PATRICIA TASCHNER GOLDENSTEIN

**Impacto da remodelação óssea sobre a transferência da massa
de cálcio durante a hemodiálise: estudo em pacientes com
hiperparatireoidismo pré e pós paratireoidectomia**

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Orientadora: Profa. Dra. Rosa Maria Affonso Moysés

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Para minha querida mãe que me incentivou a buscar sempre mais

(in memoriam).

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“Dizem que a vida é para quem sabe viver, mas ninguém nasce pronto. A vida é para quem é corajoso o suficiente para se arriscar e humilde o bastante para aprender”

Clarice Lispector

NORMATIZAÇÃO ADOTADA

Esta tese está de acordo com as seguintes normas, em vigor no momento desta publicação:

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LISTA DE ABREVIATURAS

Ca	Cálcio
Cad	Concentração de cálcio no dialisato
Cai	Cálcio iônico
DRC	Doença renal crônica
DMO-DRC	Distúrbios minerais e ósseos relacionados à doença renal crônica
HPT	Hiperparatireoidismo
PTH	Paratormônio
PTx	Paratireoidectomia
TFG	Taxa de filtração glomerular
VDRA	Ativadores seletivos dos receptores da vitamina D

LISTA DE SÍMBOLOS

%	Percentual
>	Maior
≥	Maior ou igual
≤	Menor ou igual
>	Maior
<	Menor
±	Mais ou menos
g/L	Gramas por litro
Kg	Kilograma
mg/dl	Miligramas por decilitro
ng/ml	Nanogramas por microlitro
pg/ml	Picogramas por microlitro
UI	Unidade Internacional
U/L	Unidades por litro
µg	Micrograma

RESUMO

Goldenstein PT. *Impacto da remodelação óssea sobre a transferência da massa de cálcio durante a hemodiálise: estudo em pacientes com hiperparatireoidismo pré e pós paratireoidectomia* (tese). São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2015.

Distúrbios do metabolismo mineral e ósseo são altamente prevalentes e considerados como causa relevante da morbidade e mortalidade dos pacientes com doença renal crônica. Diversas estratégias diagnósticas e terapêuticas têm sido estudadas nesses doentes; entretanto, pouco valor é dado ao cálcio do dialisato, apesar do impacto que possa exercer sobre o balanço de cálcio durante a hemodiálise. Os fatores determinantes da transferência de cálcio durante o procedimento são ainda controversos. Nesse estudo prospectivo, avaliamos a influência da remodelação óssea sobre o balanço de cálcio em dez pacientes dialíticos em três situações consecutivas: hiperparatireoidismo grave (Pré paratireoidectomia), durante a “síndrome de fome óssea” (Fome óssea) imediatamente após a paratireoidectomia e após estabilização clínica (Paratireoidectomia tardia). Durante cada fase os participantes foram submetidos a três sessões randômicas de hemodiálise com diferentes concentrações de cálcio no dialisato: 2,5; 3,0 e 3,5 mEq/L. Todos os pacientes foram submetidos à biópsia óssea para análise histomorfométrica e quantificação de proteínas ósseas no início do estudo. A transferência de cálcio variou grandemente entre os pacientes em cada fase do estudo mesmo usando o mesmo cálcio no dialisato, com valores negativos de medianas no Pré paratireoidectomia e Fome óssea (-161mg e -218mg, respectivamente) e discretamente positivo no Paratireoidectomia tardia (39mg; $p < 0,05$ versus Pré paratireoidectomia e Fome óssea). Análise de regressão multivariada mostrou que o gradiente de cálcio entre o cálcio iônico sérico inicial e o cálcio do dialisato, a diferença entre o cálcio iônico sérico final e o

inicial e a forma não carboxilada da osteocalcina foram preditores independentes da transferência de cálcio ($R^2=0.48$; $p<0.05$). Pelo fato da remodelação óssea também influenciar os níveis séricos de cálcio iônico e suas variações durante a diálise, nesse estudo demonstramos que o esqueleto tem papel fundamental no balanço de cálcio e essas variáveis devem ser consideradas na individualização do cálcio do dialisato de nossos pacientes.

Descritores: Diálise renal; Cálcio; Remodelação óssea; Osteocalcina; Doenças ósseas metabólicas, Hiperparatireoidismo secundário; Hormônio paratireóideo; Biópsia.

ABSTRACT

Goldenstein PT. *Effects of bone remodelling on calcium mass transfer during hemodialysis: study of patients with hyperparathyroidism pre and post parathyroidectomy* [thesis]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2015.

Disturbances in mineral and bone metabolism are highly prevalent and are a major cause of morbidity and mortality in chronic kidney disease patients. Different diagnostic and therapeutic strategies have been studied in these patients. However, little attention is paid to the calcium concentration in the dialysate, despite the impact it could exert over calcium balance during dialysis. The variables that determine calcium transfer during hemodialysis are still controversial. In this study, we have prospectively investigated the influence of bone remodeling on calcium balance in ten dialysis patients in three consecutive situations: severe hyperparathyroidism (Pre parathyroidectomy), during “hungry bone syndrome” (Hungry bone) right after surgery and after stabilization of clinical status (Late parathyroidectomy). During each phase participants were submitted to 3 random hemodialysis sessions, with different dialysate calcium: 2.5, 3.0 and 3.5 mEq/L. Bone biopsy for histomorphometric analysis and bone proteins quantification were performed in all patients at baseline. Calcium mass transfer varied widely among patients in each study phase even using the same dialysis calcium with negative median values in Pre parathyroidectomy and Hungry Bone (-161 and -218mg,

respectively) and slightly positive in Late parathyroidectomy (39mg; $p<0.05$ versus Pre parathyroidectomy and Hungry Bone). Multiple regression analysis showed that calcium gradient between initial serum ionic calcium and the dialysate calcium, the difference between final and initial serum ionic calcium and serum undercarboxylated form of osteocalcin were independent predictors of calcium mass transfer ($R^2=0.48$; $p<0.05$). As bone remodeling also influences the serum levels of ionic calcium and its variance during dialysis, in this study we have added new data by demonstrating that the skeleton plays a key role on calcium balance and these variables must be considered when individualizing calcium dialysate for our patients.

Descriptors: Renal dialysis; Calcium; Bone remodeling; Osteocalcin; Bone diseases, metabolic; Hyperparathyroidism, secondary; Parathyroid hormone; Biopsy.

1. INTRODUÇÃO

A doença renal crônica (DRC) é um problema de saúde pública em esfera internacional na medida em que 5 a 10% da população mundial apresenta esta afecção (1). A progressão da DRC leva a uma deterioração do metabolismo mineral relacionada a anormalidades englobando paratormônio (PTH), cálcio (Ca), fósforo, 25 vitamina D, 1,25 vitamina D e fator de crescimento de fibroblasto 23 (FGF23). As funções minerais e endócrinas alteradas na DRC são fundamentais na regulação da formação óssea durante o crescimento e também durante o remodelamento ósseo na idade adulta. Como resultado, anormalidades ósseas são encontradas na maior parte dos pacientes estágios 3 a 5 de DRC e de forma quase universal em pacientes com DRC dialítica, ou seja, estágio 5D (2).

Nos últimos anos, estudos observacionais realizados comprovaram que alterações no metabolismo mineral e ósseo em pacientes com DRC resultam não somente em maior risco de fraturas, mas também em um maior risco de doença cardiovascular e redução de sobrevida (3-6). Isso levou à proposta de um novo conceito: “Distúrbios Minerais e Ósseos relacionados à DRC (DMO-DRC)”. Desta forma, foi descrita uma condição sistêmica que além das anormalidades minerais e ósseas inclui em seu espectro a doença cardiovascular, sendo todos intimamente relacionados, de modo a serem os maiores responsáveis pela elevada morbidade e mortalidade dos pacientes com DRC (7).

O estudo DOPPS (*Dialysis Outcomes and Practice Patterns Study*), a mais importante coorte observacional envolvendo pacientes em diálise de diversos

países, mostrou uma associação entre presença de níveis séricos de PTH acima de 600 pg/ml e um aumento de 21% no risco de mortalidade por todas as causas (8). Entretanto, a maior parte dos estudos observacionais compararam pacientes dialíticos com altos níveis de PTH a outros com níveis teoricamente adequados de PTH, levantando a questão se a mortalidade encontrada estava relacionada ao hiperparatireoidismo (HPT) grave ou a outros fatores de risco associados (9, 10). Além disso, faltam dados avaliando se o tratamento do HPT com paratireoidectomia (PTx) de fato reverteria esse aumento de mortalidade. Esse estudo seria difícil em países onde estão amplamente disponíveis tratamentos clínicos alternativos como o cinacalcete ou análogos da vitamina D, já que o grupo controle não poderia deixar de ser tratado por questões antiéticas.

Atualmente no Brasil, temos um cenário com aproximadamente 90.000 pacientes com DRC em diálise, sendo a maior parte dos tratamentos financiados pelo serviço de saúde público (11). O PTH é medido apenas duas vezes por ano e o tratamento proporcionado pelos serviços públicos de saúde para o HPT conta apenas com quelantes de fósforo à base de Ca e calcitriol. O sevelamer é distribuído em poucos Estados ou apenas em condições de hipercalcemia mantida, enquanto o paricalcitol e o cinacalcete não estão disponíveis. Por isso, ainda nos tempos atuais, temos no Brasil uma proporção significativa de pacientes em diálise com formas graves de HPT e a única opção terapêutica permanece sendo a PTx (12).

Para piorar a situação, poucos serviços médicos no Brasil realizam essa cirurgia, levando a uma fila de espera que muitas vezes supera um ano (13).

O ambulatório de DMO-DRC do Hospital das Clínicas da Universidade de São Paulo é um dos maiores serviços de referência de pacientes portadores de doença óssea de difícil controle, especialmente o HPT grave. Os serviços de diálise do Estado e muitas vezes também de outros locais encaminham seus pacientes na busca por soluções do especialista, que muitas vezes resultam na indicação da PTx e inscrição do paciente ao final da fila cirúrgica e acompanhamento peri-operatório.

No início do meu trabalho de pós-graduação e durante o processo de seleção de pacientes para o meu projeto de pesquisa, pude observar a significativa perda de qualidade de vida dos portadores de HPT grave. Além disto, surgiu em nosso grupo a sensação de que diversos pacientes morriam antes de ter acesso à PTx. Dessa forma, tive a oportunidade de estudar se realmente o tratamento oferecido mudaria a mortalidade desses pacientes. Como dito anteriormente, por não dispormos de outras opções de tratamento para esses pacientes graves, tivemos a chance de comparar o grupo de indivíduos submetidos à PTx a um grupo controle de igual gravidade e indicação cirúrgica mas que ainda estava aguardando uma oportunidade de tratamento cirúrgico, o que seria considerado antiético em qualquer lugar onde outro tratamento estivesse disponível.

Conforme descrito no anexo A (14), dos 300 pacientes encaminhados ao ambulatório de 2005 a 2009 (já excluindo pacientes transplantados e DRC em tratamento conservador), 251 tinham indicação de PTx por HPT grave. Os pacientes entravam em uma fila por ordem cronológica para serem submetidos à

PTx; entretanto, alguns pacientes com dor refratária, novas fraturas ou ruptura de tendão tinham a cirurgia antecipada.

Após tempo de seguimento de 23 meses, apenas 123 dos 251 pacientes haviam sido operados. A mortalidade geral foi de 29%, sendo 51 no grupo não submetido à PTx (16 óbitos por 100 pacientes-ano) e 21 no grupo operado (7 óbitos por 100 pacientes-ano; $p < 0,0001$). Para entendermos os fatores associados à mortalidade separamos os pacientes entre sobreviventes e não sobreviventes. O primeiro grupo era mais jovem, em sua maioria homens e menos frequentemente diabéticos ($p < 0,05$). O grupo de não sobreviventes apresentava níveis de 25 vitamina D mais baixos e menor número de pacientes havia sido submetido à PTx.

Quando comparamos o grupo submetido com o não submetido à cirurgia, vimos que o grupo PTx era mais sintomático, tinha exames laboratoriais mais alterados e maior tempo em diálise; tornando-os mais graves. Apesar disso, o risco relativo de óbito foi menor nesse grupo que no grupo não PTx (0,428; IC 95% 0,28 a 0,67; $p = 0,0001$). A curva de sobrevida mostrou benefício da cirurgia (teste log-rank; $p = 0,0001$). Após ajuste para idade, sexo e presença de diabetes, a mortalidade foi dependente da idade (1,06; IC 95% 1,04 a 1,08; $p = 0,0001$) e falta de acesso à PTx (3,91; IC 95% 2,16 a 7,88; $p = 0,0001$).

Finalmente, após excluir o “viés de imortalidade”, fizemos outra análise multivariada onde consideramos a data da PTx como início do estudo nesse grupo. Após ajuste para idade, sexo e diabetes, o grupo PTx permaneceu com maior sobrevida (Log-rank $p = 0,0001$) sendo que o grupo não PTx apresentou RR de mortalidade ajustada de 2,4. Portanto, nesse estudo mostramos que, apesar de

nossos pacientes serem extremamente doentes e muito sintomáticos, eles claramente se beneficiaram da cirurgia.

Um aspecto importante dos pacientes do ambulatório refere-se à dor óssea e à fratura. De fato, cerca de 64% deles apresentavam queixas de dores ósseas, embora nem sempre tivessem fraturas diagnosticadas. Sabemos que grande parte da dor que sentem se refere ao próprio HPT e essas queixas muitas vezes desaparecem imediatamente após o retorno do paciente da PTx. Entretanto, o risco de fraturas no paciente com DRC é 1,5 a 2 vezes maior que na população geral. Além disso, ocorrem em indivíduos mais jovens e resultam em maior mortalidade.

Apesar disso, a visão de estratificação de risco de fraturas e cuidados preventivos e terapêuticos tem sido, em grande parte, negligenciada na DRC. Dados de pacientes em hemodiálise nos Estados Unidos (US Renal Data System) mostram que entre 1992 e 2009 houve um aumento significativo na incidência de fraturas com necessidade de hospitalização (15). As mesmas altas taxas de fratura foram demonstradas nos pacientes participantes do DOPPS, no qual o risco de mortalidade não ajustada foi 3,7 vezes maior nos pacientes dialíticos com fratura em relação aos não fraturados (16). O aumento de risco de fratura tem sido também observado em pacientes com DRC ainda em tratamento conservador, de forma progressiva conforme o estágio da DRC, com incidência de 15; 20,5; 24,2; 31,2 e 46,3 por 1.000 pacientes-ano nos estágios 1, 2, 3a, 3b e 4, respectivamente (17).

Apesar dos dados chamarem atenção, a estratificação de risco de fratura e seu tratamento nessa população ainda não são realizados de forma satisfatória. A biópsia óssea é ainda hoje o padrão ouro para avaliação da qualidade e remodelamento ósseo de modo dinâmico. Entretanto, a biópsia não é parte rotineira na abordagem desses pacientes e, além disso, ela nos traz informações relacionadas a um dado momento em que foi realizada. De fato, não existem diretrizes sobre quais métodos não invasivos deveríamos utilizar nessa investigação e como estratificar o risco nessa população de modo a implementar medidas preventivas (18).

Desde a publicação do KDIGO de 2009 sobre DMO-DRC (2), diversos artigos foram publicados e uma nova convenção do KDIGO em Madri, Espanha, estabeleceu a necessidade de revisão da literatura para posicionar-se a esse respeito (19). Diante de tantos pacientes portadores de doença óssea e elevado risco de fratura, por vezes subvalorizada, subdiagnosticada, e talvez subtratada, sentimos a necessidade de revisar os recentes artigos relacionados e o que poderíamos acrescentar aos nossos pacientes. Dessa decisão surgiu então a publicação mostrada no Anexo B (20).

