatro grupos (21,79,102,107) apresentaram resultados animadores, no entanto com número reduzido de pacientes e tempo relativamente curto de seguimento.

CONCLUSÕES

A DC permanece um desafio médico em termos de diagnóstico etiológico. Entretanto, a melhor maneira de prever a remissão e a recorrência da doença em longo prazo constitui-se em desafio ainda maior. Devido à raridade de estudos analisando os pacientes com DC no PO da CTE sem uso de corticoide (21,79,102,107), com diferentes pontos de corte discriminatórios, pequeno número de pacientes estudados, além de diferentes momentos de mensuração do cortisol, considerou-se importante a avaliação de uma coorte de pacientes que não receberam glicocorticoide exógeno no transoperatório e que foram acompanhados a médio-longo prazo para que se pudesse obter uma

melhor compreensão sobre os determinantes de remissão e recidiva da DC após a CTE.

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Tabela 1: Avaliação do cortisol sérico sem uso de corticoide no transoperatório de cirurgia hipofisária para tratamento de Doença de Cushing

Autor, ano	n	Cortisol PO	Critério de remissão: Cortisol (µg/dl)	S/E (%)	Seguimento (meses)
Simmons, 2001	27	6/6h 1°dia	10 e < 24h	95/100	27
Rollin, 2004	26	6/6h 1° dia	10	90/100	56
Esposito, 2006	40	8h 1°, 2°dia	5	97/97	32
Acebes, 2007	44	8h 1° dia	21	97/90	49

n: número de pacientes; PO: pós-operatório; S: sensibilidade; E: especificidade.

24h = cortisol da meia-noite no pré-operatório.

Capítulo II

ARTIGO ORIGINAL

Cushing´s disease long term remission based on serum cortisol dynamic early after transsphenoidal surgery evaluation

Cushing´s disease long-term remission based on serum cortisol dynamic early after transsphenoidal surgery evaluation

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Disclosure summary: The authors have no relevant conflict of interest to disclose.

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ABSTRACT

Cushing´s disease (CD) remission and recurrence predictors are still a matter of debate. This study evaluated the serum cortisol dynamic after transsphenoidal pituitary surgery (TSS) and its ability to predict remission and recurrence of CD. A cohort of 103 CD patients was prospectively analyzed at 111 TSS. Eighteen patients (18 surgeries) received routine glucocorticoids in transoperative and had serum cortisol measured at 10-12 days after TSS (Protocol I). Eighty-six patients (93 surgeries) had serum cortisol measured at each 6h in the first 24h, at 48h and 10-12 days after TSS, and glucocorticoid administration only if adrenal insufficiency was confirmed (Protocol II). Remission was defined as the absence of clinical hypercortisolism plus cortisol < 3 µg/dl (82.8 nmol/l) in overnight dexamethasone test and/or normal free urinary 24hr cortisol during follow-up. Recurrence was defined as the loss of remission criteria at least a year after TSS. Remission was found in 75.7% of the patients, and recurrence in 8.1% of them. The serum cortisol nadir of 10.7 µg/dl (295.3 nmol/l) at 24h after TSS had sensitivity of 92% and specificity of 87%, and the serum cortisol nadir of 5.7 µg/dl (157.3 nmol/l) at 10-12 days after surgery had sensitivity of 100% and specificity of 92% in predicting remission at 6.0 ± 4.8 years of follow-up. After adjustments, only serum cortisol levels were still associated with CD remission. Recurrence could not be predicted by serum cortisol early after TSS. These results state the importance of serum cortisol values after TSS in predicting CD remission.

INTRODUCTION

Active Cushing Disease (DC) mortality reaches 5-6 times higher than in general population. However, a mortality rate similar to the general population, adjusted for sex and age, can be reached when the disease is remitted (1-3).

Transsphenoidal pituitary surgery (TSS) with selective resection of the corticotroph adenoma is the treatment of choice for CD. Surgical success rates range from 53 to 96% according to different centers (4-12). The definition of disease remission varies among different research groups. They vary from clinical evaluation to laboratory parameters, such as: normalization of postoperative urinary free cortisol (UFC) (3,4,11,13-15), adrenal insufficiency with glucocorticoid dependence (16,17), cortisol suppression after dexamethasone overnight (5,18) and sometimes a group of these different criteria are used. This variability makes it difficult to compare data about remission rates and recurrence between different publications.

Considering the high morbidity and mortality of hypercortisolism in CD patients after TSS failure, the early and efficient identification of the active disease optimizes clinical care and appropriate management.

Postoperative cortisol level has been proposed as the standard criteria to predict surgical remission after TSS. Serum cortisol has been analyzed on early and immediate postoperative (PO) (until two weeks after TSS) (6,12,15,16,18-30) and or latter PO (between one to six months after TSS) (4,19,22,23,31). Noteworthy, this variable suffers numerous interferences as exogenous glucocorticoids administration and recent preoperative use of steroidogenesis inhibitors. Few casuistics are available without the use of routine glucocorticoid on transoperative time (18,20,26,30). Moreover, these few publications evaluated after surgery cortisol levels at different protocols and moments on the early PO with a limited number of patients and suggested an important role for serum cortisol in predicting long-term remission (18,20,26,30).

An interesting point that has been discussed is the routine administration of transoperative glucocorticoids in all patients who underwent TSS. This handling is supported by the possible risk of an adrenal crisis during surgical stress in CD patients. However, five different groups (18,20,26,30,32) have showed the safety of glucocorticoid administration only in patients with clinical

or laboratorial evidence of adrenal insufficiency. The detection of Adrenocorticotrophic Hormone (ACTH) and cortisol levels even in patients who are considered in remission early after TSS supports the use of glucocorticoid only in these situations (33). Another possible argument for the selective cortisol use is the persistent ACTH release from partially suppressed normal corticotrophs as a result of surgery and anesthesia stress or by mechanical manipulation of pituitary gland (33). There is also the hypothesis that peripheral receptors of glucocorticoids are full of hormone in CD patients (18), what could explain the absence of hemodynamic damages in patients with postoperative cortisol levels between 5 and 10 µg/dl (138 and 276 nmol/) (30,34). The immediate and early postoperative cortisol dynamic evaluation could give information to provide an adequate glucocorticoid replacement and avoid steroid abuse in hypercortisolemic patients.

Acebes et al (20) and Esposito et al (26), that have not used exogenous glucocorticoid as routine on perioperative time studied plasma ACTH at PO to predict CD remission. Their results showed plasma ACTH inferior performance in predicting CD remission when compared to serum cortisol (20, 26). On the other hand, Pereira et al (23) suggested that ACTH plasma levels after TSS are accurate in predicting CD remission.

The aims of this study were to evaluate the dynamics of serum cortisol in a cohort of patients with CD, to confirm the safety of glucocorticoid use at transoperatory time in CD patients only with adrenal insufficiency evidence and to predict the factors associated to remission and recurrence of the disease after TSS.

PATIENTS AND METHODS

Patient Cohort

One hundred eight CD patients who have undergone TSS at our institution and had cortisol serum evaluated at some time until 10-12 days after surgery were assessed since 1989. Five patients dropped out, three of them were lost to follow-up and the other two had a short follow-up period, which prevented these patients to be classified regarding the remission status.

