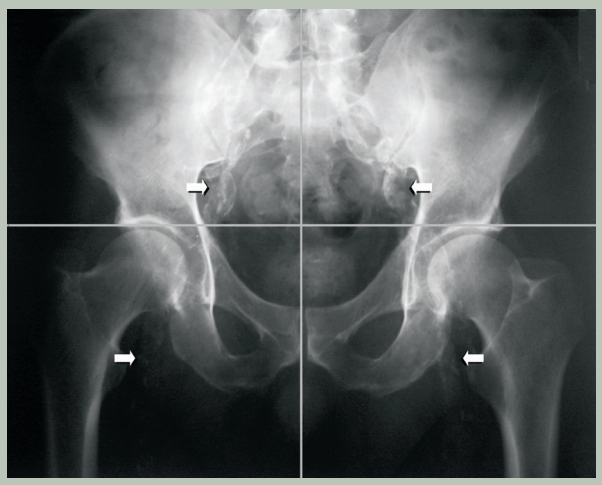
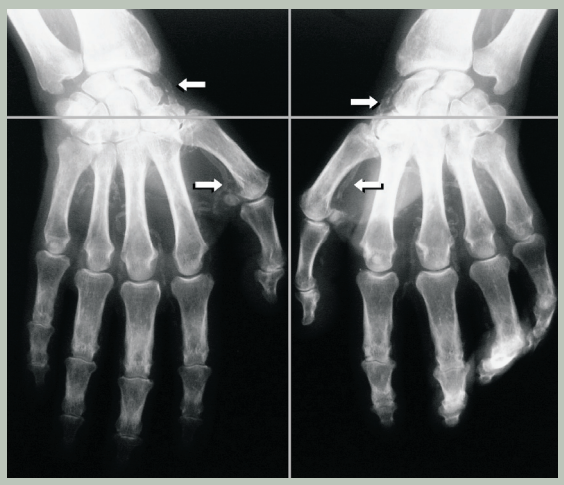
CALCIFICAÇÕES VASCULARES NOS DOENTES EM DIÁLISE: ELO DE LIGAÇÃO ENTRE DOENÇA ÓSSEA E DOENÇA VASCULAR





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ÍNDICE

Segundo o acordo ortográfico em vigor.

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CAPÍTULO 1

PREFÁCIO

Calcificações vasculares nos doentes em diálise: Elo de ligação entre doença óssea e doença vascular

Apesar dos grandes progressos no tratamento dos doentes renais crónicos em diálise, a mortalidade mantém-se muito elevada, verificando-se em algumas séries que a sobrevida aos 5 anos pode ser de apenas 30%[1]. A principal causa de morte é a cardiovascular, que atinge valores 100 vezes superiores aos dos indivíduos da população geral do mesmo grupo etário[2].

Esta elevada mortalidade cardiovascular dos doentes renais crónicos não é totalmente explicada pelos fatores de risco tradicionais[2,3], e múltiplos fatores não tradicionais parecem contribuir para este elevado risco nesta população. O esclarecimento destes processos poderá representar um dos avanços mais significativos no tratamento do doente renal crónico.

Em 2001 foi publicado o primeiro estudo que mostrou que as calcificações vasculares nos doentes em diálise são preditoras de mortalidade[4] e, no momento atual, é universalmente reconhecido que as calcificações vasculares são um fator de risco cardiovascular na doença renal crónica[5]. As alterações do metabolismo fosfocálcico e a patologia óssea destes doentes são alguns dos fatores que se associam ao desenvolvimento e à progressão das calcificações vasculares.

A presente dissertação para tese de doutoramento apresenta o desenvolvimento e a validação de um método simples e original para o diagnóstico de calcificações vasculares em doentes em diálise, utilizando um *score* semiquantitativo obtido em RX simples da bacia e das mãos criado por nós. Este trabalho iniciou-se no ano 2000 e incluiu a avaliação, ao longo de vários anos, deste *score* de calcificação em diferentes populações de doentes em diálise e da sua relação com a mortalidade, com o risco cardiovascular, com a rigidez arterial e com a doença óssea. Em inúmeros trabalhos publicados demonstramos que este *score* é preditor, nos doentes em diálise, de mortalidade cardiovascular e global, de internamentos cardiovasculares, de doença vascular e de doença arterial periférica[6,7]. É igualmente preditor de rigidez arterial avaliada por velocidade de onda de pulso e por pressão de pulso[7]. Este *score* de calcificação apresenta uma relação inversa com a densidade mineral óssea avaliada por DXA (*dual-energy x-ray absorptiometry*)[8]. Demonstramos que este *score* é um instrumento

útil e barato para a avaliação de calcificações vasculares, permitindo de forma simples identificar os doentes com mais elevado risco cardiovascular.

Salienta-se que dois dos trabalhos incluídos nesta dissertação foram referenciados nas *guidelines* publicadas pela KDIGO (*Kidney disease improving global outcomes*) em 2009 para validar a associação entre calcificações vasculares e mortalidade cardiovascular nos doentes renais crónicos (KDIGO 2009: Tabela suplementar 12; Fig.3.7)[5]. O primeiro trabalho foi publicado em 2004 e demonstrou a existência de uma associação entre este *score* vascular simples de calcificação e a mortalidade cardiovascular num grupo de doentes em diálise [6]. O segundo trabalho foi publicado em 2009 e demonstrou que o *score* vascular simples se associa diretamente à pressão de pulso e à velocidade de onda de pulso e que estes três fatores são preditores de mortalidade [7].

O diagnóstico de calcificações vasculares tem um interesse prático para os doentes renais crónicos. A presença de calcificações vasculares é um sinal de alerta para a existência de um elevado risco cardiovascular, e esta informação pode ser utilizada para modificar a terapêutica nestes doentes. No momento atual podemos atuar em alguns destes fatores, nomeadamente nas alterações do metabolismo mineral e ósseo, como sugerido nas *guidelines* KDIGO de 2009 [5].

A apresentação desta dissertação será dividida em diferentes capítulos, cada um deles correspondendo às distintas vertentes resultantes da análise do *score* de calcificação vascular simples na sua relação com os vários desfechos e parâmetros clínicos avaliados por nós: mortalidade, risco cardiovascular, rigidez arterial e doença óssea. Cada um destes temas foi analisado em diversos trabalhos apresentados em congressos nacionais e internacionais e publicados na revista *Portuguese Journal of Nephrology and Hypertension* e em revistas internacionais de elevado prestígio.

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CAPÍTULO 2

CRIAÇÃO DE UM *SCORE* SEMIQUANTITATIVO PARA AVALIAR A CALCIFICAÇÃO VASCULAR NOS DOENTES EM DIÁLISE

Nos doentes em diálise, as calcificações vasculares e as alterações do metabolismo fosfocálcico têm sido associadas de forma consistente em diversos estudos a uma maior mortalidade, sendo consideradas atualmente fatores de risco cardiovascular nesta população. Em 1998, foi publicado o primeiro estudo observacional[1] que mostrou que nos doentes em diálise existe uma associação independente entre as frequentes alterações do metabolismo mineral, como a hiperfosfatemia, a hipercalcemia e as alterações da paratormona (PTH), e a mortalidade. Após este primeiro estudo, muitos outros se sucederam revelando a existência de uma associação consistente entre a hiperfosfatemia e a mortalidade nos diferentes estádios da doença renal crónica (3 a 5 e 5D)[2-4].A associação entre hiperfosfatemia e mortalidade não é exclusiva da doença renal crónica, pois também em doentes sem insuficiência renal se verificou que níveis mais elevados de fósforo sérico, embora ainda dentro dos limites normais, são preditores de mortalidade [5].

Os mecanismos que associam a hiperfosfatemia e a hipercalcemia à mortalidade são parcialmente explicados por estudos *in vitro* realizados em células musculares lisas em cultura provenientes de aorta humana[6,7]. Nestes estudos foi demonstrado que níveis elevados de fósforo e de cálcio promovem a calcificação vascular através da expressão de um fator de transcrição genético Cbfa1 (*core binding factor alpha 1*), atualmente conhecido por RunX2 (*runt related transcription factor 2*), pertencente à família dos fatores de transcrição RunX. Este fator codifica uma proteína essencial para a diferenciação osteoblástica. Verifica-se, deste modo, que a alteração da ativação de determinados genes torna as células musculares lisas capazes de sintetizar matriz óssea e de promover a calcificação dessa matriz. Demonstrou-se assim que a calcificação vascular é um processo ativo que resulta de uma diferenciação das células musculares lisas em osteoblastos em resposta a diferentes estímulos.

Além destas alterações de expressão genética, sabemos atualmente que a calcificação vascular resulta de um equilíbrio complexo entre fatores indutores e inibidores, verificando-se o aumento de alguns fatores indutores e por vezes também a diminuição de fatores inibidores. Nos doentes em diálise, a hiperfosfatemia e a hipercalcemia são alguns dos fatores indutores de calcificação. Verifica-se igualmente o défice de diversos fatores inibidores da calcificação, entre os quais se identificam o défice de fetuína A[8] geralmente

em relação com inflamação, e o défice de matrix-gla em consequência de um défice de vitamina K[9].

A hiperfosfatemia e a hipercalcemia são frequentes nos doentes em diálise e surgem habitualmente em duas situações opostas: no hiperparatiroidismo secundário e na doença óssea adinâmica. No hiperparatiroidismo o osso apresenta uma elevada remodelação óssea e liberta cálcio e fósforo para a circulação. Na doença adinâmica, o osso é incapaz de incorporar cálcio e fósforo por apresentar uma baixa remodelação óssea e o cálcio e o fósforo que o doente recebe na sua dieta habitual (ou no caso do cálcio também na terapêutica) acumulam-se. Quer num caso quer noutro a consequência desta alteração do metabolismo do cálcio e do fósforo é o seu depósito extraósseo nos tecidos moles e na parede dos vasos.

Estas são as bases para a presente hipótese da existência de um elo de ligação entre a doença óssea e a doença vascular nos doentes em diálise. A iniciativa mundial KDIGO (*Kidney disease improving global outcomes*), que tenta unificar recomendações para o tratamento da doença renal crónica, propôs em 2006[10] que as calcificações vasculares fossem incluídas no diagnóstico da doença mineral e óssea da doença renal crónica. As *guidelines* publicadas pela KDIGO em 2009[11,] referentes à doença mineral e óssea da doença renal crónica consideram que os doentes renais crónicos com calcificações vasculares sejam reconhecidos como apresentando elevado risco cardiovascular e sugerem que o RX simples de perfil da aorta abdominal e o ecocardiograma sejam usados para detetar, respetivamente, a presença de calcificações vasculares e valvulares nestes doentes.

Tipos de calcificações vasculares

Existem dois tipos histológicos de calcificações arteriais: calcificações da íntima e calcificações da camada média. Estes dois tipos de calcificações têm sido considerados como duas entidades distintas com diferente significado clínico e diferente prognóstico. A calcificação da íntima ocorre durante a evolução da placa aterosclerótica e relaciona-se com a dislipidemia. A calcificação da média está presente na arteriolosclerose e desenvolve-se sobretudo em doentes renais crónicos, diabéticos e em indivíduos idosos.

Foi levantada a hipótese de estes dois processos poderem corresponder a um fenómeno contínuo da patologia vascular[12] mas, nos doentes renais crónicos, a calcificação da camada média pode preceder o aparecimento de placas ateroscleróticas e surge na ausência de depósitos lipídicos, tendo esta hipótese sido contestada[13].

As manifestações clínicas da calcificação da íntima relacionam-se com a presença de placas ateroscleróticas que estenosam as artérias devido a fenómenos de rotura da placa ou de trombose.

Considera-se que a carga de cálcio se relaciona com a carga aterosclerótica e o *score* de cálcio avaliado pelo método de Agatston é usado como preditor do risco cardiovascular na população geral[14].

A calcificação da média não causa estenose arterial e, por esta razão, foi considerada no passado como um achado radiológico sem consequências clínicas. Sabemos atualmente que este tipo de calcificação modifica as propriedades da parede arterial e é um dos fatores que contribui para a rigidez arterial. A rigidez arterial provoca uma perda de distensibilidade aórtica. A onda de reflexão sanguínea habitual acontece mais precocemente devido à perda de elasticidade da aorta e encontra a válvula aórtica ainda aberta, contribuindo para o aumento do volume do ventrículo esquerdo. A consequência é o aumento da pressão sistólica e uma diminuição da pressão diastólica que se manifestam clinicamente por hipertensão sistólica e por aumento de pressão de pulso[15,16]. O aumento da pressão sistólica contribui para o desenvolvimento de hipertrofia ventricular esquerda. A diminuição da pressão diastólica compromete a perfusão coronária que ocorre durante a diástole[17]. Deste modo, a calcificação da camada arterial média pode associar-se a doença coronária sintomática, mesmo na ausência de lesões estenóticas das artérias coronárias. A compreensão desta fisiopatologia é fundamental para interpretar os resultados por vezes contraditórios entre a coronariografia e a avaliação quantitativa das calcificações coronárias nos doentes renais crónicos[18].

O diagnóstico diferencial entre calcificação da íntima e da média é feito através do exame histológico. Letho *et al*[19] propuseram uma metodologia simples, usando RX simples para avaliação das calcificações da camada média em doentes diabéticos, e este grupo foi o primeiro a demonstrar que estas calcificações não estenosantes se associavam a maior morbilidade. London *et al*[20] utilizaram esta mesma metodologia nos doentes renais crónicos e demonstraram que a calcificação da média e a calcificação da íntima eram preditores independentes de mortalidade nesta população.

O *score* de Agatston, avaliado por tomografia computorizada de feixe de eletrões ou por tomografia axial computorizada multicorte, não distingue a calcificação da íntima e da média. Estes dois tipos de calcificação podem estar presentes no mesmo doente e no mesmo vaso. O *score* de calcificação vascular simples criado por nós[21] considera apenas a presença ou ausência de calcificação vascular nos diferentes setores, sem distinguir estes dois tipos de calcificação. Esta metodologia simplifica a sua utilização pelo nefrologista, pois não é necessária a interpretação por um radiologista.

Desenvolvimento do *score* de calcificação vascular simples

Apercebemo-nos cedo da elevada prevalência da doença arterial periférica nos doentes em diálise e das suas consequências clínicas dramáticas: claudicação incapacitante, úlceras

isquémicas e elevado número de amputações[22]. No estudo radiológico simples do esqueleto, que nessa altura era feito rotineiramente para avaliar as manifestações ósseas do hiperparatiroidismo secundário dos doentes em diálise, era frequente observar o que se denominava "arteriografias espontâneas" e que resultavam da presença de calcificações vasculares desenhando as paredes vasculares.

A existência de uma aparente discordância entre as manifestações clínicas de doença arterial periférica e os dados laboratoriais do metabolismo mineral e ósseo nos doentes em diálise foram a razão e o incentivo para a criação de um método simples e acessível para avaliar as calcificações vasculares nestes doentes.

No início da década de 90, a existência de calcificações vasculares e de partes moles observada nos doentes em diálise era habitualmente explicada como sendo devida ao hiperparatiroidismo secundário, provavelmente em relação com o elevado produto fosfocálcico destes doentes. No entanto, só em 2000 ficou demonstrado, num modelo celular *in vitro,* usando células musculares lisas da aorta humana, que a hiperfosfatemia causava calcificação por um mecanismo ativo de transcrição genética[6]. Só em 2004, no mesmo modelo, foi feita uma demonstração semelhante para a hipercalcemia[23]. A arteriolopatia urémica cálcica, ou calcifilaxis, uma entidade clínica catastrófica causada por calcificações vasculares das arteríolas subcutâneas e que origina extensas áreas de necrose cutânea, era também classicamente atribuída ao hiperparatiroidismo secundário. Preconizava-se a paratiroidectomia de urgência nestes doentes para reverter este quadro clínico, muitas vezes fatal. Contudo, os níveis de paratormona (PTH) destes doentes nem sempre eram elevados, contrariamente ao que estava descrito.

A existência de valores baixos ou normais de PTH em doentes com extensas calcificações vasculares e com manifestações clínicas de calcifilaxis ou de doença arterial periférica foram a base para a criação de um método para avaliar as calcificações vasculares e de partes moles e tentar correlacioná-lo com as alterações do metabolismo mineral e ósseo dos doentes em diálise.

Com base nas calcificações detetadas no RX simples do esqueleto, realizamos um primeiro estudo transversal em que relacionamos a presença de calcificações vasculares e de partes moles com as alterações do metabolismo mineral e ósseo dos doentes em diálise. Nesse estudo, as calcificações das partes moles foram pesquisadas na região periarticular das articulações acromioclavicular e coxofemoral. As calcificações vasculares foram avaliadas nas artérias ilíacas e femorais. A hipótese de avaliar as artérias dos membros inferiores foi também considerada, mas, devido aos inúmeros casos de amputações, consideramos que não seria um método comparativo fiável. Optamos então por pesquisar as calcificações vasculares noutro território com artérias distais, tendo por esta razão incluído a avaliação das artérias das mãos.

Com base nestes estudos radiológicos criamos um *score* de calcificação global (vascular e partes moles) que resultava da presença de calcificação em cada território avaliado. Neste

primeiro estudo, apresentado no ano 2000 no Congresso da Sociedade Portuguesa de Nefrologia[24], foram estudados 183 doentes em diálise, e neste grupo de doentes, em análise multivariada, verificamos uma correlação inversa entre o *score* de calcificação e os níveis de PTH e de albumina e uma correlação positiva com os valores de pressão sistólica e com o tempo de permanência em diálise. Não encontramos correlação entre o *score* de calcificação e os níveis de fósforo ou cálcio séricos, e estes resultados foram considerados discordantes dos conhecimentos prevalentes à data.

No entanto, no ano seguinte (2001), Blacher demonstrou pela primeira vez uma associação independente entre as calcificações vasculares e a mortalidade nos doentes em diálise[25]. Neste estudo, as calcificações vasculares tinham sido avaliadas por ecografia na aorta e nas artérias carótidas, ilíacas e femorais. Os autores não encontraram igualmente nenhuma correlação entre as calcificações e os níveis séricos de cálcio ou de fósforo. Encontraram uma associação directa com a mortalidade e, tal como nós, uma relação inversa com os níveis de albumina sérica.

Num estudo prévio deste mesmo grupo, Guérin *et al* tinham mostrado uma associação independente entre as calcificações vasculares e a rigidez arterial nos doentes em diálise[26]. Neste estudo, os valores de PTH apresentavam uma correlação inversa com as calcificações vasculares e também não havia correlação entre as calcificações e o produto fosfocálcico. Estes dois estudos foram a confirmação da veracidade dos nossos resultados prévios.

Decidimos então avaliar prospetivamente a mortalidade associada às calcificações vasculares avaliadas por RX simples e criamos, para este fim, um método semiquantitativo para avaliar as calcificações vasculares nos mesmos territórios que tínhamos previamente analisado. O RX da bacia foi dividido por duas linhas imaginárias em quatro setores: uma linha vertical sobre a coluna vertebral e uma linha horizontal tangencial à extremidade superior da cabeça dos fémures. O RX das mãos foi dividido em cada mão por uma linha horizontal tangencial à extremidade superior dos metacarpos. Ficaram assim definidos quatro setores no RX da bacia e quatro no RX das mãos (Fig 2.1). A presença de alguma calcificação vascular em qualquer setor era contada como 1 e a ausência como 0. Os limites deste *score* de calcificação eram 0 e 8.

Avaliamos as calcificações vasculares num grupo de 123 doentes em diálise. Após um seguimento de 37 meses, verificamos que um *score* vascular superior a 3 se associava a maior mortalidade cardiovascular e que este *score*, em análise multivariada, se associava a maior risco de morte cardiovascular, a internamentos cardiovasculares, a doença arterial periférica e a doença vascular em geral. Este trabalho foi publicado como artigo original na revista *Nephrology Dialysis and Transplantation* em 2004 [21].

Estava assim definido um método simples e económico para avaliar as calcificações vasculares e predizer o risco cardiovascular nos doentes em diálise. Foi este método que

constituiu a base de um trabalho de investigação clínica que avaliou a existência de correlações entre as calcificações vasculares e a doença vascular e óssea nos doentes em diálise.

***Score* de calcificação vascular simples (SCVS)**

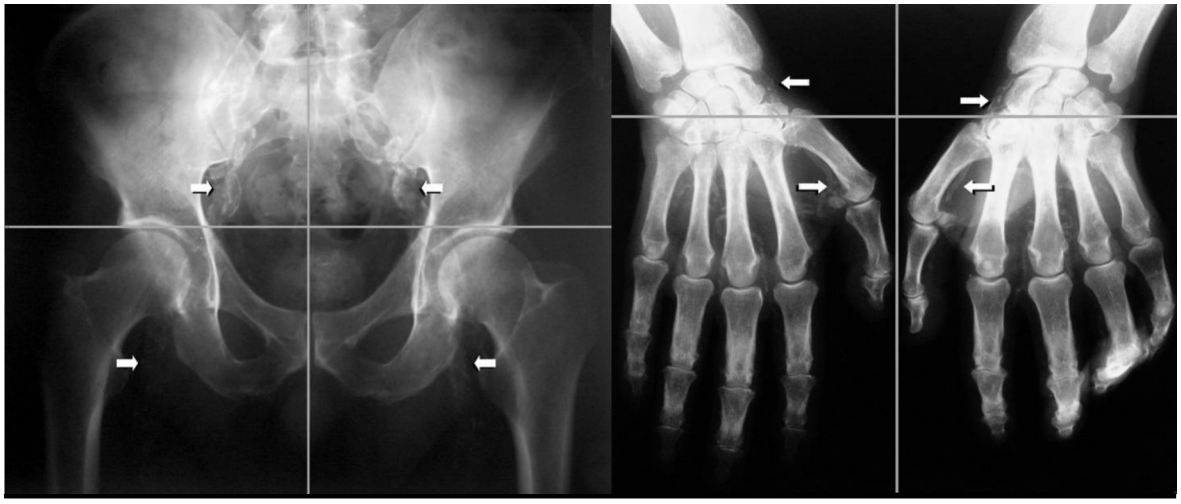

Fig. 2.1. Calcificações vasculares no RX bacia (artérias ilíacas e/ou suas colaterais e artérias femorais) e no RX das mãos (artérias radiais e digitais). O SCVS é a soma da presença (1) ou ausência (0) das calcificações vasculares em cada setor definido pelas linhas horizontais e verticais. Neste exemplo, o *score* da bacia = 1+1+1+1=4 e o *score* das mãos = 1+1+1+1=4. O *score* total é o SCVS = 8.