As diretrizes do KDIGO de 2009 recomendam uso de densitometria para avaliação de risco de fraturas em pacientes com DRC estágios 1 até o início do 3 (Taxa de filtração glomerular (TFG) maior ou igual 45 ml/min/m^2) e não de forma rotineira a partir do estágio 3B em diante (TFG menor que 45 ml/min/m^2) (2). Entretanto, estudos recentes, prospectivos, mostraram correlação entre baixa densidade mineral óssea com risco aumentado de fraturas incidentes, ajustado

para diversos fatores (21-23). O manejo desses pacientes é complexo e pouco estudado, ao contrário da população saudável, em que vários agentes estão aprovados principalmente para mulheres na pós-menopausa com osteoporose (24, 25). Análises “post-hoc” de populações com DRC incluídas em alguns desses estudos mostraram uma perspectiva de uso de drogas como raloxifeno, um modulador do receptor de estrogênio, em pacientes com TFG até 20 ml/min/m² (26, 27); do denosumab, um anticorpo monoclonal que se liga ao receptor ativador do ligante do fator nuclear Kappa-B em osteoblastos e osteócitos, em pacientes com TFG de até 15 ml/min/m² (28, 29) e do teriparatide, uma proteína recombinante do PTH humano (30, 31). Novos agentes como Romosozumabe, um inibidor da esclerostina, também têm sido estudado como estimuladores da formação óssea (32, 33). Estudos futuros, prospectivos, são ainda necessários para confirmar essas informações preliminares tanto em relação ao uso da densitometria e outros métodos preditores de fratura, quanto em relação ao uso de medicações que reduzam esse risco na população portadora de DRC.

Neste momento, as orientações para o manejo da doença mineral óssea são controle da hiperfosfatemia com restrição dietética e uso de quelantes de fósforo, controle da calcemia e redução dos níveis de PTH com calcitriol e mais recentemente com o uso de ativadores seletivos dos receptores da vitamina D (VDRA), calcimiméticos e às vezes PTx (34, 35). Entretanto, pouco se tem valorizado o Ca utilizado no dialisato (Cad) durante o procedimento da hemodiálise. A escolha do Cad ideal é altamente complexa e controversa na literatura desde o início dos procedimentos dialíticos.

De fato, no início da hemodiálise na década de 60 e 70, existia uma grande preocupação em controlar os níveis de Ca para prevenir doença óssea. O quelante de fósforo utilizado na época era o hidróxido de alumínio. Apesar de o Cad considerado adequado na época fosse 2,5 mEq/L por ser um Ca próximo ao valor sérico de Ca iônico (Cai) não havia uniformidade de conduta e dialisatos de até 4,5 mEq/L eram utilizados.

Entretanto, muitos pacientes apresentavam hipocalcemia e hiperparatireoidismo secundário o que resultou em uma mudança na recomendação para Cad 3,5 mEq/L visando a supressão do PTH através da sobrecarga de Ca (36, 37). A disponibilização de calcitriol oral nos anos 70 melhorou a hipocalcemia e reduziu a necessidade de ganho de Ca através da diálise; entretanto, a maior parte dos nefrologistas continuaram a utilizar o Cad de 3,5 mEq/L na tentativa de manter a concentração sérica de Ca no limite superior e suprimir o PTH. No final da década de 80 os quelantes de fósforo a base de alumínio foram progressivamente substituídos por quelantes a base de Ca e a hipercalcemia tornou-se frequente, levando vários nefrologistas a retornar para o Cad de 2,5 mEq/L. A favor desta conduta, estudos mostraram o papel de calcificações extra-ósseas, especialmente a calcificação vascular, sobre a mortalidade e mostraram a associação do balanço positivo de Ca com a presença ou progressão desta calcificação (38, 39).

Atualmente, temos recomendações distintas entre as diversas sociedades da nefrologia, muitas delas baseadas em opinião de especialistas. O KDOQI orienta

no geral uso de Cad de 2,5 mEq/L (40), e uso de Cad ainda mais baixo em situações de níveis séricos baixos de PTH associados à doença óssea adinâmica, enquanto o KDIGO sugere o uso de Cad entre 2,5 e 3,0 mEq/L, embora enfatize a necessidade de individualização do Cad com objetivo de manter um balanço próximo ao neutro (2). Diretrizes japonesas recomendam Cad 2,5 mEq/L para maior parte dos pacientes (41) e no Brasil a recomendação é, no geral, semelhante ao KDIGO (12), embora a maioria das unidades de diálise ainda utilize um Cad de 3,5 mEq/L. O grau de evidência para as recomendações das diretrizes acima citadas foi 2D (qualidade muito baixa) e fortemente baseado em opinião.

Sabemos que a escolha do Cad influencia o balanço de Ca durante a diálise (42, 43); entretanto, não sabemos exatamente quanto um paciente irá ganhar ou perder de Ca com um determinado Cad. Sabe-se que um balanço positivo de Ca pode levar a hipercalcemia ou mesmo sobrecarga de Ca com Ca sérico normal, resultando em calcificação vascular e doença óssea adinâmica (44-46). Entretanto, um balanço negativo aumenta o risco de hipotensão relacionada à diálise e arritmias, além de poder piorar o hiperparatireoidismo. (44, 46, 47).

Há poucos estudos na literatura avaliando a transferência de Ca durante a hemodiálise e alguns foram realizados na década de 60 e 70, com diferentes metodologias, dificultando comparações (37, 48, 49). Além disso, houve grande avanço no conhecimento e tecnologias em relação à época em que estes estudos foram realizados e mudanças no procedimento dialítico com máquinas, filtros de diálise, prescrições de fluxos, frequência e mesmo tempo muito diferentes dos

atuais, dificultando ainda mais a extrapolação de dados da época. Poucos estudos mediram efetivamente o balanço de Ca (42, 43, 50-52). Isso se deve a dificuldades técnicas em obter uma medida acurada do Cad total ou uma alíquota proporcional dele. Por esses motivos novos estudos são necessários.

Susan Hou e cols. publicou estudo clássico em 1991 avaliando balanço de Ca em diferentes concentrações de Cad. Foi demonstrado que pacientes submetidos à hemodiálise com concentração de Ca de 2,5 mEq/L faziam balanço neutro (ganho de cerca de 216 mg de Ca por sessão), enquanto aqueles que usavam Cad de 3,5 mEq/L faziam balanço positivo (ganho de cerca de 879 mg de Ca em uma sessão) e Ca de 1,5 mEq/L balanço negativo (perda de cerca de 231 mg de Ca por sessão). Este trabalho serviu de base para diversas diretrizes e é muito citado ainda hoje; entretanto ele englobou apenas 7 pacientes, o que limita muito suas conclusões (42). Outros estudos também com número pequeno de pacientes demonstraram resultados conflitantes (44, 47).

Posteriormente, Sigrist e cols. avaliaram 52 pacientes em hemodiálise com uma concentração fixa de Ca 2,5 mEq/L no dialisato e observaram também um balanço de Ca ligeiramente negativo (média de -187 mg). Entretanto, alguns pacientes apresentavam ganho de Ca maior que 500 mg na sessão de diálise enquanto outros perdiam 500 mg no mesmo período, mesmo diante da mesma concentração de Cad. Foi estabelecida uma relação entre o gradiente de Ca pré diálise (diferença entre C_{ai} menos o Cad) e o ganho ou perda de Ca avaliado ao

final do procedimento. Ou seja, quanto maior o gradiente de Ca, maior o fluxo de Ca e, portanto, a troca de Ca durante a hemodiálise (43).

Alguns estudos demonstraram os efeitos da concentração de Cad sobre a secreção de PTH e consequentemente sobre a remodelação óssea a longo prazo (44, 45, 47). Entretanto, os efeitos da remodelação óssea sobre a transferência de Ca na diálise são raramente considerados. Nosso grupo, em 2010, foi o primeiro a estudar a influência do remodelamento ósseo sobre a massa transferida de Ca durante a hemodiálise. Foi demonstrado em 23 pacientes que o balanço de Ca variou de forma dependente do Cad, tendo o uso de dialisato com concentração de Ca 3,0 mEq/L atingido, em média, balanço neutro de Ca.

No entanto, quando em vigência do mesmo Cad, foi encontrada uma grande variação no balanço deste elemento entre os pacientes, havendo associação entre a transferência de massa de Ca com o gradiente de Ca e com o remodelamento ósseo do paciente, inferido através do PTH e da osteocalcina (OC). Este estudo mostrou que outros fatores individuais, que vão além do Ca utilizado no dialisato, estão envolvidos de forma independente na transferência da massa de Ca.

Pacientes com alta remodelação óssea, com PTH maiores que 300 pg/mL apresentaram maior perda de Ca durante as sessões de hemodiálise se comparados aos pacientes com baixa remodelação óssea inferida através de PTH abaixo de 300 pg/mL (50). Se a reabsorção óssea mediada pelo PTH ocorresse apenas por mecanismos celulares ela levaria horas ou dias, o que parece não ter sido o ocorrido no estudo anteriormente citado. Os achados desse estudo vão de acordo com a teoria de Talmage (53), que sugeriu que a remodelação óssea se

desse em duas fases sequenciais. A primeira o Ca iria do fluido extra-celular para o osso por diferença de concentração entre eles. Na segunda fase, a presença de proteínas não colágenas com alta afinidade pelo Ca ao redor da superfície óssea, como a OC, funcionariam como um local de troca de Ca entre o osso, o extra celular e o plasma de modo a manter um equilíbrio entre os diversos compartimentos. Essas proteínas serviriam como verdadeiros reservatórios para trocas rápidas desse elemento.

Dependendo da quantidade dessas proteínas, a perda de Ca seria maior ou menor. De acordo com a teoria, a ação do PTH ocorreria de duas formas: através da reabsorção óssea (um evento mediado por estimulação osteoclástica) e também através da regulação dessas proteínas, alterando sua conformação, quantidade ou removendo substâncias interferentes de modo a aumentar sua disponibilidade no tecido ósseo. A remodelação óssea parece ser o maior determinante da OC no osso (54).

Em acordo com a teoria, nosso grupo previamente mostrou que a OC foi um fator preditor independente de transferência de massa de Ca. Entretanto, o papel exato da OC na remodelação ainda não foi totalmente explicado e seu uso como marcador bioquímico de remodelação óssea permanece controverso.

De fato a OC é a principal proteína não colágena no osso. Ela é composta das frações GLA (carboxilada) e GLU (não carboxilada). A fração GLA tem alta afinidade pela hidroxiapatita e por isso está diretamente envolvida na remodelação óssea. No entanto, ainda há controvérsias se ela atuaria como um estimulante ou como inibidor da formação óssea. A fração GLU é produto da reabsorção óssea e

tem baixa afinidade pela hidroxiapatita, apresentando comportamento hormonal envolvendo metabolismo energético (55) e também tem sido relacionada a um maior risco de fraturas em mulheres idosas (56).

O estudo de Karohl e cols. (50), apesar de achados significativos, apresentou algumas limitações: a avaliação da OC foi realizada através da dosagem sérica, e não há estudos que comprovem a existência de uma relação entre a OC sérica e o seu conteúdo ósseo. Além disso, a remodelação óssea foi estimada a partir de marcadores bioquímicos como o PTH, sendo a biópsia óssea o padrão ouro para este diagnóstico. Finalmente, a comparação foi realizada entre diferentes indivíduos e, apesar da análise multivariada, alguns outros fatores individuais que não foram considerados também poderiam interferir na transferência de Ca.

Desta forma, um estudo ideal para comprovar o papel da remodelação óssea e da OC na transferência de Ca deveria ser realizado em um mesmo indivíduo em situações de remodelação óssea distintas com diagnóstico realizado através de biópsia óssea e com a quantificação de proteínas ósseas não colágenas no osso e no plasma. Uma situação que se parece com esta pode ser encontrada em pacientes com HPT posteriormente submetidos à PTx, onde um estado de alta é seguido por baixa remodelação óssea.

Baseado nisso, decidimos avaliar a influência da remodelação óssea na transferência de Ca durante a hemodiálise em pacientes com HPT secundário. Esses pacientes eram submetidos à biópsia óssea e sessões consecutivas e randômicas de diálises com diferentes Cad antes da PTx, durante a fome óssea e após a estabilização da doença óssea (PTx tardio), conforme descrito no anexo C.

2. OBJETIVO

Este estudo teve por objetivo avaliar os efeitos da remodelação óssea e do conteúdo ósseo de proteínas não colágenas sobre a transferência da massa de Ca durante a hemodiálise em pacientes portadores de hiperparatireoidismo secundário antes e após a PTX.

ANEXO A: Parathyroidectomy Improves Survival in Patients with Severe Hyperparathyroidism: A Comparative Study. PLOS ONE 2013; 8(8):e68870.

Parathyroidectomy Improves Survival In Patients with Severe Hyperparathyroidism: A Comparative Study

Patricia Taschner Goldenstein¹, Rosilene Motta Elias¹, Lilian Pires de Freitas do Carmo¹, Fernanda Oliveira Coelho¹, Luciene Pereira Magalhães¹, Gisele Lins Antunes¹, Melani Ribeiro Custódio¹, Fábio Luiz de Menezes Montenegro², Silvia Maria Titan¹, Vanda Jorgetti¹, Rosa Maria Affonso Moysés^{1*}

¹ Nephrology Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, ² Head and Neck Surgery Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Abstract

Background and objectives: Secondary hyperparathyroidism (SHPT) in CKD is associated with an increased risk for mortality, but definitive data showing that parathormone control decreases mortality is still lacking. This study aimed to compare the mortality of patients with severe SHPT submitted to parathyroidectomy (PTX) with those who did not have access to surgery.

Methods: This is a retrospective study in a cohort of 251 CKD patients with severe SHPT who were referred to a CKD-MBD Center for PTX from 2005 until 2012.

Results: Most of our patients had indication of PTX, but only 49% of them had access to this surgical procedure. After a mean follow-up of 23 months, 72 patients had died. Non-survivors were older; more often had diabetes, lower serum 25 vitamin D and mostly had not been submitted to surgery. The relative risk of death was lower in the PTX patients (0.428; 95% CI, 0.28 to 0.67; $p < 0.0001$). After adjustments, mortality risk was dependent on age (1.04; 95% CI, 1.01 to 1.07; $p = 0.002$), 25 vitamin D (0.43; 95% CI, 0.24 to 0.81; $p = 0.006$) and no access to PTX (4.13; 95% CI, 2.16 to 7.88; $p < 0.0001$). Results remained the same in a second model using the PTX date as the study start date for the PTX group.

Conclusions: Our data confirms the benefit of PTX on mortality in patients with severe SHPT. The high mortality encountered in our population is significant and urges the need to better treat these patients.

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* E-mail: rosa.moyses@uol.com.br

Introduction

Mineral and bone metabolism disorders (CKD-MBD) are found almost universally in patients with chronic kidney disease (CKD) requiring dialysis. Secondary hyperparathyroidism (SHPT) is one of the most important and well-recognized of these disorders and data from observational studies have shown that CKD patients presenting high levels of serum parathormone (PTH) have an increased risk for mortality, as well as a higher morbidity [1,2,3]. The Dialysis Outcomes and Practice Patterns Study (DOPPS) is the most important ongoing observational study and its results indicated that serum PTH higher than 600 pg/ml was associated with a 21% increase in all-cause mortality risk [3].

However, most of these observational studies compared patients with high levels of PTH with those presenting adequate levels of this hormone [4,5] raising the question whether this mortality is related to SHPT itself or to other associated risk-factors. In addition, data that shows that controlling PTH in dialysis patients

decreases mortality is still lacking [6], since most of the studies compared different strategies of therapy for SHPT, considering that not offering any therapeutic strategy would be considered as non-ethical.

Currently in Brazil, approximately 90,000 patients are being treated with dialysis, most of them reimbursed by the public health system [7]. Serum PTH is measured only two times per year and the current therapy for SHPT provided by this public system is calcium-based phosphate binders and calcitriol. Sevelamer is offered only to patients who present sustained hypercalcemia and paricalcitol or cinacalcet are not available yet. The consequence is that a significant proportion of our patients develop severe forms of SHPT and the only option for their therapy is surgical parathyroidectomy (PTX). However, few services in our country perform this surgery, leading to a waiting list in the reference centers, which is, at the biggest University in Latin America, of 563 days. This dramatic condition gave us the unique opportunity to test the hypothesis whether SHPT therapy, in this case through

Figure 1. Flow chart of study design. Refractory hyperparathyroidism was defined as initial intact PTH greater than 800 pg/ml on calcitriol or in the presence of hyperphosphatemia and/or hypercalcemia, which prevented the use of calcitriol.
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surgical PTX, is able to decrease mortality. To that end, we prospectively followed dialysis patients that were referred to our center for PTX and compared the mortality of those that were submitted to PTX with those who did not have access to this surgical therapy over time.