Therefore, 103 patients were included in this analysis. Eight patients were submitted to a second TSS at our institution, five of them because remission was not achieved at the first surgery and the other three due to the recurrence of CD at 4.5, 5 and 5.5 years. These 8 patients were analyzed at postoperative period of both surgeries. Therefore, there are data from 111 postoperative periods of 103 different patients. Figure 1 shows the flowchart of this study.

Two patients were not on remission at their first TSS at another center and were submitted to a second TSS at our institution. One of the 103 patients had multiple endocrine neoplasia type 2A comprising CD, gastrinoma and primary hyperparathyroidism. The study protocol was approved by Ethic Committee and all the patients provided written informed consent.

Biochemical diagnosis of Cushing's disease

Cushing's syndrome was diagnosed after admission as previously described (18) and according to the guidelines from Endocrine Society 2008 (35). Clinical findings and at least two of the following laboratorial criteria were considered: increased levels of at least two out of three urinary free cortisol (UFC), loss of diurnal rhythm :midnight serum cortisol > 7.5 µg/dl (207 nmol/l), absence of cortisol suppression after low dose (1 mg) of dexamethasone overnight test cortisol < 5 µg/dl (138 nmol/l), and/or no suppression of UFC and serum cortisol after oral low-dose dexamethasone suppression testing: Liddle I [UFC < 20 µg/24h (55 nmol/24h); and/or serum cortisol < 5 µg/dl (138 nmol/l) after oral administration of 0.5 mg dexamethasone every 6 h for 48 h].

Diagnosis of pituitary-dependent CD was based on: suppression of UFC and serum cortisol in the oral high-dose dexamethasone suppression test: Liddle II (UFC and/or serum cortisol suppressed > 50% of baseline after 2 mg dexamethasone 6/6 h for 48 h); unsuppressed plasma ACTH; an ACTH increase of at least 50% after DDAVP (desmopressine) administration in some patients; and a pituitary low-intensity/density lesion on magnetic resonance or computed tomography pituitary scan. Since 2002, the inferior petrosal sinus sampling (IPSS) has been performed with DDAVP or CRH in those patients

whose no tumor was identified by pituitary images or for those who did not present suppressed serum or urinary cortisol levels by Liddle II test. The criteria adopted and considered for diagnosis were a basal ACTH gradient central/periphery ≥2 or stimulated ≥ 3. Before 2002, the CD diagnosis of normal pituitary imaging patients was confirmed by a long-term follow-up after TSS.

Surgical approach and glucocorticoid replacement perioperative protocols

The surgery was performed by the same surgeon (N.P.F.) using sublabial selective adenomectomy at least 2 weeks after the diagnostic work-up (including dexamethasone suppression tests) and routinely early in the morning (until 8:00 a.m.). All the patients received prophylactic heparin during hospital admission. Two patients received ketoconazole before surgery and one of them also received mitotane for severe CD. However, the administration of these drugs were interrupted at least a month before the surgery was performed. All the patients remained at an Intensive Care Unit during the first three days after TSS and were closely monitored during this period.

The postoperative assessment of the first 18 surgeries consisted of measurement of serum and urinary cortisol at 10–12 days after the procedure (Protocol I). These patients received intravenous hydrocortisone intraoperatively and prednisone was prescribed as routine after surgery for 18 patients (18).

After the first 18 surgeries, the postoperative assessment protocol was changed (Protocol II). The next 85 patients had serum cortisol measured preoperatively and at 6, 12, and 24 h after the end of the TSS. These subjects received glucocorticoid only in case of clinically diagnosed adrenal insufficiency or in laboratory adrenal insufficiency evidence [serum cortisol < 5 µg/dl (138 nmol/l)]. After the first 24 h after surgery, serum cortisol was measured at 48h after surgery if glucocorticoid therapy was not initiated. Ten to 12 days after surgery, serum and urinary cortisol were also assessed (18). When it was considered necessary a serum and urinary additional samples were collected 28-30 days PO. From this group, three patients were treated according to both protocols, as they underwent TSS twice. Thus, the additional 93 surgeries

followed Protocol II, including eight patients that underwent TSS twice by this Protocol II (Figure 1).

Plasma ACTH was collected 10-12 and 28-30 days PO in all patients. When it was considered relevant, the serum cortisol was additionally analyzed in some patients at 30 days PO.

Patients with low or undetectable serum and/or urinary cortisol levels on both Protocols (I-II) maintained glucocorticoid therapy until recovery of the pituitary-adrenal axis. Patients underwent subsequent overnight oral 1 mg dexamethasone suppression testing at least once a year.

Remission criteria

Long-term remission after TSS was defined by the presence of clinical and laboratory signs of adrenal insufficiency, postoperative normal UFC 24h, and/or serum cortisol levels less than 3.0 µg/dl (82.8 nmol/l) at 08:00 a.m. after administration of 1 mg oral dexamethasone at midnight during the follow-up for at least a year after TSS, and resolution of signs and symptoms of hypercortisolism. The presence of glucocorticoid dependence was previously considered an obligatory criterion to define remission after TSS by our group (18). However, it was observed that a group of patients who had never used glucocorticoid during follow-up were cured and maintained remission up to the end of follow-up.

Recurrence criteria

The recurrence of CD was defined as the loss of the remission criteria at least a year after the TSS. Therefore, some patients were initially considered in remission and then presented recurrence during follow-up; their duration of remission was considered the time of TSS until the last medical evaluation before the CD recurrence had been identified.

Biochemical methodology

Urinary cortisol was measured until April 2004 by RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX) with intra- and interassay coefficients of variation of 8.3% and 9.8%, respectively, and a lower detection limit of 0.3 μg/dl (8.3 nmol/l). The normal range for 24-h UFC was 20–90 μg (55-248 nmol) From 2004 to March 2010 the methodology was changed to electrochemiluminescence immunoassays (ECLIA) Kit (Modular Analytics E 170, Roche Diagnostics GmbH, D-68298 Mannheim, Germany) with intra- and interassay coefficients of variation of 1.7% and 2.8%, respectively; a lower detection limit of 0.3 μg/dl (8.3 nmol/l). The normal range for 24-h UFC was 36–137 μg (99-102 nmol) From March 2010 up to the last assessments the UFC was measured by chemiluminescent immunoassay (ADVIA Centaur XP Immunoassay System, Siemens Diagnostics, Tarrytown, NY) with intra- and interassay coefficients of variation of 4.8 % and 5.5%, respectively, and a lower detection limit of 0.2 μg/dl (5.5 nmol/l). The normal range for 24-h UFC was 55.5-286 μg (153-789 nmol). A competitive chemiluminescent immunoassay (Automated Chemiluminescence System, Bayer Diagnostics, Tarrytown, NY) was used to measure serum cortisol during the first 24–72 h after surgery [intra- and interassay coefficients of variation of 6.0% and 8.4%, respectively; a lower detection limit of 0.2 μg/dl (5.5 nmol/l)]. ACTH was measured by a chemiluminescent enzyme immunometric assay (Immulite, Siemens Healthcare Diagnostics Products Ltd. Lanberis, Gwynedd, UK), with intra- and interassay coefficients of variation of 9.5% and 10%, respectively, and a lower detection limit of 5 pg/ml (1.1 pmol/l).