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CAPÍTULO 3

CALCIFICAÇÕES VASCULARES NOS DOENTES RENAIS CRÓNICOS: PREVALÊNCIA E ASSOCIAÇÃO COM MORTALIDADE E MORBILIDADE

Prevalência de calcificações vasculares em doentes em diálise

A prevalência de calcificações vasculares é elevada nos doentes renais crónicos, variando com o estádio da doença renal crónica e com o método utilizado no seu diagnóstico. Métodos quantitativos, como a tomografia axial computorizada helicoidal multicorte ou a tomografia computorizada de feixe de eletrões, mostraram que nos doentes prevalentes em diálise as calcificações coronárias estão presentes em cerca de 80% dos casos[1,2]. Utilizando métodos menos sensíveis, como a ecografia, Blacher *et al* diagnosticaram calcificações vasculares em 66% de um grupo de 110 doentes em diálise[3]. Em quatro estudos analisando diferentes populações de doentes e utilizando o *score* de calcificação vascular simples avaliado no RX simples da bacia e das mãos, verificamos a presença de calcificações vasculares em 75% de 123 doentes[4], em 76% de 101 doentes[5], em 70% de 219 doentes[6] em hemodiálise e em 61% de 70 doentes em diálise peritoneal[7].

Para avaliação da prevalência das calcificações vasculares nos doentes renais crónicos, as KDIGO 2009[8] selecionaram 22 estudos publicados entre 2003 e 2009, entre os quais foi também incluído o nosso estudo, publicado em 2004, no qual definimos o *score* de calcificação vascular simples[4] (KDIGO 2009: Tabela suplementar 10; Fig. 3.6). Para avaliação da associação entre calcificações e mortalidade, as KDIGO 2009 selecionaram apenas 12 estudos publicados entre 2001 e 2009, nos quais foram também incluídos o nosso primeiro estudo[4], que mostrou uma associação entre o *score* de calcificação vascular simples e mortalidade, e um segundo estudo, publicado em 2009[5], mostrando que o *score* vascular simples é preditor de rigidez arterial (KDIGO 2009: Tabela suplementar 12).

A inclusão de estudos observacionais nas presentes recomendações KDIGO 2009[8] baseou-se em conceitos definidos pelo sistema GRADE[9] (*Grades of recommendation, assessment, development, and evaluation*). O sistema GRADE considera que os estudos observacionais podem ter elevada qualidade de evidência se avaliarem parâmetros de segurança e de eficácia importantes, se mostrarem uma associação forte com estes parâmetros e se a metodologia utilizada for de elevada qualidade. A inclusão dos nossos estudos nas referências das KDIGO 2009, em que foi utilizado um método de selecção muito exigente, valida o interesse científico do nosso trabalho.

Associação entre calcificações vasculares e mortalidade nos doentes em diálise

A mortalidade cardiovascular nos doentes renais crónicos é superior à da população geral, quer nos doentes em diálise, quer nos doentes em estádios pré-diálise[10,11]. Os fatores de risco tradicionais não explicam integralmente este elevado risco cardiovascular[12], e considera-se que inúmeros fatores de risco não tradicionais possam contribuir para este resultado.

Nos últimos anos, um novo fator foi acrescentado à já extensa lista de fatores de risco cardiovascular dos doentes renais crónicos. Demonstrou-se que nestes doentes as calcificações vasculares apresentam uma associação consistente com a mortalidade. Blacher *et al*[3], em 2001, foram os primeiros a demonstrar esta associação. O método que utilizaram para diagnosticar as calcificações vasculares foi a ecografia de grandes vasos: aorta, carótidas, artérias ilíacas e femorais. Em 110 doentes, com um seguimento de 80 meses, estes autores demonstraram que, quanto maior o número de vasos afetados, menor a sobrevida dos doentes. London *et al*[13], em 2003, demonstraram que calcificações vasculares avaliadas por RX simples eram preditoras de mortalidade. Neste estudo de London, as calcificações foram avaliadas em 202 doentes em diálise, nas artérias ilíacas e femorais visualizadas num RX simples do abdómen. Utilizando uma metodologia previamente descrita por Letho em doentes diabéticos[14], estes autores distinguiram calcificação da íntima e calcificação da média e verificaram que, durante um seguimento de 80 meses, estes dois tipos de calcificação foram preditores independentes de mortalidade global e cardiovascular nos doentes em diálise. Alguns meses após esta publicação, publicamos o nosso estudo, que utilizou um *score* de calcificação vascular avaliado em RX simples da bacia e das mãos[4]. Verificamos que em 123 doentes prevalentes em diálise, durante um seguimento de 37 meses, um *score* de calcificação superior ou igual a 3 se associou a 14 mortes cardiovasculares, enquanto nos doentes com um *score* de calcificação inferior a 3 se verificaram apenas três mortes (Fig. 3.1). Esta associação foi confirmada em análise multivariada, ajustando para idade, sexo, tempo em hemodiálise, diabetes, produto fosfocálcico, níveis séricos de PTH e albumina. Um *score* de calcificação vascular simples superior ou igual a 3 associou-se a um risco 3,9 vezes superior de morte cardiovascular, quando comparado com um *score* inferior a 3 (HR=3,9; IC 95% 1,1 a 13,4, p=0,03) (Fig. 3.2).

Noutra população de 101 doentes em diálise, em que analisamos a associação entre calcificações vasculares e rigidez arterial avaliada por velocidade de onda de pulso e pela pressão de pulso, verificamos que um *score* de calcificação superior a 3 foi preditor de mortalidade de causa global [5]. Em análise multivariada e ajustando para a idade, a duração da hemodiálise, a diabetes, a doença vascular prévia, o índice de massa corporal, a pressão sistólica, as doses de calcitriol e o carbonato de cálcio, os doentes com *score* de calcificação vascular superior a 3 apresentaram um risco de morte 3,3 superior, quando comparados com

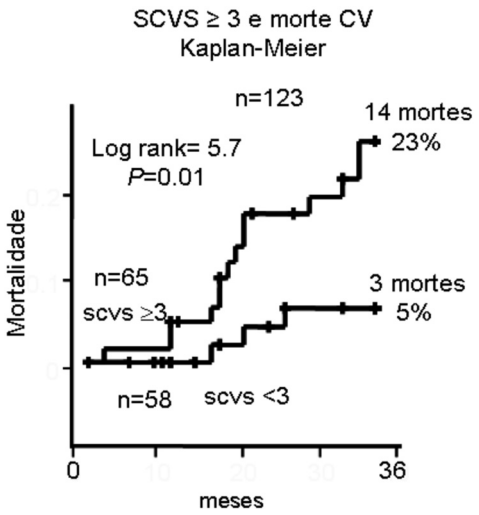


Fig. 3.1. O *score* de calcificação vascular simples, SCVS ≥ 3, associou-se a uma maior mortalidade cardiovascular

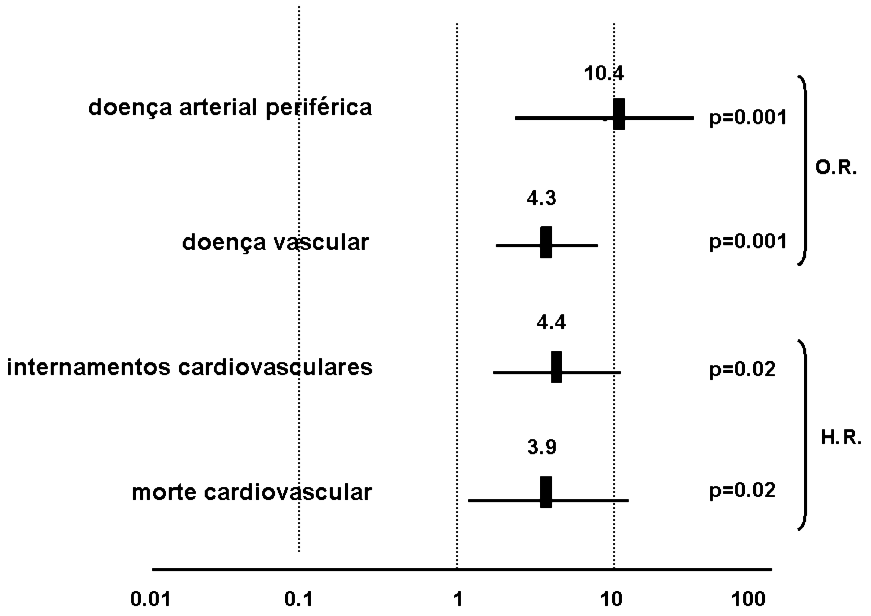


Fig. 3.2. Risco cardiovascular em relação com o *score* de calcificação vascular simples, SCVS≥3, sendo a referência o SCVS <3. O risco foi calculado por *odds ratio* com regressão logística (doença arterial periférica e doença coronária) e por *hazard ratio* com regressão COX (internamentos cardiovasculares e morte cardiovascular)

os doentes com *score* inferior ou igual a 3 (HR=3,3; IC 95% 1,1 a 9,8, p=0,03). Outros estudos, utilizando diferentes metodologias, confirmaram esta associação entre calcificações vasculares e mortalidade[2,15,16].

Associação entre calcificações vasculares e morbilidade nos doentes em diálise

Neste primeiro estudo, publicado em 2004[4], demonstrámos também que o *score* de calcificação vascular simples foi um preditor independente de morbilidade cardiovascular avaliada pelos internamentos cardiovasculares e pela presença de doença coronária, doença arterial periférica e doença vascular em geral (Fig. 3.2). As doenças coronária, arterial periférica e cerebrovascular foram definidas por critérios clínicos. Diagnosticamos doença coronária nos doentes com sintomas inequívocos de angina de peito ou com uma prova de esforço ou ecografia de *stress* positivos ou que tivessem tido um enfarte do miocárdio ou sido submetidos a angioplastia coronária ou cirurgia de revascularização coronária. Consideramos a existência de doença arterial periférica nos doentes com história de claudicação intermitente ou úlceras isquémicas ou com diagnóstico de obstrução arterial por doppler ou angiografia ou que tivessem sido submetidos a revascularização arterial ou a amputações distais dos membros inferiores. Consideramos a existência de doença cerebrovascular nos doentes com o diagnóstico de acidente vascular cerebral ou acidente isquémico transitório ou com evidência de um enfarte na tomografia axial computorizada cranioencefálica. No final do estudo, 43 doentes (35%) tinham o diagnóstico de doença coronária, 33 (27%) o diagnóstico de doença arterial periférica, 16 (13%) o diagnóstico de doença cerebrovascular e 61 doentes (50%) tinham pelo menos um destes três tipos de doença vascular.

Em análise multivariada e ajustando para idade, sexo, tempo em hemodiálise, diabetes, produto fosfocálcico, níveis séricos de PTH e albumina, um *score* superior ou igual a 3 associou-se a um risco aumentado de internamentos cardiovasculares (HR=4,4; IC 95% 2,1 a 10,2, p=0,02), de doença arterial periférica (OR=10,4; IC 95% 2,7 a 40,3), de doença coronária (OR=3,2; IC 95% 1,4 a 7,4) e de doença vascular em geral (OR=4,3; C 95% 1,9 a 9,9) (Fig. 3.2).

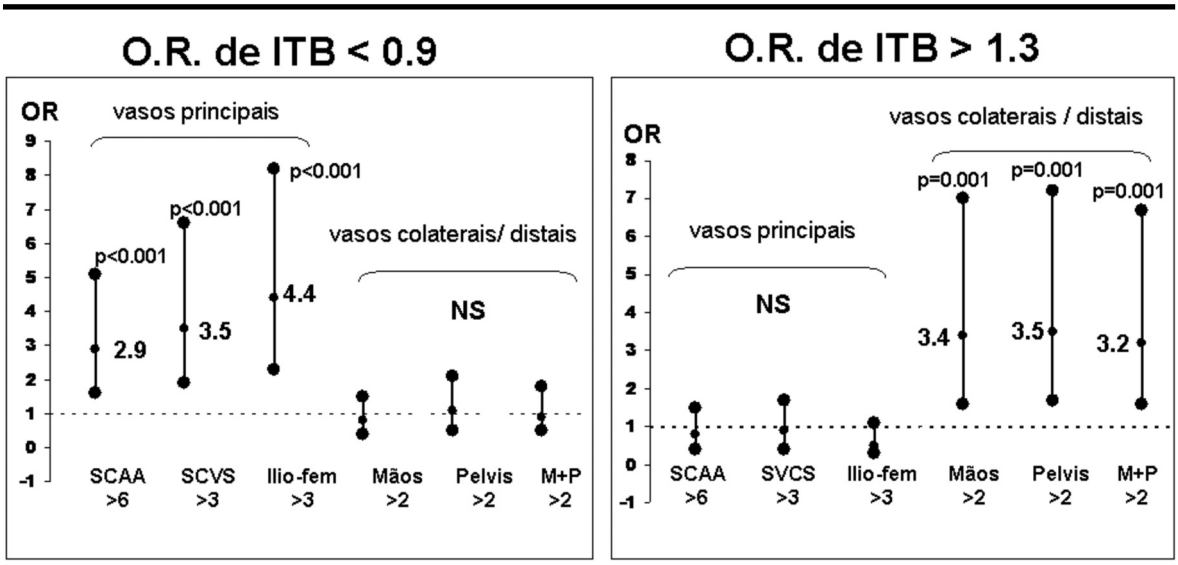
Calcificações vasculares e doença arterial periférica

Desde logo, na primeira avaliação do *score* de calcificação vascular simples, detectamos que este *score* se relacionou fortemente com a doença arterial periférica[4]. Os doentes com um *score* superior ou igual a 3 apresentaram um risco 10,4 vezes aumentado de associação a doença arterial periférica sintomática (Fig. 3.2). Neste primeiro estudo, a doença arterial periférica foi diagnosticada com base em critérios clínicos. Nos doentes em diálise, a doença

arterial periférica é frequentemente de diagnóstico tardio, porque a neuropatia periférica mascara os sintomas de claudicação intermitente e as artérias rígidas distais continuam a apresentar pulso palpável. Um método simples e não invasivo de avaliar a doença arterial periférica é através do índice tornozelo-braço. Nos doentes em diálise existe até ao momento apenas um estudo que demonstrou associação entre mortalidade e este índice[17], verificando-se maior mortalidade nos doentes com um índice inferior a 0,9 ou superior a 1,3. Um índice superior a 1,3 é considerado um falso negativo no diagnóstico de doença arterial obliterativa e resulta de artérias rígidas e não compressíveis, provavelmente devido à presença de calcificações vasculares.

Iniciamos em 2008 um estudo com o objetivo de analisar a correlação entre calcificações vasculares avaliadas por RX simples e o índice tornozelo-braço (ITB) avaliado por doppler. Os resultados preliminares deste estudo foram apresentados sob a forma de *poster* no EDTA Congress of Nephrology 2010[6]. Neste estudo analisamos 219 doentes em hemodiálise. As calcificações vasculares foram avaliadas por RX simples usando dois métodos diferentes: o *score* de calcificação vascular simples no RX simples da bacia e das mãos e o *score* de calcificação aórtico avaliado no RX simples de perfil da aorta abdominal, de L1 a L4, descrito por Kauppila L[18]. Em análise multivariada e ajustando para a idade, o sexo, a duração de hemodiálise, a diabetes, os hábitos tabágicos, a pressão de pulso e os níveis séricos de albumina verificamos um associação independente entre as calcificações vasculares das grandes artérias (aorta, ilíacas e femorais) e um ITB <0,9 e entre as calcificações vasculares de artérias colaterais da bacia e periféricas das mãos com um ITB >1,3 (Fig. 3.3).

Neste grupo de doentes, um ITB <0,9 e um ITB >1,3 associaram-se a um aumento de risco de morte ajustando para idade, duração da hemodiálise, diabetes e níveis de albumina sérica. O tipo de calcificações nas grandes artérias é irregular e corresponde a calcificações da íntima[13], às quais se podem associar ou não calcificações da média. O tipo radiológico das calcificações nas artérias colaterais e periféricas é linear e contínuo, o que, segundo Letho[14] e London[13], corresponde a calcificação da camada média da parede arterial. A associação das calcificações de grandes vasos com um ITB < 0,9 sugere uma contribuição da aterosclerose na doença arterial obliterativa. A associação das calcificações das artérias colaterais e periféricas com um ITB >1,3 sugere que este tipo de calcificações da camada média arterial pode contribuir para a rigidez das artérias distais, que se tornam não compressíveis, razão pela qual apresentam um aumento do índice tornozelo-braço.



OR, *odds ratio*; ITB, índice tornozelo-braço; SCAA, *score* de calcificação na aorta abdominal; SCVS, *score* de calcificação vascular simples; M+P, calcificações vasculares mãos ou pélvis.

Fig. 3.3. Risco de ITB <0,9 associou-se a calcificações vasculares nos grandes vasos. Risco de ITB > 1,3 associou-se a calcificações vasculares nos vasos colaterais ou distais (regressão logística).

Este estudo permitiu ainda a comparação entre o *score* vascular simples de calcificação e o *score* de calcificação da aorta abdominal (SCAA). Esta comparação é atualmente importante, pois a avaliação da calcificação na aorta abdominal por RX simples é o método sugerido pelas *guidelines* KDIGO 2009 para a avaliação das calcificações vasculares como alternativa razoável à tomografia computorizada. Verificamos que valores mais elevados do *score* vascular simples se associam a valores mais elevados do SCAA (Fig. 3.4) e, em análise de curvas ROC, o *score* vascular simples de calcificação superior a 3 apresentou uma alta sensibilidade (73%) e especificidade (86%) para detetar um SCAA > 6 (Fig. 3.5).

Noutro estudo, ainda não publicado, avaliamos os padrões histológicos da doença arterial periférica distal em 56 doentes submetidos a amputações distais. Trinta e nove destes doentes faziam diálise, 10 eram diabéticos não em diálise e 7 não tinham nem diabetes *mellitus* nem doença renal crónica. Em análises histológicas em 65 amostras de material de amputação verificamos a presença de placas ateroscleróticas classe II/III em 24 (38%), de calcificações vasculares da camada média em 31 (48%), e não encontramos evidência de calcificação da íntima. As calcificações vasculares associaram-se a placas ateroscleróticas em 50% dos casos. As calcificações da média, as placas ateroscleróticas ou ambas apresentaram uma distribuição semelhante nas diferentes entidades clínicas. Estes dados confirmam a associação de calcificações da camada média à doença arterial crónica distal[19,20].

Nestes dois estudos, ainda não publicados, quer com avaliação clínica quer histológica da doença arterial periférica, constatamos que as calcificações vasculares se associam a esta patologia. No caso particular dos doentes renais crónicos, em que as alterações do metabolismo mineral e ósseo se associam ao desenvolvimento e à progressão das calcificações vasculares, surge a possibilidade de um novo campo de intervenção terapêutica na doença arterial periférica diferente do da população geral. É necessário avaliar se o manejo das alterações do metabolismo mineral e ósseo pode ter um papel na evolução e no prognóstico da doença arterial periférica nos doentes renais crónicos.

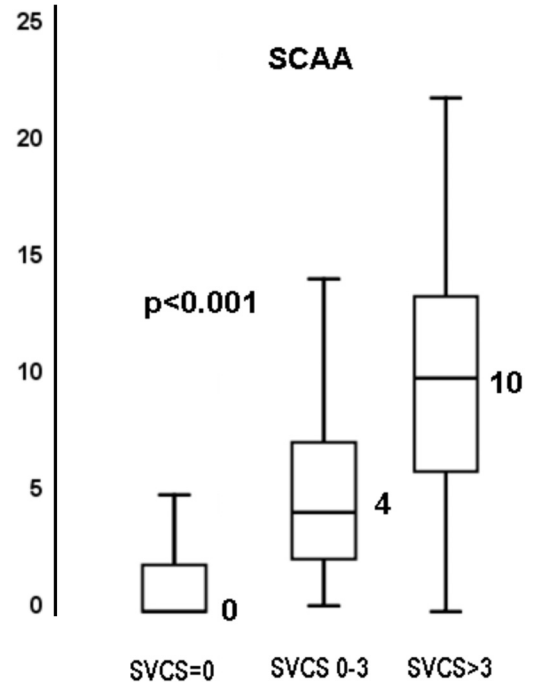


Fig. 3.4. Valores mais elevados de *score* de calcificação vascular simples SCVS associam-se a valores mais elevados de score de calcificação na aorta abdominal SCAA (Kruskal-Wallis)

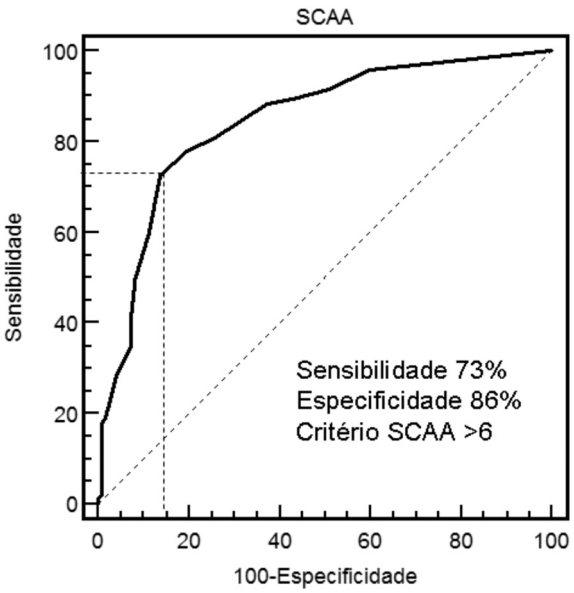


Fig. 3.5. Um SCVS>3 teve uma sensibilidade de 73% e uma especificidade de 86% de diagnosticar um SCAA>6 (análise de curvas ROC)

Em conclusão, nestes diferentes estudos[4,5,7,19,20] demonstramos que o *score* de calcificação vascular simples é um método acessível e barato que permite identificar o risco cardiovascular nos doentes em diálise. A simplicidade e baixo custo deste *score* tornam a sua utilização passível de ser aplicada em larga escala, fornecendo dados muito importantes para o acompanhamento dos doentes insuficientes renais.