Materials and Methods

This historical analytical cohort encompassed CKD patients who attended the CKD-MBD clinic in a tertiary care hospital in São Paulo, Brazil (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo), from January 1, 2005 to December 31, 2009. This service is a reference center that receives

Table 1. Characteristics of the Patients at Baseline.

Variable		Reference Values
Male n (%)	163 (54.3)	-
Age (ys)	48 (13.5)	-
Diabetes n (%)	37 (12.6)	-
Hypertension n (%)	163 (58.6)	-
Ionized calcium (mg/dl)	5.1 (3.7–7.1)	4.6–5.3 mg/dl
Phosphorus (mg/dl)	5.5 (1.62)	2.7–4.5 mg/dl
AP (U/L)	224 (46–3215)	40–129 U/L
PTH (pg/ml)	1,294 (10–7368)	150–300 pg/ml
25 vitamin D (ng/ml) [#]	24 (3–97)	30–100 ng/ml
Dialysis vintage (months)	72 (3–288)	-
Pain n (%)	178 (63.7)	-
Fractures n (%)	37 (13.2)	-
Calcitriol Therapy (%)	112 (40.8)	-

Data expressed as mean (SD), median (min-max), or percentage.

PTH = parathormone, AP = Alkaline phosphatase.

[#] 1 ng/mL = 2.5 nmol/L;

^a data available for 218 patients.

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patients from different dialysis services in São Paulo area for further evaluation and treatment. After excluding kidney transplant and predialysis patients, 300 individuals on dialysis were evaluated. Those patients that on initial evaluation had serum levels of intact PTH greater than 800 pg/ml on calcitriol or in the presence of hyperphosphatemia and/or hypercalcemia, which prevented the use of calcitriol, were considered as having refractory hyperparathyroidism and were referred to surgical PTX (n = 251). Patients selected for PTX were then entered in a waiting list according to the chronological order. A flow chart of the study design is shown in Figure 1. Even though the original

Table 2. Comparison between groups of patients with indication versus no indication of PTX.

	Need of PTX (n = 251)	No need of PTX (n = 47)
Male gender n (%)	114 (45)	24 (51)
Age (ys)	47 (12.8)	51.2 (16.5)
Diabetes n (%)	27 (10.7)	9 (19.1)
Hypertension n (%)	139 (59.4)	24 (58.5)
Ionized calcium (mg/dl)	5.1 (3.7–7.1)	5.2 (4.2–6)
Phosphorus (mg/dl)	5.7 (1.6)	4.9 (1.5)*
AP (U/L)	262 (47–3215)	105.5 (46–522)*
PTH (pg/ml)	1457 (810–7368)	241 (10–794)*
25 vitamin D (ng/ml) [#]	24 (3–93)	27 (4–97)
Pain n (%)	159 (67)	17 (42.5) *
Fractures n (%)	33 (13.9)	4 (10)
On Calcitriol n (%)	92 (39.6)	19 (47.5)
Dialysis vintage (mo)	72 (3–288)	42 (3–168)*

*p<0.05 Data expressed as mean (SD), median (min-max), or number (percentage). PTH = parathormone. PXT = parathyroidectomy, AP = Alkaline phosphatase.

[#] 1 ng/mL = 2.5 nmol/L;

^a data available for 218 patients.

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Table 3. Characteristics of survivors versus non-survivors.

	Survivors (n = 179)	Non-Survivors (n = 72)
Male gender n (%)	88 (49.1)	26 (26)*
Age (ys)	44.6 (12.3)	53.9 (12)*
Diabetes n (%)	12 (6.7)	15 (20.8%)*
Hypertension n (%)	98 (57.9%)	41 (62.1)
Ionized calcium (mg/dl)	5.1 (3.7–7.1)	5.1 (4.3–6.5)
Phosphorus (mg/dl)	5.7 (1.5)	5.5 (1.6)
AP (U/L)	244 (69–2574)	296 (47–3215)
PTH (pg/ml)	1460 (810–7368)	1419 (811–5797)
25 vitamin D (ng/ml) [#]	30.1 (SD 17.2)	(SD 10.9)*
Pain n (%)	110 (64.3)	48 (72.7)
Fractures n (%)	22 (12.8)	11 (16.6)
On Calcitriol n (%)	67 (39.6)	25 (39.6)
Parathyroidectomy n (%)	102 (56.9)	21 (29.1)*
Dialysis vintage (mo)	72 (3–264)	84 (8–288)

*p<0.0001 Data expressed as mean (SD), median (min-max), or percentages.

PTH = parathormone, AP = Alkaline phosphatase.

[#] 1 ng/mL = 2.5 nmol/L;

^a data available for 182 patients.

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Table 4. Characteristics of patients submitted versus not submitted to PTX.

	Submitted to PTX (n = 123)	Not submitted to PTX (n = 128)
Male gender n (%)	57 (46.3%)	57 (44.5%)
Age (ys)	46 (37–54)	50 (40–58)*
Diabetes n (%)	7 (5.6%)	20 (15.6%)*
Hypertension n (%)	62 (50.4%)	77 (60.1%)
Ionized calcium (mg/dl)	5.2 (4.95–5.5)	5 (4.8–5.3)*
Total calcium (mg/dl)	10 (9.37–10.63)	9.7 (9.22–10.1)*
Phosphorus (mg/dl)	5.7 (5–6.9)	5.3 (4.2–6.6)*
AP (U/L)	335 (161.5–559)	246 (135–441)*
PTH (pg/dl)	1554 (1168–2300)	1360 (1026–1967)*
25 vitamin D (ng/ml) ^{#,‡}	23.5 (15.25–38.5)	24 (16–32.5)
Pain n (%)	82 (66.6%)	77 (60.1%)
Fractures n (%)	21 (17%)	12 (9.3%)
On Calcitriol n (%)	40 (32.5%)	52 (40.6%)
Dialysis vintage (mo)	84 (48–120)	72 (36–120)*

*p<0.05 ; Data expressed as mean (SD), median (min-max), or percentages. PTH = parathormone; PTX = parathyroidectomy, AP (Alkaline Phosphatase).

[#]1 ng/mL = 2.5 nmol/L;

[‡]data available for 182 patients.

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criterion for surgery was time on the waiting list, the severity of symptoms (such as worsening of pain, tendon rupture, new fractures) was actually a main guide to anticipate surgery.

Total parathyroidectomy with autotransplantation in the forearm is the procedure of choice at our Institution. Intraoperative PTH levels were obtained before and after resection of each enlarged parathyroid gland with the goal to attain a final intraoperative PTH less than 100 pg/mL.

All patients of this cohort were followed until March 1, 2012, when a survival analysis was performed. Clinical assessments were performed on all patients. At baseline, serum PTH, total calcium, ionized calcium, phosphorus, alkaline phosphatase, vitamin D levels and medical record was queried.

This study was approved by the Ethics Committee of the Hospital das Clínicas da Universidade de São Paulo. As this was a retrospective study, our University Ethics Committee did not require an informed consent.

All data are expressed as mean $\pm$ SD for normally distributed data, median (min-max) for skewed data, and frequency (%) for categorical data. Comparisons between continuous variables and categorical variables were made using the t-test and chi-square test, respectively. The Kaplan-Meier method was used for the estimation of survival, and differences between the curves (PTX and non-PTX groups) were compared using the log-rank test. Data for survival analysis were censored at the time of renal transplantation or end of study (March 1st, 2012). Forward Stepwise (Likelihood Ratio) cox proportional hazards regression analysis was used to estimate the hazard ratio (HR) of non-PTX in the risk of death (variables in the model: sex, age, PTX, diabetes and 25 vitamin D). Lastly we performed a second analysis to exclude the “immortal time” bias, using the PTX date as the study start date for the PTX group. The same variables were entered on the adjusted models. Statistical analysis was performed using SPSS 17.0.1 (SPSS Inc, Chicago, Ill) and Graphpad prism 5 (CA, USA). A two-sided *P* value less than 0.05 was considered significant.

Results

As shown in Table 1, patients that were referred to our center were relatively young, with a mean age of 47.9 ± 13.5 years, with a slight predominance of men. We also had few diabetics, but more than 50% were hypertensive. Most had elevated ionized calcium and phosphate, as well as high alkaline phosphatase. Median PTH was high, whereas median 25 vitamin D was lower than normal [defined as ≥ 30 ng/ml (75 nmol/L)] and 22% of the patients were considered as having vitamin D deficiency [defined as < 15 ng/ml (45 nmol/L), whereas vitamin D insufficiency was defined as serum 25 vitamin D levels between 15 and 30 ng/ml]. Patients were symptomatic, as confirmed by the presence of continuous pain in more than 60% of them, although self-referred fracture was detected only in 13%. The only affordable therapy, calcitriol, was being used in 40% of the patients.

Most of the patients (84.2%) had indication of PTX. When compared to those that did not need PTX, we could observe that these patients with refractory hyperparathyroidism presented higher serum phosphorus, alkaline phosphatase and serum PTH. They were also more symptomatic and had been on dialysis therapy during a longer period of time (Table 2).

After a mean follow-up of 23 months, only 123 out of the 251 patients with indication of PTX were submitted to this surgical procedure. We observed that 72 patients from this group of refractory hyperparathyroidism died [21 in the group that was submitted to PTX (7 deaths per 100 patient-year and 51 in the group that did not have access to the surgery (16 deaths per 100 patient-year); $p < 0.0001$]. To better understand the factors that could be associated with an increased risk of death in this population of patients with severe hyperparathyroidism, we compared survivors and non-survivors, as shown in Table 3. The first group was younger, with a higher prevalence of males, and less frequently had diabetes. Ionized calcium, phosphate, alkaline phosphatase, as well as serum PTH were not able to differentiate groups. However, mean 25 vitamin D was lower in the non-survivor group, as well as the prevalence of patients that were submitted to PTX. We could also observe that the mortality

Figure 2. Kaplan-Meier survival curves according to PTX. A higher median survival time was observed for PTX group (2185 days) [superior dashed line] than non-PTX group (1357 days) [continuous line]. Log-rank $p < 0.0001$ for curves A (unadjusted), B (adjusted for age, gender and diabetes) and C (adjusted for age, gender, diabetes and vitamin D). doi:10.1371/journal.pone.0068870.g002

rates were 19.4%, 30.7% and 47.5% in vitamin D replete, insufficient and deficient patients, respectively. However, a significant difference in terms of mortality was found only between the first and last groups of patients. Conversely, we could not find any beneficial effect of calcitriol on survival, since mortality rates were of 27.2% and 27.1% in patients that have been treated or not with this drug, respectively, at the time they were referred to our center.

The comparison between patients submitted to PTX to those who did not have the chance to undergo surgery disclosed that the first group was younger and less frequently diabetic; however, they were more symptomatic, more often referring pain and fractures (Table 4). They also had higher serum calcium, phosphate, alkaline phosphatase and PTH and a longer dialysis vintage, turning them into more seriously ill patients. The analysis of the relative risk of death showed that it was lower in the PTX group than in the non-PTX group (0.428; 95% confidence interval, 0.28 to 0.67; $p < 0.0001$).

Survival curve showed a benefit of PTX, as depicted in Figure 2 (log-rank test $p < 0.0001$). In unadjusted model the relative hazard in the non-PTX group vs. the PTX group was 4.13; 95% confidence interval [CI], 2.46 to 6.92; $p = 0.0001$. After adjustment for age, gender and presence of DM, mortality risk was dependent on age (1.06; 95% CI, 1.04 to 1.08; $p < 0.0001$) and no access to PTX (3.91; 95% CI, 2.30 to 6.66; $p < 0.0001$). After adjustment for age, gender, DM and 25 vitamin D, the variables that remained significant were age (1.04; 95% CI, 1.01 to 1.07; $p = 0.002$), 25 vitamin D (0.43; 95% CI, 0.24 to 0.81; $p = 0.006$) and no-PTX (4.13; 95% CI, 2.16 to 7.88; $p < 0.0001$). Finally, we performed another multivariable analysis, in order to rule out the “immortal time” bias, and we observed that the relative hazard in the non-PTX group vs. the PTX group was 2.5, 2.2 and 2.4 using the same models described before (Figure 3). Therefore, the benefits of PTX were still confirmed in this unique population with severe form of hyperparathyroidism.

Discussion

Secondary hyperparathyroidism (SHPT) is a common complication in patients on chronic hemodialysis. Conventional medical therapy with first generation of vitamin D receptor activators (VDRAs), such as calcitriol and alfacalcidol, lack adequate long-term control of intact parathyroid hormone (iPTH) in a large group of patients, resulting in vascular calcification [8] and an increase of cardiovascular mortality [9].

The exact mechanisms by which altered mineral metabolism increases mortality and morbidity remain unclear, but vascular calcification has been proposed as a possible link [8,10]. It has been described that serum phosphorus stimulates the transformation of vascular smooth muscle cells into osteoblast-like cells, creating an environment susceptible to calcification [11,12]. Similarly, hypercalcemia and uremia cause vascular calcification by active processes suggesting the importance of strict management of uremia, serum minerals and PTH in dialysis patients [8,13,14].

Parathyroidectomy is the treatment of choice for patients who become resistant to medical therapy. A small clinical study has shown slowing of calcification progression after PTX [15], but there has been a few reports of improved survival post-PTX

[16,17,18]. This surgical procedure seems to improve high blood pressure, anemia, nutritional state, immunity, glucose and lipid metabolism, muscle strength and quality of life, relieves insomnia and even increases cognitive function [19,20,21,22,23].

The mechanisms responsible for these remarkable beneficial effects of PTX are not certain; however, the dramatic reductions in PTH and better control of serum minerals are certainly involved. Iwamoto et al [4] performed a retrospective comparison of survival and mineral metabolism between 88 patients who had undergone PTX and 88 control dialysis patients with no indication of PTX. The overall survival rate was 90.4% in the PTX group and 67.4% in the control group, whereas the cardiovascular death-free survival rate was significantly better in the PTX group (94.6% versus 76.3%). They found that besides reducing PTH, calcium levels were significantly lower in the PTX group compared to the control group and suggested that these effects could contribute to the decrease of cardiac afterload and inhibition of the further progression of vascular calcification resulting in a better prognosis of this group of patients. Sharma et al also have described an overall improvement in mortality from cardiovascular diseases in 86 patients who had this time undergone near total parathyroidectomy compared to matched controls [5]. However, both of them performed retrospective studies and their control group was composed of dialysis patients with no indication of PTX, which makes the groups distinct, and the presence of other confounding factors contributing to this benefit of PTX could not be ruled out.

In our center, although more than two surgeries were performed each month, the number of patients referred to us and the mean time on waiting list were astoundingly elevated. As a consequence, sometimes we had to anticipate surgery in those patients that were more symptomatic and severely ill. The final result is that the group that was submitted to PTX was younger, but with a more severe manifestation of SHPT. Nevertheless, the adjusted survival curve was able to confirm the benefit of therapy in this group of patients that currently is rarely seen in the US, Japan or Western Europe.

During the follow up, we found a mortality rate of 29% in the group patients with refractory hyperparathyroidism, which was even higher in the group of patients who needed, but was not submitted to PTX (40%). The median non-PTX patients' follow-up time was shorter than PTX patients' follow-up time (493 vs. 763 days; $p = 0.15$), but did not reach statistical significance. If we considered the PTX date as the study start date for the PTX group, the results basically remained the same, although the HR of mortality for non-PTX group had diminished from 4.0 (as previously showed) to 2.4 (unadjusted) and 2.1 (adjusted for age, gender and presence of diabetes).