Since there were changes on UFC normal range during the years of follow-up, the UFC levels were adjusted by percentage over the upper limit of normal range (ULN).

The variation (Δ) of plasma ACTH was defined trough of the difference at determinate time of postoperative value from baseline plasma ACTH levels.

Statistical analysis

Statistical evaluation was carried out by Student's t-test and Mann-Whitney's test for unpaired data and ANOVA for repeated measures. χ^2 test was used to compare categorical variables. Sensitivity, specificity were

calculated according to standard statistical methods. The ROC (receiver operator characteristic) curve was used to quantify the postoperative serum cortisol time and levels diagnostic efficacy for remission. Cox regression over time was used to evaluate the factors/predictors associated with remission after TSS. A *P* value of 0.05 was considered to be significant. SPSS 16.0 (Chicago, IL) was used for these analyses.

RESULTS

The cohort of 103 CD patients analyzed consisted of 79 (76.6%) female patients, mean age was 35.6 ± 12.3 years (range: 12-64), and nine patients were aged under 18 years. Preoperative body mass index [(BMI), weight (kg)/height m^2] was 31.3 ± 6.7 kg/m^2 (range: 20.6-51.2).

The laboratory characteristics were UFC (mean of the three samples) 481 ± 380 µg/24h (range: 40 - 2838) [1329 ±1050 nmol/24h (range 110 – 7833)], or 4.6 ± 3.1 times ULN (range: 3.98 - 5.22); median of serum cortisol after 1 mg dexamethasone overnight: 14.4 µg/dl (range: 3.5 - 70) [397 nmol/l (range 97 – 1932)]; median of midnight serum cortisol: 21.2 µg/dl (range: 9.4 - 120) [585 nmol/l (range 259 – 3312)]; median after Liddle I test for serum cortisol: 12.4 µg/dl (range: 6.4 - 95) [342 nmol/l (range: 177 – 2622)]; and for UFC: 55.5 µg/24h (range: 7.7 - 984) [153 nmol/24h (range: 21 – 2716)]; median of plasma ACTH: 58.2 pg/ml (range: 9.9 - 199) [12.8 pmol/l (range: 2 – 44)]. Liddle II test was positive for cortisol in 79% of patients and for UFC in 41% of the patients. The median serum cortisol for Liddle II was 4.5 µg/dl (range: 0.48 - 31.5) [124 nmol/l (range 13 – 869)] and UFC for this test was 35.1 µg/24h (range: 5 - 1155) [97 nmol/24h (range 14 – 3188)].

Of all 111 pituitary images analyzed, adenomas were not observed in 28 (25%) patients through pituitary CT or MRI scans, and three of those presented empty sella. Thirty one (28%) patients had macroadenoma and 52 (47%) patients had microadenoma. The diagnosis of micro- or macroadenoma was based on pituitary imaging findings in this study. However, adenoma was identified in all patients during TSS.

The histopathologic evaluations confirmed adenoma in 85.4% patients and 1 had hyperplasia, while the others had normal pituitary tissue. Positive immunohistochemistry ACTH was present in 75% of patients.

The post operative BMI of the cohort at the last visit was: 28.1± 3.1 kg/m^2 (range 16.4 - 59.5) and the mean time of follow-up was 6.0 ± 4.8 years. Remission was achieved in 84 (75.6%) patients, according to the data obtained during follow-up. After TSS, the remission group presented a mean time of glucocorticoid dependence of 3.0 ± 4.4 years. Six out of 84 remission patients had not received glucocorticoid after TSS. All the patients considered non-cured or in surgery failure had never received glucocorticoid, except for those evaluated by Protocol I. Of note, all patients with cortisol measured during the first 48h hours after surgery had received glucocorticoid only if laboratorial evidence of adrenal insufficiency was identified and proved.

There were 84 remissions and 27 surgery failures, and the preoperative characteristics are described in Table 1. There were no differences between patients in remission or in failure according to the preoperative clinical and laboratorial characteristics. Patients who presented remission had higher frequency of microadenoma and lower frequency of macroadenoma than patients considered in failure. Moreover, subjects in surgery failure had higher frequencies of normal pituitary images than subjects in remission.

After the 111 TSS, 12 (10.8%) patients had panhypopituitarism (more than two pituitaries deficiencies), 35 (31.5%) patients had hypopituitarism (one or two pituitary axes injured including permanent diabetes insipidus), and isolated corticotrophin axe deficiency was observed in 22 subjects.

The perioperative mortality (until a month after TSS) of the cohort was 1.8%. One patient died from acute myocardial infarction (Protocol I) and other from septicemia (Protocol II). In addition, two patients presented stroke soon after TSS; one of them (Protocol I) became hemiplegic and the other (Protocol II), who had an invasive macroadenoma, became hemiparetic and with third cranial nerve palsy soon after TSS. There were three thromboembolic events during Protocol II: two thrombophlebitis in the arm, and one deep vein thrombosis, which needed anticoagulation treatment. Two patients presented a cerebrospinal fluid leak that required surgical repair (Protocol II). One patient presented a cardiopulmonary arrest without clinical sequel.

Table 2 presents the postoperative characteristics of patients with and without remission. It was observed that patients in remission group had a lower BMI in the last evaluation, higher frequency of adenoma seen in the histopathologic analysis, higher frequency of ACTH positive at immunohistochemistry assessment, and suffered more hypopituitarism than failure group.

Figure 2 shows the curve of the PO serum cortisol for remission and surgery failure group. There was no difference for pre-operative cortisol values between the groups. However, after this period all subsequent levels were different between the groups, patients in remission had lower cortisol levels.

Figure 3 presents the dynamic of cortisol in each patient during transoperative time and it shows the important inter and intra group variability.

Table 3 displays the cut offs for serum cortisol on different times after TSS with their better sensitivity and specificity to predict remission in this cohort of patients. Notably, the accuracy of the serum cortisol improved after 24h PO and the performance of 24h PO cortisol nadir of 10.7 µg/dl (295.3 nmol/l) had sensitivity of 92 % and specificity of 87%. The accuracy of the 48h PO cortisol nadir of 5.4 µg/dl (149 nmol/l) was very similar: sensitivity of 95 % and specificity of 87%. Some patients had a delayed fall of cortisol level, therefore serum cortisol nadir at 10-12 days PO of 5.7 µg/dl (149 nmol/l) or at 28-30 days PO of 5 µg/dl (138 nmol/l) presented the same and the best performance of the PO serum cortisol curve, with sensitivity of 100 % and specificity of 92 %. At 10-12 days PO de nadir of 10.2 µg/dl (281.5 nmol/l) presented specificity of 100% with a sensitivity of 83%.

Figure 4 shows the different times of cortisol nadir in differentiating remission from surgical failure patients, the best accuracy of the nadir at 10-12 days PO comparing to 24h PO, and also the superiority of 28-30 days PO nadir cortisol from 48h PO nadir cortisol in discriminating patients in remission and surgical failure.