Supplemental table 10. Overview Table of selected studies presenting data on calcification prevalence

Author, Year Study Design Country	N Population	Representative Test (s)	Prevalence of Calcification
Hernandez, 200559 Chart Review Spain	1117 CKD 5D, At transplant	Plain X-ray of abdomen and pelvis	24.4%
Honkanen, 200860 Cross-sectional Belgium, Netherlands, Sweden, Denmark, Finland, Norway	933 CKD 5D on HD/PD	Abdominal aorta calcification by lumbar (L1-L4) radiographs	81%
Ix, 200761 Cross-sectional USA	653 CKD 3-5	Valvular calcification by MSCT/EBCT	20/25%
Okuno, 200762 Prospective Japan	515 CKD 5D, HD	Plain X-ray	56.5%
Adeney, 200863 Retrospective US	439 CKD Stage 3-5	CAC and valvular calcifications by EBCT or multi-detector CT	67% coronary 49% aortic 20% mitral valve 25% aortic valve
Panuccio, 200464 Prospective Italy	202 CKD 5D, HD	Valvular calcification by echocardiography	23.3%
London, 200365 Prospective France	202 CKD 5D, HD	Calcification score by ultrasound and conventional X-ray	63.9%
Wang, 200366 Prospective Hong Kong	193 CKD 5D, PD	Valvular calcification by echocardiography	32.3%
Rodriguez-Garcia, 200949 Prospective Spain	193 CKD 5D, HD	Vascular calcifications by thoracic, lumbar spine, pelvic and hand X-rays	79%
Jean, 200967 Prospective France	161 CKD 5D on HD	Vascular calcification by plain radiograph	83%
Kronenberg, 200368 Prospective Austria	155 CKD 5D on Incident HD and PD	Conventional X-ray of pelvis and calves	67%
Sharma, 200769 Prospective UK	140 CKD 4-5D, Predialysis, HD and PD on transplant waiting list	Valvular calcification by echocardiography	40%
Varma, 200570 Chart review USA	137 CKD 5D, HD	Valvular calcification by echocardiography	47%

(Continua)

(Supplemental table 10. – Continuação)

Author, Year Study Design Country	N Population	Representative Test (s)	Prevalence of Calcification
Sigrist, 200652 Sigrist, 200771 Prospective UK	134 CKD 4, 5D on HD/PD	Arterial calcification by MSCT	CKD 4: 47% PD: 71% HD 73% + higher scores
Adragão, 200472 Prospective Portugal	123 CKD 5D, HD	Calcification score by conventional X-ray	61% iliac 60% femoral 36% radial 5% digital
Garland, 200873 Cross-sectional Canada	119 CKD 3-5	CAC by MSCT	83.2%
Blacher, 200174 Prospective France	110 CKD 5D, HD	Calcification score by ultrasound and conventional X-ray	66.4%
Matsuoka, 200475 Prospective Japan	104 CKD 5D, HD	CAC by EBCT	81.7%
Russo, 200776 Prospective Italy	90 CKD Stage 3-5	CAC by MSCT	83%
Shroff, 200755 Retrospective USA	85 Children aged 5-18 y CKD 5D	CAC by MSCT	20%
Chertow, 200277 Raggi 200478 RCT USA, Germany, Austria	70 CKD Stage 5D on HD	CAC by EBCT Valvular calcification by EBCT	83% coronary 80% aortic 46% mitral valve 33% aortic valve
Stompor, 200656 Prospective Poland	61 CKD 5D on PD	Total CAC by MSCT	>51% (51% w/ a CAC score of <10, median 0)
Russo, 200779 Prospective Italy	53 CKD Stage 3-5	CAC by MSCT	51%
Block, 200580 RCT USA	53 CKD 5D on Incident HD	CAC by EBCT	63%/69%
Nitta, 200458 Prospective Japan	53 CKD 5D on HD	CAC by MSCT	92.5%

CAC, coronary artery calcification; CKD, chronic kidney disease; CT, computed tomography; EBCT, electron-beam CT; HD, hemodialysis; MSCT, multislice spiral computed tomography; N, number of subjects; PD, peritoneal dialysis; RCT, randomized controlled trial.

Fig. 3.6. KDIGO *Guidelines* 2009: Tabela suplementar 10

Supplemental table 12. Overview table of selected studies demonstrating the risk relationship between vascular calcification and mortality in CKD

Study Design Country	N Population Follow-up	Vascular Calcification		Mortality Categorization
		Technique	Categorization	
Hernandez, 200559 Chart Review Spain	1117 At transplant Median 49 mo	Plain X-ray of abdomen and pelvis	– Vascular calcification – None	– All cause – Cardiovascular
Okuno, 200762 Prospective Japan	515 HD 51 mo	Plain	– Abdominal aortic calcification – None	– All cause – Cardiovascular
London, 200365 Prospective France	202 HD Up to ~100 mo	B-mode US	– Arterial medial – Arterial intimal – None	– All cause – Cardiovascular
Rodriguez-Garcia, 200949 Prospective Spain	193 HD 24 mo	X-ray of thoracic, lumbar spine, pelvis and hands	– Vascular calcifications – Abdominal aortic calcifications	– All-cause
Block, 200783 RCT USA	127 HD Median 44 mo	EBCT	CAC score 0, 1-400, >400	– All cause
Adragão, 200484 Prospective Portugal	123 HD 37 mo	Plain X-ray of pelvis and hands	Vascular calcification score <or ≥ 3	– Cardiovascular
Blacher, 200174 Prospective France	110 HD 53 mo	US	Arterial calcification score 0-4	– All cause – Cardiovascular
Matsuoka, 200475 Prospective Japan	104 HD 44 mo	EBCT	CAC score <or ≥ 200	– Cardiac – Stroke – Infection – Other
Adragão, 200972 Prospective Portugal	101 HD 43 mo	Plain X-ray of pelvis and hands	Vascular calcification score < or ≥3	– All-cause
Kushiya, 200585 Prospective Japan	84 HD 24 mo	CT	ACI > or <0.3	– All cause

ACI, aortic calcification index; AVC, aortic valve calcification; CAC, coronary artery calcification; CKD, chronic kidney disease; CT, computed tomography; EBCT, electron-beam CT; HD, hemodialysis; N, number of subjects; PD, peritoneal dialysis; RCT, randomized controlled trial; US, ultrasound.

Fig. 3.7. KDIGO *Guidelines* 2009: Tabela suplementar 12

Referência: KDIGO, Clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney disease: Improving global outcomes (KDIGO). Kidney Int 2009; 76 (Suppl): S113. [Supplementary tables.]

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Original Article

Nephrology Dialysis Transplantation

A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients

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Abstract

Background. Cardiovascular morbidity and mortality are highly prevalent in haemodialysis (HD) patients and have been recently associated with vascular calcifications. The objective of our study was to assess the value of a simple vascular calcification score for the prediction of cardiovascular death, cardiovascular hospitalizations and fatal and non-fatal cardiovascular events in HD patients, and to correlate this score with cardiovascular disease and with other known predictors of vascular disease.

Methods. In this observational, prospective study 123 chronic HD patients (75 males and 48 females; 20% diabetic) were included, who were on low-flux HD treatment for 46.6 ± 52 months (mean $\pm$ SD). We set up a simple vascular calcification score based on plain radiographic films of pelvis and hands. Brachial pulse pressure and mean arterial pressure (MAP) were measured and cardiovascular events and hospitalization episodes were assessed.

Results. During an observational period of 37 months there were 17 cardiovascular deaths; 28 patients needed cardiovascular hospitalizations and 32 patients suffered fatal and non-fatal cardiovascular events. Coronary artery disease was diagnosed in 43 patients (35%), peripheral arterial disease in 33 patients (26.8%), cerebrovascular disease in 16 patients (13%) and vascular disease (coronary artery disease or peripheral arterial disease or cerebral vascular disease) in 61 patients (49.6%). By binary logistic regression, diabetes ($P = 0.01$), male sex ($P < 0.001$), age ($P = 0.02$), HD duration ($P = 0.02$) and MAP ($P = 0.03$) were independently associated with a vascular score ≥ 3. This score ≥ 3 was independently associated with coronary artery disease ($P = 0.008$), peripheral arterial disease ($P < 0.001$) and vascular disease ($P = 0.001$). Patients with a vascular calcification score ≥ 3 had a 3.9-fold higher risk of cardiovascular mortality ($P = 0.03$), a 2.8-fold higher risk of cardiovascular hospitalizations ($P = 0.02$) and a 2.3-fold higher risk of fatal or non-fatal cardiovascular events ($P = 0.04$).

Conclusions. The present vascular calcification scoring represents a simple tool for the assessment of cardiovascular risk related with vascular calcifications in chronic HD patients.

Keywords: haemodialysis; mortality; vascular calcification; vascular disease

Introduction

Cardiovascular mortality is the main cause of death in haemodialysis (HD) patients and can be 20-fold higher than in the general population, with greater differences in the younger population [1,2].

There are two main types of vascular calcifications in HD patients: (i) common atherosclerosis, with intimal patchy calcifications of atherosclerotic plaques and (ii) mediasclerosis with medial linear calcifications that seem to be related to mineral metabolism disturbances [3,4]. It has been recently demonstrated that mediasclerosis is an active cellular process, similar to bone formation [4,5] and is not the result of a passive metastatic calcification. Vascular muscle cells can differentiate into osteoblasts due to different stimuli, one of which may be hyperphosphataemia [3,4,6]. Deposition of bone matrix proteins can precede vascular calcification [5]. Different vascular calcification scores have been evaluated in HD patients by various methods, mainly using B-mode ultrasonography [7] and electron beam computed tomography [8,9]. These scores have been related to oral calcium load [9], cardiovascular disease [9,10]

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and mortality [7,11] in HD patients. Medial artery calcifications have already been differentiated from intimal calcifications by plain radiography in non-insulin diabetic patients and have been associated with all-cause and cardiovascular mortality [12]. In HD patients, these different calcification patterns were both associated with cardiovascular and all-cause mortality and intimal artery calcifications were associated with a lower survival [13]. The early diagnosis of vascular calcifications and the identification of their cause raise the hope for a possible direct therapeutic intervention that might reduce cardiovascular disease in HD patients. It has already been demonstrated that calcium carbonate and calcium acetate are associated with the progression of vascular calcification, a phenomenon that can be attenuated or arrested by sevelamer, a phosphorus binder that does not increase calcium levels and also reduces LDL-cholesterol [14,15].

The main objective of this study was to evaluate the usefulness of a simple vascular calcification score based on plain radiographic films, for prediction of cardiovascular mortality in HD patients. Secondary objectives were to correlate this score with cardiovascular hospitalizations, fatal and non-fatal cardiovascular events, cardiovascular disease, with simple arterial properties [pulse pressure (PP) and mean arterial pressure (MAP)], and with calcium, phosphorus, calcium and phosphorus product and intact parathormone (iPTH) levels.

Patients and methods

Study design

An observational, prospective, single-centre study of a cohort of prevalent patients treated with HD was used.

Population

One hundred and twenty-three patients, 75 males and 48 females, treated with low flux HD, without previous parathyroidectomy, constituted the study population (Table 1); 25 patients (20.3%) were diabetic. On the day of the vascular score evaluation, mean age was 62 ± 14 years (24–91) and mean HD duration was 46.6 ± 52 months (6–271). During an observational period of 37 months, 36 patients died and one patient received a renal transplant. Mean follow-up was 32 ± 9 months (4–37).

The primary endpoint after 37 months was cardiovascular mortality. Secondary endpoints were cardiovascular hospitalizations, fatal and non-fatal cardiovascular events, and the diagnosis of vascular disease (coronary artery disease and/or cerebral vascular disease and/or peripheral arterial disease). The diagnosis of vascular disease was based on a query answered by the attending physicians of the study patients and was dependent on clinical manifestations. Coronary artery disease was diagnosed if the patient had developed typical angina pectoris, had a positive stress test, suffered a myocardial infarction, or underwent a percutaneous coronary intervention or coronary bypass surgery; diagnosis of cerebral vascular disease was based on the occurrence of

Table 1. Univariate analysis

	All patients	Score ≥3	Score <3	Significance (*P*)
Number of patients	123	65 (53%)	58 (47%)	NS
Age (years)	62.9 (14.5)	64.5 (12.8)	61.3 (16.2)	NS
HD (months)	46.6 (52.)	53.0 (57.1.)	39.4 (44.1)	NS
Male gender (%)	75 (61%)	51 (79%)	24 (41%)	
Female gender (%)	48 (39%)	14 (22%)	34 (58%)	<0.001
Diabetes (%)	25 (20%)	18 (28%)	7 (12%)	0.02
Ca (mg/dl)	9.9 (0.7)	9.9 (0.7)	9.8 (0.9)	NS
P (mg/dl)	4.9 (1.4)	4.8 (1.5)	4.9 (1.3)	NS
CaXP $(mg/dl)^2$	48.8 (15.4)	49 (16)	49 (15)	NS
iPTH (pg/ml)	302 (379)	292 (354)	313(408)	NS
Al	18.6 (7.0)	18.8 (7.2)	18.1 (6.7)	NS
Albumin (g/dl)	3.72 (0.36)	3.76 (0.32)	3.68 (0.28)	NS
Haemoglobin (g/dl)	11.3 (1.2)	11.5 (1.4)	11.2 (1.1)	NS
Kt/v	1.38 (0.19)	1.37 (0.18)	1.42 (0.21)	NS
PP (mmHg)	67.3 (17)	69.0 (18)	65.4 (15.7)	NS
MAP (mmHg)	105.3 (14)	107.4 (14.9)	102.9 (12.4)	NS
Initial CAD (%)	32 (26%)	20 (31%)	12 (21%)	NS
Final CAD (%)	43 (35%)	28 (43%)	15 (26%)	0.03
Initial CVD (%)	6 (5%)	4 (6%)	2 (3%)	NS
Final CVD (%)	16 (13%)	11 (17%)	5 (9%)	NS
Initial PAD (%)	16 (13%)	12 (19%)	4 (7%)	0.06
Final PAD (%)	33 (27%)	28 (43%)	5 (9%)	<0.001
Initial VASCD (%)	36 (29%)	22 (34%)	14 (24%)	NS
Final VASCD (%)	61 (50%)	43 (66%)	18 (31%)	<0.001
CV deaths (%)	17 (14%)	14 (22%)	3 (5%)	0.008
CV hospitalizations (%)	28 (23%)	22 (34%)	6 (10%)	0.002
CV events (%)	32 (26%)	24 (37%)	8 (14%)	0.004

Results in mean values (SD); CAD, coronary arterial disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; VASCD, vascular disease; CV, cardiovascular; NS, not significant.

stroke or transient ischaemic attack or the detection of an old cerebral infarction using computed tomography; peripheral arterial disease was diagnosed if there was claudication, ischaemic ulcers, lower limbs amputation, revascularization or diagnosis of obstruction by Doppler or angiography. Vascular disease diagnosis was performed in two steps: at baseline and at the end of the follow-up. For each patient, hospitalizations during the observational period were classified as cardiovascular, non-cardiovascular or absent. Calcium, phosphorus, haemoglobin, albumin and Kt/V were evaluated every month and iPTH and aluminum every 3 months, in the 6 months that preceded vascular calcification score evaluation. Levels of iPTH were determined by a first generation immunochemiluminometric assay. PP and MAP were evaluated once a month, based on blood pressure (BP) measurement before HD, on the day of the blood chemistry analysis, during the same 6 months period. PP was calculated by the formula $PP = SBP - DBP$; $MAP = DBP + (SBP - DBP)/3$ (SBP, systolic blood pressure; DBP, diastolic blood pressure).

HD duration was evaluated on the day of vascular calcification assessment. A simple vascular calcification score was evaluated in all patients during a 4 month period and marked the beginning of the study for each patient. This vascular calcification score was evaluated in plain radiographic films of pelvis and hands, performed in the same centre. The pelvis radiographic films were divided into four sections by two imaginary lines: a horizontal line over the upper limit of both femoral heads and a median vertical line over the vertebral column. The films of the hands were divided, for each hand, by a horizontal line over the upper limit of the metacarpal bones. The presence of linear calcifications in each section was counted as 1 and its absence as 0. The final score was the sum of all the sections, ranging from 0 to 8. Vascular calcifications were deliberately evaluated only in muscular arteries: iliac, femoral, radial and digital. Pelvis films evaluated iliac and femoral arteries (Figure 1); hand films evaluated radial and digital arteries (Figure 2). The analysis of all the radiographic films was performed by one single experienced clinician blinded to patient information. Only linear calcifications, with or without patchy calcifications, were considered for the final calcification score, because they outline the vessel wall and have undoubtedly vascular localization. Patchy isolated calcifications that may be associated with intimal calcifications were not considered in this score because they may be confused with other types of extra-vascular calcifications, for instance, phleboliths.

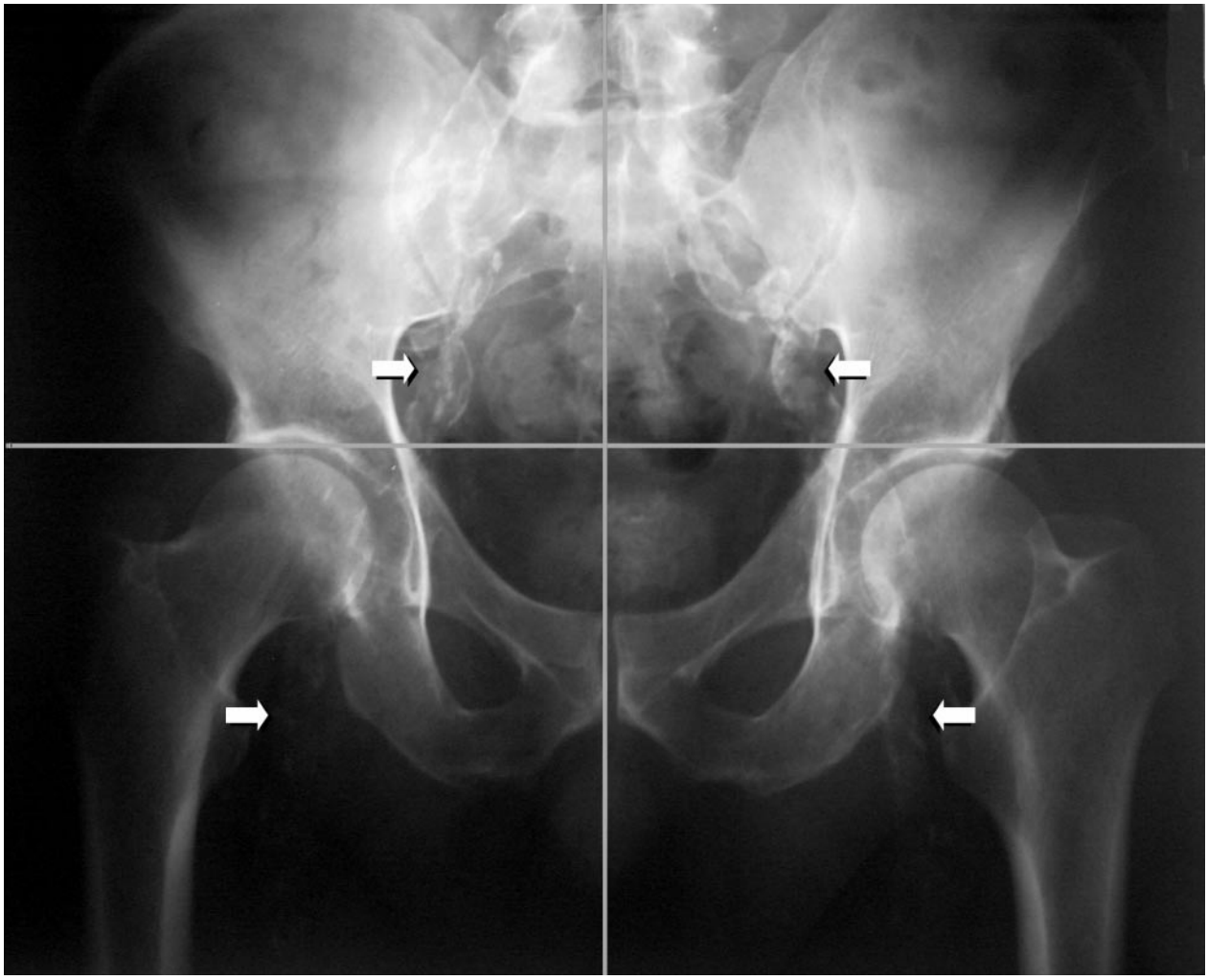

Fig. 1. Calcification score is the sum of the presence (1) or absence (0) of parallel linear calcifications in each section. In the example, pelvis score $= 1 + 1 + 1 + 1 = 4$.

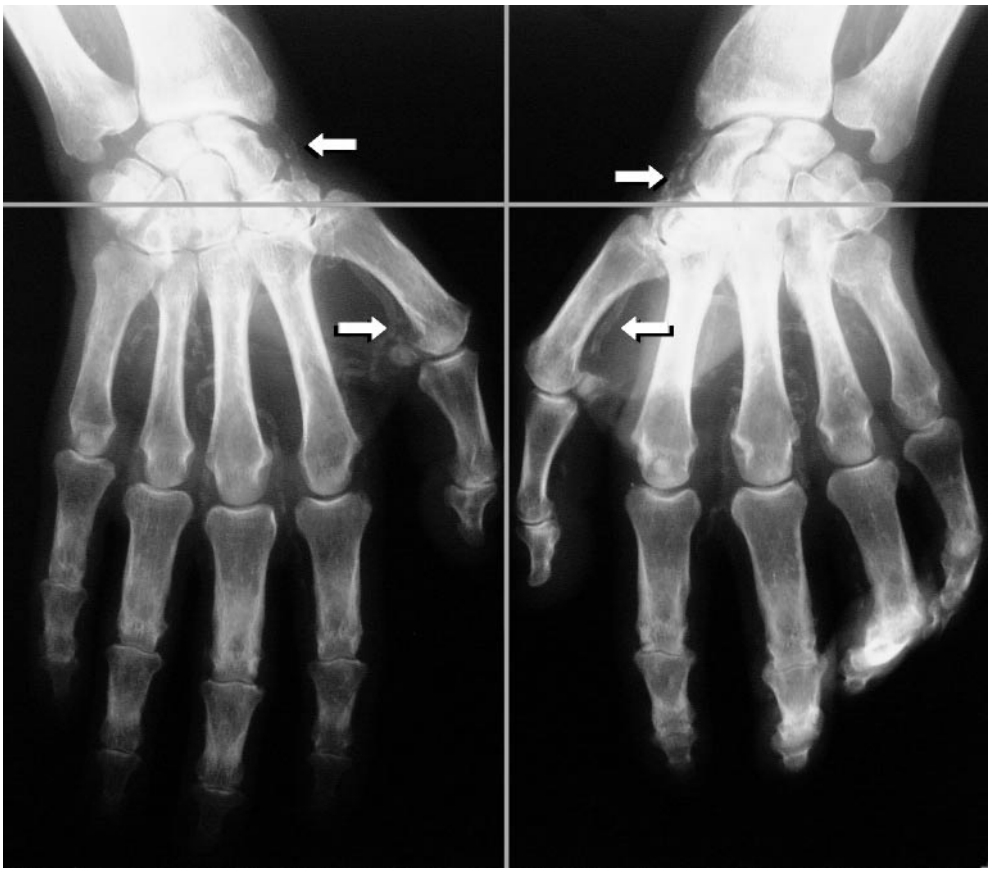

Fig. 2. Hands score in this example is 4; total score is the sum of pelvis and hands score [8].