Kestenbaum et al [24] have demonstrated that long-term relative risks of death among patients undergoing PTX were estimated to be 10% to 15% lower than those of matched control patients not undergoing surgery. Median survival was higher in the PTX group than in matched control group. Although these authors also found a benefit of PTX, this study differs from ours in several aspects: first, the control group did not have indication for PTX; second, the patients had full access to medication; third, the PTX was not delayed due to extensive waiting list; finally, the above mentioned study compared patients and controls from many centers, which may have some difference in clinical approach, as well as indication of PTX. The more pronounced

Figure 3. Kaplan-Meier survival curves according to PTX in the second model, using the PTX date as the study start date for the PTX group. A higher median survival time was observed for PTX group [superior dashed line] than non-PTX group [continuous line]. Log-rank $p < 0.0001$ for curves A (unadjusted), B (adjusted for age, gender and diabetes) and C (adjusted for age, gender, diabetes and vitamin D). doi:10.1371/journal.pone.0068870.g003

benefit of PTX in our study is probably related to the differences mentioned above.

When we compared survivors and non-survivors we could observe that survivors were younger and mostly males with less frequent diabetes. Interestingly, neither serum minerals not even PTH was able to differentiate groups. On the other hand, parathyroidectomy and mean levels of 25 vitamin D were crucial to differentiate the group of survivors versus non-survivors. This association of serum 25 vitamin D and survival has already been shown in other studies [25,26]. In our study, we could observe a progressive increase in mortality rate as the serum levels of 25 vitamin D decreased. However, a significant difference in terms of mortality was found only between vitamin D replete in comparison to vitamin D deficient patients. Curiously, after the initial evaluation, all patients with hypovitaminosis D were prescribed with cholecalciferol, what would normalize their vitamin D status, at least in theory. Another interesting finding is related to the absence of any significant effect of calcitriol administration on mortality. Previous studies have shown that active vitamin D administration decreases mortality in dialysis patients with hypovitaminosis D [25]. However, in our sample, mortality rates were similar between those treated or not with calcitriol. One possible explanation is that those non-classical benefits of calcitriol therapy could not be observed in these patients with a severe form of SHPT.

In the literature, there is still an intense debate on the better strategy for SHPT management. Some authors believe that it should start with VDRA [27], whereas others prefer calcimimetics [28]. A recent published study, which compared the effects of calcimimetics with conventional therapy with VDRA, showed inconclusive results [29]. Curiously, one possible explanation for the EVOLVE disappointing results was the high rate of PTX in the control group, which might have decreased the number of events in those patients, i.e. the effect of cinacalcet could be masked by that imbalance in PTx between groups. A dangerous conclusion that might be drawn from the EVOLVE results [6] is to whether there is any benefit of cinacalcet or any SHPT therapy in terms of mortality and morbidity. Our data, generated from a critical situation in the Brazilian health system comes to confirm

that not giving any therapeutic option to these patients increases significantly their risk of death. In Brazil and other developing countries, dialysis is affordable for most of the CKD patients and this could give them a chance to a better survival. However, if this access to dialysis does not come with medical therapy for CKD-MBD and other CKD complications, our patients will still be exposed to a high risk of mortality, as well as of fractures and pain. One piece of information that could not be given by our data is whether surgical PTX is better than current conservative therapy, which includes new generations of VDRA and calcimimetics, since any of our patients have the opportunity to be treated with these drugs.

This work has several limitations. The number of patients is relatively small; this is a cohort study, in which patients were not randomized to treatment with PTX or not, and the more seriously ill were eventually submitted to surgery before. Like other observational studies, the present data is also prone to selection bias, implying the risk of residual confounding. Additional limitations are some unmeasured patient characteristics, such as serum 25 vitamin D and presence of undetected clinical comorbidities, which limit the adjustments and inferences that can be made. Notwithstanding, we had the unique opportunity to have a control group of real life with indication, but no therapy to their disease, which would not be accepted in a classical clinical study. We believe that the high mortality encountered in our population is significant and urges the need to treat these patients.

Conclusion

The definitive information that we can provide is that, although being seriously ill, symptomatic, with high serum phosphorus, very elevated PTH and low vitamin D, in an extreme situation, our patients were clearly benefited from PTX.

Author Contributions

Conceived and designed the experiments: PTG RME SMT VJ RMAM. Performed the experiments: PTG LPFC FOC LPM GLA FLMM MRC. Analyzed the data: PTG RME SMT VJ RMAM. Wrote the paper: PTG RME RMAM.

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ANEXO B: Fractures in chronic kidney disease: pursuing the best screening and management. *Curr Opin Nephrol Hypertens.* 2015;24(4):317-23.

Fractures in chronic kidney disease: pursuing the best screening and management

Patrícia T. Goldenstein^a, Sophie A. Jamal^b, and Rosa M.A. Moysés^{a,c}

Purpose of review

Osteoporotic fractures are common and cause increased sickness and death. Men and women with chronic kidney disease (CKD) are at particularly high risk of osteoporotic fractures. Currently, however, there are no guidelines concerning noninvasive methods to assess fracture risk in CKD. Further, approved treatments to prevent fractures in otherwise healthy men and women are only recommended for use with caution in those with CKD. This review focuses on the recent data that support the use of noninvasive methods to assess fracture risk in CKD and highlights new therapies that could be used in fracture prevention in CKD.

Recent findings

Data from prospective studies demonstrate that low bone mineral density predicts fracture in CKD patients. Post-hoc analyses demonstrate that agents approved for the treatment of postmenopausal osteoporosis (bisphosphonates, denosumab and teriparatide) when given to those with CKD are well tolerated and potentially efficacious with respect to fracture risk reduction.

Summary

To date, patients, and nephrologists taking care of them, have largely ignored fracture risk assessment and treatment in CKD. This should change given recent data. Further studies are needed, specifically bone histomorphometric studies, which will increase our understanding of CKD-mineral bone disease (MBD) pathophysiology, and randomized clinical trials of therapy in patients with CKD.

Keywords

bone disease, bone histomorphometry, CKD-MBD, fracture, osteoporosis, osteoporosis therapy

INTRODUCTION

Among otherwise healthy men and women, one in three women and one in five men are at risk for fractures from osteoporosis. Osteoporosis is defined as a compromised bone strength (made up of two features – bone density and bone quality) predisposing to an increased risk of fracture. Osteoporotic fractures cause increased morbidity, mortality, and healthcare costs. The adverse outcomes associated with fractures have fuelled numerous studies that have identified both well accepted methods to assess fracture risk (most commonly dual energy X-ray absorptiometry – DXA) and treatments to reduce fractures [1–3].

Diagnosing and treating osteoporosis in the setting of chronic kidney disease (CKD) is complex, due in part to metabolic and hormonal abnormalities associated with CKD that result in impairment of bone turnover, mineralization, and volume – so called CKD-mineral bone disease (MBD). CKD-MBD is associated with an increased risk of fracture; indeed, the risk of fracture among those with CKD is

1.5 to 2-fold times higher than the general population. Further, in those with CKD (again compared with otherwise healthy men and women), fractures occur at a younger age and are associated with a higher mortality. Despite this, fracture risk assessment and treatment has, for the most part, been neglected in CKD. For example, despite an improvement in renal bone care, data from US hemodialysis patients [4[¶]] showed that between 1992 and 2009 there was an overall significant increase in the incidence of fractures requiring hospitalization. The US Renal Data System (USRDS) also showed that

^aNephrology Division, Universidade de São Paulo, São Paulo, Brazil,

^bWomen's College Research Institute, University of Toronto, Toronto, Canada and ^cMedicine Master Degree Program, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

Correspondence to Rosa M. A. Moysés, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil, Rua Iperoig, 690 ap 121, ZIP CODE 05016-000, São Paulo, SP, Brazil. E-mail: rosa.moyses@uol.com.br

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KEY POINTS

- Fracture-risk assessment and treatment is still a challenge in chronic kidney disease (CKD).
- Data from recent prospective studies demonstrate that low bone mineral density predicts fracture in CKD patients.
- Recognition of the current understanding of CKD-MBD pathophysiology, and judicious use of therapy, could help us to definitively prevent fractures in CKD patients in the near future.

fractures increased 43% in 2004 and decreased non-significantly in 2009 in incident patients [5]. The same high rates of fractures were demonstrated in dialysis patients participating in the cohort of Dialysis Outcomes and Practice Patterns Study (DOPPS). Compared with those without fractures, those with CKD and fractures had 3.7 times unadjusted higher death [6[•]]. The increased risk of fracture was also observed among CKD patients not on dialysis with a progressive fracture incidence per 1000 person-years of 15, 20.5, 24.2, 31.2, and 46.3 for patients with CKD stages 1–2, 3a, 3b, and 4, respectively [7^{••}]. Excess fractures are also seen after kidney transplant [8]. Over the first 3 years of transplantation, hip fracture is 34% higher than in dialysis patients, and over the first 5 years one-fourth will have a fracture [9].

To reduce the burden of illness associated with fractures in CKD it is crucial we identify and treat those at high fracture risk. However, the 2009 Kidney Disease Improving Global Outcomes (KDIGO) guidelines do not recommend using DXA to assess fracture risk in CKD-MBD [10]. The rationale for this recommendation is the lack of prospective data demonstrating that low DXA increased fracture risk and the fact that bone mineral density (BMD) does not provide information as to the underlying MBD.

Since the publication of the 2009 CKD-MBD KDIGO guidelines there have been some new data concerning fracture risk assessment and treatment in CKD-MBD. In light of these new data, in 2014 KDIGO convened a controversies conference in Madrid, Spain to establish which guidelines concerning CKD-MBD should be revisited. Areas that were revisited include the use of DXA in CKD-MBD and new pharmacologic agents approved for the treatment of postmenopausal osteoporosis, which may be well tolerated and efficacious in early stages of CKD. These recommendations will be reviewed for the next KDIGO initiative [11[•]]. Some of the recent data considered by KDIGO in revising the

guidelines are contemplated below and summarized in Table 1.

METHODS OF EVALUATION OF BONE DENSITY AND QUALITY

Dual energy X-ray absorptiometry

DXA is obtained by aiming two radiograph beams with different energy levels at the patient's bones. After subtracting the soft tissue absorption, the BMD is determined [12]. DXA is the most widely used noninvasive technique for measuring BMD in the general population. Low BMD by DXA is a robust and consistent risk factor for fracture and treatments that increase BMD reduce fracture risk. The 2009 KDIGO guidelines recommended DXA for the assessment of fracture risk in CKD stages 1 through early 3, and that it should not be performed routinely in CKD stages 3–5 due to lack of evidence for fracture prediction in these patients [10]. This recommendation was based on the fact that there were no longitudinal studies demonstrating that low BMD predicted fracture risk and the fact that cross-sectional studies reported inconsistent associations between low BMD and fracture [13].

Since the KDIGO publication there have been studies reporting that low DXA can predict fractures in CKD. A 5-year prospective cohort study of 485 participants on hemodialysis found that low BMD at the total hip or femoral neck was associated with excess risk of incident fractures independent of age, sex, diabetes, and dialysis vintage [14]. A longitudinal study that analyzed the effect of CKD on fracture risk prediction by DXA in 587 older adults with CKD enrolled in the Health, Aging, and Body Composition Study reported that, with a follow-up of 11.3 years, each SD decrease in BMD at the femoral neck was associated with greater than a two-fold increased risk of fracture in patients with CKD compared with those without [15]. A prospective study of participants with stages 3–5 CKD found that over 2 years of follow-up, BMD by DXA at baseline was significantly lower at all sites among those with incident fractures versus those without. For example, after adjusting for age and weight, for every SD decrease in BMD at the lumbar spine there was a two-fold increase in the risk of fracture; the authors found a similar relationship between total hip BMD and fracture [16^{••}]. Finally, a study in 238 transplant patients reported an increase in the risk of fracture by 2.7 fold when osteopenia was diagnosed and 3.5 fold when osteoporosis was diagnosed by DXA [17]. Considered together, these data demonstrate that low BMD predicts fracture in predialysis, dialysis, and transplant CKD.

Table 1. Weaknesses, strengths, and perspectives of screening methods and therapeutic options

	Pros	Cons	Perspectives
Screening method			
DXA	Noninvasive	Does not provide bone turnover and mineralization information	Future observational studies confirming fracture risk predictive value in other CKD populations
	Low cost, easy to perform, available worldwide	Does not differentiate cortical from trabecular bone	Could be used in interventional studies in an immediate future
HR-pQCT	Fracture risk prediction confirmed		
	Noninvasive	High cost, not available worldwide	Higher availability in an immediate future
Bone biopsy	High definition and sensibility	Does not provide bone turnover and mineralization information	Future observational studies evaluating fracture risk predictive value in CKD
	Differentiates cortical from trabecular bone	Fracture risk prediction confirmed by a single study	
	High definition and sensibility	Invasive	New research groups with bone biopsy experts are needed
	Differentiates cortical from trabecular bone	High cost, not available worldwide	Future observational and interventional studies comparing DXA, HR-pQCT, and bone biopsy
	Provides bone turnover and mineralization information	Fracture risk prediction not evaluated	
Management			
Bisphosphonates	Low cost, worldwide available	Extended half-life in CKD	Future studies evaluating different drugs, doses, time of administration in patients with different stages of CKD
	Decreases fracture in predialysis CKD in post-hoc analyses	Potential risk of toxicity	
Denosumab	Not cleared by the kidney	Potential risk of ABD	Future prospective studies in patients with different stages of CKD in an immediate future
	Decreases fracture in predialysis CKD in post-hoc analyses	Higher cost, not available worldwide	
Teriparatide	Not cleared by the kidney	Hypocalcemia	Future prospective studies in patients with different stages of CKD and ABD suspicion in an immediate future
	Increases bone mass and turnover; no potential risk of ABD	Potential risk of ABD	
Romosozumab	Not cleared by the kidney	Fracture risk decrease not evaluated	
	Increases bone mass and turnover, no potential risk of ABD	Still on phase 3 studies in the general population	Future prospective studies in patients with different stages of CKD and ABD suspicion in an immediate future
		Fracture risk decrease not evaluated	If benefits confirmed in the general population, would be a first-choice drug for predialysis CKD

ABD, adynamic bone disease; CKD, chronic kidney disease; DXA, dual energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitated tomography.

High-resolution peripheral quantitated tomography

High-resolution peripheral quantitated tomography (HR-pQCT) has a resolution of $82 \mu\text{m}^3$ and provides details of bone geometry and microarchitecture by quantifying trabecular number and thickness and cortical porosity. Cross-sectional studies

demonstrated that low HR-pQCT parameters at the distal radius and tibia were associated with fractures [18–21]. However, only one recent study published prospective data showing that HR-pQCT predicts fracture. Moreover, in this cohort, HR-pQCT did not demonstrate a better performance than DXA in terms of fracture prediction [16²²]. This

fact, combined with the limited availability of this technique, currently limits the use of HR-pQCT for fracture risk assessment in CKD. Nevertheless, this method is able to detect structural changes, differentiating cortical from trabecular compartments, and could potentially provide important information in future interventional studies.

OTHER NONINVASIVE TECHNIQUES

We recognize that there are other noninvasive imaging techniques that are currently being used in research studies. These include MRI, high resolution MRI, Raman spectroscopy, Fourier transform infrared spectroscopy, and quantitative backscatter electron imaging [22–24]. To date, none of these techniques can be recommended for use in clinical care.

BONE BIOPSY

Tetracycline double-labeled transiliac bone biopsy with histomorphometry is the gold standard to assess bone quality and strength, and uniquely bone turnover; note that all the other noninvasive measures give static assessments of bone turnover. Unfortunately, bone biopsy is not routinely performed, mainly because it is invasive, and requires specialized expertise, both to obtain the biopsy and interpret the histomorphometric findings [10,25]. Further, bone biopsy is limited as it only gives information about bone at one point in time and from one site, and intersample variation should be taken in to account to assess the minimal difference required in order to affirm that changes noted in a repeat biopsy are significant [25,26].

That said, bone histomorphometry can provide information about the pathophysiology of bone loss in CKD that may, ultimately, increase our understanding of fracture risk in CKD. Specifically, bone histomorphometry can evaluate cortical bone compartments, this is highly relevant as previous noninvasive studies have shown a faster bone loss in the cortical area in CKD patients [27]. For example, bone histomorphometry confirmed data from HR-pQCT, demonstrating that increased cortical porosity and erosion are present in dialysis patients with hyperparathyroidism and with a higher trabecular bone turnover [28]. In pediatric CKD patients, similar findings were described. However, this difference was present primarily in external, not internal, cortical bone. The investigators also found a higher osteoid accumulation in the external cortical bone of children with hyperparathyroidism, suggesting that this site is the most active in terms of metabolism and response to parathyroid hormone (PTH)

[29]. We recently measured cortical porosity in bone biopsies from dialysis patients who have been followed for one year. An increase in cortical porosity was observed, which was more pronounced in black patients and in those with high PTH [30]. If confirmed by other studies, our findings show direct effects of CKD-MBD management on cortical bone, challenging the effects of conventional CKD-MBD therapy on the risk for fracture.