Table 4 describes plasma ACTH values in remission and surgical failure patients. Remission patients presented lower plasma ACTH, but it did not present a good performance in predicting CD remission (data not showed). The best cut off for ACTH to predict remission in this cohort was with Δ ACTH at 10-12 days: - 23.9 pg/ml (-5.2 pmol/l) with sensitivity of 70% and specificity of 75%

and at 28-30 days: -18.2 pg/ml (-4.0 pmol/l) with sensitivity of 80% and specificity of 78%.

We analyzed the 24h UFC performance by ROC curve in predicting remission at 10-12 and at 28-30 days PO, but the performance was worse than serum cortisol levels (data not showed).

Additionally, when we defined patients in remission with mild CD according to the UFC levels less than 2 times the ULN, we observed that they presented a higher peak of cortisol levels at 48h PO. Patients with mild disease presented a median of the cortisol peak at 48h of 39.2 ± 10.6 μg/dl (1081.9 ± 292.6 nmol/l) vs. cortisol peak of 27.2 ± 10.6 μg/dl (750.7 ± 292.6 nmol/l) in patients above 2 times the ULN, p<0.04 for the difference between the two groups.

Cox regression was performed to evaluate predicting factors for remission. Initially, we performed models considering remission as the dependent variable, and considering either nadir of 24h PO serum cortisol and micro-and macroadenoma at baseline, or normal pituitary image, or presence of adenoma at histopathology, or ACTH positive at immunohistochemistry, or occurrence of hypopituitarism after TSS, as independent variables. In all models, the nadir of 24h PO serum cortisol was the only variable that remained associated with remission. All the other adjustments lost their association. Table 5 displays these results.

The same analyses were performed replacing the nadir of 24h PO serum cortisol for nadir at 10-12 days PO serum cortisol. Here, the last remained associated with remission after the adjustments for normal pituitary image, presence of adenoma at histopathology, and presence of hypopituitarism after TSS (in individual's models). However, it was not a predictive factor in the model adjusted for micro- and macroadenoma at baseline or ACTH positive at immunohistochemistry. Table 5 also displays these analyses.

Recurrence during all follow-up was observed in only 9 (8.1%) patients with a mean time of 3.9 ± 1.2 years (range: 1.8-5.5) for the occurrence of this event. Table 6 describes the PO serum cortisol of patients according to recurrence. The PO cortisol levels were different for all measurements after TSS; patients with recurrence had higher values than patients with no recurrence. Undetectable cortisol levels: < 2 μg/dl (55.2 nmol/l) at 48h PO did

not accurately exclude recurrence as they presented sensitivity of 67% and specificity of 0% at 24h PO, and sensitivity of 60% and specificity of 40% at 48h PO.

There was no difference according to sex, age, suppression on Liddle II, micro- and macroadenoma or normal pituitary image, presence of adenoma in histopathologic assessments, presence of positive ACTH in immunohistochemistry evaluations, presence of hypopituitarism and Δ ACTH in subjects who had CD recurrence from subjects who did not have (data not showed).

The duration of glucocorticoid dependence in recurrence group was 1.4 ±1.6 years and it was not different from those who did not present recurrence (p = 0.53). Although two out of six patients in remission group that did not need to use glucocorticoid on PO presented recurrence after 37 and 53 months after surgery, it was not efficient in predicting recurrence of CD (p = 0.063).

DISCUSSION

In this study, we found 75.7% of CD remission after TSS, and the nadir of the cortisol at 24h and 10-12 days after surgery were the best predictors of long-term remission in this group of CD patients. Patients with mild CD and in remission had a higher peak of cortisol at 48h after TSS. Undetectable cortisol levels at the first 48h during the curve of cortisol showed no accuracy to predict protection against recurrence over time.

Exogenous glucocorticoids were used, in the most cases, only when adrenal insufficiency was detected clinical or laboratorial. None of our patients from Protocol II presented adrenal crisis. One of them presented hypotension without consequences, some patients presented vague adrenal insufficiency symptom (headache, malaise, nausea, fatigue, prostration) and some remained asymptomatic even with undetectable cortisol level and these patients have received glucocorticoid in the PO time. These informations support the safety of no use of glucocorticoid as a routine after the surgery, as suggested by other authors (20,26,30).

The usefulness of the measurements of early cortisol levels dynamic after the TSS was demonstrated by its ability of predicting long-term remission,

with a cortisol nadir at 24h PO of 10.7 μg/dl (295.3 nmol/l) presenting an interesting performance (sensitivity of 92% and specificity of 87%), although the best point was observed at 10-12 days after TSS [sensitivity of 100% and specificity of 92% for 5.7 μg/dl (157.3 nmol/l), as cortisol nadir in 10-12 days PO] with similar result for the nadir of 5 μg/dl (138 nmol/l) at 28-30 days PO. However, the 10-12 days PO cortisol nadir remained as a remission predictor when adjusted for almost all confusion criteria.

The best point of cortisol serum PO analysis in predicting remission seems to be at 28-30 days PO, but only 53 patients were analyzed at 30 days: especially those whose cortisol levels fall was delayed or those who presented no fall in cortisol serum level (failure group). So, it is difficult to believe this is the best point for the use in clinical practice. Furthermore, the performance of cortisol nadir at 30 days (considering all PO points) was identical to the cortisol nadir at 10-12 days performance. Of note, we have performed Cox regression for 28-30 days PO, and serum cortisol was not significantly associated with the outcome at this time point (data not showed).

The various criteria used to define remission make it difficult to compare different results of previous studies. In this cohort, a remission rate of 75.7% was found, which is similar to other reports (23,26,30). In accordance to other authors (1,3,10-13,17,18), we found that patients on remission group had a lower percentage of macroadenoma. On the other hand, Bochicchio et al (5) did not find association between tumor size or invasiveness and surgical outcomes. Our patients with adenoma identified at pituitary images had higher remission rates than those with normal pituitary images, which is also in accordance to previous publications (5,36). We observed that adenoma identified at histopathologic assessments and positive tumor immunohistochemistry for ACTH were predictive for CD remission, as also reported by other authors (5,10,11,14). Chee et al (19) identified that the presence of an adenoma at histopathology predicted remission only when associated with an adenoma identified at pituitary images. However, in our cohort, when these variables were analyzed by Cox regression, the serum cortisol levels after surgery were more strongly associated with remission than with these other characteristics.

Immediate and early PO serum cortisol has been proposed as important tool to predict remission on CD patients. Firstly, Treiner et al (16), reinforced by

McCance et al (24), suggested than a cortisol level ≤ 1.8 µg/dl (49.7 nmol/l) after surgery was indispensable to achieve long-term remission of CD. After that, some publications showed that patients with undetectable serum cortisol after TSS presented recurrence of CD (5,10,22,31) and other reports stated that some patients with detectable cortisol levels early after surgery could have a slowly and delayed fall of cortisol level across the time after surgery, and remain in CD remission for long time (23,37). However, these trials (5,10,16,22-24,27,31) used exogenous glucocorticoid as routine in the perioperative period.