Statistics

Variables were expressed as frequencies, percentages for discrete factors, and mean value for normally distributed continuous factors. Statistical comparison of baseline characteristics and endpoints was performed using the two-tailed chi-square test with Yates' correction or Fisher exact test when appropriate, for categorical variables, and the two-tailed Student *t*-test for continuous variables. Kaplan–Meier survival curves of patients with vascular calcification score ≥ 3 and <3 were compared by log-rank test. The independent variables associated with cardiovascular death, cardiovascular hospitalizations and fatal and non-fatal cardiovascular events were identified by Cox regression models. The covariates in Cox regression models were age, sex, HD duration, diabetes, CaXP, iPTH, albumin, vascular disease at baseline and vascular score. The independent variables associated with PP and MAP were identified by linear regression models. Variables included in these models were: age, sex, diabetes, HD duration, albumin, Ca, P, CaXP, iPTH and vascular calcification score. The independent variables associated with vascular calcifications, vascular disease at baseline and vascular disease at the end of follow-up were identified by binary logistic regression models. Covariates for vascular calcification analysis were: age, sex, diabetes, HD duration, albumin, Ca, P, CaXP and iPTH. Covariates for vascular disease analysis were the above plus PP, MAP and vascular calcification score. To identify those patients at highest risk for the study endpoint, the vascular calcification score values and the corresponding endpoint rates were related via a receiver operating characteristic (ROC) curve. The value associated with the highest accuracy was considered as the cut-off point for defining an elevated cardiovascular risk. The risk estimates for death, cardiovascular hospitalizations and fatal and non-fatal cardiovascular events were the adjusted hazard ratios (HRs) obtained by Cox regression. Statistical analyses were performed with the SPSS system 10.0 (SPSS Inc., Chicago, IL) and the Medcalc program version 6.0 (Medcalc software; Mariakerke, Belgium). For all comparisons, a *P*-value <0.05 was considered statistically significant.

Results

During an observational period of 37 months, there were 36 all-cause deaths and 17 cardiovascular deaths; 28 patients needed cardiovascular hospitalizations, and 32 patients suffered fatal or non-fatal cardiovascular events. At baseline, clinical vascular disease was diagnosed in 36 patients (29%): coronary artery disease in 32 patients (26%), peripheral arterial disease in 16 patients (13%) and cerebral vascular disease in six patients (5%); at the end of the follow-up coronary artery disease had been diagnosed in 43 patients (35%), peripheral arterial disease in 33 patients (27%), cerebral vascular disease in 16 patients (13%) and vascular disease in 61 patients (50%) (Table 1). Cardiovascular

death was associated with coronary artery disease in 12 patients, peripheral arterial disease in three patients and cerebral vascular disease in two patients. Coronary artery disease in 14 patients, peripheral arterial disease in 12 patients and cerebral vascular disease in three patients led to cardiovascular hospitalizations. One patient experienced two hospitalization episodes, one for coronary artery disease and one for cerebral vascular disease.

Vascular calcification score frequency

Anatomical calcification distribution was the following: iliac calcifications were present in 75 patients (61%), femoral calcifications in 74 patients (60%), radial calcifications in 45 patients (36.6%) and digital calcifications in six patients (5%). The distribution of vascular calcification score in the 123 patients was the following: score 0 in 31 patients (25.2%), score 1 in eight patients (6.5%), score 2 in 19 patients (15.4%), score 3 in two patients (1.6%), score 4 in 25 patients 20.3%, score 5 in eight patients (6.5%), score 6 in 26 patients (21.1%), score 7 in zero patients and score 8 in four patients (3.3%). This parameter does not present a normal distribution mainly due to the less frequent cases of unilateral vascular calcification: unilateral iliac calcifications in three patients, unilateral femoral calcifications in five patients, unilateral radial calcifications in 12 patients and unilateral digital calcifications in one patient.

By ROC curve analysis, the vascular calcification scores of the four anatomical regions (iliac, femoral, radial and digital) showed a similar relationship with cardiovascular mortality: iliac score >0 (AUC $=0.613$; 95% CI 0.522–0.699); femoral score >0 (AUC $=0.630$; 95% CI 0.539–0.715); radial score >0 (AUC $=0.604$; 95% CI 0.512–0.690); digital score >0 (AUC $=0.589$; 95% CI 0.498–0.677).

Univariate analysis

ROC curve analysis identified vascular calcification score ≥ 3 as the best cut-off value associated with cardiovascular mortality (AUC $=0.716$; 95% CI 0.629–0.793) and cardiovascular events (AUC $=0.687$; 95% CI 0.598–0.766). The vascular score ≥ 3 was measured in 65 patients (53%) (Table 1) and was more frequent among men and diabetic patients. Furthermore, a vascular score ≥ 3 was more frequently associated with cardiovascular hospitalizations, cardiovascular mortality and fatal and non-fatal cardiovascular events, coronary artery disease, peripheral arterial disease and vascular disease at the end of the follow-up period (Table 1). By univariate analysis, there were no significant differences between higher score and lower score patients with regards to age, HD duration, calcium, phosphorus and iPTH levels (Table 1). By Kaplan–Meier analysis, the cumulative hazard for cardiovascular death at 37 months was higher in patients with a vascular score ≥ 3: 23 *vs* 5%, log-rank $=5.7$; $P=0.01$ (Figure 3).

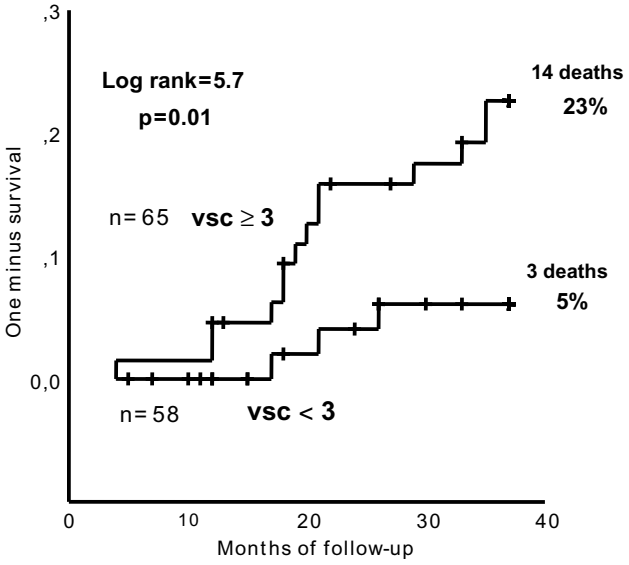

Fig. 3. Higher cardiovascular death risk in patients with vascular calcification score ≥ 3.

Multivariate analysis

Evaluation of the association of calcium, phosphorus and iPTH with vascular calcification score and vascular disease. Calcium, phosphorus and iPTH levels were evaluated during the 6 months that preceded the vascular calcification score assessment. Calcium levels were independently associated with iliac calcifications ($P=0.03$) (Table 2) and with PAD ($P=0.01$) (Table 3); phosphorus levels were independently associated with CAD ($P=0.01$) (Table 3). PTH levels were not correlated with vascular calcifications or with vascular disease in these patients.

Factors independently associated with PP and MAP. By multiple linear regression, PP was associated with diabetes ($B=12.8$; 95% CI $=5.8$–19.7; $P<0.001$) and correlated with age ($B=0.33$; 95% CI $=0.13$–0.52; $P=0.001$). MAP was associated with a vascular calcification score ≥ 3 (B $=5.6$; 95% CI $=0.8$–10.4; $P=0.02$). PP was an independent predictor of PAD ($P<0.001$) (Table 3).

Factors independently associated with vascular calcification score. Binary logistic regression (Table 2) showed that a vascular calcification score ≥ 3 was associated with diabetes ($P=0.01$), male sex ($P<0.001$), age ($P=0.02$), HD duration ($P=0.02$) and MAP ($P=0.03$). There was no correlation between final vascular score and calcium metabolism factors, but when analysing the different vascular calcification regions, in the same model, calcium levels were independently associated with iliac calcifications ($P=0.03$) (Table 2).

Factors independently associated with cardiovascular morbidity and mortality. By binary logistic regression (Table 3), vascular disease at the end of the follow-up was associated with vascular calcification score

Table 2. Vascular calcification score: multivariate analysis

Dependent variable	Independent variable	Risk	CI (95%)	Significance (*P*)
Vsc ≥3	Diabetes	4.2	1.4–13.1	0.01
	Male gender	7.47	2.9–19.1	0.000
	Age	1.04	1.008–1.077	0.02
	HD duration (months)	1.01	1.002–1.021	0.02
	MAP	1.04	1.004–1.074	0.03
Iliac score >0	Diabetes	4.6	1.4–14.7	0.01
	Male gender	3.5	1.5–8.3	0.004
	Age	1.04	1.012–1.072	0.005
	Calcium	1.8	1.050–3.143	0.03

Vsc, vascular calcification score.

Table 3. Cardiovascular morbidity and mortality: multivariate analysis

Dependent variable	Independent variable	Risk	CI (95%)	Significance (*P*)
Final CAD	Vsc >0	1.3	1.091–1.555	0.003
	Phosphorus	1.5	1.094–1.967	0.01
Final PAD	Vsc >0	1.7	1.278–2.259	0.000
	Diabetes	13.7	3.132–56.360	0.000
	PP	1.1	1.024–1.101	0.001
	Calcium	2.9	1.205–7.343	0.02
Final VASCD	Vsc >0	1.4	1.168–1.698	0.000
	Diabetes	3.3	1.071–10.044	0.04
	Age	1.04	1.008–1.074	0.01
CV hospitalizations	Vsc >0	1.3	1.093–1.554	0.003
CV events	Vsc >0	1.2	1.054–1.446	0.009
CV death	Vsc >0	1.4	1.107–1.803	0.01

Final, at the end of the follow-up; CAD, coronary artery disease; PAD, peripheral artery disease; VASCD, vascular disease; CV, cardiovascular; Vsc, vascular calcification score.

($P < 0.001$), age ($P = 0.01$) and diabetes ($P = 0.04$); coronary artery disease at the end of the follow-up was associated with phosphorus levels ($P = 0.01$) and vascular calcification score ($P = 0.003$); peripheral artery disease at the end of the follow-up was associated with diabetes ($P < 0.001$), vascular calcification score ($P < 0.001$), PP ($P = 0.001$) and calcium levels ($P = 0.02$). Vascular disease at baseline was not associated with any of these factors. Fatal and non-fatal cardiovascular events were associated with vascular calcification score ($P = 0.009$); cardiovascular hospitalizations were associated with vascular calcification score ($P = 0.003$); cardiovascular mortality was associated with vascular calcification score ($P = 0.01$). All-cause mortality was inversely associated with albumin levels ($P < 0.001$).

For a better evaluation of the cardiovascular risk, a vascular calcification score cut-off defined by ROC curve analysis was applied to the same statistical tests. The cardiovascular risk evaluation was the HR adjusted for sex, age, HD duration, diabetes, calcium and phosphorus product, iPTH levels and albumin. In patients with a vascular calcification score ≥3, the HR was 2.8 (95% CI 1.151–7.012; $P = 0.02$) for cardiovascular hospitalizations, 2.3 (95% CI 1.044–5.182; $P = 0.04$) for fatal and non-fatal cardiovascular events, and 3.9 (95% CI 1.108–13.422; $P = 0.03$) for cardiovascular mortality (Figure 4). In patients with a vascular calcification score ≥3 the odds ratio was 3.2 (95% CI 1.358–7.448; $P = 0.008$) for association with CAD, was 10.4 (95% CI 2.676–40.381; $P = 0.001$) for association with PAD and was 4.3 (95% CI 1.884–9.929; $P = 0.001$) for association with vascular disease (Figure 4).

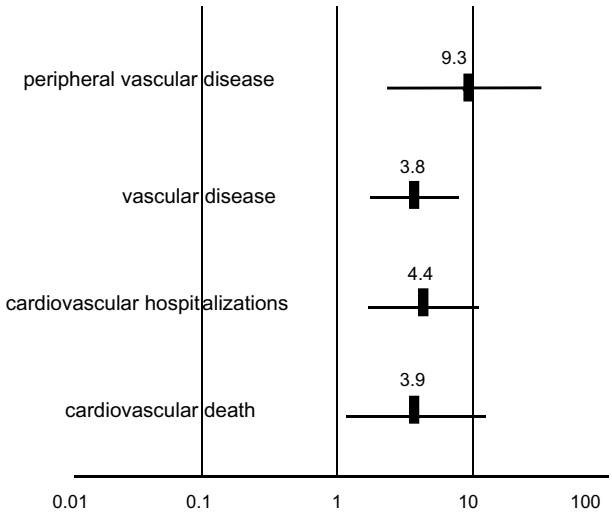

Fig. 4. Cardiovascular risk for vascular calcification score ≥3.

In summary, this simple vascular calcification score was independently associated with coronary artery disease, peripheral artery disease and vascular disease present at the end of the follow-up. A vascular calcification score ≥ 3 was an independent predictor of cardiovascular mortality, cardiovascular hospitalizations, and fatal or non-fatal cardiovascular events.

Discussion

Vascular calcifications in HD patients have already been related to arterial stiffness [7,11,16], cardiovascular disease [8,10] and cardiovascular mortality [7,11], and are more common than in the general population [8]. These extensive vascular calcifications, found even in young HD patients [9], may represent one of the factors contributing to the extremely high cardiovascular mortality for HD patients when compared with the general population [1].

The diagnosis of vascular calcification is usually made with very expensive and highly technical devices like electron beam computed tomography or multislice computed tomography [8,17,18]. The use of plain radiographic films of bone has already been suggested in the recent KDOQI clinical practice guidelines for bone metabolism and disease [19], not for bone disease evaluation but for vascular calcification assessment. In our score, vascular calcifications were deliberately evaluated only in muscular arteries: iliac, femoral, radial and digital, because muscular arteries are more prone to linear calcification in contrast with elastic arteries that are more prone to intimal calcification. Our objective was to have a simple tool for the evaluation of peripheral muscular arteries calcifications. Lower limb arteries were not chosen because of the high prevalence of amputations in HD patients. The radial artery was included in our score based on a previous evaluation by Mourad *et al.* [20] who demonstrated that the radial artery, a muscular artery devoid of atherosclerosis, had an increased stiffness that was independent of the BP level. Intimal calcifications have already been differentiated from medial calcifications by plain radiography and these different calcification patterns have been associated with different cardiovascular and all-cause mortality results [13]. In our score, only linear calcifications that outline the vessel wall, with or without patchy calcifications, were considered because they have an obvious vascular origin. Patchy isolated calcifications were not considered because of their possible non-vascular origin. These linear calcifications may correspond to the linear railroad calcifications described by Lehto *et al.* [12] and by London *et al.* [13] and identified by these authors as medial calcifications. However, vascular calcifications were evaluated in four vascular territories, and medial and intimal calcifications may coexist in the same patient and in the same vessel. Our vascular score does not exclude association of intimal and medial calcifications. This would explain why this score was a good predictor of cardiovascular mortality. In another study, this vascular calcification score was also correlated in univariate and multivariate analysis with pulse wave velocity measured by Complior® [21]. This finding confirms the value of this simple score, suggesting that linear calcifications identified by this method are associated with changes of the arterial wall properties. This calcification score that we have developed is a very simple tool, easy to use by the attending physician without assistance of a radiologist and may help identify patients at higher cardiovascular risk.

Changes of arterial wall properties with age are associated with SBP increase and DBP decrease. PP increase has been established as a cardiovascular disease risk factor in the general population [22] and in HD patients as well [23,24]. In our study, PP in multivariate analysis was not correlated with vascular calcification score but was correlated with age and diabetes and was independently associated with peripheral arterial disease. However, MAP, which is related to cardiac output and vascular resistance, was independently associated with a vascular calcification score ≥ 3. The fact that BP was evaluated immediately before an HD procedure, in a clinical situation of hypervolaemia, may have contributed to this result.

It has already been shown that PTH levels are overestimated with the common iPTH assays because they detect not only PTH (1–84), but also evaluate C-terminal fragments, which can have inhibitory activity. Newer 'whole PTH' assays can detect exclusively PTH (1–84), but the predictive power of these new assays is still unknown [19]. In the present study, vascular calcification score was independent of PTH values. This has already been demonstrated in other studies [8,16]. Hyperphosphataemia and high calcium phosphate product are predictors of mortality in HD patients [25]. In our study, there is some evidence for a relationship between calcium or phosphorus levels and vascular disease: phosphorus levels were correlated with coronary artery disease and calcium levels were correlated with peripheral arterial disease. The final vascular calcification score was not correlated with calcium, phosphorus or iPTH levels, but calcium levels were correlated with the presence of iliac calcifications. Similarly, Guérin *et al.* [16] did not find any association between a semi-quantitative vascular calcification score evaluated by B-mode ultrasonography and calcium, phosphorus and iPTH levels [16]. Previous hyperphosphataemia episodes that would have initiated this vascular calcification process may have been missed because of the time limited monitoring phase in these patients.

Vascular disease, present at the end of the follow-up, correlated with the vascular calcification score. Conversely, clinical vascular disease diagnosed at baseline did not predict vascular mortality or vascular events and did not correlate with the vascular calcification score. Vascular disease is oligosymptomatic in this population and sub-clinical vascular disease was not diagnosed at baseline, reflecting what happens in current practice. As many patients do not have a very

active life style, symptoms of cardiovascular disease may be delayed, explaining the absence of correlation of clinical vascular disease at baseline with cardiovascular death and cardiovascular events. Clinical evidence of vascular disease at the end of the follow-up, however, was strongly associated with the vascular calcification score. This association strongly supports the usefulness of this simple vascular tool at a stage of sub-clinical vascular disease.

Limitations of this vascular calcification score

This vascular calcification score is not quantitative and therefore is not adequate for accurately assessing the progression of calcifications, as opposed to the scores evaluated by electron beam computed tomography [14,15]. However, in a retrospective study of long-term HD patients where the authors also used a vascular calcification score based on radiographic films [26], it was possible to evaluate regression and aggravation of the calcifications over time.

Conclusion

Cardiovascular morbidity and mortality in this cohort of HD patients were related, among other factors, to a simple vascular calcification score evaluated by plain radiographic films of pelvis and hands. This simple vascular calcification score was independently associated with coronary artery disease, peripheral artery disease and vascular disease diagnosed at the end of the follow-up. Male sex, diabetes, age, HD duration and MAP were independently associated with a vascular calcification score ≥ 3. A vascular calcification score ≥ 3 was an independent predictor of cardiovascular mortality, cardiovascular hospitalizations, and fatal or non-fatal cardiovascular events. This vascular calcification score represents a simple and inexpensive tool for assessing the cardiovascular risk related to vascular calcifications in HD patients.

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CAPÍTULO 4

CALCIFICAÇÃO VASCULAR E RIGIDEZ ARTERIAL

A rigidez arterial resulta da modificação das propriedades da parede arterial e é preditora de risco cardiovascular nos doentes com e sem doença renal crónica[1,2]. Diversos fatores contribuem para o aumento da rigidez arterial, como idade avançada, diabetes, hipertensão arterial e calcificações vasculares. Nos doentes em diálise, todos estes fatores apresentam elevada prevalência, e a rigidez arterial é um achado comum nesta população. Em estudos anteriores, a rigidez arterial foi associada a maior risco de mortalidade[2], mas esta associação não é universal[3].

As calcificações vasculares provocam uma diminuição da elasticidade da parede da aorta responsável por uma onda de reflexão mais precoce, encontrando ainda a válvula aórtica aberta. Esta ocorrência contribui para o aumento do volume sistólico com consequente hipertensão sistólica e diminuição da pressão diastólica. O aumento da pressão sistólica contribui para o aumento da pós-carga cardíaca e para o desenvolvimento da hipertrofia ventricular esquerda. A diminuição da pressão diastólica pode comprometer a perfusão coronária que se faz predominantemente durante a diástole[4]. Objetivamente, o aumento da pressão sistólica e a diminuição da pressão diastólica traduzem-se por um índice clínico facilmente mensurável, o aumento da pressão de pulso, que se avalia simplesmente pela diferença aritmética entre o valor da pressão sistólica e da pressão diastólica. Na população geral já foi demonstrado que, em doentes com idade superior a 60 anos, é o aumento da pressão de pulso que se associa a maior risco cardiovascular, enquanto em indivíduos mais novos o aumento da pressão diastólica é que apresenta maior associação ao risco cardiovascular[5]. Também em doentes em diálise o aumento da pressão de pulso avaliado no início ou no final de uma sessão de hemodiálise se associou a maior mortalidade em doentes não diabéticos[6] ou em todos os doentes[7].

O grupo de trabalho de imagiologia da iniciativa KDIGO 2006[8] propôs a investigação clínica de diversas questões, na altura ainda sem resposta, entre as quais a análise da associação entre rigidez arterial avaliada por velocidade de onda de pulso e pressão de pulso e a existência de calcificações vasculares avaliadas por métodos radiológicos. Em 2000, Guérin *et al* tinham sido os primeiros a demonstrar que calcificações vasculares avaliadas por ecografia em grandes artérias se associavam a rigidez arterial nos doentes em diálise[9]. Após a proposta de investigação

clínica feita pela KDIGO 2006, Raggi *et al* analisaram a associação entre a rigidez arterial avaliada por velocidade de onda de pulso carotidofemoral com calcificações coronárias e da aorta abdominal[10]. As calcificações coronárias foram avaliadas por tomografia computorizada de feixe de eletrões e as calcificações da aorta abdominal por RX simples utilizando um *score* de calcificação semiquantitativo descrito por Kauppila[11]. Neste estudo verificou-se que as calcificações da aorta abdominal se associaram a um aumento da velocidade de onda de pulso carotidofemoral. Em análise multivariada, as calcificações coronárias não se associaram ao aumento de rigidez arterial.