It is important to note that in contrast to DXA, there are no studies that report on the ability of bone histomorphometry to predict incident fractures in CKD. That said, bone histomorphometric studies have contributed substantially to our understanding of the pathophysiology of bone aberrations in CKD-MBD and, ultimately, this may increase our understanding of the pathophysiology of increased fracture risk in CKD. Future research should include a prospective study designed to compare and contrast DXA, HR-pQCT, and bone histomorphometry.

New therapeutic evidence

In the general population, management of osteoporosis involves both nonpharmacological, including changes in lifestyle and dietary habits, and pharmacological approaches in patients at high risk for fracture and presenting with an established osteoporosis. Guidelines recommend supplementation of calcium and vitamin D, as well as, inhibitors of bone resorption (bisphosphonates, denosumab, and selective estrogen receptor modulators), stimulators of bone formation (teriparatide, romosozumab) or chemical entities decreasing bone resorption and stimulating bone formation (strontium ranelate, available only in Europe and South America) [31].

KDIGO guidelines recommend that osteoporosis management and treatment for patients with stages 1–3 CKD should be the same as for patients without CKD, as long as they do not present with features of CKD-MBD [10]. For patients with later stages of CKD, a bone biopsy is required to accurately identify the type of bone disease prior to initiating treatment, keeping in mind that most FDA approved pharmacological choices for osteoporosis lack evidence for fracture reduction in advanced CKD (stages 4–5).

ANTIRESORPTIVE DRUGS

There are no data on the use of estrogen to prevent fractures in CKD. Of note, in healthy postmenopausal women the use of estrogen has been associated with an increased incidence of breast cancer and cardiovascular disease. Raloxifene, a systemic estrogen receptor modular, has been shown, in a

post-hoc analysis to reduce the risk for vertebral fracture but not of nonvertebral or hip fracture in those women with CKD and eGFR more than 20 ml/min [32].

Bisphosphonates are structural analogues of inorganic pyrophosphate, a substance present in body fluids that prevents soft tissue calcification and plays a regulatory role in bone mineralization processes. These agents inhibit osteoclast activity and stabilize calcium-phosphorus surface [33]. They remain in the bone in the remodeling resorption cavity depending on remodeling space, bone turnover and GFR. Note, that all the bisphosphonates carry warnings or contraindications for patients with low creatinine clearance (CrCl); for instance, the labels for risedronate and alendronate warn against use in patients with CrCl less than 30 ml/min and less than 35 ml/min, respectively, and the label for zoledronic acid indicates a contraindication for patients with CrCl less than 35 ml/min [34]. That said, these studies included some women with stages 3 and 4 CKD and post-hoc analyses of risedronate [35] and alendronate [36] in these women demonstrated that both agents reduced fractures and were not associated with an increase in renal adverse events.

Denosumab, approved for the treatment of postmenopausal osteoporosis, is a fully human monoclonal antibody that binds to receptor activator of nuclear factor kappa-B ligand (RANKL) in osteoblasts and osteocytes inhibiting it from binding to the osteoclast receptor RANK, and therefore, inhibiting osteoclastogenesis [37]. A post-hoc analysis of the pivotal denosumab fracture prevention trial showed an increase in BMD and a reduction in vertebral and nonvertebral fractures in postmenopausal CKD women with GFR as low as 15 ml/min [38] treated with this drug. Of particular relevance to those with CKD is the fact that denosumab is not cleared by the kidney. However, in hemodialysis patients, denosumab may induce significant hypocalcemia [39]. This fact, together with the fact that denosumab may make low turnover bone disease worse, highlights the concept that this agent should be used with caution.

STIMULATORS OF BONE FORMATION

Teriparatide is a recombinant protein of the 34 first amino acids of human PTH, an anabolic agent approved by the FDA for the treatment of postmenopausal osteoporosis. The pivotal fracture trial of teriparatide (dose 20 mcg/day or 40 mcg/day) included some women with an eGFR as low as 30 ml/min [40]. Post-hoc analyses demonstrate that there was an increase in BMD in all subsets of CKD stage and fractures, but numbers were too small to

reach statistical significance. There was no greater risk of nephrolithiasis and serum uric acid increased with no clinical consequence. Of note, there are also some case reports of teriparatide use in bone biopsy-confirmed adynamic bone disease demonstrating that the agent increases bone turnover and is well tolerated [41].

Romosozumab is an antisclerostin antibody. Sclerostin, an osteocyte-secreted glycoprotein, inhibits Wnt and bone morphogenic protein signaling pathways, which are promoters of bone formation. By inhibiting sclerostin, romosozumab could potentially stimulate bone formation [42]. A phase 2, prospective study in postmenopausal women has shown benefits of romosozumab bone mineral density, as well as transitory increase in bone-formation markers and sustained decrease in a bone-resorption marker, in comparison to alendronate and teriparatide [43]. The potential benefits of this drug include the nonrenal clearance, and the recent findings of increased bone expression [44,45] and serum levels of sclerostin in CKD patients [45,46,47]. If the current belief that sclerostin is increased in early CKD stages and could be associated with CKD-related bone resistance to PTH is correct, a new avenue for therapy of bone disease in CKD is being opened. However, although very promising, all these therapeutic agents should be tested in randomized clinical trials in CKD patients to demonstrate fracture risk reduction and safety.

CONCLUSION

Management of bone disease in CKD, more specifically low BMD, is still challenging, as CKD-MBD pathophysiology is complex and not completely elucidated. However, recent studies have shown that BMD by DXA can predict those with CKD who will be at high fracture risk. Future, prospective studies are needed to confirm these preliminary observations, and also to compare the predictive value of different techniques, such as DXA, HR-pQCT, and bone biopsy. In addition, studies determining which agents can increase BMD and decrease MBD in CKD are needed. This research will help us to identify and treat those with CKD at high fracture risk. Ultimately, these studies will enable us to decrease the sickness and death associated with fractures in CKD.

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Conflicts of interest

S.A.J. has received honoraria for lectures and is on Board of Amgen and Sanofi. R.M.A.M. has received honoraria for lectures and is on the speaker's bureau for Amgen. For the remaining author no conflicts were declared.

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ANEXO C: The Skeleton as a Determinant of Intradialytic Calcium Balance: Effects of Parathyroidectomy on Calcium Mass Transfer.

**The Skeleton as a Determinant of Intradialytic Calcium Balance:
Effects of Parathyroidectomy on Calcium Mass Transfer**

Patricia Taschner Goldenstein¹, Fabiana Giorgeti Graciolli¹, Gisele Lins Antunes¹, Wagner Vasques Dominguez¹, Luciene Machado dos Reis¹, Sharon Moe², Rosilene Motta Elias¹, Vanda Jorgetti¹, Rosa Maria Affonso Moysés^{1,3*}

***Corresponding author: Rosa Maria Affonso Moysés**

1. Nephrology Division, Universidade de São Paulo, São Paulo, Brazil
2. Indiana University School of Medicine and Roudebush Veterans Administration Medical Center, Indianapolis, USA
3. Medicine Master Degree Program, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

Corresponding Author:

Rosa M. A. Moysés

Universidade Nove de Julho (UNINOVE), São Paulo, Brazil

Rua Iperoig, 690 ap 121

ZIP CODE 05016-000

São Paulo, SP, Brazil

Running Title: Bone Remodeling and Calcium Mass Transfer

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Abstract

Although it is recognized that the dialysate calcium concentration ($d[Ca]$) is an important factor that may impact calcium (Ca) mass transfer during hemodialysis, little is known about the variables that might determine it. We have prospectively investigated the influence of bone remodelling on Ca transfer in 10 dialysis patients in three consecutive situations: severe hyperparathyroidism before parathyroidectomy (Pre PTx), during “hungry bone syndrome” post PTx (Hungry Bone), and after stabilization of clinical status (Late PTx). During each phase participants were submitted to 3 hemodialysis sessions using different $d[Ca]$: 1.25, 1.5 and 1.75 mmol/L in random order. A bone biopsy for histomorphometric analysis and bone protein quantification was performed in all patients at baseline. Ca mass transfer varied widely among patients in each study phase even using the same $d[Ca]$, with negative median values in Pre PTx and Hungry Bone (-161 and -218mg, respectively) and slightly positive in Late PTx (39mg; $p < 0.05$ versus Pre PTx and Hungry Bone). Multiple regression analysis showed that Ca gradient between initial serum iCa and $d[Ca]$, difference between final and initial serum iCa and serum undercarboxylated osteocalcin were independent predictors of Ca mass transfer ($R^2 = 0.48$; $p < 0.05$). As bone remodeling also influences the serum levels of iCa and its variance during dialysis, in this study we have added new data by demonstrating that the skeleton plays a key role on Ca balance and these variables must be kept in mind at the time while deciding which $d[Ca]$ should be chosen for a specific patient.

Introduction

Disturbances in mineral and bone metabolism in chronic kidney disease patients (CKD-MBD) are highly prevalent and are a major cause of morbidity and mortality. CKD with secondary hyperparathyroidism (SHPT) results in increased risk of fractures and cardiovascular events.^{1,2} Treatment strategies to control SHPT include phosphate binders, calcitriol and vitamin D analogues, calcimimetics and when refractory, parathyroidectomy (PTX).² However, little attention has been paid to the choice of the dialysate Ca concentration ($d[Ca]$) and its role to both manage SHPT and treat the hungry bone syndrome after PTX.

Currently, there is still a lot of controversy in the literature about the optimal $d[Ca]$, and recommendations of a $d[Ca]$ of 1.25, 1.5 or 1.75 mmol/L are mostly opinion-based. Most nephrologists and current guidelines believe that an ideal $d[Ca]$ should provide a near-neutral calcium (Ca) balance during dialysis.^{2,3} Yet, there are only limited studies that quantify of calcium that moves between patient and dialysate (calcium mass transfer).⁴⁻⁹ This is mainly due to technical difficulties in obtaining an accurate measurement of Ca in the spent dialysate.

Multiple factors are involved in the calcium mass transfer during a dialysis treatment. First, the gradient between $d[Ca]$ and serum Ca plays a major role in Ca mass transfer. The skeleton also impacts intra-dialytic Ca balance: there is an exchangeable Ca pool on the bone surface composed of non-collagenous proteins with high Ca affinity that could act as a reservoir for rapid exchanges of Ca with the extracellular Ca.¹⁰ Our group has previously shown that Ca balance is dependent not only on Ca gradient, but also on bone turnover, as differences in mass transfer were observed in patients with high compared to low bone remodeling.⁴ A limitation of this study was the indirect assessment of bone remodeling as circulating biomarkers, rather than bone biopsy were used. Finally, patient specific factors such as age, gender and mobility likely play a role.

To overcome these limitations, we designed a cross over study in dialysis patients with known different states of bone turnover: Pre and post

PTX, where patients go from high to low turnover. These patients were submitted to bone biopsy and to consecutive dialysis sessions with different $d[Ca]$ before PTX, during hungry bone syndrome immediately post PTX, and after stabilization of bone disease (Late PTX).

Results

For study purposes, only the 10 patients who completed the 3 phases of the protocol were analyzed as described in Figure 1. They were relatively young, 60% were men and most of them had been on dialysis for more than 4 years, and had clinical and laboratory manifestations of SHPT. Even though no patient had a history of bone fractures, more than half complained of bone pain (Table 1).

Bone Biopsy Results

a. Histomorphometric analysis

Table 2 shows the histomorphometric parameters of bone biopsies done pre-PTX. We observed that most patients had reduced trabecular thickness, increased osteoid surface, accompanied by abnormal mineralization and increased osteoblast and osteoclast surface. In general, the biopsies showed high turnover, normal volume and abnormal mineralization.

b. Bone and serum protein analysis

As described in Table 3, the most abundant protein quantified in bone was osteocalcin (OC), representing almost 2% of bone samples protein content. The carboxylated form of OC (GLA) predominated over the undercarboxylated form (GLU). The second most abundant protein in bone was sclerostin. Quantification of serum proteins also showed OC as the most prevalent protein, even though in this compartment, the GLU was more expressive, composing almost 50% of circulating OC pool. Bone proteins that correlated with their corresponding serum levels were sclerostin and fibroblast growth factor 23 (FGF-23), as shown in Figure 2. Serum OC, as well as its fractions GLU and GLA, decreased 1 year after

surgery, in Late PTx phase, as seen in Table 3. Conversely, serum sclerostin increased immediately after PTx, probably due to the significant decrease of its inhibitor PTH, and persisted elevated in Late PTx phase. No significant changes were observed in the serum concentration of any other protein.

Ionized calcium and PTH curves during dialysis

There were no adverse events during any of the dialysis sessions. The variation in ionized calcium (iCa) and PTH from pre to post dialysis are shown in Table 4. A significant decrease in PTH was seen in the Pre PTX phase, whereas a significant increase in iCa was seen in all dialysis with 1.75 mmol/L. At a d[Ca] of 1.5 mmol/L, a slightly positive Δ iCa was seen in the Pre PTx and Hungry Bone phases reaching significance at Late PTx phase. In the same direction, Δ PTH was negative in all phases. At a d[Ca] of 1.25 mmol/L, a significantly negative Δ iCa, accompanied by a significantly positive Δ PTH, were observed only at the Pre PTX phase. Figure 3 shows serum ionized Ca (iCa) and PTH curves during hemodialysis. Comparisons among different d[Ca]s disclosed that Δ iCa and Δ PTH were significantly different in a 1.25 than in a 1.75 mmol/l d[Ca] in all phases. Based on these findings, we would expect a negative Ca balance in the 1.25 d[Ca] dialysis in the Pre PTx phase, neutral in the 1.25 d[Ca] dialysis in the Hungry Bone phase and positive in all other situations tested in our protocol.

Ca mass transfer and gradients during dialysis

However, there was a wide variation on Ca mass transfer among patients. The amplitude of variation occurred even at similar d[Ca] and changed through the study phases, confirming the hypothesis that is very difficult to predict the Ca mass transfer based exclusively on the d[Ca] (Figure 4a).

As shown in Table 4 and Figure 4b, the three d[Ca] used determined statistically different Ca gradients in each of the 3 phases. Interestingly, the same d[Ca] resulted in a less negative Ca gradient in Pre PTx than in Late PTx, which was attributed to differences in pre dialysis serum iCa between these phases.

During all phases of the study, we observed that the more negative the gradient between serum iCa and d[Ca], the more positive was the Ca mass transfer. Consequently, when d[Ca] rose, the gradient became more negative and the mass transfer tended to be more positive. However, as shown in Figure 5, even at similar Ca gradient, patients presented different Ca mass transfer. Furthermore, Ca transfer tended to obey the phase of the study: negative for Pre PTx and Hungry Bone (median values of -161 and -218 mg, respectively), and slightly positive for Late PTx (median value of 39 mg; $p < 0.05$ vs. previous phases).

Analysis of each of the three phases

In Pre PTx, d[Ca] of 1.25 mmol/L and 1.5 mmol/L resulted in more negative calcium mass transfer than d[Ca] of 1.75 mmol/L, and only a d[Ca] of 1.75 mmol/L really turned Ca transfer neutral or even positive. (Figure 4 and Table 4). Ca mass transfer in this phase was associated with initial PTH ($r = -0.4$; $p = 0.028$), Ca gradient ($r = -0.64$; $p < 0.0001$); Ultrafiltration volume ($r = -0.38$; $p = 0.04$), bone GLA ($r = 0.35$; $p = 0.056$), serum GLU ($r = -0.39$; $p = 0.036$), and ΔiCa ($r = 0.46$; $p = 0.01$). In multivariate analysis, ΔiCa and serum GLU remained as independent predictors of Ca mass transfer, explaining 41% of Ca transfer (Table 5).