In a memorable appointment (38), Newell-Price suggested that exogenous glucocorticoid administration could interfere in serum cortisol values measured after surgery. Nowadays, four groups had published about serum cortisol evaluation after TSS without routine use of exogenous glucocorticoids (18,20,26,30).

Simmons et al (30) was the first group to analyze PO cortisol dynamic without the routine use of exogenous glucocorticoids in a group of 27 patients with cortisol levels measured at each 6h for 3 PO days, started at 6:00 p.m. on the day the surgery was performed. They found that a cortisol level lower than midnight preoperative cortisol level plus a nadir < 10 µg/dl (276 nmol/l) on day 1 after surgery correctly classified 21 out of 22 long-term remission patients, during a follow-up of 2.25 years. They showed that 6 out of 22 long-term remission patients had one or more cortisol levels greater or equal to 10 µg/dl (276 nmol/l), suggesting the importance of more than one measurement of serum cortisol. Actually, the authors used combined measurements for evaluation.

Rollin et al (18) suggested a protocol for evaluating CD patients with the measurement of cortisol at each 6h during first 24h after TSS and then at 48h and 10-12 days PO, with a group of patients that had not received routine administration of glucocorticoid on transoperatory period (Protocol II). They found a sensitivity and specificity of 100% for a cortisol nadir PO at 10-12 days of 7.5 µg/dl (207 nmol/l)and a great accuracy for a cortisol nadir of 10 µg/dl (276 nmol/l) at 24h PO with sensitivity of 90% and specificity of 100 % in predicting CD remission .

Acebes et al (20) did not use routine transoperative glucocorticoid in 44 patients and proposed a protocol measuring a unique serum cortisol at 8:00

a.m. on the day after TSS and found sensitivity of 100% and specificity of 90% for a serum cortisol < 21 µg/dl (579.6 nmol/l) in predicting CD remission during a follow-up of 4 years.

Esposito et al (26) did not use routine glucocorticoid in 40 CD patients and suggested a protocol measuring serum cortisol at 8:00 a.m. of the first and second days after TSS and found that a cortisol nadir of 5 µg/dl (138 nmol/l) between these two days had a sensitivity of 100% and specificity of 97% for sustained remission during 2.75 years of follow-up.

In contrast with these studies (20,26,30), we have measured the cortisol at each 6h after surgery for the first 24h and then at 10-12 days PO, and at 30 days PO when deemed necessary. We believe that this protocol is better than the others (20,26,30) because measuring serum cortisol 6/6h after surgery better represents the relationship between pituitary-adrenal axis and corticotrope adenoma withdrawal. This also seems to be better when compared to the measurements of serum cortisol at fixed 6/6h of the day as proposed by Simmons et al (30).

Actually, our findings support the Protocol II suggested by Rollin et al (18) in a larger cohort of patients. A good accuracy of serum cortisol was found by the nadir of the measures at 10-12 days of 5.7 µg/dl (157.3 nmol/l) with better accuracy than unique sample collected at 10-12 days, justifying the necessity of collecting cortisol each 6h PO. The same accuracy of 10-12 days nadir was found with the nadir of 5 µg/dl (138 nmol/l) at 28-30 days PO

Mild CD patients in remission presented a higher peak cortisol along the postoperative period in comparison to other remission patients of this cohort, probably because of a lower previous suppression of normal corticotrope.

Recurrence rate of CD is variable in different publications as a consequence of different remission criteria and distinct time of follow-up in different centers. There were nine cases of recurrence in this cohort during a follow-up of 6 years, which is very similar to longer CD cohorts data (3,23,28), that described 9-12% of recurrence during 7-11 years of follow-up.

In this cohort, the longer period of glucocorticoid dependence was not protective to CD recurrence. Although some authors have not reached the same results (5, 12), another group (17) have already showed that the time of

glucocorticoid dependence in CD patients with macroadenoma had no correlation with the permanence of remission along the time.

Unfortunately, we have not identified a good marker as predictor of recurrence over the follow-up of this cohort, including the undetectable serum cortisol levels at 24-48h after TSS

In contrast to other groups (20,26,30) and even to Rollin et al (18), we found lower accuracy in predicting remission with serum cortisol in the first 24-48h. Probably, this was observed because our cohort was expressively larger, possibly composed by more heterogeneous patients regarding the disease's severity, and with a higher number of patients with delayed fall of serum cortisol. The longer follow-up could have contributed to these different results.

Another point of possible limitation could be the inclusion of patients with exogenous glucocorticoid routine administration (Protocol I) and of patients with no exogenous glucocorticoid routine administration in the same analysis at 10-12 days PO (Protocol II). However, in the first 48h PO only patients from protocol II were analyzed and these data represent the largest cohort described until now without glucocorticoid routine administration on transoperative TSS.

In conclusion, the evaluation of cortisol dynamic on PO after TSS showed to be relevant in predicting long-term remission in patients with CD. Our data reinforce the use of exogenous glucocorticoids in transoperative of TSS only when adrenal insufficiency has been proved and the requirement of long term follow-up for patients with CD.

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Table 1: Preoperative data of Cushing´s disease patients with remission or surgical failure.

	Remission (n=84)	Failure (n=27)	p
Female	66 (78)	20 (77)	0.938
Age (years)	36 ± 12	33 ±11	0.190
BMI (kg/m²)	31 ± 6	32 ± 7	0.559
24h-UFC (µg/24h)	380 (40-1384)	421 (61-2838)	0.161
Overnight cortisol (µg/ dl)	14 (3.5-70)	17 (7.4-55.2)	0.241
Midnight cortisol (µg/dl)	20 (9.4-120)	23 (10-45.3)	0.314
Cortisol 8h µg/ dl	25 (11.3-71.8)	29 (8.3-100)	0.881
Cortisol 8 mg < 50%*	41 (80)	8 (73)	0.571
UFC 8 mg < 20 µg/ 24h **	19 (45)	2 (22)	0.203
Plasma ACTH (pg/ml)	60 (23-199)	55 (10-111)	0.529
Macroadenoma	21 (25)	10 (37)	0.010
Microadenoma	47 (56)	5 (19)	0.010
Normal pituitary image	16 (19)	12 (44)	0.008

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

BMI: body mass index; UFC: urinary free cortisol; Overnight cortisol overnight: serum cortisol after 1mg dexamethasone overnight; Cortisol/UFC 8 mg: Liddle II test.

*n= 51patients in remission group and n=11 patients in failure group.

**n= 42 patients in remission group and n= 9 patients in failure group. (To convert serum cortisol µg/dl to nmol/dl multiply by 27.6 and plasma ACTH pg/ml to pmol/l multiply by 0.22).

Table 2: Postoperative data of Cushing´s disease patients with remission or surgical failure.

	Remission (n=84)	Failure (n=27)	p
BMI (kg/m²)	27 ± 6	33 ± 9	0.004
Adenoma in histopathology	76 (90)	18 (70)	0.012
ACTH in immunohistochemistry*	26 (90)	5 (42)	0.001
Hypopituitarism **	34 (40)	1 (8)	0.021
Panhypopititarism **	12 (14)	0	0.117
Follow-up (years)	6.1 ± 4.9	5.8 ± 4.3	0.878

Data were displayed as mean ± Standard Deviation, or absolute number and (%).BMI: body mass index at the last visit of follow-up.