Em 2005, apresentamos, no XVII Congresso Português de Nefrologia, um estudo em que verificamos que a velocidade de onda de pulso era preditora de mortalidade num grupo de doentes em hemodiálise e em que demonstramos que o *score* de calcificação vascular simples se associava à velocidade de onda de pulso. Este estudo ganhou o prémio para a melhor comunicação oral na área da hemodiálise desse ano e foi apresentado como *poster* na 38th Annual Renal Week Meeting, da Sociedade Americana de Nefrologia. Aceitando a proposta da iniciativa KDIGO 2006, aprofundamos este estudo para poder responder a todas as questões levantadas e dispúnhamos já de um tempo de seguimento que nos permitiu também avaliar a associação entre a rigidez arterial e a mortalidade. A rigidez arterial foi avaliada por velocidade de onda de pulso carotidofemoral com um método não invasivo usando o Complior (Artech Medical, Pantin, France) e pela pressão

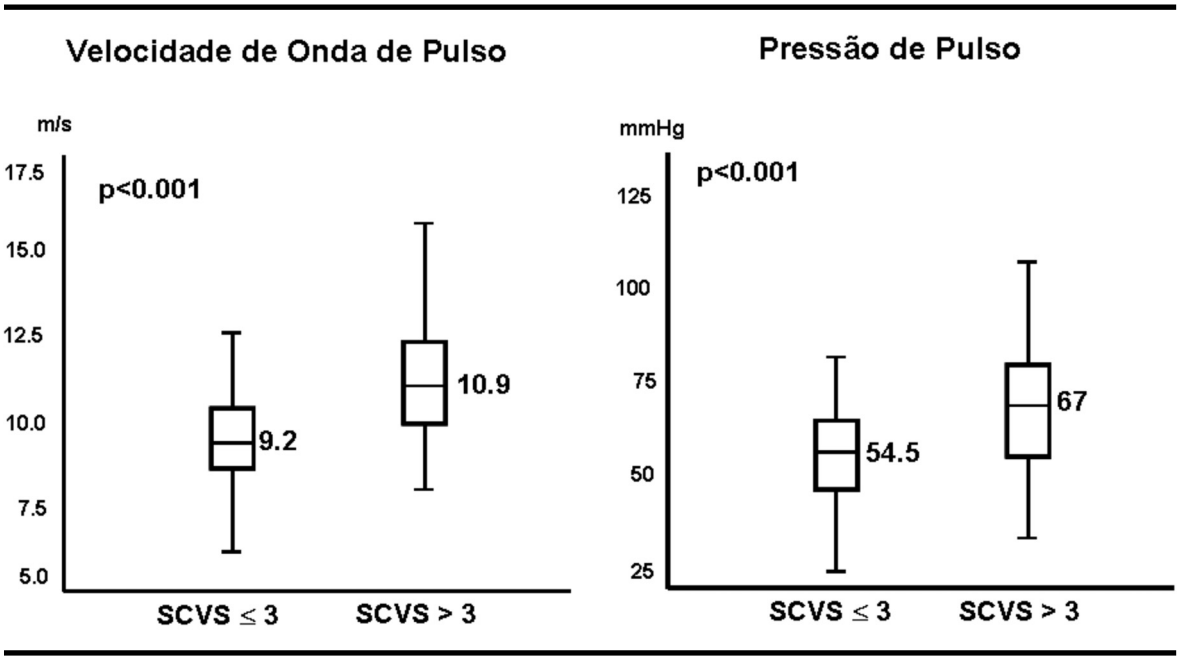

Fig. 4.1. Um *score* de calcificação vascular simples (SCVS) > 3 associou-se a valores mais elevados de velocidade de onda de pulso e de pressão de pulso (teste *T* de S*tudent*)

de pulso. A pressão de pulso, calculada pela diferença entre a pressão sistólica e a pressão diastólica, correspondeu ao valor médio da pressão de pulso medida no início de uma sessão de hemodiálise durante os 6 meses que precederam a medição da velocidade de onda de pulso. Nos nossos doentes verificamos que o *score* de calcificação vascular simples superior a 3 se associou a valores mais elevados da velocidade de onda de pulso e da pressão de pulso (Fig. 4.1).

Esta análise foi ajustada para idade, duração de hemodiálise, diabetes, níveis de colesterol, pressão sistólica, índice de massa corporal e doses de calcitriol e de carbonato de cálcio. A idade e a pressão sistólica foram os outros fatores preditores da velocidade de onda de pulso. A diabetes *mellitus* e a dose de carbonato de cálcio foram igualmente preditores da pressão de pulso. Verificamos ainda que valores de pressão de pulso superiores a 70 mmHg, valores de velocidade de onda de pulso superiores a 10,4 m/s e um *score* de calcificação vascular simples superior a 3 se associaram a maior mortalidade (Fig. 4.2), com semelhante área sob a curva na análise de curvas ROC (*receiver operating characteristic*) (Fig. 4.3).

Em análise multivariada, o risco de morte ajustado para inúmeras variáveis foi semelhante para estes três fatores (Tabela 4.1), mas a velocidade de onda de pulso foi preditora de mortalidade apenas nos doentes não diabéticos. Este achado pode ser explicado pelo facto de, nos doentes diabéticos, a mortalidade ter sido elevada e ter-se verificado tanto nos doentes com velocidade de onda de pulso alta como normal.

Calcificações vasculares, rigidez arterial e mortalidade

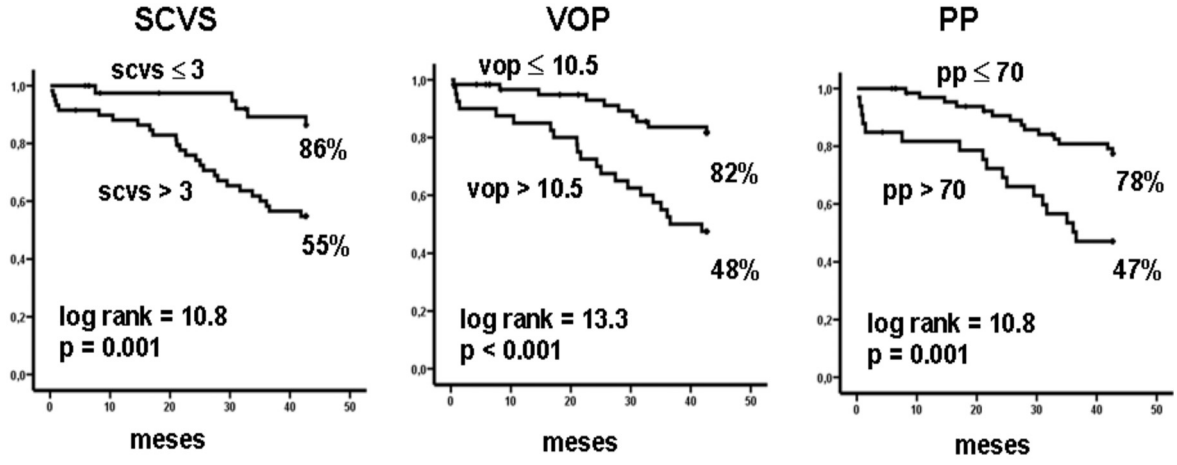

Fig. 4.2. Um SCVS >3, uma velocidade de onda de pulso (VOP) >10,4 m/s e uma pressão de pulso (PP) > 70 mmHg associaram-se a menor sobrevida (Kaplan-Meier)

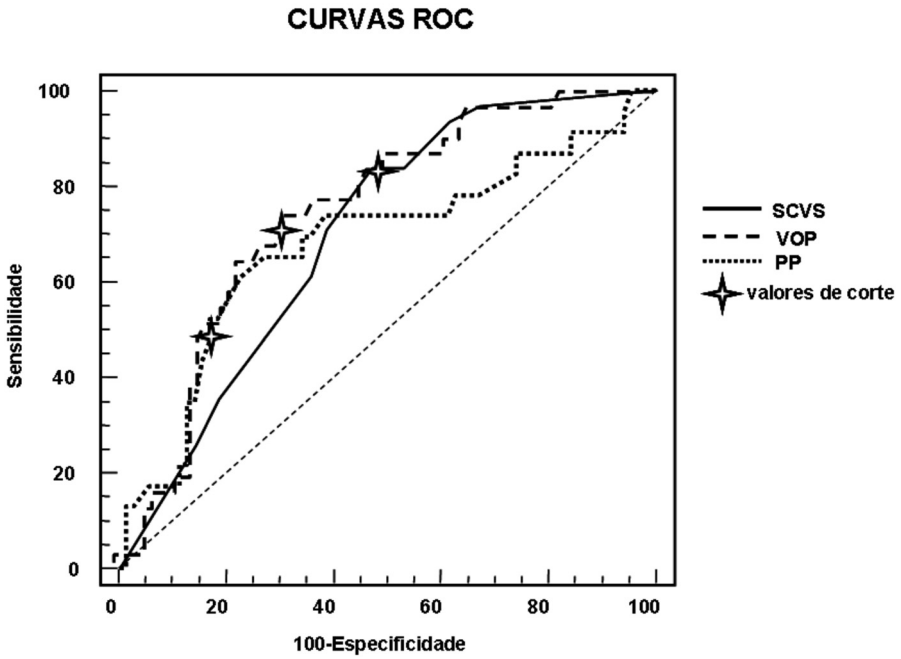

Fig. 4.3. A área sob a curva em relação à mortalidade foi semelhante para o *score* de calcificação vascular simples (SCVS), para a velocidade de onda de pulso (VOP) e para a pressão de pulso (PP) (análise de curvas ROC)

Risco de morte avaliado por *Cox regression*

		B	HR	IC 95%	Sig.
Todos os doentes	SCVS >3	1,196	3,308	1,109-9,863	0,032
Todos os doentes	PP >70 mmHg	1,171	3,227	1,114-9,347	0,031
Não diabéticos	VOP > 10,5 m/s	1,092	2,981	1,013-8,775	0,047

HR, *hazard ratio***; IC, intervalo de confiança.**

Tabela 4.1. HR ajustado para idade, duração de hemodiálise, diabetes *mellitus*, doença vascular prévia, índice de massa corporal, pressão arterial sistólica e doses de carbonato de cálcio e de calcitriol

Em resumo, demonstramos que calcificações vasculares avaliadas por RX simples se associam a um aumento da rigidez arterial. Este estudo foi publicado na revista *Nephrology Dialysis and Transplantation* em 2009[12] e foi um dos estudos selecionados pelas *guidelines* KDIGO 2009 para mostrar a associação entre calcificações vasculares e mortalidade, confirmando assim a utilidade e a validade do *score* de calcificação vascular simples na identificação dos doentes com mais elevado risco cardiovascular.

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A plain X-ray vascular calcification score is associated with arterial stiffness and mortality in dialysis patients

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Abstract

Background. Vascular calcifications are highly prevalent in dialysis patients and are associated with arterial stiffness and mortality. The use of simple and inexpensive methods to evaluate arterial stiffness and vascular calcifications is desired. The objective of this study was to evaluate the relationship of a simple vascular calcification score (SVCS) with pulse wave velocity (PWV) and pulse pressure (PP) and to evaluate their association with all-cause mortality.

Methods. 101 haemodialysis patients (71 men; 19% diabetic) were evaluated. At baseline, arterial stiffness was measured by PP and by PWV with Complior. SVCS was evaluated in plain X-ray of pelvis and hands.

Results. During a 43-month observational period, 31 patients died. By Kaplan–Meier analysis, SVCS >3 ($P = 0.001$), PP > 70 mmHg ($P = 0.001$) and PWV > 10.5 m/s ($P < 0.001$) were found to be associated with lower cumulative survival. Adjusting for multiple variables, association with mortality was maintained for SVCS >3 (HR = 3.308, $P = 0.032$) and PP > 70 mmHg (HR = 3.227, $P = 0.031$) in all patients and for PWV > 10.5 m/s (HR = 2.981, $P = 0.047$) in non-diabetic patients. Age ($P < 0.001$), systolic pressure ($P = 0.004$) and SVCS > 3 ($P = 0.032$) were associated with PWV. Diabetes ($P = 0.031$), calcium carbonate dose ($P = 0.009$) and SVCS > 3 ($P = 0.012$) were associated with PP.

Conclusion. Higher SVCS, PWV and PP were associated with higher mortality in this population. SVCS was associated with arterial stiffness. Simple and inexpensive methods such as PP or SVCS may be used to detect mortality risk and to provide important information that may be relevant for guiding therapeutic intervention in dialysis patients.

Keywords: arterial stiffness; haemodialysis; mortality; vascular calcification

Introduction

It is clearly demonstrated that dialysis patients have a much higher cardiovascular mortality when compared with the general population [1]. This high cardiovascular risk in chronic kidney disease (CKD) patients is only partly explained by traditional risk factors [2]. Vascular calcifications evaluated by ultrasonography [3], plain X-ray [4], electron beam computed tomography [5] or multislice computed tomography [6] have been associated with mortality in dialysis patients. KDIGO has recommended a new classification for mineral and bone disorder of chronic kidney disease patients (CKD-MBD) that includes the evaluation of vascular calcifications [7]. Arterial stiffness is an alteration of the arterial wall properties with multiple causes, some of which are old age, diabetes, hypertension and medial calcification. All these features are highly prevalent in dialysis patients, and arterial stiffness is a common finding in this population. In dialysis patients, arterial stiffness has been associated with all-cause and cardiovascular mortality [8], but this finding is not universal [9]. One of the KDIGO Imaging Work Group research questions is the evaluation of the relationship between the radiological vascular calcification assessment and the measurement of vascular stiffness by pulse wave velocity (PWV) and pulse pressure (PP) [7]. Vascular calcifications evaluated by ultrasonography [10] and by plain X-ray [11] have already been associated with arterial stiffness in dialysis patients. We developed a simple vascular calcification score (SVCS) evaluated in plain X-ray of pelvis and hands that was a predictor of cardiovascular mortality and was associated with higher risk of coronary disease, peripheral artery disease and cardiovascular hospitalizations [12]. The objective of this study was to evaluate the relationship of this SVCS with PWV and PP and to evaluate the association of vascular calcification and arterial stiffness with all-cause mortality in our patients.

Study design

This study was a cross-sectional analysis performed in a group of prevalent haemodialysis (HD) patients to evaluate the relationship between a SVCS evaluated in plain X-ray

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with PWV and PP. This group of patients was followed prospectively during a period of 43 months for evaluation of the impact of vascular calcifications and arterial stiffness in all-cause mortality.

Population

One hundred and one patients (71 men and 30 women), treated with HD for at least 6 months, with a mean age of 58.8 ± 15.5 (26–89) years and mean HD duration of 55.8 ± 54.8 (6–289) months, from a single HD unit, accepted to participate in this study that was approved by the institutional scientific board; 19 patients (18.8%) were diabetic. In the 6 months before PWV evaluation, 89 and 41 patients were treated with calcium carbonate and calcitriol, respectively. The mean calcium carbonate and calcitriol prescribed doses in these patients were 2.5 ± 1.3 [1–8] g/day and 1.1 ± 0.6 [0.25–3] μg/week, respectively. Oral or intravenous calcitriol was administered in 25 and 16 patients, respectively. No patients received sevelamer or cinacalcet as they were not available at the time of the study; 71 patients received anti-hypertensive treatment (one single medication in 25 patients and two different medications in 46 patients). At baseline, diagnosis of vascular disease was based on a query answered by the attending physicians, concerning previous clinical manifestations and test results, according to the usual standard of care. Coronary artery disease was diagnosed if the patient had a positive stress test, had suffered a myocardial infarction or had been submitted to a percutaneous coronary intervention or coronary bypass surgery. Diagnosis of cerebral vascular disease was based on the occurrence of stroke or transient ischaemic attack or the detection of an old cerebral infarction in computed tomography. Peripheral arterial disease was diagnosed if there was claudication, ischaemic ulcers, lower limb amputation, revascularization or diagnosis of obstruction by Doppler or angiography. Coronary artery disease was diagnosed in 20 patients (19.8%), peripheral artery disease was present in 16 patients (15.8%) and cerebral vascular disease was identified in 4 patients (4%). During a period of 43 months, 31 patients died and 8 patients received a kidney transplant. No patient was lost for follow-up.

Vascular calcifications and arterial stiffness

Vascular calcifications were evaluated at baseline by a single observer blind to clinical data, in plain X-ray of pelvis and hands by a method previously described in detail [12]. Pelvis films were divided into four sections by two imaginary lines: a horizontal line over the upper limit of both femoral heads and a median vertical line over the vertebral column. Hands films were divided for each hand by a horizontal line over the upper limit of the metacarpal bones. Pelvis films evaluated iliac and femoral arteries (ileo-femoral score) and hands films evaluated radial and digital arteries (hands score). Any vascular calcification lining the vessel walls, either in an irregular pattern or in a linear pattern, was considered. The presence of vascular calcifications in each section was rated as 1 and its absence as 0. The total vascular calcification score was the sum of all sections and ranged from 0 to 8. Arterial stiffness was evaluated by PWV and by PP. PWV was evaluated twice, at baseline, 24 h after a HD session, using a non-invasive automated device, Complior. Complior measures the propagation of the same individual pulse wave between two arterial points. The proximal and distal sensors were located in the carotid and femoral arteries, respectively. The velocity of the pulse wave is calculated with the formula distance/time, where the distance corresponds to the distance between the suprasternal notch and the femoral artery pulse at the groin, and the time corresponds to the time that takes a pulse wave that originates in the heart to reach the femoral artery. The Complior software calculates the velocity of conduction. PP was evaluated by the formula (systolic blood pressure − diastolic blood pressure). The mean values of PP were time averaged for the 6-month period preceding PWV evaluation, corresponding to the mid-week HD session in the day of the monthly blood sample collection. PP was calculated from the predialysis evaluation of blood pressure.

Biochemical analysis

Mid-week Kt/V and predialysis serum levels of the following biochemical parameters were evaluated and time averaged for the 6 months preceding the evaluation of PWV. Kt/V, Ca, P, Ca × P product, alkaline phosphatase, albumin and C-reactive protein (CRP) were evaluated every month. Ca levels were adjusted to albumin levels. Total intact iPTH (iPTH) was evaluated every 3 months by immunochemiluminescence (three evaluations per patient) using a second generation assay, Elecsys 2010 from Roche Diagnostics, Basel, Switzerland. CRP was evaluated with an immunoturbidimetric assay. Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were evaluated twice.

Statistical analysis

Data are presented as frequencies for categorical variables and mean values with SD for continuous variables. Comparison between groups was performed by the independent samples *t*-test, chi-square and Fisher exact test when appropriate. Correlation was performed using the Pearson correlation coefficient. Survival curves were estimated by Kaplan–Meier analysis and compared by the log-rank test. A Cox regression model was used to identify predictors of mortality. Variables in this model, besides the vascular calcification score, PWV and PP, were age, HD duration, diabetes, presence of previous vascular disease, body mass index (BMI), systolic pressure and calcitriol and calcium carbonate doses. Separate Cox regression models using the enter method were applied to evaluate the adjusted hazard ratio mortality of vascular calcification score, PWV and PP. Independent association of vascular calcification with PWV and with PP was evaluated in linear regression models adjusting for age, HD duration, diabetes, cholesterol levels, systolic pressure, BMI, and calcitriol and calcium carbonate doses. The absence of collinearity among explanatory factors was checked in all models based on standard procedures. The receiver operating characteristic (ROC) curve

Table 1. Demographic, biochemical and clinical factors

	All patients	Pulse wave velocity (m/s)		Pulse pressure (mmHg)		Plain X-ray score	
		≤10.5	>10.5	≤70	>70	≤3	>3
N (%)	101	61 (60%)	40 (40%)	68 (67%)	33 (33%)	42 (42%)	58 (58%)
Age (years)	58.9 ± 15.5	53.1 ± 15.2	**67.6 ± 11.3****	56.2 ± 15.8	**64.3 ± 13.6***	51.1 ± 15.9	**64.4 ± 12.7****
Male gender (*N*, %)	71 (70%)	45(74%)	26 (65%)	47 (69%)	24 (73%)	27 (64%)	44 (75%)
Diabetes (*N*, %)	19 (19%)	6 (10%)	**13 (33%)***	8 (12%)	**11 (33%)***	3 (7%)	**16 (27%)***
HD duration (months)	55.8 ± 54.8	56.7 ± 59.0	57.4 ± 45.6	54.2 ± 51.8	62.7 ± 58.3	47.8 ± 48.6	63.5 ± 56.8
Systolic pressure (mmHg)	145.1 ± 25.7	141.3 ± 24.4	151.0 ± 26.8	133.3 ± 19.8	**169.4 ± 18.5****	136.521.4	**151.3 ± 26.9****
Diastolic pressure (mmHg)	82.7 ± 13.7	84.2 ± 14.9	80.6 ± 11.5	81.0 ± 14.0	86.3 ± 12.5	82.2 ± 13.7	83.1 ± 13.8
Mean arterial pressure (mmHg)	103.5 ± 16.3	103.2 ± 17.1	104.0 ± 15.1	98.5 ± 15.3	**114.0 ± 13.1****	100.3 ± 15.5	105.8 ± 16.6
Ca (mg/dL)	9.3 ± 0.9	9.3 ± 0.9	9.4 ± 0.8	9.2 ± 0.9	9.5 ± 0.8	9.2 ± 1.0	9.4 ± 0.7
P (mg/dL)	5.2 ± 1.4	5.4 ± 1.3	4.9 ± 1.5	5.4 ± 1.4	4.9 ± 1.4	5.4 ± 1.2	5.1 ± 1.5
iPTH (pg/mL)	476.4 ± 442	526.2 ± 483.7	400.3 ± 362.2	495.7 ± 441.9	436.4 ± 446.5	452.5 ± 394.3	493.3 ± 475.7
Total cholesterol (mg/dL)	203.4 ± 49.3	201.6 ± 49.8	206.4 ± 49.1	208.4 ± 50.0	192.0 ± 46.7	207.449.5	200.6 ± 49.4
Albumin (g/dL)	4.4 ± 0.6	4.4 ± 0.7	4.3 ± 0.4	4.4 ± 0.6	4.4 ± 0.4	4.4 ± 0.7	4.4 ± 0.4
CRP (mg/dL)	1.28 ± 0.6	1.23 ± 0.3	1.35 ± 0.8	1.25 ± 0.2	1.33 ± 0.9	1.25 ± 0.36	1.31 ± 0.7
Kt/V	1.41 ± 0.2	1.43 ± 0.2	1.38 ± 0.2	1.40 ± 0.2	1.41 ± 0.1	1.43 ± 0.2	1.38 ± 0.2
Body mass index (Kg/cm^2)	24.2 ± 4.8	24.1 ± 5.2	22.9 ± 3.9	24.8 ± 5.1	23.1 ± 4.0	24.9 ± 5.7	23.7 ± 4.1
Ca dose (g/day)	2.45 ± 1.31	2.47 ± 1.22	2.41 ± 1.49	2.33 ± 1.16	2.72 ± 1.61	2.52 ± 1.55	2.39 ± 1.13
Calcitriol dose (μg/week)	1.14 ± 0.6	1.13 ± 0.66	1.15 ± 0.52	1.14 ± 0.65	1.13 ± 0.53	1.17 ± 0.74	1.12 ± 0.53
Anti-HTA drugs (≥2) (*N*, %)	46 (46%)	26 (43%)	20 (50%)	20 (29%)	**26 (79%)****	12 (29%)	**34 (74%)****
CAD (*N*, %)	20 (20%)	7 (12%)	**13 (33%)***	12 (18%)	8 (24%)	7 (17%)	13 (22%)
PAD (*N*, %)	16 (16%)	4 (7%)	**12 (30%)***	4 (6%)	**12 (36%)****	2 (5%)	**14 (24%)***
Vascular disease (*N*, %)	32 (32%)	11 (18%)	**21 (53%)****	16 (24%)	**16 (49%)***	8 (19%)	**24 (41%)***
All-cause death (*N*, %)	31 (31%)	10 (16%)	**21 (53%)****	14 (20%)	**17 (52%)****	5 (12%)	**26 (44%)****

CRP = C-reactive protein; CAD = coronary artery disease; PAD = peripheral artery disease.
*$P < 0.05$; **$P < 0.01$.

analysis allowed the identification of the best cut-off values in relation with all-cause mortality for plain X-ray score (SVCS >3), PWV (>10.5 m/s) and PP (>70 mmHg). These cut-off values were used to compare groups in univariate analysis and to compare survival.