In Hungry Bone phase, a significantly difference in Ca mass transfer was found only between d[Ca] of 1.25 and 1.75 mmol/L. Unexpectedly, we found a negative Ca mass transfer during most hemodialysis procedures, even with a d[Ca] of 1.75 mmol/L, being even more negative with a d[Ca] 1.25 mmol/L (Table 4). Ca mass transfer in this phase correlated with PTH pre hemodialysis procedure ($r = 0.49$; $p = 0.024$), P ($r = 0.47$; $p = 0.01$),

ΔiCa ($r = 0.62$; $p < 0.0001$) and Ca gradient ($r = -0.74$; $p < 0.0001$). A significant correlation was also found between Ca mass transfer and some histomorphometric parameters of bone remodeling, such as osteoid ($r = -0.49$; $p = 0.006$), osteoblastic ($r = -0.47$; $p = 0.009$) and osteoclastic surfaces ($r = -0.42$; $p = 0.02$). As shown in Table 5, in multivariate analysis only Ca gradient remained significant, however contributing to 64% of Ca mass transfer.

Dialysis in late PTx phase resulted in practically neutral or positive Ca mass transfer in all three different $d[Ca]$ s. It increased as $d[Ca]$ raised, becoming progressively more positive as the gradient became more negative, but no statistical significance was achieved among the different $d[Ca]$ in terms of Ca mass transfer (Table 4). PTH ($r = -0.4$; $p = 0.027$), Ca gradient ($r = -0.45$; $p = 0.012$), bone eroded surface at baseline bone biopsy ($r = -0.47$; $p = 0.005$), alkaline phosphatase ($r = -0.44$; $p = 0.016$), serum DKK1 ($r = -0.574$; $p = 0.001$), serum FGF-23 ($r = -0.71$; $p = 0.0001$), P ($r = -0.38$; $p = 0.042$), serum GLA ($r = 0.53$; $p = 0.002$) and ΔiCa ($r = 0.48$; $p = 0.007$) correlated with Ca transfer. In multivariate analysis, serum GLA was the only variable that remained a significant predictor of Ca mass transfer, contributing alone to 21% (Table 5).

Combined analysis of all phases

At this time, we gathered all 90 dialysis sessions and tested which factors were related to Ca mass transfer. Univariate analysis showed that initial iCa ($r = -0.52$; $p < 0.0001$), alkaline phosphatase ($r = -0.4$; $p < 0.0001$), bone alkaline phosphatase ($r = -0.33$; $p = 0.001$), serum GLU ($r = -0.39$; $p < 0.0001$), OC ($r = -0.29$; $p = 0.006$), Ca gradient ($r = -0.69$; $p < 0.0001$), and ΔiCa ($r = 0.61$; $p < 0.0001$) correlated with Ca transfer. Multivariate analysis showed that Ca gradient, GLU and ΔiCa remained significant predicting 48% of Ca mass transfer (Table 5). A logistic regression including Ca gradient, ultrafiltration volume, study phase and serum GLU showed that a higher Ca gradient and a serum GLU above the median were associated

with a lower probability of a positive Ca mass transfer. Repeated measures general linear model found a significant difference among $d[\text{Ca}]$, $p=0.013$, among phases ($p=0.040$) and also an interaction ($p=0.028$), as showing in Figure 6.

Discussion

Decisions regarding the best choice of $d[\text{Ca}]$ for CKD patients are still controversial. Ideally, it should be chosen according to the expected Ca balance and individual needs at that time. However, predictors of Ca mass transfer are still poorly understood. Besides, individual factors and bone turnover were almost never taken into account. Our findings show that Ca mass transfer varied widely in our patients and it was dependent on Ca gradient, $\Delta i\text{Ca}$ and serum GLU, such that less negative calcium gradient, lower $\Delta i\text{Ca}$ and higher GLU resulted in a lower probability of a positive Ca mass transfer. Together they were able to predict almost 50% of Ca transfer.

The fact that the same $d[\text{Ca}]$ resulted in different Ca balances between Pre PTx and Late PTx phases could be attributed to different initial serum $i\text{Ca}$ through study phases. This finding empowered the Ca gradient as an important factor affecting Ca transfer, which was quite different from Pre to Late PTx phases. In fact, Sigrist et al.⁶ using a fixed $d[\text{Ca}]$ of 1.25 mmol/L, partially explained a marked inter-patient variability in Ca balance by different Ca gradients during dialysis. Indeed, Ca gradient in Hungry Bone phase contributed, by itself, to 58% of Ca balance. As Ca gradient is determined by the difference between $d[\text{Ca}]$ and initial $i\text{Ca}$, the significant variation in the basal $i\text{Ca}$ in the same patient among the 3 phases of our study could be explained by different doses of Ca salts or calcitriol, but also by PTx, which dramatically changed serum PTH and bone remodeling. When patients were switched to a low turnover status, or hypoparathyroidism, most of them could not maintain a serum $i\text{Ca}$ similar to that presented before surgery. In summary, Ca gradient is major component of Ca balance and $d[\text{Ca}]$ exerts a crucial role on this parameter, but also the initial serum $i\text{Ca}$, which is a function of PTH actions on.¹¹

The $\Delta i\text{Ca}$ was also an important factor that determined the Ca balance, such that an initial $i\text{Ca}$ will present different Ca balances depending on how much their serum $i\text{Ca}$ varies. The intriguing question is

which is the compartment “buffering” the extravascular during the dialysis procedure to maintain a constant Ca gradient and, consequently, a continuous Ca mass transfer. Our hypothesis is that the skeleton is the most important part of such compartment. As we demonstrated, and confirming the classical findings of Kurz et al.,¹² the higher the bone remodeling, the higher the refilling and the negative balance in low d[Ca]s, as seen in the Pre PTx phase. Therefore, bone may also exert its influence on Ca balance through ΔiCa .

Indeed, our group⁴ has previously shown that bone remodeling, inferred through PTH and OC, could predict Ca balance. In the present study we found serum GLU fraction of OC to be an independent predictor of Ca transfer although the exact mechanism still deserves investigation. Ca is continuously removed and incorporated into bone in a regulated process that maintain bone integrity and serum Ca levels.¹³ According to Talmage,¹⁰ bone proteins, such as OC, would function as an exchangeable pool of Ca that would provide equilibrium between Ca in bone, extra cellular fluid and plasma. OC is the main non-collagenous hydroxyapatite binding protein in bone. GLA is the gamma carboxylated fraction of OC and its importance resides on its high affinity binding to hydroxyapatite and, therefore, GLA is directly involved in bone remodeling, especially bone formation. GLU residue is the undercarboxylated fraction of osteocalcin. GLU is a product of bone resorption and it has low affinity to hydroxyapatite, acting as a hormone involved in energetic metabolism,¹⁴ and related to higher risk of fractures in elderly women.¹⁵ Bone turnover seems to be the major determinant of OC in bone.¹⁶

Talmage suggested that PTH, besides stimulation of bone osteoclast resorption, could act by either changing the conformation, the amount of OC, or by removing interfering substances.¹⁰ Any of these three mechanisms would alter the level of iCa . In the present study, we found that bone OC and its fractions GLA and GLU, extracted through biopsy in a situation of severe SHPT, were far higher than any other measured protein in bone. Furthermore, bone GLA was associated with Ca balance in Pre PTx phase. Even though neither bone OC nor its fractions correlated with

their serum levels, they were still the most prevalent proteins quantified in serum in Pre PTx phase. Additionally, serum OC and its fractions were significantly higher in Pre PTx and Hungry Bone than in Late PTx phase, when bone turnover is expectedly lower. This difference could justify, at least in part, the more negative calcium balance we found in Pre PTx and Hungry Bone phase compared to Late PTx phase.

The second most prevalent protein found in our bone biopsies was sclerostin. This protein, as well as DKK1, is an inhibitor of Wnt signaling pathway, which plays an important role in bone formation and regeneration.^{14,17} The expression of sclerostin is relatively restricted to osteocytes,¹⁸ although recently Zhu et al.¹⁹ showed its expression in calcified vascular smooth muscle cells and aortas, highlighting the complexity of this signaling pathway. We observed that serum sclerostin levels increased from Pre PTx to Post PTx and to Late PTx, probably as a consequence of PTx, leading to a subtle reduction in PTH, which is sclerostin main inhibitor.²⁰ DKK1 is expressed within osteoblasts and osteocytes²¹ but expression outside of bone has been shown in many other places.²² Leptin²³ and osteoprotegerin²⁴ are produced mainly outside the bone structure and their serum levels, as well as serum FGF23, did not change significantly over the study phases. Their roles in Ca transfer during dialysis are not clarified.

Using a specific protocol, we could measure the concentration of some bone proteins.²⁵ This is an important step to better understand the process involved in regulating bone resorption and formation through activities of osteoclasts and osteoblasts, which is the subject of ongoing research.^{26, 27} In this way, we could demonstrate, for the first time, using this new technique, that serum levels of sclerostin and FGF-23 correlate with their respective bone content in CKD patients. Looking from a practical perspective, we might assume that serum levels changes of these proteins are reflecting changes in their local expression.

Our study has some limitations. The number of patients that actually concluded the whole protocol was lower than expected. However, we could

partially overcome this problem as patients were their own controls. As there is no previous data on bone proteins measurements in the literature, it was difficult to ascertain normal range values. Neither we had performed another biopsy at the end of the study to confirm some of our findings. Also, as we enrolled only patients with SHPT, our results may not apply to patients with minor disease. Our study has also several strengths as provided new insights in the Ca mass transfer process during hemodialysis, describing not only the role of Ca gradient but also showing the variation inter- and intra-patient according to bone turnover status. In addition, our findings are based on bone biopsy and bone concentration proteins.

In summary, our study demonstrated that the skeleton plays a key role on Ca balance. In clinical practice, this must be kept in mind at the time of deciding which $d[Ca]$ should be chosen for a specific patient. Last, but not least, the final question must be what we want in terms of Ca balance for a patient, negative, neutral or positive. Keeping these basic questions in mind would help us to decrease our chances of making a bad choice and worsen their already risky clinical situation.

Concise Methods

Study design

This was a prospective cohort study, where patients were assigned to a bone biopsy and were then evaluated during 3 consecutive phases of the study:

1. Pre PTx: SHPT while waiting for PTX (within 90 days pre PTx)
2. Hungry Bone: right after PTX (within 14 days), during the “hungry bone syndrome”, defined as a necessity of calcium carbonate supplementation p.o. and/or more than 2 capsules of calcitriol p.o. per day.
3. Late PTX: after stabilizing bone remodeling, defined as no need for calcium supplements or normalization of serum alkaline phosphatase

During each of the phases participants were submitted to 3 randomly assigned, consecutive bicarbonate-based hemodialysis sessions, with different $d[Ca]$: 1.25 mmol/L, 1.5 mmol/L and 1.75 mmol/L. Each session lasted 4 hours, with blood and dialysate flow of 350 and 800 ml/min, respectively. Ultrafiltration was adjusted according to each patient dry weight.

Participants

Patients were included between July 2011 and July 2013. All subjects gave informed written consent to participate in the study, which was approved by our Institutional Review Board in accordance with the Declaration of Helsinki. Inclusion criteria were: age 18 or older, CKD patients on dialysis for more than 3 months who attended the CKD-MBD clinic at Hospital das Clínicas of University of São Paulo and the presence of severe SHPT with clinical indication for a PTX.

Bone Biopsy

All patients underwent an iliac crest bone biopsy using a 7-mm Bordier trephine after a course of double-labeling tetracycline (20 mg/kg/day) for 3 days, separated by an interval of 10 days. The biopsy was performed 2–5 days after the last dose of antibiotic.²⁸

The bone was stored in DMEM - Dulbecco's modified eagle medium (Gibco BRL, Rockville, EUA) and subsequently sliced in a sterile environment.

Undecalcified bone was submitted to standard processing and sectioning for histological studies²⁹ and bone histomorphometry using a semiautomatic technique in the Osteomeasure software (Osteometrics, Atlanta, GA, USA). The static and dynamic parameters were examined according to the standards established by the American Society of Bone and Mineral Research.³⁰ The reference ranges (RR) used for static parameters were obtained from our normal laboratory controls,³¹ whereas the ranges for the dynamic parameters were the same as those described elsewhere.³²

Another fragment was immediately frozen at -80°C for protein quantification. Total protein was extracted with Trizol reagent (Invitrogen, USA) as we have previously published.²⁵ Bone proteins were then quantified through Multiplex Milliplex map kit – Human Bone Magnetic Bead Panel - HBNMAG-51K (EMD Millipore Corporation, MA, USA®) assay that quantified Dkk1, Leptin, FGF-23, sclerostin, osteoprotegerin and total OC. Bone GLA and GLU were measured through ELISA method from Takara (Japan).

Biochemical Parameters:

Blood samples were collected every 30 min during each dialysis session for biochemical analysis, which included: total Ca (tCa), iCa, P, urea, and PTH. Blood samples for the measurement of FGF 23, sclerostin, leptin, FGF-23, DKK1, osteoprotegerin and OC were collected pre-dialysis of the first dialysis session of each of the three phases of the study. The samples were centrifuged, aliquoted in cryovials, and stored at -80° C. Serum levels of iCa (RR: 4.6–5.3 mg/dl), tCa (RR: 8.4–10.2 mg/dl), P (RR: 2.7–4.5 mg/dl), total alkaline phosphatase (RR: 40–129 U/l), urea (RR: 10–50 mg/dl) and albumin (RR: 3.4–4.8 g/dl) were determined using routine laboratory techniques. Serum iPTH (RR: 10–65 pg/ml) was measured using a chemiluminescence assay (DPC; Medlab, San Antonio, TX, USA) and serum 25(OH) vitamin D (RR: 30–100 ng/dl) was measured using a radioimmunoassay (DiaSorin, Stillwater, MN, USA). The same Multiplex Milliplex map kit (EMD Millipore Corporation, MA, USA®) was used to measure the serum proteins. Serum GLA and GLU fractions of OC and bone alkaline phosphatase, were measured through ELISA method from Takara (Japan) and Quidel (Ca, USA) manufacturers, respectively.

Dialysate samples were collected from fresh dialysate and from a homogenous sample of spent dialysate from the preceding 30 min of each dialysis session. The samples were then combined to determine mass transfer. This procedure has previously been validated in literature.^{33, 34}

Ca Mass Transfer and Ca Gradient

Ca dialysate mass transfer (net amount of calcium put into or taken out of dialysate) was calculated using the formula:

Ca Mass Transfer = [final dialysate volume (L)* final dialysate calcium (mg/dl)] – [dialysate volume (L)]* [pre capillary fresh dialysate calcium (mg/dl)].

Final dialysate volume = dialysate volume (L) + ultrafiltrate volume (L).
 Dialysate volume = 4hs x 800ml/min = 192 L.

Ca Gradient was calculated through the following formula:

Ca Gradient = ionized serum calcium pre dialysis (mg/dl) – initial pre capillary fresh dialysate calcium (mg/dl) (Difference between blood and dialysate calcium concentrations).

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation or median and percentiles (25; 75), according to the D'Agostino & Pearson omnibus normality test. Categorical variables were expressed as N and percentage. Analysis of variance for repeated measures with Tukey 's post-test or alternative nonparametric Friedman test with Dunns post-test were used to compare variables in the three different dialysate concentrations (Ca 1.25, 1.5 and 1.75, mmol/L) in each phase of the study. ANOVA or the alternative Kruskal-Wallis were used to compare variables among the three phases. Relationship between independent variables and calcium balance was performed by Spearman correlation. In each phase, multiple stepwise regression analysis ($p < 0.05$ to enter the model and > 0.1 to exit the model) was undertaken with calcium balance as the dependent variable and independent variables selected from the univariate analysis, always including calcium gradient and ultrafiltration volume. A final model, which included the three phases with all different dialysate combined were also examined with Ca balance as dependent variable, in a linear stepwise model and also in a logistic analysis. General linear model (GLM) repeated measures were run to determine mean calcium mass transfer differences among the three D[Ca] over time, and to examine differences between phases. Bonferroni correction was used for multiple comparisons. Where repeated measures analyses showed significant associations, post hoc univariate GLM analysis was used to localize effects. Statistical analysis was performed with GraphPad Prism 5.0 (Ca, USA) and SPSS 21.0 (SPSS Inc. one Chicago, IL). Significance was assigned at p values < 0.05 .