*n= 29 patients in remission group and n=12 patients in failure group

**n= 81 patients in remission group and n=13 patients in failure group, because radiotherapy patients were excluded.

Table 3: Sensitivity and Specificity (%) of serum cortisol (µg/dl) as remission criterium in different times after surgery

	Best cut-off (µg/dl)	Sensitivity (%)	Specificity (%)
Pre-OP	20.3	60	52
6h PO	25.4	87	63
12h PO	14	87	71
24h PO	10.7	91	83
24h nadir PO	10.7	92	87
48h PO	6.6	94	80
48h nadir PO	5.4	95	87
10-12d PO	6.8	100	84
10-12d nadir PO	5.7	100	92
30d PO	21	100	96
30d nadir PO	5	100	92

OP: operative; PO: postoperative; d: days. (To convert serum cortisol µg/dl to nmol/l multiply by 27.6).

Table 4: Postoperative Adrenocorticotropic Hormone values in remission and failure groups.

ACTH (pg/ml)	Remission (n=84)	Failure (n=27)	p
ACTH 10-12 day	10 (2 — 63)	38 (12 — 163)	<0.001
ACTH 28-30 day	10 (5 — 62)	38 (31 — 42)	0.012
Δ ACTH 10-12 day	-35 (-156 — +2.5)	+14 (-37 — +66.2)	0.002
Δ ACTH 28-30 day	-33 (-170 — +5.2)	-0.85 (-27 — +19.1)	0.013

Data were displayed as median and range. ACTH: Adrenocorticotropic Hormone; Δ: delta from postoperative and baseline plasma ACTH value.(To convert plasma ACTH pg/ml to pmol/l multiply by 0.22)

Table 5: Relative risk for postoperative cortisol as Cushing's disease predictor, in different adjusted models.

	RR	CI (95%)	P
Model 1			
Nadir 24 hrs PO	0.96	0.93-0.99	0.035
Model 2			
Nadir 24 hrs PO	0.96	0.92-0.99	0.006
Model 3			
Nadir 24 hrs PO	0.95	0.92-0.99	0.007
Model 4			
Nadir 24 hrs PO	0.91	0.85-0.98	0.013
Model 5			
Nadir 24 hrs PO	0.95	0.92-0.98	0.004
Model 1			
Nadir 10-12 days PO	0.91	0.83-1.0	0.063
Model 2			
Nadir 10-12 days PO	0.88	0.80-0.96	0.005
Model 3			
Nadir 10-12 days PO	0.89	0.81-0.97	0.008
Model 4			
Nadir 10-12 days PO	0.77	0.58-1.0	0.069
Model 5			
Nadir 10-12 days PO	0.88	0.81-0.96	0.004

Model 1: adjustment for microadenoma or macroadenoma in pituitary image

Model 2: adjustment for normal pituitary image

Model 3: adjustment for presence of adenoma at histopathology

Model 4: adjustment for presence of Adrenocortotrophic Hormone at immunohistochemistry

Model 5: adjustment for presence of hypopituitarism after transsphenoidal surgery

PO: postoperative; RR: Relative Risk; CI: Confidence Interval

Table 6: Postoperative cortisol median in recurrence and not recurrence patients.

Cortisol (µg/dl)	Recurrence (n=9)	Not Recurrence (n=75)	p
Pre-OP	20.0 (11.5-50.2)	20.4 (11.6-42.1)	0.928
6h PO	38.2 (15.7-52.6)	18.3 (1.5-67.8)	0.024
12h PO	27.4 (4.4-56.8)	4.7 (0.4-53.6)	0.003
24h PO	15.3 (2.1-39.9)	2.3 (0.2-39.6)	0.004
24h nadir PO	15.3 (2.1-39.9)	2.3 (0.2-30.0)	0.002
48h PO	9.5 (4.1-22.8)	1.9 (0.01-23.8)	0.010
48h nadir PO	5.2 (1.7-22.8)	1.4 (0.01-15.1)	0.001
10-12d PO	3.2(1.2-26.0)	1.6 (0.1-17.5)	0.040
10-12d nadir PO	2.8 (1.2-17.1)	1.1 (0.10-9.7)	0.026
30d PO	19.4 (4.2-26.8)	1.1 (0.04-23.8)	0.002
30d nadir PO	3.2 (1.2-17.1)	0.8 (0.04-9.7)	0.006

Data were displayed as median and range

OP: operative; PO: postoperative. (To convert serum cortisol µg/dl to nmol/l multiply by 27.6).
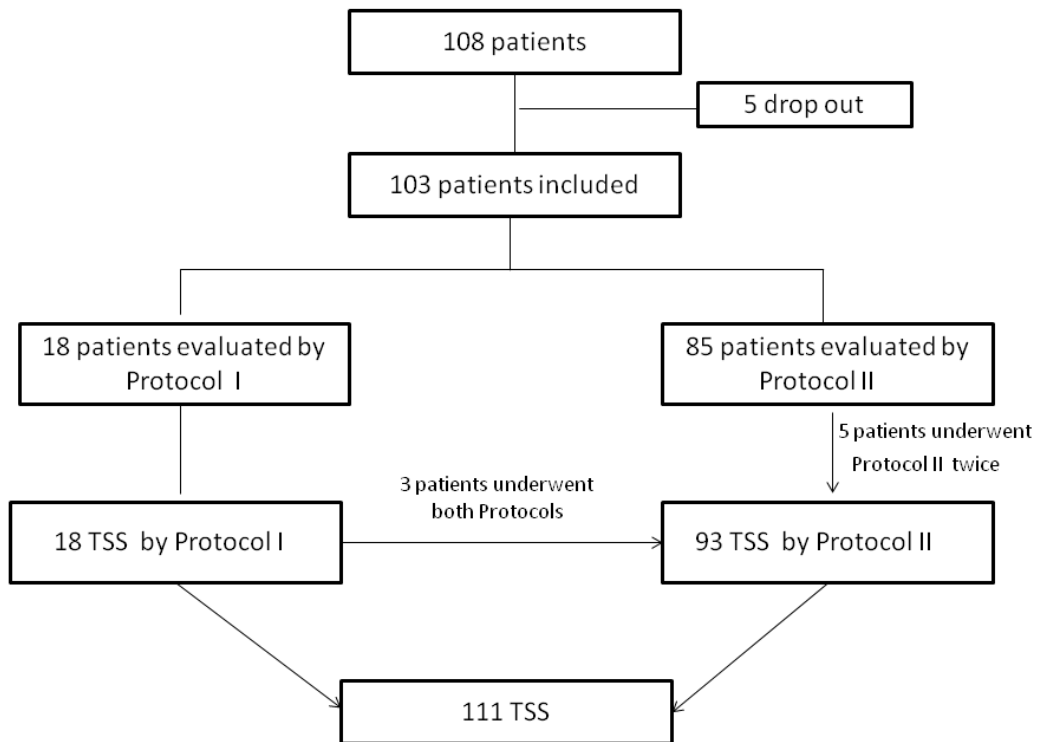
Figure 1: Flowchart of patients included in the study.