Statistical analyses were performed with the SPSS system 15.0 (SPSS Inc., Chicago, IL, USA) and with the Medcalc program version 6.0 (Medcalc software, Mariakerke, Belgium). For all comparisons and statistical tests, a *P*-value < 0.05 implied the rejection of the null hypothesis and the result was considered statistically significant.

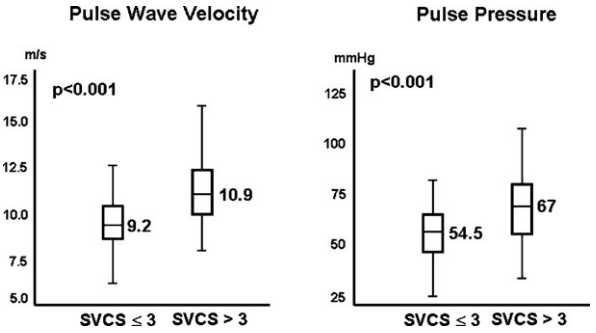

Fig. 1. A simple vascular calcification score >3 was associated with higher pulse wave velocity and with higher pulse pressure.

Results

Descriptive and univariate analysis

In this group of 101 patients, vascular calcifications were present in 77 patients. A SVCS > 3, a PWV > 10.5 m/s and a PP > 70 mmHg were observed in 59, 42 and 33 patients, respectively. In univariate analysis (Table 1), higher PWV, higher PP and higher vascular calcification score were associated with older age and with higher prevalence of diabetes, vascular disease and all-cause mortality. The calcium carbonate dose was correlated with systolic pressure ($r = 0.277$; $P = 0.005$) and with PP ($r = 0.226$; $P = 0.023$). Patients with SVCS > 3, when compared with patients with SVCS ≤ 3, had a higher PWV (11.2 ± 1.9 versus 9.3 ± 1.5 m/s; $P < 0.001$) and a higher PP (68.2 ± 20.4 versus 54.3 ± 13 mmHg, $P < 0.001$) (Figure 1).

Table 2. Vascular calcification and arterial stiffness (linear regression)

Dependent variable	Independent variables	*B*	CI	Significance	R^2
Pulse wave velocity (all patients)	Age	0.055	0.032–0.079	<0.001	0.424
	Systolic pressure	0.021	0.007–0.035	0.004	
	SVCS >3	0.845	0.074–1.615	0.032	
Pulse pressure (all patients)	Diabetes	10.102	0.926–19.278	0.031	0.268
	Calcium carbonate (g/day)	3.281	0.844–5.719	0.009	
	SVCS >3	10.103	2.315–17.890	0.012	

SVCS = simple vascular calcification score; PP = pulse pressure; PWV = pulse wave velocity.

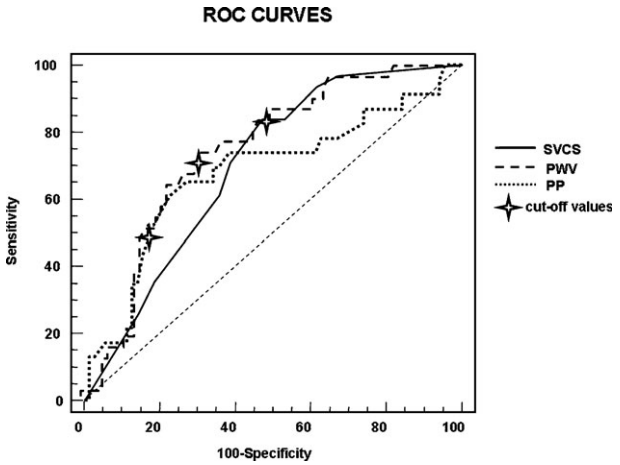

Fig. 2. ROC curves of simple vascular calcification score, pulse wave velocity and pulse pressure in relation with all-cause mortality.

Factors independently associated with PWV and with PP

In all patients, using linear regression with the enter method and adjusting for multiple factors (Table 2), a higher vascular calcification score was directly associated with PWV ($P = 0.032$) and with PP ($P = 0.012$). Other factors explaining PWV were age ($P < 0.001$) and systolic pressure ($P = 0.004$). Diabetes ($P = 0.031$) and calcium carbonate dose ($P = 0.009$) were directly associated with PP.

ROC curve analysis of mortality

During a 43-month observational period, 31 (30.7%) patients died. All-cause mortality was associated with a SVCS >3 (AUC = 0.701; 95% CI [0.602–0.788]; 84% sensitivity, 53% specificity, 88% negative predictive value and 1.78 positive likelihood ratio), with a PWV >10.5 m/s (AUC = 0.738; 95% CI [0.641–0.820]; 71% sensitivity, 69% specificity, 85% negative predictive value and 2.26 positive likelihood ratio) and with a PP >70 mmHg (AUC = 0.640; 95% CI [0.539–0.733]; 48% sensitivity, 81% specificity, 78% negative predictive value and 2.61 positive likelihood ratio) (Figure 2). There was no difference in the AUC between ROC curves.

Cumulative survival and all-cause mortality risk

Lower cumulative survival (Figure 3) was observed in patients with a SVCS >3 (32.7 versus 40.8 months; log rank = 10.8; $P = 0.001$), with a PP >70 mmHg (30.9 versus 38.8 months; log rank = 10.8; $P = 0.001$) and with a PWV >10.5 m/s (31.3 versus 39.3; log rank = 13.3; $P < 0.001$). In diabetic patients, higher PWV was not associated with higher mortality: 4 deaths in 6 patients with PWV ≤10.5 m/s (67%) versus 8 deaths in 13 patients with PWV >10.5 m/s (62%). In Cox regression analysis, using the enter method (Table 3), the mortality-adjusted hazard ratio was 3.308 ($P = 0.032$) for SVCS >3, 3.227 ($P = 0.031$) for PP > 70 mmHg in all patients and was 2.981 ($P = 0.047$) for PWV >10.5 m/s in non-diabetic patients. Entering vascular calcification score, PWV and PP in the same model and using the enter method, SVCS > 3 (HR = 4.247, $P = 0.015$), PP > 70 mmHg (HR = 3.795, $P = 0.031$), lower BMI (HR = 0.856, $P = 0.017$) and vascular disease at baseline (HR = 2.551, $P = 0.047$) were associated with mortality (Table 4).

Discussion

In general population, it was demonstrated that, in patients older than 60 years of age, higher PP was associated with higher cardiovascular risk [13]. In dialysis patients it was demonstrated that a higher PP evaluated before or after HD was associated with higher mortality in non-diabetic patients [14] or in all patients [15] and that higher aortic PWV was associated with mortality [8]. Vascular calcifications, evaluated by different methodologies [3–6,12], have been associated with mortality in dialysis patients. In this study, we tried to answer one of the research questions suggested by the KDIGO Imaging Work Group: the evaluation of the relationship between the radiological vascular calcification assessment and the measurement of vascular stiffness by PWV and by PP [7]. Vascular calcifications evaluated by ultrasonography have already been associated with increased stiffness of large elastic-type arteries [10]. The Kauppila score, [16] evaluating calcifications in the abdominal aorta, which is an elastic artery, was the first plain X-ray calcification score to be correlated with arterial stiffness, evaluated by carotid-femoral PWV [11]. In this study, we used a SVCS evaluated in plain X-ray of pelvis and hands that has been previously associated with higher cardiovascular mortality, cardiovascular disease and cardiovascular hospitalizations [12]. We verified that this score, although evaluating only muscular arteries, was associated both with carotid-femoral PWV and with PP. In the same dialysis population,

Table 3. Adjusted hazard ratio of all-cause mortality (Cox regression)

	Independent variables	*B*	HR	CI	Significance
All patients	SVCS >3	1.196	3.308	1.109–9.863	0.032
All patients	PP >70 mmHg	1.171	3.227	1.114–9.347	0.031
Non-diabetic patients	PWV >10.5 m/s	1.092	2.981	1.013–8.775	0.047

SVCS = simple vascular calcification score; PP = pulse pressure; PWV = pulse wave velocity; HR = adjusted hazard ratio.

Table 4. Predictors of all-cause mortality (Cox regression)

Dependent variable	Independent variables	B	HR	CI	Significance
All-cause mortality (all patients)	Age	0.004	1.004	0.966–1.044	0.824
	HD duration (months)	0.000	0.999	0.992–1.006	0.805
	Diabetes	0.348	1.416	0.508–3.948	0.506
	Vascular disease at baseline	0.936	**2.551**	1.014–6.419	**0.047**
	Systolic pressure (mmHg)	−0.022	0.978	0.955–1.002	0.077
	Body mass index (kg/cm^2)	−0.156	**0.856**	0.753–0.972	**0.017**
	Calcium carbonate (g/day)	−0.100	0.905	0.651–1.259	0.554
	Calcitriol (μg/week)	−0.161	0.851	0.455–1.594	0.615
	SVCS >3	1.446	**4.247**	1.319–13.673	**0.015**
	PP >70 mmHg	1.334	**3.795**	1.132–12.722	**0.031**
	PWV >10.5 m/s	−0.026	0.974	0.367–2.589	0.958

SVCS = simple vascular calcification score; PP = pulse pressure; PWV = pulse wave velocity; HR = adjusted hazard ratio.

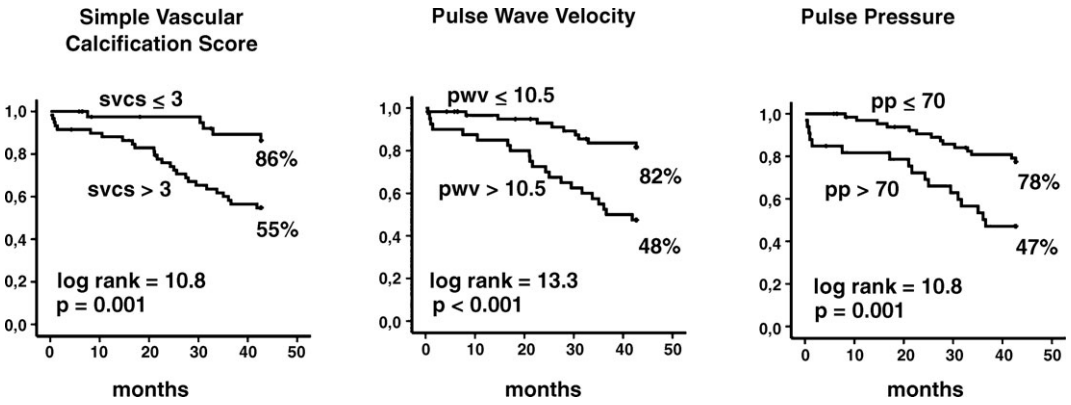

Fig. 3. A simple vascular calcification score >3, a pulse wave velocity >10.5 m/s and a pulse pressure >70 mmHg were associated with lower survival.

higher vascular calcification score, higher PWV and higher PP were associated with mortality with comparable hazard ratios and with similar AUC in ROC curve analysis. In our study, PWV was an independent predictor of mortality only in non-diabetic patients, probably because, in diabetic patients, mortality was equally high with higher or lower PWV. In a study evaluating a large cohort of HD patients, Tozawa *et al.* [14] observed that PP was also a predictor of mortality only in non-diabetic patients. Covic *et al*. [9] verified an opposite situation: in a group of young HD patients with a low prevalence of cardiovascular disease, arterial stiffness evaluated by the augmentation index was not a predictor of mortality. PWV evaluation requires a specific device and is not widely available. In our study, simple and inexpensive methods such as the evaluation of PP or the assessment of the SVCS with plain X-ray were enough to detect higher cardiovascular risk. Diagnosis of arterial stiffness and vascular calcification has the advantage of also providing important information that can be used for guiding therapeutic intervention in dialysis patients. Identification of patients with higher PP may orientate the choice of anti-hypertensive treatment with special indication for inhibition of the renin–angiotensin axis and avoidance of inappropriate reduction of diastolic blood pressure that may threaten coronary reserve [17]. At the present time, it also seems possible to interfere in some factors associated with the development of vascular calcifications. Some studies in dialysis patients have already demonstrated that vascular calcifications may progress or remain stable depending on the control of mineral metabolism alterations [5,18] and that phosphate binder choice may have an impact on mortality [5]. London *et al*. showed an association between vascular calcifications and low bone turnover [19] and found a significant interaction between calcium-containing phosphate binders and aortic calcification and stiffness in the presence of adynamic bone disease [20]. The presence or extension of vascular calcifications may be an indication for an intensive hyperphosphataemia control, for an adequate choice of

phosphate binder and for avoidance of PTH oversuppression. In several studies, association of a calcium carbonate dose with vascular calcifications [3–5,18,20,21] and with PWV [8,20] has already been described and in our patients we have also verified a correlation between the calcium carbonate dose and PP. We have not verified any association between calcium carbonate and calcitriol treatment with survival.

Different methods can be used to evaluate vascular calcifications in dialysis patients. Electron beam computed tomography and multislice computed tomography are considered to be the gold standard for the evaluation of coronary calcifications. They perform a quantitative assessment of coronary calcification that permits the evaluation of calcification progression but are very expensive. Screening vascular calcifications in dialysis patients may be performed by different and inexpensive plain X-ray methods [4,11,12,22] that have the advantage of being easily interpreted by the attending physician. The SVCS and Kauppila score are semi-quantitative scores with cut-off values associated with higher cardiovascular risk. A cardiovascular calcification index using the Kauppila score and valvular calcification evaluated by echocardiography has been demonstrated to be associated with coronary calcification in HD patients [23]. Simpler methods to evaluate vascular calcifications seem to be an attractive option but the final choice would depend on the available tests and on the preference of the nephrologist.

In summary, a SVCS evaluated in plain X-ray and PP were associated with higher mortality in dialysis patients. PWV was associated with higher mortality in non-diabetic patients. In this study, we demonstrate that a SVCS evaluated in plain X-ray of pelvis and hands is associated with the arterial stiffness evaluated by PWV and PP. Identification of vascular calcifications in dialysis patients is included in the classification of CKD-MBD, and simple and inexpensive plain X-ray methods are available for that purpose. Arterial stiffness or the presence of vascular calcifications are an alert sign for an increased mortality risk, and this information may be relevant for guiding therapeutic intervention in dialysis patients, such as selecting the most adequate anti-hypertensive regimen or achieving an effective mineral metabolism management.

Conflict of interest statement. Teresa Adragao has received research grants from Genzyme, has received lecture fees from Amgen, Genzyme, Abbot and Novartis and consultancy fees from Genzyme and Abbot. The other authors have no conflict of interest of any type (personal, commercial, political, academic or financial) in the elaboration and presentation of this work.

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Vascular calcification, cardiovascular risk and arterial stiffness in hemodialysis patients

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INTRODUCTION

Intimal and medial calcification, the two main types of arterial calcification, are associated, respectively, with arteriosclerosis and mediosclerosis. In non-dialysis patients, both intimal[1] and medial calcification[2] may be the result of an active regulated process with different aetiologies, where local cells, such as macrophages in the intima or vascular smooth cells in the media, differentiate into osteoblasts[3]. In haemodialysis patients it has also been demonstrated that medial calcification is an active cellular process, similar to bone formation[4,5]. Vascular smooth muscle cells can differentiate into osteoblasts due to different stimuli, some of which may be hyperphosphatemia or hypercalcemia[6]. A passive calcification mechanism is, however, not excluded. High calcium phosphorus product may also contribute to the matrix mineralization[6]. Reduction of calcification inhibitors in dialysis patients, such as fetuin-A or matrix-Gla protein, may be other factors associated with the development of calcification[7]. It has already been demonstrated that calcium carbonate and calcium acetate are associated with the progression of vascular calcification, a phenomenon that can be attenuated or arrested by sevelamer, a phosphorus binder that does not increase calcium levels and also reduces LDL-cholesterol[8,9]. In a rat model of hyperparathyroidism treatment with calcitriol or cinacalcet reduced PTH but, unlike calcitriol, cinacalcet did not produce hypercalcemia, increase in calcium phosphorus product or aortic calcification[10].

VASCULAR CALCIFICATION AND CARDIOVASCULAR RISK

Blacher et al.[11] showed for the first time that a vascular calcification score evaluated by B-mode ultrasonography in large arteries was associated with an increased risk of mortality in dialysis patients. Brawn et al.[12] had previously demonstrated that dialysis patients, when compared with non-dialysis patients, presented a higher coronary calcification score evaluated by electron beam computed tomography (EBCT). In general population this score, the Agatston score, is mainly caused by intimal calcification and is related with arteriosclerosis and coronary stenosis[13,14]. The higher values of this score in dialysis patients may be explained by the presence of both intimal and medial calcification. Medial calcification is not occlusive but modifies the properties of the arterial wall and may also contribute to coronary ischemia. The quantitative significance of Agatston score may be different from that described for the general population where a score greater than 400 is associated with a very high cardiovascular risk. In a study evaluating 43 dialysis patients a mean score of 559±255 was found in patients with normal coronary angiographies while abnormal coronary angiographies were associated with a mean score of 2869±417[15]. In dialysis patients, high values of Agatston score may occur in the absence of occlusive coronary atherosclerosis[16] but, like other calcification scores, Agatston score was an independent predictor of death in haemodialysis patients[16]. The discriminative significance of Agatston score in dialysis patients remains, however, to be identified.

Multislice computed tomography (MSCT)[17] has already been employed for the diagnosis of vascular calcifications in dialysis patients, but, as well as EBCT, it is very expensive to be used in a routine way. Evaluation of vascular calcifications in plain radiographs has been proposed by KDOQI guidelines[18]. We have already verified that a simple vascular calcification score based on plain radiographs of pelvis and hands was a predictor of cardiovascular mortality, cardiovascular hospitalizations and fatal and non-fatal cardiovascular events[19]. Radial, iliac and femoral arteries were the arteries evaluated for the estimation of this score. This score may be used as a simple and inexpensive tool for the assessment of cardiovascular risk in haemodialysis patients.

Development of a numeric cardiovascular calcification index for cardiovascular risk evaluation in dialysis patients has been recently suggested[20]. The methodology recommended for the assessment of this calcification index should be non-invasive and low-cost, in order to be widely accessible, such as conventional blood pressure measurements for pulse pressure evaluation, standard radiographs for vascular calcifications assessment and echocardiography for valvular calcifications diagnosis.

Vascular[11] and valvular[21] calcification are demonstrated risk factors for cardiovascular death in dialysis patients. Pulse pressure increase is associated with vascular stiffness and has been related with cardiovascular risk in general population[22] and in haemodialysis patients as well[23].

We have developed, in a cohort of haemodialysis patients, a combined cardiovascular score based on pulse pressure and on vascular and valvular calcifications and compared it with the previously described simple vascular calcification score. This combined cardiovascular score was a stronger predictor of cardiovascular risk than the simple vascular calcification score[24]. We verified that the addition of a valvular and of a pulse pressure score to the simple vascular calcification score allowed a more accurate prediction of cardiovascular risk in this group of patients.

CLINICAL SIGNIFICANCE OF INTIMAL AND MEDIAL CALCIFICATION IN HAEMODIALYSIS PATIENTS

Intimal and medial calcifications are highly prevalent in dialysis patients. Intimal calcification corresponds to the type Vb of atherosclerotic plaques (American Heart Association classification) and medial calcification is associated with mediosclerosis. Standard radiographs can be employed to differentiate these two different types of arterial calcification[25] since other more sophisticated diagnostic techniques such as ultrasonography, EBCT or MSCT can not discriminate these calcification types. Using this methodology, London et al. demonstrated that intimal and medial calcification are independent predictors of cardiovascular and all-cause death in dialysis patients[26]. Intimal calcification was associated with older age and lower survival when compared with medial calcification. Medial calcification was associated with haemodialysis duration, diabetes, hyperphosphatemia and with calcium carbonate dose treatment.

VASCULAR CALCIFICATION CONTRIBUTES TO ARTERIAL STIFFNESS IN HAEMODIALYSIS PATIENTS

Arterial stiffness is an alteration of the arterial wall properties with multiple causes, some of which are old age, diabetes, hypertension and medial calcification. All these features are highly prevalent in dialysis patients and arterial stiffness is a common finding in this population. Some of the manifestations of arterial stiffness are isolated systolic hypertension, pulse pressure increase and high pulse wave velocity. Pulse pressure is the difference between systolic and diastolic pressure. The arterial wall stiffness creates an early aortic pulse wave reflection that finds the aortic valve still opened and causes a systolic blood pressure increase and a diastolic blood pressure decrease. Increase in systolic pressure provides an increase in after load that can contribute to the development left ventricular hypertrophy. Decrease in diastolic pressure decreases coronary perfusion which occurs during diastole and may aggravate coronary ischemia.

Pulse pressure increase has already been connected to cardiovascular risk in the general population[22] and in haemodialysis patients[23].