Table 1 – Patient baseline characteristics

Characteristics	Values	Reference Values
Male sex (n;%)	6 (60%)	-
Age (years)	48.1 ± 11.0	-
Dialysis vintage (Mo)	57.6 ± 16.8	-
Hypertension (n;%)	8 (80%)	-
Diabetes (n)	0	-
Bone pain (n;%)	6 (60%)	-
Fracture (n;%)	0	-
PTH	1896 ± 648	12-65 pg/ml
Total Calcium	9.5 ± 0.3	8.6-10.2 mg/dl
Ionized calcium	4.9 ± 0.2	4.6-5.3 mg/dl
Phosphorus	6.2 ± 1.1	2.7-4.5 mg/dl
Alkaline Phosphatase	149.5 (102.0; 316.5)	40-129 U/L
25 vitamin D	30.0 ± 13.1	30-100 ng/ml
Bicarbonate	21.0 ± 2.4	23-27 mmol/l
Albumin	4.16 ± 0.33	3.4-4.8 g/dl
Urea	120.8 ± 33.2	10-50 mg/dl
Calcitriol use (n;%)	0	-
Sevelamer use (n;%)	8 (80%)	-
CaCO₃ use (n;%)	1 (10%)	-

HAS = hypertension, DM = diabetes mellitus, PTH = parathormone, CaCO₃ = calcium carbonate. Results expressed as mean ± standard deviation or median (25;75%), as appropriate.

Table 2 – Baseline Bone Histomorphometry (Pre PTX)

Parameter	Values	Reference Values	
		Women	Men
BV/TV	23.8 ± 9.5	21.8 ± 7.2	24.0 ± 6.1
TbN	2.2 ± 0.9	1.76 ± 0.52	1.89 ± 0.42
Tb.Th	123.1 (97.9; 135.8)	126 ± 28.8	127.9 ± 29.7
OS/BS	48.3 ± 8.4	9.2 ± 8.4	16.1 ± 12.6
Ob.S/BS	26.0 ± 9.15	1.2 ± 3.2	1.2 ± 1.4
Oc.S/BS	2.7 ± 1.35	0.03 ± 0.06	0.03 ± 0.11
ES/BS	16.7 ± 4.9	2.3 ± 2.4	1.75 ± 1.21
BFR	0.15 (0.1; 0.2)	0.07 ± 0.03	0.13 ± 0.07
T (H/L)	10/0	-	-
M (A/N; n)	7/3	-	-
V (L/N/H; n)	1/9/0	-	-

BV/TV- Bone volume (%); TbN- trabecular number (n/mm); Tb.Th- trabecular thickness (µm); OS/BS- osteoid surface (%); Ob.S/BS- osteoblastic surface (%); Oc.S/BS- osteoclastic surface (%); ES/BS- reabsorption surface (%); BFR- bone formation rate (µ³m/µ²m/dia); Mlt- mineralization lag time (days); T- turnover (H/L)- high/low (%); M- mineralization (A/N)- abnormal/normal (%); V- volume (L/N/H)- low/normal/high (%). Results expressed as mean ± SD or median (25; 75%), as appropriate.

Table 3 – Bone and serum proteins according to the phase

Proteins	Bone	Serum		
		Pre PTx	Hungry bone	Late PTx
OC	19,294 (10,932; 30,071)	161.6 ± 48.7	188.6 ± 45.1**	116.1 ± 40.5
GLA	2278 (641.1; 2856)	245.5 (110.2; 321.9) **	151.5 (82.1; 404.0) **	11.84 (0.7; 104.3)
GLU	112.3 (77.6; 379)	221.7 (103.6; 609.9) **	159.5 (89.8; 435.8) **	10.0 (4.2; 32.1)
SCL	199 (120.1; 353.6)	1.57 ± 0.62*,**	2.12 ± 0.57	2.30 ± 0.45
Leptin	6.31 (0.9; 24.8)	22.6 (17.0; 50.3)	26.3 (13.9; 82.0)	39.8 (20.5; 96.1)
FGF23	2.34 (0.8; 3.9)	9.1 ± 3.7	6.1 ± 2.9	7.2 ± 6.3
DKK1	1.25 (0.7; 2.0)	4.5 ± 1.8	4.4 ± 0.8	4.6 ± 1.0
OPG	0.06 (0.0; 0.2)	3.0 ± 1.0	3.6 ± 1.0	3.8 ± 0.9

Bone protein concentrations are expressed in ng/mg of the total proteic lysate. Serum proteins concentration are expressed in ng/ml. OC- osteocalcin, GLA- carboxylated osteocalcin, GLU- undercarboxylated osteocalcin, SCL- sclerostin, FGF23- fibroblast growth factor 23, DKK1- Dikkopf 1, OPG- osteoprotegerin. Results are expressed as mean ± SD ou median (25;75%), as appropriate.*p<0.05 versus Hungry Bone; ** p<0.05 versus Late PTx.

Table 4 – Calcium gradients, balances and variations

d[Ca] (mmol/L)	Δ iCa (mg/dL)	Δ PTH (pg/mL)	Ca Gradient (mg/dL)	Ca Balance (mg)
Pre PTx phase				
1.25	-0.21 (-0.47; -0.08) [#]	126 (-128; 606) ****, [#]	-0.07 ± .24 ^{*,#,##}	-358 (-561; -247) ^{*,#}
1.5	0.32 (0.02; 0.50)	-632 (-879; -379) ****	-1.07 ± 0.28 ^{*,#}	-422 (-585; -69) ^{*,#}
1.75	0.80 (0.22; 0.94) ^{***}	-829 (-1120; -547)	-1.92 ± 0.19 ^{***}	51 (11; 266)
Hungry Bone phase				
1.25	-0.08 (-0.75; 0.23) ^{*,#}	5 (-4; 24) [#]	-0.23 ± 0.73 ^{*,##}	-308 (-1044; -218) ^{*,#}
1.5	0.31 (-0.14; 0.77)	-6 (-25; 7)	-1.17 ± 0.77 [#]	-107 (-576; 182)
1.75	0.47 (-0.08; 1.17) ^{***}	-19 (-88; 0)	-2 ± 1.31	-58 (-296; 448)
Late PTx phase				
1.25	0.33 (-0.11; 0.71) [#]	4 (-18; 48) [#]	-0.81 ± 0.60 ^{*,##}	-12 (-170; 297)
1.5	0.71 (0.35; 1.07)	-34 (-99; -2)	-1.74 ± 0.67 [#]	120 (-133; 353)
1.75	1.27 (1.07; 1.58)	-94 (-125; 31)	-2.97 ± 0.65	153 (-143; 412)

Results expressed as mean ± SD or median (25;75%), as appropriate. Δ iCa, and Δ PTH= difference between final and initial values of iCa and PTH during dialysis, respectively. *p<0.05 versus d [Ca] of 1.25 mmol/L Late PTx phase; **p<0.05 versus d[Ca] of 1.5 mmol/L Late PTx phase; ***p<0.05 versus d[Ca] of 1.75 mmol/L Late PTx phase; ****p<0.05 versus d[Ca] of 1.5 mmol/L Hungry Bone phase; [#]p<0.05 versus d[Ca] of 1.75 mmol/L of the same phase; ^{##}p<0.05 versus d[Ca] of 1.5 mmol/L of the same phase.

Table 5- Multivariate analysis: predictors of Ca balance

Model	B coefficient	95% CI	p value	Adjusted R²
Linear				
Pre PTx phase				0.41
Serum GLU	-0.560	-1.1; -0.023	0.041	
ΔiCa	318.33	141.34; 495.32	0.001	
Hungry Bone phase				0.64
Ca gradient	-349.64	-460.73; -238.55	<0.001	
Late PTx				0.21
Serum GLA	1.94	0.59; 3.29	0.007	
All Phases				0.48
Ca gradient	-150.91	-252.14; -49.69	0.004	
Serum GLU	-0.31	-0.57; -0.07	0.015	
ΔiCa	188.61	37.5; 339.7	0.015	
Logistic (-2 Log likelihood 73.53) for all Phases				Exp (B)
Ca gradient	0.148	0.064; 0.34	0.004	0.148
Serum GLU	0.310	0.098; 0.986	0.047	3.222
PTx, parathyroidectomy; GLU, undercarboxylated osteocalcin; ΔiCa = difference between final and initial values of iCa				

Figure Legends

Figure 1. Patients flow chart.

Figure 2. Correlation of bone content and serum levels of Sclerostin and FGF-23
Scl, Sclerostin; FGF-23, fibroblast growth factor 23.

Figure 3. Calcium and PTH curves throughout the phases of study.

Red line represents d[Ca] 1.25, blue line represents d[Ca] 2.5 and black line represents d[Ca] 1.75 mmol/l.

Differences are marked when significant as follow: * for $p < 0.05$ vs. time 0 (intra-group comparisons), # for $p < 0.05$ vs. d[Ca] 1.75 mmol/l and • for $p < 0.05$ vs. d[Ca] 1.5 mmol/l (inter-group comparisons).

Figure 4. Calcium gradients and balances in the three phases of the study.

* represents $p < 0.05$ vs. d[Ca] 1.75 mmol/l in the same phase.

Figure 5. Correlations between calcium gradient and calcium balance in the three phases of the study (black line in Pre PTX, blue line in Hungry Bone and red line in Late PTX phase).

Figure 6. Plot generated from repeated measures GLM, showing the entire group in all phases, corresponding to 90 dialysis sessions. There was a significant difference among dialysate calcium content ($p = 0.013$), among phases (0.040), and also an interaction between these factors (D[Ca] * phases), with a p value 0.028.

* Dialysate calcium content 1.75 was different from 1.25 and 1.5, by Bonferroni post-test.

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Figure 1- Patients flow chart

Figure 2 – Correlation of bone content and serum levels of Sclerostin and FGF-23

Figure 3. Calcium and PTH curves throughout the phases of the study

* $p < 0.05$ vs. Time 0 (intra-group comparison); # $p < 0.05$ vs. 1.75 mmol/L; • $p < 0.05$ vs. 1.5 mmol/L (inter-group comparisons)

Figure 4. Ca gradients and mass transfer in the 3 phases of the study

* $p < 0.05$ vs. a 1.75 mmol/L d[Ca] at the same phase

Figure 5. Correlations Between Ca gradient and Ca mass transfer in the 3 phases of the study

Ca gradient vs. mass transfer in the 3 phases of the study; all dialysates

Ca gradient vs. mass transfer in the 3 phases; Cad 1.25mmol/L

Ca gradient vs. mass transfer in the 3 phases; Cad 1.5mmol/L

Ca gradient vs. mass transfer in the 3 phases; Cad 1.75 mmol/L

Figure 6- Plot generated from repeated measures general linear model

5. CONCLUSÕES GERAIS

Os resultados desse estudo mostraram que nossos pacientes apresentavam doença óssea extremamente grave caracterizada por alto turnover e reduzida mineralização óssea confirmada por biópsia.

Pela primeira vez na literatura pudemos quantificar as principais proteínas ósseas, e encontramos uma correlação entre as dosagens ósseas e séricas de FGF-23 e esclerostina nesses pacientes. Confirmamos o predomínio da osteocalcina e pudemos também quantificar suas frações carboxilada e não carboxilada no osso e no plasma, o que consideramos um passo a mais no melhor entendimento da formação e reabsorção óssea e da fisiopatologia envolvida nos distúrbios do metabolismo ósseo.

Os resultados desse estudo mostraram curvas significativamente diferentes de Cai e PTH entre o Cad de 2,5 e o de 3,5 mEq/L. Apesar disso, e contrário às expectativas, o balanço de cálcio em muitas vezes não seguiu as tendências do Ca sérico e do PTH, apresentando uma grande variação entre os pacientes e as fases do estudo, mostrando a dificuldade em se prever o balanço baseado exclusivamente no Cad.

Durante a fase Pré PTx, o balanço de Ca foi dependente da variação do Cai (ΔCai) e GLU sérica, explicando 41% da transferência de Ca. Durante a Fase de fome óssea, o balanço foi dependente do gradiente de Ca, explicando 64% do balanço de Ca. Nas diálises na fase PTx tardio, o balanço foi dependente da GLA

sérica, explicando 21% da transferência de Ca. A junção das 90 diálises realizadas no estudo mostrou, por análise multivariada, que o gradiente de cálcio, a GLU sérica e o ΔCai foram preditores independentes do balanço de cálcio e juntos explicaram 48% da transferência de cálcio.

Em resumo, o presente estudo mostrou que o esqueleto teve papel fundamental no balanço de cálcio uma vez que as frações da osteocalcina foram fatores determinantes no balanço de cálcio, ao mesmo tempo em que a remodelação óssea influenciou diretamente os níveis de cálcio sérico em cada uma das fases de nosso estudo e portanto também influenciou o gradiente de Ca.

Na prática clínica devemos considerar, portanto, a remodelação óssea para escolhermos o melhor Cad para cada paciente especificamente. Além disso, devemos nos questionar o que queremos em termos de balanço de cálcio para dado paciente: negativo, neutro ou positivo. Desta forma, poderíamos reduzir as chances de escolhermos um Cad que poderia piorar ainda mais a situação clínica de nossos pacientes.

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5. APÊNDICE

5.1 MÉTODOS

5.1.1 Amostra

Como não há na literatura qualquer trabalho prévio semelhante, e considerando que os pacientes envolvidos seriam seus próprios controles, consideramos que seria necessário ao menos 10 pacientes para o estudo. Estudo prévio do nosso grupo com resultados positivos utilizou 23 pacientes sendo 10 considerados portadores de hiperparatireoidismo e 13 de doença adinâmica (50).

Foram, portanto, selecionados pacientes maiores de 18 anos, em hemodiálise há mais de 3 meses com doença renal crônica em programa de diálise e que faziam acompanhamento no grupo de doença óssea da disciplina de Nefrologia da Faculdade de Medicina da USP, com diagnóstico de hiperparatireoidismo refratário em programação cirúrgica e que concordaram em participar do estudo. Os pacientes foram incluídos entre julho de 2011 e julho de 2013.

5.1.2 Comissão de ética

O projeto foi desenvolvido em acordo com as normas éticas da Faculdade de Medicina da Universidade de São Paulo e previamente autorizado pela Comissão de Ética para Análise de Projetos de Pesquisa (CAPPesq) sob o nº de protocolo 0002/11 em 06/04/2011. Todos assinaram o termo de consentimento livre e esclarecido para participação no estudo, previamente aprovado pela mesma Comissão.

5.1.3 Critérios de exclusão

Pacientes que não preenchiam os critérios exigidos acima ou que apresentavam doença inflamatória aguda, instabilidade hemodinâmica, doença coronariana instável, qualquer outra doença que não a insuficiência renal que potencialmente levasse à hipercalcemia, PTX prévia, uso de corticosteróides ou anti convulsivantes e pacientes em diálise diária.

5.1.4. Desenho do estudo

Este foi um estudo de coorte prospectivo onde pacientes com DRC em hemodiálise foram inicialmente submetidos à biópsia óssea e posteriormente às 3 fases do estudo:

- a. Pré PTx: pacientes com HPT grave aguardando PTx

- b. Fome Óssea: imediatamente após a PTx, durante a fase de “fome óssea,” definida pela necessidade de suplementação de Ca via endovenosa ou via oral e/ou mais de 2 cápsulas de calcitriol por dia.
- c. PTx tardio: após estabilização da remodelação óssea, definida pela ausência de necessidade de suplementação de Ca via oral ou normalização da fosfatase alcalina sérica.