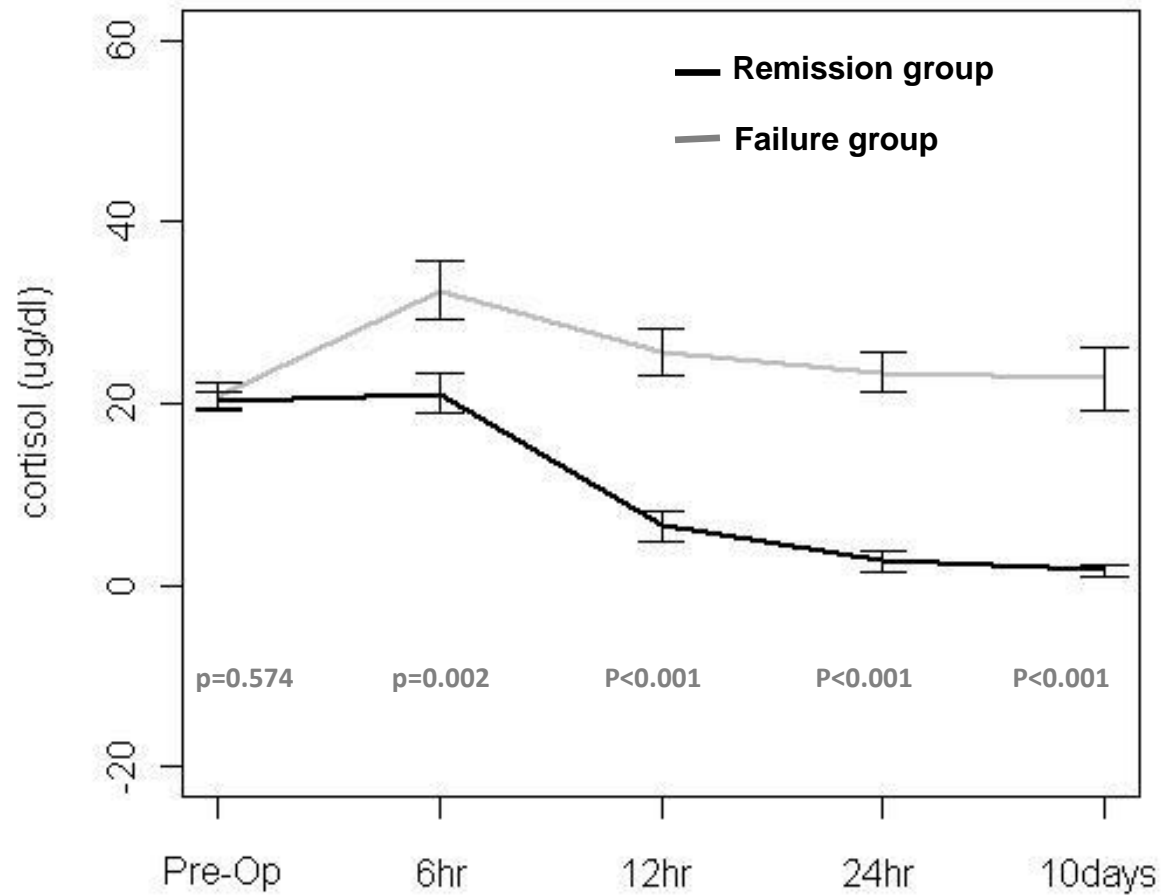**TSS: transsphenoidal pituitary surgery**.