Pulse wave velocity is also a marker of arterial stiffness. The loss of elasticity of the arterial wall increases the velocity of the blood flow through the arterial system. Guérin et al demonstrated that, in haemodialysis patients, vascular calcifications were associated with increased stiffness of elastic type arteries like the aorta and common carotid artery[27]. In this study vascular calcifications were evaluated by ultrasonography. We have also verified in a cohort of haemodialysis patients that the simple vascular calcification score, assessed in plain radiographs of hands and pelvis and evaluating not elastic arteries but muscle arteries such as radial, iliac and femoral arteries, was independently and directly associated with pulse wave velocity[28]. This study was another evidence of the usefulness of this simple vascular calcification score in this population.

PERIPHERAL ARTERY DISEASE IN DIALYSIS PATIENTS

There is a remarkably high prevalence of peripheral artery disease among patients with renal insufficiency. Its prevalence may reach 24% of patients with creatinine clearance < 60ml/min versus 3.7% of persons with creatinine clearance $\geq$ 60 ml/min[29]. Both moderate and

severe renal insufficiency are associated with an increased risk of death in patients with peripheral artery disease[30]. In this study, the percentages of patients who presented with gangrene or ischemic ulceration rather than rest pain increased with declining renal function. Lower limb amputations are a major problem in dialysis patients. Histological patterns found in 11 dialysis patients submitted to distal amputations (figures 1 to 3) reflect the diversity of lesions present in this population that may contribute to the chronic distal artery disease[31]. Occlusive lesions consisted of luminal thrombi and cholesterol emboli. Arteriosclerosis plaques and medial calcification may be present in the same patient and even in the same artery. In other evaluation of 56 patients submitted to lower limb amputations (non-published data) medial calcification was present in 48% of the samples and was associated with atherosclerotic plaques type II or III (American Heart Association classification) in 50% of cases. In this group of patients submitted to lower limb amputations there was no histological evidence of intimal calcification in any sample. This data confirm the association of medial calcification with distal artery disease. Dialysis vintage, diabetes, calcium carbonate dose and hyperphosphatemia have already been associated with medial calcification[26]. In a registry study, hyperphosphatemia was independently associated with future amputations[32]. These observations raise the hope of modifying the evolution of peripheral artery disease in dialysis patients by the correction of some of these factors.

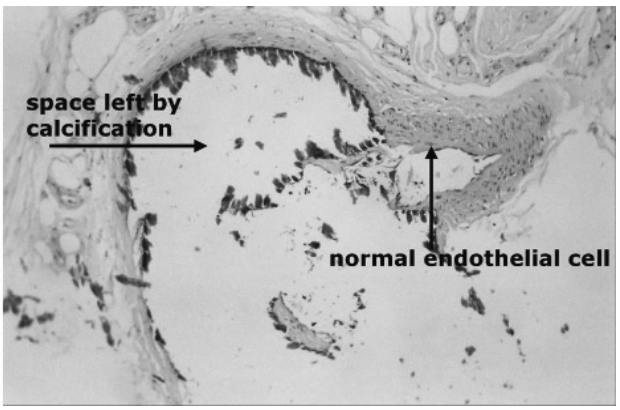

Fig. 2 – Exuberant medial calcification. (Hemotoxilin Eosin x 100)

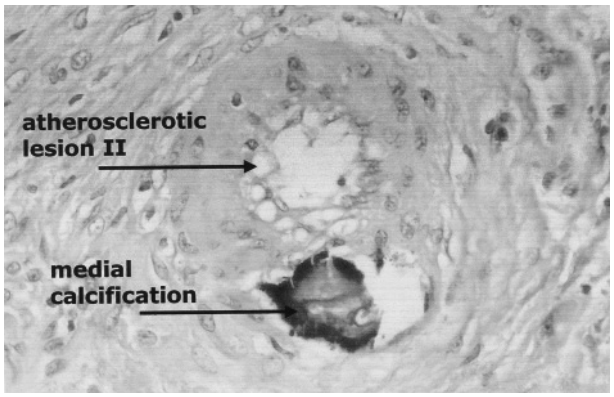

Fig. 3 – Type II atherosclerosis lesion and medial calcification. (Hemotoxilin Eosin x 100)

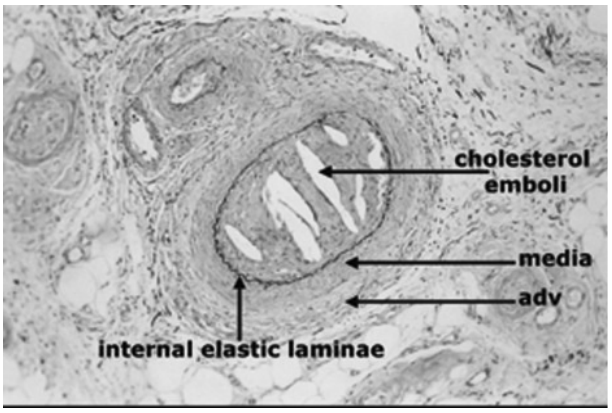

Fig. 1 – Muscular artery: cholesterol emboli. (Elastic van Giesen x 100)

In summary, vascular calcifications are highly prevalent in dialysis patients, and contribute to cardiovascular mortality and morbidity. Plain

radiographs are a simple and inexpensive tool for the assessment of cardiovascular risk. Arteriosclerosis and mediosclerosis are the histological setting for the development of vascular calcifications. Intimal calcification is associated with arteriosclerosis and medial calcification with mediosclerosis. In dialysis patients, medial calcification has been associated with diabetes, dialysis vintage, hyperphosphatemia and treatment with calcium carbonate. Decrease of calcification inhibitors facilitates this process. Sevelamer can arrest vascular calcification progression. In a rat model of hyperparathyroidism Cinacalcet, unlike calcitriol, was not associated with vascular calcification development. It is necessary to demonstrate that the correction of those factors contributing to vascular calcification is associated with a better cardiovascular outcome in dialysis patients.

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CAPÍTULO 5

CALCIFICAÇÃO VALVULAR CARDÍACA NOS DOENTES EM DIÁLISE

A calcificação valvular cardíaca foi durante muito tempo considerada um processo degenerativo passivo de lesão valvular. Contudo, nos últimos anos foram identificados mecanismos de calcificação valvular associados a um fenómeno de ativação osteoblástica. Vários fatores habitualmente expressos nos osteoblastos foram também identificados em válvulas cardíacas calcificadas, como osteopontina[1], BMP (*bone morphogenic protein*) 2 e 4,[2] ou Runx2 (*runt related transcription factor 2*) e osterix[3]. Em alguns casos foram mesmo identificadas verdadeiras zonas ossificadas com formação de osso lamelar apresentando atividade osteoblástica e osteoclástica, traduzindo a existência de remodelação óssea[2]. Atualmente considera-se que a calcificação valvular cardíaca não é um processo passivo mas ativo que resulta da mutação fenotípica de células valvulares locais ainda não identificadas, provavelmente miofibroblastos ou pericitos que se transformam em osteoblastos[2,3]. Os mecanismos responsáveis por estas alterações ainda não são totalmente conhecidos, mas apresentam algumas semelhanças com o processo aterogénico vascular. Estas lesões valvulares, como as lesões ateroscleróticas, podem ser precedidas por ruptura da membrana basal, infiltração por células inflamatórias, depósito de lípidos e podem estar associadas a diabetes, hipercolesterolemia, hipertensão arterial ou consumo de tabaco[4]. Esta formação de tecido ósseo descrito nas válvulas cardíacas calcificadas assemelha-se ao processo de calcificação ativa também descrito nas artérias e resultante da transformação das células musculares lisas em osteoblastos[5].

A prevalência de calcificações valvulares é elevada nos doentes em diálise, variando entre 32% e 47% em diferentes estudos[6-9]. Na população geral[10] e também nos doentes em diálise[7-9] comprovou-se que as calcificações valvulares se associam a mortalidade mais elevada. Nos doentes em diálise já foi demonstrado que a presença de calcificações valvulares se associa a marcadores clássicos de aterosclerose como o aumento da espessura íntima-média carotídea, placas carotídeas e aumento da proteína C-reactiva[11], mas as alterações do metabolismo fosfocálcico também parece estarem implicadas, como foi demonstrado por Ribeiro S, *et al*[6].

Num grupo de 127 doentes em hemodiálise analisamos a prevalência das calcificações das válvulas aórtica e mitral, o risco de mortalidade destas calcificações valvulares e a sua relação com as calcificações arteriais diagnosticadas pelo *score* de calcificação vascular

simples[12]. As calcificações valvulares cardíacas mitrais ou aórticas foram diagnosticadas por ecocardiografia e estavam presentes, respectivamente, em 37 (29%) e em 19 doentes (15%); 39 doentes (31%) apresentavam calcificações valvulares. A presença de calcificações valvulares associou-se a menor sobrevida, avaliada ao longo de 48 meses (Fig 5.1).

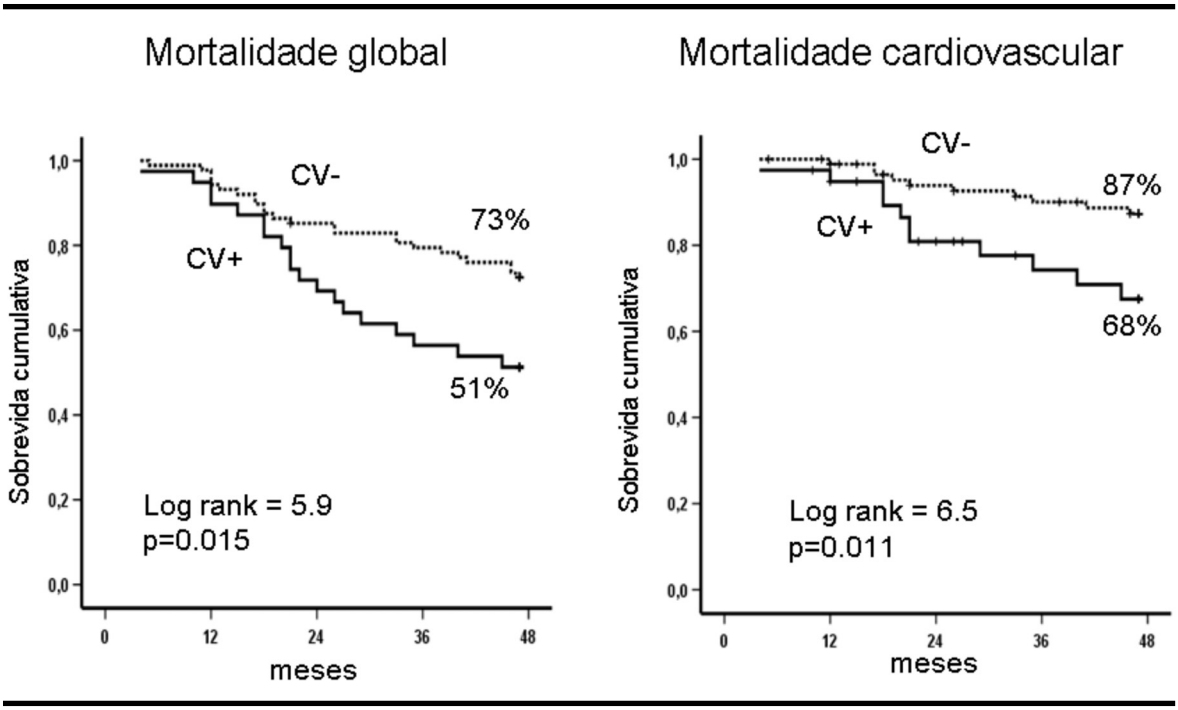

Fig. 5.1. A presença de calcificações valvulares (CV+) associou-se a maior mortalidade de causa global ou cardiovascular (Kaplan Meier)

As calcificações vasculares avaliadas pelo *score* de calcificação vascular simples estavam presentes em 72% dos doentes. O *score* de calcificação vascular foi um preditor independente de calcificação das válvulas aórtica ou mitral. Por cada aumento de 1 ponto no *score* de calcificação houve um aumento, respectivamente, de 46% e de 66% de risco de apresentar calcificação valvular aórtica ou mitral. Verificou-se também uma associação direta entre os valores da paratormona e a calcificação da válvula aórtica e os valores do fósforo sérico e a calcificação da válvula mitral.

Estes dados sugerem que a calcificação valvular possa partilhar mecanismos patogénicos semelhantes aos da calcificação vascular e que nos doentes em diálise a calcificação valvular faça parte de um processo sistémico de calcificação.

As *guidelines* KDIGO 2009[13] consideram que o rastreio das calcificações nos doentes renais crónicos possa ser feito através de RX simples ou através da avaliação das calcificações valvulares por ecocardiografia. A presença de calcificações vasculares ou valvulares identificam os doentes com maior risco cardiovascular, e esta informação pode ser usada para orientar a

terapêutica nestes doentes. Podemos tentar corrigir os mecanismos associados ao desenvolvimento e progressão das calcificações, nomeadamente tentar corrigir as alterações do metabolismo fosfocálcico, entre outros. Verificámos que nos nossos doentes a prevalência de calcificações valvulares é bastante inferior à prevalência de calcificações vasculares. Em análise por curvas ROC, avaliamos que a presença de calcificações valvulares apresenta um valor preditivo positivo de 87% e preditivo negativo de 34% para indicar presença de calcificações vasculares. Isto significa que, no caso de haver calcificações valvulares, existe 87% de probabilidades de também haver calcificações vasculares. No caso de não haver calcificações valvulares, a probabilidade de não haver calcificações vasculares é de apenas 34%. Esta limitação deve ser tida em consideração se só usarmos o ecocardiograma para rastreio das calcificações nos doentes em diálise, pois podemos subavaliar o risco cardiovascular destes doentes. O ecocardiograma não substitui a utilização do RX simples para identificar o risco cardiovascular associado às calcificações.

Em resumo, o *score* de calcificação vascular simples foi um preditor independente de calcificações valvulares, sugerindo que o processo de calcificação cardiovascular é sistémico, podendo partilhar fatores patogénicos comuns. É provável que a terapêutica utilizada no controlo da progressão das calcificações vasculares possa também afetar a progressão das calcificações valvulares. É necessário avaliar se o manejo das alterações do metabolismo mineral e ósseo pode ter um papel na evolução e no prognóstico da calcificação valvular destes doentes. O estudo ADVANCE, apresentado durante o XLVVII ERA-EDTA Congress 2010, em Munique, demonstrou que a terapêutica com Cinacalcet, agente calcimimético usado no tratamento do hiperparatiroidismo secundário, e baixas doses de vitamina D se associaram a uma redução significativa da progressão das calcificações na válvula aórtica.

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ORIGINAL ARTICLE

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Vascular and valvular calcifications in dialysis patients: the same pathogenesis?

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ABSTRACT

Osteoblastic bone formation has been described in cardiac valves resembling the active calcification process already demonstrated in arteries, in relation to osteoblastic transformation of vascular smooth muscle cells. The aim of our study was to evaluate the mortality risk of cardiac valvular calcification in haemodialysis patients and to analyse the association of valvular calcification with arterial vascular calcification. Valvular calcification (VC) was diagnosed by B-Mode echocardiography. A simple vascular calcification score (SVCS) was evaluated in plain X-ray of hands and pelvis (0-8). We studied 127 prevalent HD patients (75 males and 52 females) with a mean HD duration of 48±53 months. Aortic VC (AVC) was diagnosed in 19 patients (15%), mitral VC (MVC) in 37 patients (29%) and AVC or MVC in 39 patients (31%); SVCS › 0 was diagnosed in 91 patients (71%) and SVCS ≥ 3 in 63 patients (50%). After 48 months follow-up there were 43 all cause deaths and 21 cardiovascular deaths. Vascular and all valvular calcifications were associated with lower cumulative survival. The adjusted risk of all cause death was 3.7 fold higher in AVC ($p<0.001$). For each unit increment of the SVCS there was 56% increase in cardiovascular death risk ($p<0.001$). SVCS was associated with aortic ($p=0.002$) and mitral ($p<0.001$) valvular calcification. In conclusion, in these patients valvular and vascular calcifications were independent predictors of mortality. Vascular calcification was independently associated with cardiac valvular calcification, suggesting that these two types of calcification may share common characteristics.

Key-Words:
Haemodialysis; mortality; valvular calcification; vascular calcification.

INTRODUCTION

Vascular calcifications are highly prevalent in dialysis patients and have been associated with an increased risk of total and cardiovascular death[1]. Some mechanisms linking vascular calcifications with cardiovascular risk, such as the association between vascular calcifications and arterial stiffness, have already been recognised[2]. Loss of arterial distensibility is associated with increased pulse pressure[3], left ventricular hypertrophy and decrease of coronary perfusion during diastole. It has been demonstrated that vascular calcification in dialysis and non-dialysis patients is an active cellular process, similar to bone formation[4-6]. Vascular smooth muscle cells can differentiate into osteoblasts due to different stimuli, which, in dialysis patients, may be hyperphosphataemia and hypercalcaemia[7]. Reduction of calcification inhibitors, such as fetuin-A or matrix-Gla protein, may be another factor associated with the development of calcification[8]. Valvular calcification is an independent predictor of cardiovascular death in the general population[9] and ectopic bone

formation has been also identified, most likely originating from differentiated myofibroblasts[10], resembling the active calcification process already demonstrated in arteries. It has already been demonstrated that valvular calcification is also a predictor of cardiovascular mortality in peritoneal dialysis patients[11]. The aim of this study was to evaluate in a group of haemodialysis patients the risk of all cause death and of cardiovascular death related to cardiac valvular calcification and to analyse the association of cardiac valvular calcification with arterial vascular calcification.

PATIENTS AND METHODS

Study design

An observational, prospective, single-centre study of a cohort of prevalent haemodialysis patients was used.

Population

One hundred and twenty seven patients, 75 males and 52 females, without previous parathyroidectomy were evaluated. Twenty six patients (21%) were diabetic. At baseline, mean age was 62±15 years (24-91) and mean haemodialysis duration was 47±56 months (4-271). During an observational period of 48 months, 43 patients (34%) died. The diagnosis of vascular disease at baseline was based on previous clinical manifestations and test results. Coronary artery disease was diagnosed if the patient had typical angina pectoris, a positive stress test, suffered a myocardial infarction, or underwent a percutaneous coronary intervention or coronary bypass surgery. Diagnosis of cerebral vascular disease was based on the occurrence of stroke or transient ischaemic attack or the detection of an old cerebral infarction in computed tomography. Peripheral arterial disease was diagnosed if there was claudication, ischaemic ulcers, lower limbs amputation, revascularisation or diagnosis of obstruction by Doppler or angiography. Coronary artery disease was diagnosed in 33 patients (25%). Peripheral artery disease was present in 17 patients (13%) and 6 patients (5%) had had a previous stroke.

Vascular and valvular calcifications

Vascular calcifications were evaluated in plain X-ray of pelvis and hands by a method previously described[12]. Pelvis films were divided into four sections by two imaginary lines: a horizontal line over the upper limit of both femoral heads and a median vertical line over the vertebral column (Fig. 1). Hand films were divided for each

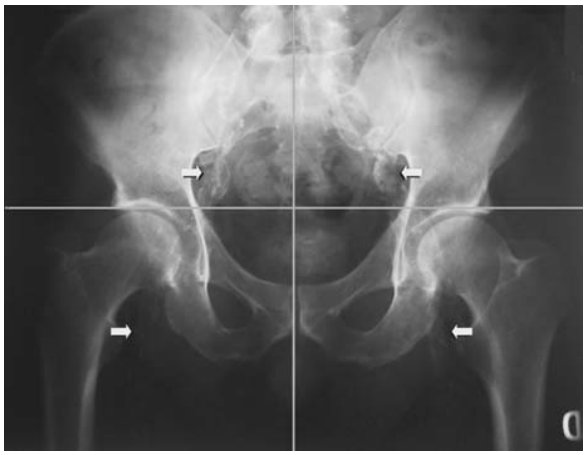

Figure 1

Ileo-femoral score evaluates the presence of vascular calcifications in iliac and femoral arteries. Calcification score is the sum of the presence (1) or absence (0) of vascular calcifications. In this example, pelvis score (1+1+1+1) = 4

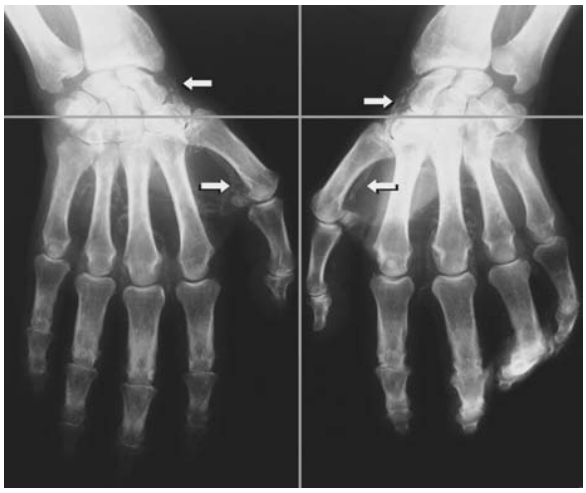

Figure 2

Hand score evaluates the presence of vascular calcifications in radial and digital arteries. In this example hand score (1+1+1+1) = 4. Total score is the sum of pelvis and hand score (8)

hand by a horizontal line over the upper limit of the metacarpal bones (Fig. 2). Pelvis films evaluated iliac and femoral arteries (ileo-femoral score) and hand films evaluated radial and digital arteries (hand score). Any vascular calcification lining the vessel walls either in an irregular pattern or in a linear pattern was considered. The presence of vascular calcifications in each section was rated as 1 and its absence as 0. Final score was the sum of all sections and ranged from 0 to 8.

Valvular calcifications of mitral and aortic valve were assessed with B-mode Echocardiography

Biochemical analysis

Serum levels of the following biochemical parameters were evaluated and time averaged for the 6 months preceding the evaluation of vascular and valvular calcifications: Ca, P, CaxP product, alkaline phosphatase, albumin, total iPTH. Total iPTH was evaluated every three months by immunochemiluminescence using a second generation assay.

Statistical analysis

Data are expressed as frequencies for categorical variables, mean values with SD for normally distributed variables. Comparison between groups was performed by Mann-Whitney U and chi-square tests. Multivariate analysis was performed by Binary logistic Regression Models. Survival evaluation was performed with Cox regression models. Survival curves were performed using Kaplan Meyer with evaluation of log rank. Variables entered in multivariate analysis were age, gender, haemodialysis duration, diabetes, CaxP, iPTH, albumin, cardiovascular disease at baseline and vascular and valvular calcifications. Statistical analyses were performed with the SPSS system 14.0 (SPSS Inc., Chicago,IL) and the Medcalc program version 6.0 (Medcalc software; Mariakerke, Belgium). For all comparisons, a P value ‹0.05 was considered statistically significant.