Durante cada uma das fases, os participantes eram submetidos a 3 sessões randômicas e consecutivas de hemodiálise Cad de 2,5; 3,0; 3,5 mEq/. Os demais constituintes do dialisato foram os mesmos em todas as sessões de hemodiálise (sódio 138 mEq/L, potássio 2 mEq/L, bicarbonato 35 mEq/L, Magnésio 1 mEq/L). Cada sessão de 4 horas foi realizada utilizando-se o sistema de diálise Fresenius Medical Care® com dialisador Fresenius 4008H (Fresenius Medical Care, Bad-Homburg, Alemanha) com dialisador FX60 (Helixone®, área de superfície 1,4m², KoA uréia 967 ml/min coeficiente de ultrafiltração de 46 ml/h mmHg, Fresenius medical Care). Os fluxos de sangue e de dialisato foram de 300 e 800 ml/min, respectivamente. A taxa de ultrafiltração foi ajustada de acordo com o peso seco de cada paciente. Foram computados dados clínicos como sexo, idade, tempo em diálise, uso de medicamentos, em especial quelantes de Ca e/ou análogos da vitamina D por dados de prontuário e questionamento aos pacientes. Todos os pacientes foram pesados antes e após cada sessão. Sinais e sintomas intradialíticos foram computados.

5.1.5 Biópsia óssea

Todos os pacientes foram submetidos à biópsia óssea com auxílio de uma trefina elétrica com cateter de Bordier de 7 mm (Gauthier Medical Rochester, EUA), após sedação, na crista ilíaca antero-posterior à direita ou esquerda. Todos os pacientes receberam previamente tetraciclina (20mg/Kg/dia por 3 dias, repetidos após 10 dias) para analisarmos os parâmetros dinâmicos do tecido. A biópsia foi realizada entre 3 a 5 dias após a última dose do antibiótico (32). O fragmento ósseo obtido foi armazenado em meio de cultura Dulbecco's MEM – DMEM (Gibco BRL, Rockville, EUA) e seccionado em ambiente estéril. Parte do material foi fixado em álcool 70% e destinado à histomorfometria óssea e parte foi congelado a -80° C para análises das proteínas ósseas.

Para histomorfometria foram realizados cortes de fragmento ósseo de 5µm de espessura, posteriormente marcados com coloração azul de toluidina. O microscópio utilizado para a leitura foi o Nikon, Labophot-2^a, cursor, placa digitalizadora e o *software Osteomeasure* (Osteometrics, Inc, Atlanta, EUA). Os parâmetros histomorfométricos foram divididos em estáticos, estruturais e dinâmicos e de acordo com a nomenclatura padronizada pela *American Society of Bone and Mineral Research* traduzida para o português, com exceção das abreviações)(57). Os valores de normalidade para os parâmetros estáticos foram obtidos de nossos próprios controles do laboratório(58) enquanto que os valores normais para os parâmetros dinâmicos foram os previamente descritos na literatura (59).

O fragmento ósseo destinado à quantificação das proteínas foi mantido no congelador a -80°C. Posteriormente as proteínas foram extraídas através do reagente Trizol (Invitrogen, USA) de acordo com as instruções do fabricante e protocolo específico (60). As proteínas foram então quantificadas através do kit Multiplex Milliplex map kit – Human Bone Magnetic Bead Panel - HBNMAG-51K (EMD Millipore Corporation, MA, USA®). No lisato foram quantificadas as seguintes proteínas: Dickkopf 1 (DKK1), Leptina, FGF-23, esclerostina, osteoprotegerina e OC (Milliplex), além das frações GLA e GLU da OC (ensaio ELISA).

5.1.6. Parâmetros bioquímicos

Amostras de sangue foram coletadas a cada 30 minutos durante as sessões de hemodiálise, sempre da linha arterial e 2 minutos após a redução do fluxo de sangue para 50mL/min e suspensão do fluxo de dialisato. A análise bioquímica incluiu: Ca total, Cai, fósforo, ureia e PTH. Amostras de sangue para quantificação de FGF-23, esclerostina, leptina, DKK1, osteoprotegerina e OC foram coletadas na primeira diálise de cada uma das 3 fases do estudo. As amostras eram centrifugadas, aliquotadas e armazenadas a -80°C. Níveis séricos de Cai (referência: 4.6–5.3 mg/dl), Cat (referência 8.4-10.2 mg/dl), fósforo ([referência: 2.7–4.5 mg/dl), fosfatase alcalina total ([referência: 40–129 UI/l), uréia ([referência: 10-50mg/dl) e albumina ([referência: 3.4–4.8 g/dl) foram determinados utilizando técnicas laboratoriais habituais. PTH intacto (referência: 10–65 pg/ml) foi

medido por quimioluminescência (DPC; Medlab, Santo Antonio, TX, E.U.A.) e a 35 vitamina D ([referência: 30–100 ng/dl) por radioimunoensaio (DiaSorin, Stillwater, MN, E.U.A.). O mesmo ensaio Multiplex Milliplex map lit (EMD Millipore Corporation, MA, E.U.A.®) foi utilizado para dosagem das proteínas séricas. As frações GLA e GLU séricas da OC e a fosfatase alcalina óssea foram dosadas por ELISA das empresas Takara (Japão) and Quidel (Ca, E.U.A) respectivamente.

Amostras do dialisato foram colhidas do dialisato inicial (fresco) e de uma alíquota do dialisato final a cada sessão de diálise. Adaptamos para isso uma mangueira de drenagem na via de saída do dialisato que iria ser desprezado, e através de uma bomba de infusão operando continuamente a uma velocidade de 1L/h pudemos adquirir amostras homogêneas do dialisato durante todo procedimento e ao final dele, conforme validado previamente na literatura (61, 62).

5.1.7. Balanço de Ca, Gradiente de Ca e outros cálculos

O balanço de Ca foi calculado de acordo com a fórmula (63):

Transferência da massa de Ca= [volume do dialisato final (L)* Ca no dialisato final (mg/dl)] – [volume do dialisato (L)]* [Ca no dialisato pré capilar (mg/dl)].

Sendo, volume dialisato final = volume de dialisato utilizado durante o procedimento (L) + volume de ultrafiltrado (L)

Dialisato pré capilar = dialisato inicial, antes de passar pelo filtro

Volume de dialisato = 4hs x 800 ml/min = 4hs x 48000ml/h= 192 L.

Cálcio dialisato final = média de 3 amostras de cálcio do dialisato ao final da

sessão.

O gradiente de Ca foi calculado pela seguinte fórmula:

Gradiente de Ca: Ca iônico sérico pré diálise (mg/dL) – Ca dialisato inicial (mg/dl)

Δ PTH= PTH final – PTH inicial (pg/mL)

Δ Cálcio = Cai final – Cai inicial (mg/dL)

5.1.8. Análise Estatística

Variáveis contínuas foram expressas como média $\pm$ desvio padrão ou mediana e percentis (25; 75), de acordo com o teste de normalidade de D'Agostino & Pearson. Variáveis categorias foram expressas como N e percentual. Análise de variância para medidas repetidas com pós teste de Tukey, ou o teste de Friedman não paramétrico com pós teste de Dunns foram usados para comparar variáveis nas três concentrações de Cad (2,5; 3,0 e 3,5 mEq/L) em cada fase do estudo. ANOVA ou o Kruskal-Wallis foram usados para comparar variáveis entre as três fases do estudo. A relação entre variáveis independentes e balanço de Ca foi analisada pela correlação de Spearman. Em cada fase, múltiplas análises de regressão *stepwise* (com $p < 0,05$ para entrar no modelo e $> 0,1$ para sair do modelo) foram realizadas considerando o balanço de Ca como variável dependente e diversas variáveis independentes selecionadas da análise univariada, sempre incluindo o gradiente de Ca e a ultrafiltração. Um modelo final, incluindo as três fases com todos os diferentes dialisatos combinados, foi analisado deixando o balanço de Ca como variável dependente. Nessa análise,

utilizamos o modelo linear *stepwise* e também a análise logística. A análise estatística foi realizada utilizando-se o GraphPad Prism 5.0 (Ca, EUA) e SPSS 21.0 (SPSS Inc. Chicago, IL). Foi considerado significativo $p < 0,05$.

5.2 APROVAÇÃO COMISSÃO DE ÉTICA

APROVAÇÃO

A Comissão de Ética para Análise de Projetos de Pesquisa - CAPPesq da Diretoria Clínica do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, em sessão de 06/04/2011, **APROVOU** o Protocolo de Pesquisa nº **0002/11**, intitulado: **"IMPACTO DA REMODELAÇÃO ÓSSEA SOBRE A TRANSFERÊNCIA DA MASSA DE CÁLCIO DURANTE A HEMODIÁLISE: ESTUDO EM PACIENTES COM HIPERPARATIREOIDISMO PRÉ E PÓS PARATIREOIDECTOMIA"**, apresentado pelo Departamento de **CLÍNICA MÉDICA**, inclusive o Termo de Consentimento Livre e Esclarecido.

Cabe ao pesquisador elaborar e apresentar à CAPPesq, os relatórios parciais e final sobre a pesquisa (Resolução do Conselho Nacional de Saúde nº 196, de 10/10/1996, inciso IX.2, letra "c").

Pesquisador (a) Responsável: **Rosa Maria Affonso Moysés**

Pesquisador (a) Executante: **Patrícia Taschner Goldenstein**

CAPPesq, 08 de Abril de 2011

PROF. DR. EUCLIDES AYRES DE CASTILHO
Coordenador
Comissão de Ética para Análise de
Projetos de Pesquisa - CAPPesq

Comissão de Ética para Análise de Projetos de Pesquisa do HCFMUSP da Diretoria Clínica do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo Rua Ovídio Pires de Campos, 225, 5º andar - CEP 05403 010 - São Paulo - SP Fone: 011 3069 6442 Fax: 011 3069 6492
e-mail: cappesq@hcnet.usp.br

5.3 TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO-HCFMUSP

DADOS DE IDENTIFICAÇÃO DO SUJEITO DA PESQUISA OU RESPONSÁVEL LEGAL

1. NOME:

DOCUMENTO DE IDENTIDADE Nº : SEXO : .M ☐ F ☐

DATA NASCIMENTO:/...../..... ENDEREÇO.....
Nº APTO:

BAIRRO: CIDADE.....

CEP:..... TELEFONE: DDD (.....).....

2. RESPONSÁVEL LEGAL.....

NATUREZA (grau de parentesco, tutor, curador etc.).....

DOCUMENTO DE IDENTIDADE :.....SEXO: M ☐ F ☐

DATA NASCIMENTO.:/...../.....ENDEREÇO..... Nº:..... APTO.....

BAIRRO:.....CIDADE:.....

CEP:.....TELEFONE: DDD (.....).....

6 DADOS SOBRE A PESQUISA

1. TÍTULO DO PROTOCOLO DE PESQUISA: Impacto da remodelação óssea sobre a transferência da massa de cálcio durante a hemodiálise: estudo em pacientes com hiperparatireoidismo secundário pré e pós paratireoidectomia

PESQUISADOR : Patricia Taschner Goldenstein

CARGO/FUNÇÃO: Médica assistente na Nefrologia, INSCRIÇÃO CONSELHO REGIONAL Nº 117229

UNIDADE DO HCFMUSP: Nefrologia

AValiação DO RISCO DA PESQUISA:

RISCO MÍNIMO ☒ RISCO MÉDIO ☐

RISCO BAIXO ☐ RISCO MAIOR ☐

4.DURAÇÃO DA PESQUISA :.....4 anos.....

**HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA
UNIVERSIDADE DE SÃO PAULO-HCFMUSP**

1 – O senhor/senhora ou seu familiar está sendo convidado (a) para participar de um estudo que visa avaliar a perda e o ganho de cálcio durante as sessões de hemodiálise que um paciente com Doença renal crônica realiza.

Como é do seu conhecimento, o(a) senhor/senhora possui uma complicação freqüente da Doença Renal Crônica, conhecida como Hiperparatireodismo Secundário. Para tratar esta complicação, o(a) senhor/senhora irá realizar uma cirurgia conhecida como paratireoidectomia. Até hoje, os médicos não sabem exatamente qual deve ser a concentração de cálcio no banho de diálise para pacientes com esta complicação, tanto antes, como após a cirurgia.

Desse modo, o(a) senhor/senhora está sendo convidado a realizar sessões de hemodiálise de 4 horas de duração, realizadas nesse hospital, 3 sessões antes da paratireoidectomia, 3 logo após a paratireoidectomia e 3 sessões cerca de 6 meses após a cirurgia, quando o(a) senhor/senhora estiver com os níveis de cálcio no sangue mais estáveis. A cada diálise iremos mudar a concentração de cálcio no seu banho de diálise. Iremos determinar a quantidade de cálcio e do hormônio que controla seus níveis nas amostras de seu sangue e do líquido que sai da máquina de hemodiálise, e que é normalmente desprezado ao longo do procedimento. Iremos também realizar biópsia óssea, ou seja, a retirada de um pequeno fragmento ósseo para melhor entendimento e determinação do seu quadro metabólico ósseo. Essas informações estão sendo fornecidas para sua participação voluntária neste estudo.

2 – A prescrição de cálcio no banho de diálise é hoje realizada sem muito conhecimento e depende da decisão do médico. Nesse protocolo de pesquisa, as sessões de hemodiálise serão realizadas de forma rotineira. O cálcio do banho de diálise será ajustado conforme explicado acima. Não haverá malefício para o senhor/senhora. O procedimento não rotineiro será as coletas de sangue para dosar o hormônio PTH, cálcio iônico, cálcio total, fósforo, fosfatase alcalina, albumina, gasometria. A biópsia óssea a ser realizada é procedimento rotineiro para os pacientes que irão realizar uma cirurgia de paratireoidectomia, o que é o seu caso.

3 – O sangue será coletado através da punção realizada para realizar a sua sessão de diálise, não sendo necessárias novas punções.

4- O único procedimento considerado de risco para o senhor/senhora é a biópsia óssea, sendo de risco baixo para complicações, podendo raramente ocorrer sangramento ou dor no local da retirada do fragmento ósseo. No entanto, este exame é necessário para avaliarmos a situação do seu

tecido ósseo antes da cirurgia e escolhermos o melhor tratamento possível para a sua recuperação após a cirurgia.

5 – Não há benefício direto para o(a) senhor/senhora, trata-se de um estudo clínico que irá beneficiar no futuro os pacientes que necessitam realizar hemodiálise. Somente no final do estudo poderemos concluir sobre a melhor dose de cálcio a ser utilizada na hemodiálise, podendo o estudo mudar a forma com que prescrevemos este elemento nos banhos de hemodiálise dos doentes que fazem hemodiálise.

6 – Não há procedimentos alternativos para o(a) senhor/senhora.

7 – Garantia de acesso: em qualquer etapa do estudo, você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de eventuais dúvidas. O principal investigador é o Dra. Patricia Taschner Goldenstein que pode ser encontrado no 7º andar do Instituto Central do HCFMUSP, Serviço de Nefrologia ou no LIM 16 na Faculdade de Medicina da USP ou pelo telefone (11) 91994922. Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Ovídio Pires de Campos, 225 – 5º andar – tel: 3069-6442 ramais 16, 17, 18 ou 20, FAX: 3069-6442 ramal 26 – E-mail: cappesq@hcnet.usp.br

8 – É garantida a liberdade da retirada de consentimento a qualquer momento e o abandono do estudo, sem qualquer prejuízo à continuidade de seu tratamento na Instituição.

9 – Direito de confidencialidade: As informações obtidas serão analisadas em conjunto com outros pacientes, não sendo divulgada a identificação de nenhum paciente.

10 – Direito de ser mantido atualizado sobre os resultados parciais das pesquisas, quando em estudos abertos, ou de resultados que sejam do conhecimento dos pesquisadores.

11 – Despesas e compensações: O paciente deverá comparecer ao Hospital das Clínicas em datas previamente agendadas por conta própria. Não há outras despesas pessoais para o participante em qualquer fase do estudo, incluindo exames e consultas. Também não há compensação financeira relacionada à sua participação. Se existir qualquer despesa adicional, ela será absorvida pelo orçamento da pesquisa.

12- Compromisso do pesquisador de utilizar os dados e o material coletado somente para esta pesquisa.

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo “Impacto da remodelação óssea sobre a transferência da massa de cálcio durante a hemodiálise: estudo em pacientes com hiperparatireoidismo secundário pré e pós paratireoidectomia ”.

para casos de pacientes menores de 18 anos, analfabetos, semi-analfabetos ou portadores de deficiência auditiva ou visual.

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste paciente ou representante legal para a participação neste estudo.

Assinatura do responsável pelo estudo Data / /