Figure 2: The curve of the postoperative serum cortisol for the remission and failure group after transsphenoidal pituitary surgery. (To convert serum cortisol μg/dl to nmol/l multiply by 27.6).
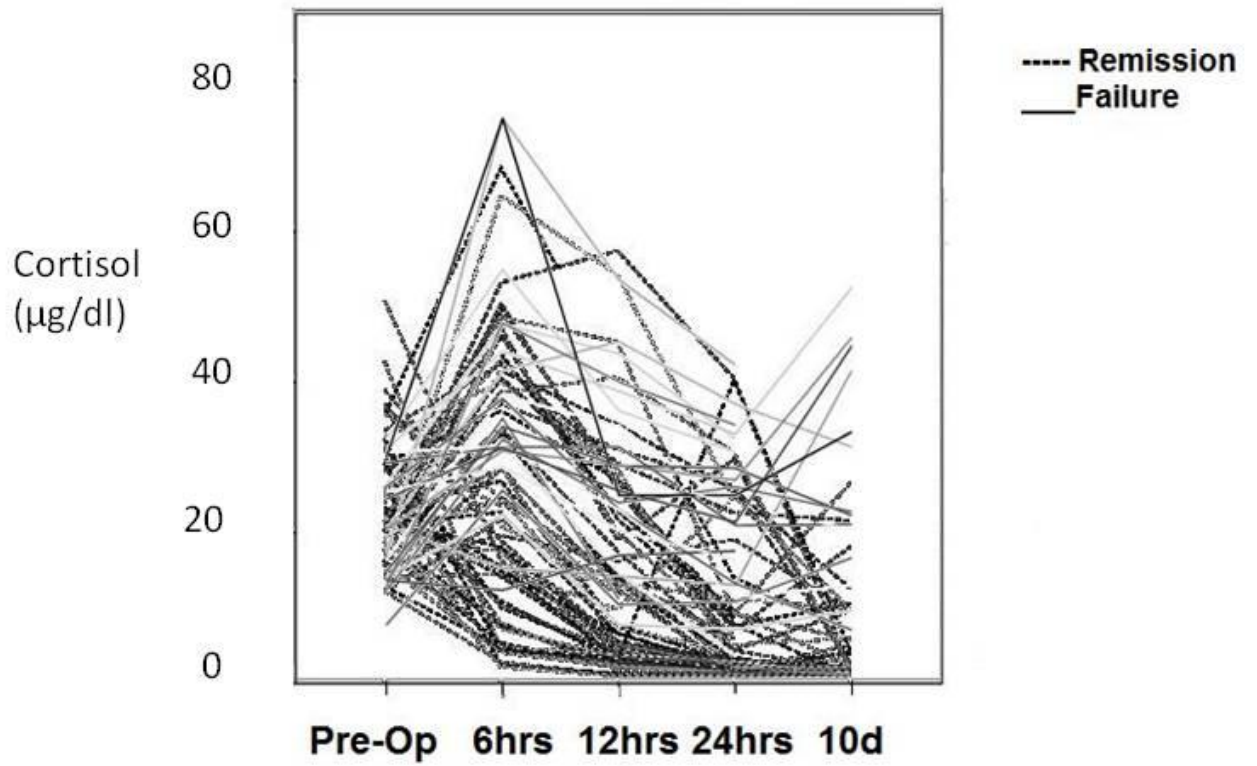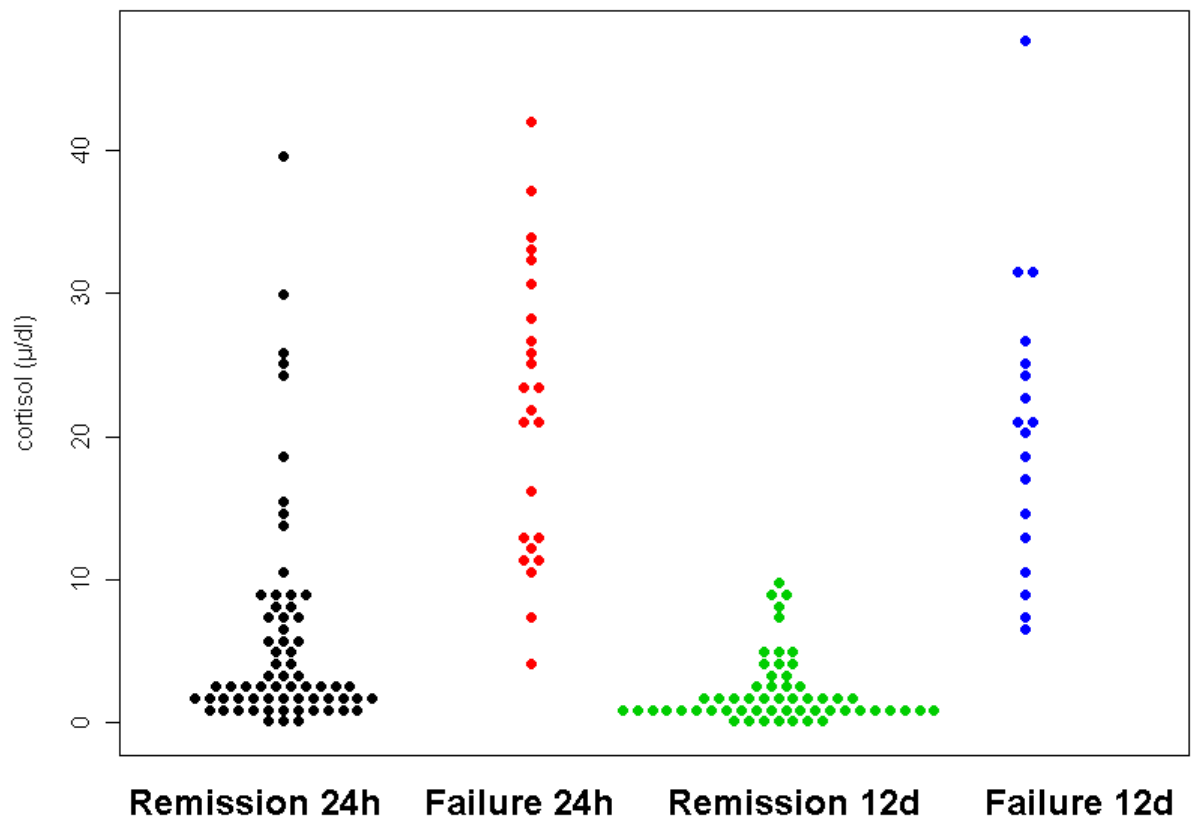
Figure 3: Transoperative serum cortisol dynamic in individual patients: remission and failure groups. (To convert serum cortisol µg/dl to nmol/l multiply by 27.6).
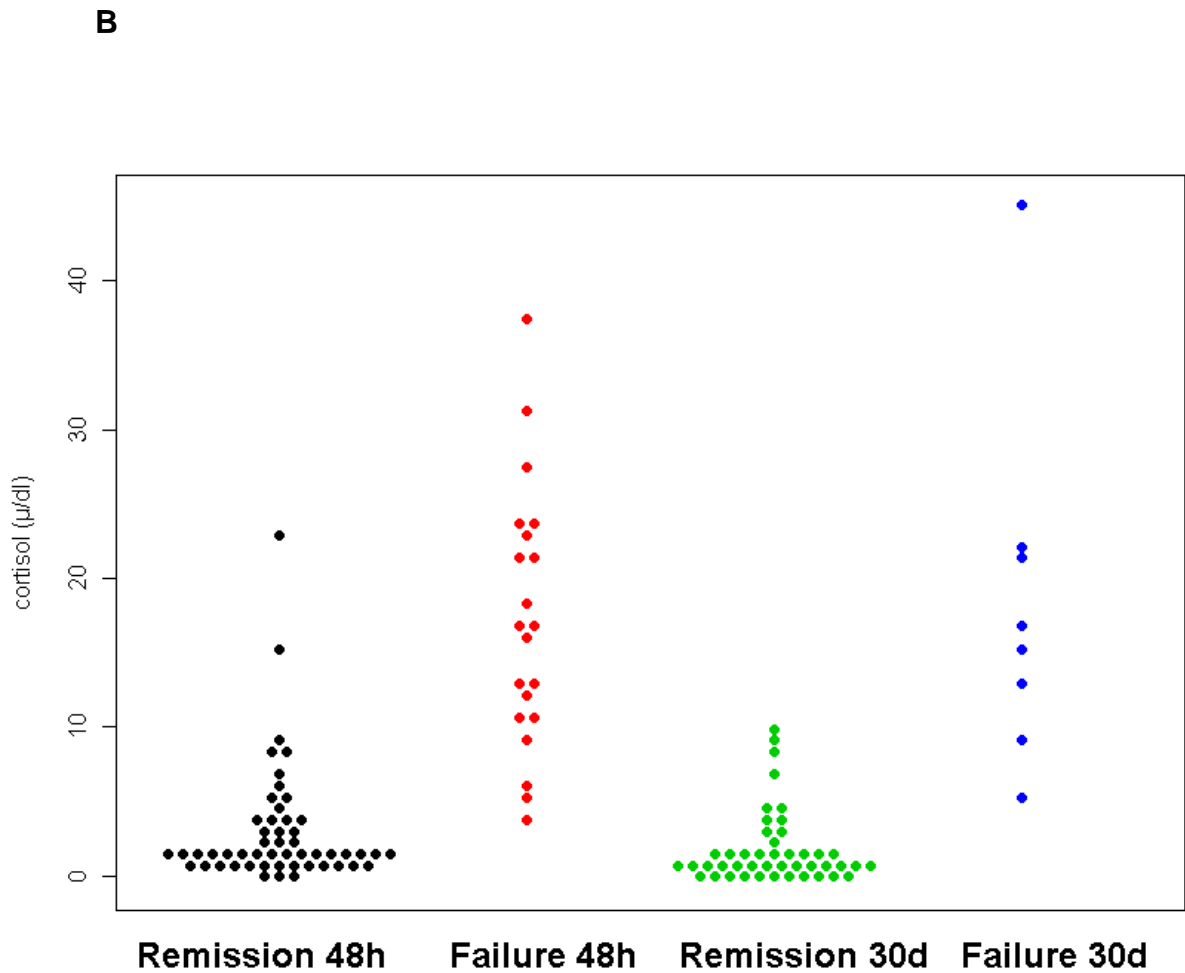
A

B

Figure 4: A, Serum cortisol (µg/dl) nadir in remission and failure group at 24h and 12 days after surgery B, Serum cortisol (µg/dl) nadir in remission and failure group at 48h and 30 days after surgery. (To convert serum cortisol µg/dl to nmol/l multiply by 27.6).