RESULTS

During an observational period of 48 months there were 43 all-cause deaths (34%) and 21 (17%) cardiovascular deaths and 34 patients (27%) needed cardiovascular hospitalisations. Demographic and biochemical values of the whole group are shown in Table 1. Aortic and mitral valvular calcification were detected in 19 (15%) and in 37 (29%) patients respectively. Aortic or mitral calcifications were detected in 39 patients (31%). Presence of vascular calcifications was identified in 91 patients (72%). A simple vascular calcification score (svcs) ≥ 3 was observed in 63 patients (49.6%). Mean calcium carbonate dose was 2.1±1.01 g/day. Thirty three patients (26%) were treated with calcitriol and the mean dose was 1.54±1.01 µg / week.

Demographic, biochemical parameters and calcifications (Table I)

Haemodialysis duration was longer in patients with aortic and mitral calcification (Table I). All cause death and cardiovascular death were more frequent in patients with valvular or vascular calcifications (Table I). Other demographic or biochemical parameters were not different in patients with or without valvular and vascular calcifications.

Mean Survival and Cumulative Survival

All cause death (Table II; Fig. 3) and cardiovascular death (Table III; Fig. 4) were responsible for significant lower mean survival and lower cumulative survival in patients with valvular and vascular calcifications.

Mortality risk of valvular and vascular calcifications (Table IV)

In separate Cox regression models, in all patients, adjusting for age, gender, HD duration, diabetes, iPTH, CaxP product, albumin and cardiovascular disease at baseline, the mortality risk for each type of valvular calcification and for the vascular calcification score was evaluated (Table IV). The adjusted risk of all cause death and of cardiovascular death was, respectively, 3.8 and 2.6 fold higher in patients with aortic valvular calcification. The adjusted risk of all cause death and of cardiovascular death was, respectively, 2.4 and 2.9 fold higher in patients with mitral valvular calcification. In non-diabetic patients, mitral valvular calcification and mitral or aortic valvular calcification were also associated with increase in all-cause mortality (Table IV).

Table I

Valvular Calcifications

	All Patients N=127	Aortic Valve		Mitral Valve		Aortic or Mitral Valve	
		AVC=0 N=108	AVC>0 N=19	MVC=0 N=89	MVC>0 N=38	AMVC=0 N=87	AMVC>0 N=40
Age	62±15	61±15	66±12	61±15	65±14	61±15	65±14
Male gender	75 (59%)	65 (60%)	10 (53%)	57 (63%)	18 (49%)	56 (64%)	19 (49%)
Diabetes	26 (21%)	23 (21%)	3 (16%)	18 (20%)	8 (22%)	18 (21%)	8 (21%)
HD duration (months)	48±53	44±52	**65±49***	**38±42**	**71±66****	**38±42**	**69±66***
Ca (mg/dL)	9.9±0.7	9.9±0.8	10±0.6	9.9±0.8	10.0±0.6	9.9±0.8	10.0±0.6
P (mg/dL)	4.9±1.4	4.8±1.4	5.3±1.3	4.8±1.4	5.2±1.4	4.8±1.4	5.2±1.4
CaXP (mg^2/dL^2)	49.3±15.6	48.6±15.7	53.6±14.0	47.7±15.6	53.2±15.0	47.8±15.6	52.7±15.1
iPTH (pg/mL)	302±380	272±313	476±624	315±394	273±347	287±320	337±492
AP (ng/mL)	30.0±22.4	30.1±23.3	29.2±17.4	30.9±22.3	28.1±23.1	30.9±22.3	28.1±23.1
Albumin (g/L)	3.7±0.3	3.7±0.3	3.7±0.3	3.7±0.3	3.7±0.3	3.7±0.3	3.7±0.3
$CaCO_3$ dose (g/day)	2.1±1.5	2.2±1.5	1.6±1.4	2.2±1.6	1.9±1.3	2.2±1.6	1.8±1.3
Ejection fraction (%)	38±7	38±6	38±7	37±6	38±7	38±6	38±7
LVPW (mm)	10.2±1.8	10.3±1.6	9.5±3.4	10.3±1.6	10±2.5	10.2±1.6	10.1±2.5
All cause death	43 (34%)	32 (30%)	**11 (58%)***	24 (27%)	**19 (51%)***	24 (27%)	**19 (49%)***
Cardiovascular death	21 (17%)	15 (14%)	6 (32%)	10 (11%)	**11 (30%)***	10 (11%)	**11 (28%)***
Cardiovascular hospitalisation	34 (27%)	26 (24%)	8 (42%)	17 (19%)	**17 (46%)****	17 (19%)	**17 (44%)****
SVCS › 0	91 (72%)	74 (69%)	17 (90%)	58 (64%)	**33 (89%)****	57 (65%)	**34 (87%)***
SVCS ≥ 3	63 (50%)	49 (45%)	**14 (74%)***	37 (41%)	**26 (70%)****	37 (42%)	**26 (67%)***

*p<0.05; **p<0.01

Comparison between means: Mann-Whitney U; comparison between frequencies: chi-square. SVCS, simple vascular calcification score; AVC, aortic valve calcification; MVC, mitral VC; AMVC, aortic or mitral VC; LVPW left ventricular posterior wall

Table II

All cause mortality (Kaplan Meier)

	Aortic Valve		Mitral Valve		Aortic or Mitral Valve		Plain X-ray score	
	AVC=0 N=108	AVC>0 N=19	MVC=0 N=90	MVC>0 N=37	AMVC=0 N=88	AMVC>0 N=39	SVCS=0 N=36	SVCS>0 N=91
Number of events	32	11	24	19	24	19	6	37
Mean survival (SE), months	40.0 (1.2)	34.5 (3.2)	41.1 (1.2)	34.5 (2.3)	40.9 (1.3)	35.2 (2.3)	42.4 (1.9)	37.9 (1.4)
Cum. survival (%)	70%	42%	73%	49%	73%	51%	83%	59%
Log Rank 5.78	7.75	5.94	5.48					
Sig. 0.016	0.005	0.015	0.019					

AVC, aortic valve calcification; MVC, mitral VC; AMVC, aortic or mitral VC; SVCS, simple vascular calcification score

Table III

Cardiovascular mortality (Kaplan Meier)

	Aortic Valve		Mitral Valve		Aortic or Mitral Valve		Plain X-ray score		Plain X-ray score	
	AVC=0 N=108	AVC>0 N=19	MVC=0 N=90	MVC>0 N=37	AMVC=0 N=88	AMVC>0 N=39	SVCS=0 N=36	SVCS>0 N=91	SVCS<3 N=64	SVCS≥3 N=63
Number of events	15	6	10	11	10	11	20	1	4	17
Mean survival (SE), months	43.6 (0.8)	40.1 (2.8)	44.5 (0.8)	39.4 (2.1)	44.5 (0.8)	39.8 (2.0)	46.1 (0.8)	41.8 (1.1)	45.5 (0.8)	40.8 (1.5)
Cum. survival (%)	85%	61%	88%	65%	87%	68%	97%	75%	93%	71%
Log Rank	4.30		7.97		6.54		6.22		8.78	
Sig.	0.038		0.005		0.011		0.010		0.003	

AVC, aortic valve calcification; MVC, mitral VC; AMVC, aortic or mitral VC; SVCS, simple vascular calcification score

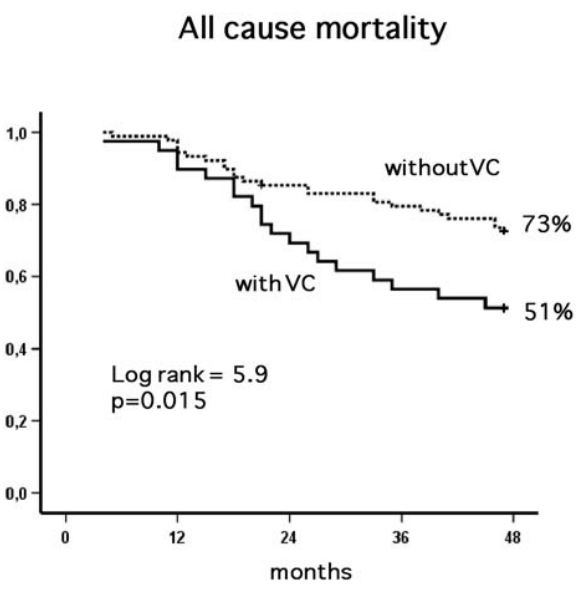

Figure 3

Lower survival in relation to all cause death in patients with valvular calcification (VC)

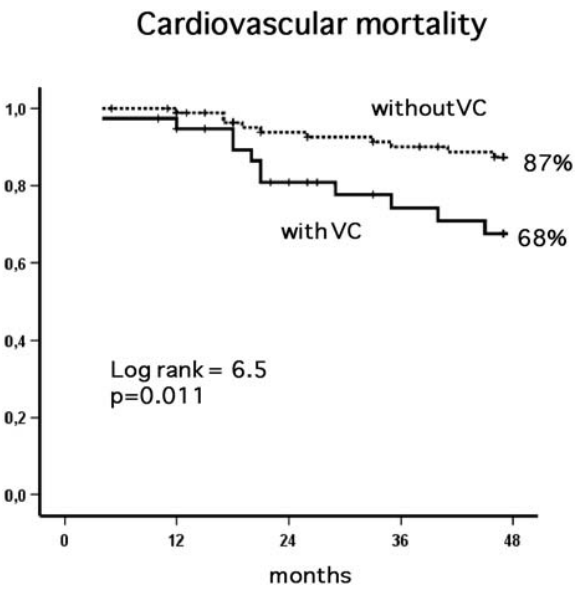

Figure 4

Lower survival in relation to cardiovascular death in patients with valvular calcification (VC)

Table IV

Mortality risk of each valvular calcification and vascular calcification score (Cox Regression)

	Dependent variable	Independent variables	B	Sig.	H.R.	95% CI
All patients	All cause mortality	Aortic valvular calcification	1.32	‹0.001	3.8	1.83 to 7.76
		Mitral valvular calcification	0.89	0.004	2.4	1.32 to 4.50
		Aortic or Mitral valvular calcification	0.93	0.003	2.5	1.36 to 4.67
		Vascular calcification score	0.19	0.003	1.2	1.06 to 1.39
	Cardiovascular mortality	Aortic valvular calcification	0.96	0.047	2.6	1.01 to 6.77
		Mitral valvular calcification	1.17	0.008	3.2	1.36 to 7.58
		Aortic or Mitral valvular calcification	1.07	0.015	2.9	1.23 to 6.84
		Vascular calcification score	0.45	‹0.001	1.6	1.26 to 1.95
Non- diabetic patients	All cause mortality	Mitral valvular calcification	0.92	0.009	2.5	1.22 to 5.04
		Aortic or Mitral valvular calcification	0.88	0.014	2.4	1.19 to 4.82
	Cardiovascular mortality	Vascular calcification score	0.64	‹0.001	1.89	1.38 to 2.58

Results are adjusted for age, HD duration, diabetes, iPTH, Ca x P product, albumin and vascular disease at baseline. Vascular calcification score and each valvular calcification were evaluated in separate models.

Factors independently associated with mortality and morbidity (Table V)

In a Cox regression model adjusting also for all types of valvular calcification and simple vascular calcification score, factors associated with all cause mortality, cardiovascular mortality and cardiovascular hospitalisations were identified (Table V). Aortic valvular calcification was directly associated with all cause mortality (HR = 3.72, 95% CI = 1.81 to 7.66, p‹0.001). The risk of all cause death was 3.7 fold higher in patients with aortic valvular calcification. Simple vascular calcification score was

Table V

Factors independently associated with mortality and morbidity (Cox Regression)

Dependent variable	Independent variables	B	Sig.	H.R.	95% CI
All cause mortality	PTH	-0.001	0.043	0.99	0.99 to 1.00
	albumin	-1.89	‹0.001	0.15	0.06 to 0.36
	aortic valvular calcification	1.32	**‹0.001**	3.72	1.83 to 7.76
Cardiovascular mortality	male gender	1.26	0.005	3.54	1.46 to 8.58
	simple vascular calcification score	0.45	**‹0.001**	1.56	1.25 to 1.94
Cardiovascular hospitalisations	diabetes	1.000	0.018	2.72	1.18 to 6.24
	albumin	-1.46	0.021	0.23	0.06 to 0.80
	Ca x P product	0.05	0.001	1.05	1.02 to 1.08
	simple vascular calcification score	0.09	**0.001**	1.34	1.13 to 1.59

Results are adjusted for age, HD duration, diabetes, iPTH, Ca x P product, albumin, vascular disease at baseline, vascular calcification score and all types of valvular calcifications.

directly associated with cardiovascular death (*HR=1.56, 95% CI = 1.25 to 1.94, p‹0.001*) and with cardiovascular hospitalizations (*HR=1.34, 95% CI = 1.13 to 1.59, p=0.001*). For each unit increment of the simple vascular calcification score there was a 56% increase of cardiovascular death risk. PTH (*p=0.04*) was a negative predictor of all-cause mortality. Albumin was a negative predictor of all-cause mortality (*p‹0.001*) and of cardiovascular hospitalisations (*p=0.02*). Diabetes (*p=0.018*) and CaxP product (*p=0.001*) were directly associated with cardiovascular hospitalisations.

Factors independently associated with valvular or vascular calcifications (Table VI)

The simple vascular calcification score was an independent predictor of aortic valvular calcification (*p=0.002*), mitral valvular calcification (*p‹0.001*) or any valvular calcification (*p‹0.001*). For each unit increase in vascular score there was, respectively, a 46% and a 66% increase in aortic and mitral valvular calcification risk (Table VI). PTH was directly associated with aortic valvular calcification (*p=0.02*). HD duration was directly associated with mitral valvular calcification (*p=0.003*) or any valvular calcification (*p=0.01*). Male gender was directly associated with mitral valvular calcification (*p=0.005*) or any valvular calcification (*p=0.006*). Phosphorus levels were directly associated with mitral valvular calcification (*p=0.04*). Age (*p=0.001*), diabetes (*p=0.01*) and HD duration (*p=0.01*) were associated with simple vascular calcification score.

DISCUSSION

The first study to demonstrate an association between valvular calcification and mortality in dialysis patients was Wang *et al* [11]. In our group of haemodialysis patients we have also verified that aortic and mitral valvular

Table VI

Factors independently associated with valvular or vascular calcification

Dependent variable	Independent variables	B	Sig.	O.R.	95% CI
Aortic valve calcification	PTH	0.001	0.020	1.00	1.000 to 1.002
	vascular calcification score	0.380	**0.002**	1.46	1.145 to 1.866
Mitral valve calcification	male gender	1.565	0.005	4.78	1.599 to 14.316
	HD duration (months)	0.012	0.003	1.01	1.004 to 1.020
	Phosphorus	0.356	0.040	1.42	1.017 to 2.003
	vascular calcification score	0.504	**‹0.001**	1.66	1.295 to 2.114
Aortic or Mitral valve calcification	male gender	1.407	0.006	4.08	1.490 to 11.195
	HD duration (months)	0.010	0.011	1.01	1.002 to 1.017
	vascular calcification score	0.410	**‹0.001**	1.51	1.211 to 1.879
Vascular calcification score	diabetes	1.730	0.012	5.64	1.467 to 21.708
	age	0.049	0.001	1.05	1.020 to 1.027
	HD duration (months)	0.015	0.011	1.015	1.004 to 1.027

Simple vascular calcification score is an independent predictor of aortic and mitral calcification

calcifications and vascular calcifications were associated with all cause death and with cardiovascular death. When valvular and vascular calcifications were evaluated in the same model, aortic valvular calcification was an independent predictor of all cause death and the simple vascular calcification score was an independent predictor of cardiovascular death and cardiovascular hospitalisations. Factors associated with valvular calcifications were the simple vascular calcification score, male gender, PTH and phosphorus levels. Ribeiro S *et al* [13] were among the first authors to verify an association between calcium phosphate product and valvular calcification, pointing out the role of mineral metabolism in the pathogenesis of valvular calcification in dialysis patients.

Many factors may contribute to the development of vascular and valvular calcifications in dialysis patients and there is increasing evidence of a link between bone disease and vascular disease in these patients. Kidney Disease: Improving Global Outcomes (K/DIGO) has recommended a new classification for mineral and bone disorder of chronic kidney disease that includes the evaluation of biochemical abnormalities (Ca, P, PTH and vitamin D levels), diagnosis of renal osteodystrophy preferably by bone biopsy and evaluation of vascular calcifications[14].

The presence of hyperphosphataemia and increased CaxP product has been considered a major pathogenic factor leading to vascular and soft tissue calcification in uraemic patients. Hyperphosphataemia can occur either with hyperparathyroidism or with adynamic bone disease and this may explain the lack of correlation between vascular calcifications and PTH levels verified in many studies. In this study we have found, as described by Wang *et al* [11], a direct association between PTH levels and valvular calcification. Other studies have also verified an association between vascular calcifications and low PTH levels[15] or an absence of association between vascular calcifications and PTH levels[2,16,17]. Giacheli CM[7] has demonstrated, in an *in vitro* model with vascular smooth muscle cells from human aorta, that increase of phosphorus and calcium outside the cell, in the culture media, leads to deposition of calcium phosphate in the matrix. This happens via two different mechanisms: an extracellular passive mechanism of direct deposition but also by an active mechanism through activation of an intracellular transcription factor, the core binding factor α-1 (Cbfa-1). This factor upregulates osteogenic genes and increases the synthesis of osteocalcin, alkaline phosphatase, collagen-rich extracellular matrix and creates the conditions for deposition of calcium-phosphate. Under these conditions, the vascular smooth muscle cell in the vessels acquires the phenotypic characteristics of an osteoblast. Some studies have also associated vascular calcification with oral calcium dose[1,15,17,18]. However, hypercalcaemia and hyperphosphataemia are not the only factors associated with vascular calcification in dialysis patients.

A deficiency in inhibitor calcification factors such as fetuin-A or matrix-Gla protein have been also associated with the development of vascular calcification[8] and it is considered that vascular calcification is the final result of the balance between calcification inhibitors and inducers.

In our patients we have not verified any association between calcium levels or calcium carbonate dose with vascular or valvular calcifications but these parameters were averaged for the six months preceding the evaluation of vascular and valvular calcifications and this period of observation may not reflect the whole period of dialysis which really determines the exposure. Low levels of 25-hydroxivitamin D have also been associated with vascular calcifications[19] and treatment with native or active vitamin D may have an impact on the development of vascular calcifications[20]. We do not evaluate vitamin D levels routinely in our patients but it is possible that this information, in the future, will be useful in the management of bone disease in CKD patients.

In non renal patients with valvular calcification, ectopic bone formation has been identified in the valves[10]. This calcification, previously considered to be the result of a passive degenerative mechanism, seems to be the result of an active mechanism most likely originated from differentiated myofibroblasts[21]. Osteoblastic bone formation and osteoclastic bone resorption were identified in these calcified valves. Osteopontin, osteocalcin, BMP 2 and BMP 4, which are potent calcification inducers, were also identified in these areas. This active process of yet unclear origin resembles what has been also described for active calcification of arterial walls in dialysis[7] and non dialysis patients[4].

In this study we have verified that a simple vascular calcification score evaluated in plain X-ray of pelvis and hands was independently associated with valvular calcification. Wang AY *et al* [22] showed that valvular calcification was associated with an increase in carotid media-thickness and carotid calcification. These results suggest that calcification is a systemic disorder in dialysis patients and that vascular and valvular calcification may share common characteristics.

Computed tomography scans are more accurate for the quantitative assessment of vascular calcifications but may be inadequate for an initial screening of vascular calcifications because of its price and its limited availability in some areas[14]. K/DOKI guidelines recommend the utilisation of plain X-ray for identification of vascular calcifications in dialysis patients[23]. This simple vascular calcification score evaluated in plain X-ray was previously demonstrated to be associated with coronary artery disease, peripheral artery disease and to be a predictor of cardiovascular mortality and cardiovascular hospitalisations in dialysis patients[12]. This vascular calcification score is an inexpensive and valuable tool that can be used for screening for the presence of vascular calcifications in dialysis patients.

Limitations of this study

This is an observational study evaluating the association of valvular calcifications with mortality and the association of vascular calcifications with valvular calcifications. It is not possible to demonstrate a cause-effect relationship between these variables.

CONCLUSIONS

In summary, in these patients, male gender, HD duration, PTH, phosphorus levels and vascular calcifications were associated with cardiac valvular calcification. Valvular and vascular calcifications were independent predictors of all cause and cardiovascular mortality. Vascular calcification was independently associated with cardiac valvular calcification, suggesting that these two types of calcification may be the result of the same pathogenic process.

Conflicts of interest. None declared.

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CAPÍTULO 6

EXISTE UM ELO DE LIGAÇÃO ENTRE O OSSO E O VASO NOS DOENTES EM DIÁLISE? (1.ª PARTE)

Calcificação vascular e análise histomorfométrica de biopsias ósseas

A insuficiência renal crónica cria um cenário fisiopatológico no qual se verificam inúmeras alterações minerais e ósseas interligadas. A diminuição da excreção urinária de fósforo desencadeia uma cascata de acontecimentos que, se não tratados, conduzem ao hiperparatiroidismo secundário. Inicialmente, o aumento dos níveis do FGF-23 (*fibroblast growth factor*) e da paratormona mantém o controlo do metabolismo mineral, mas à medida que a doença renal progride perde-se este equilíbrio e os níveis elevados da paratormona tornam-se prejudicais, com repercussão a nível de vários órgãos-alvo, entre os quais o osso. A osteodistrofia renal é uma entidade complexa que duma maneira simplista pode ser descrita como estando entre duas situações extremas de remodelação óssea: alta remodelação óssea, que corresponde ao hiperparatiroidismo secundário, e baixa remodelação óssea, que corresponde à doença óssea adinâmica. Entre estes dois extremos existe uma escala de alterações. Se às alterações de remodelação se associarem alterações de mineralização, surgem novas entidades: a osteomalacia, uma situação de baixa remodelação e défice de mineralização, e a osteodistrofia urémica mista, uma situação de alta remodelação com défice de mineralização.

Nos anos 80, o principal diagnóstico feito por biopsia óssea era a osteodistrofia urémica mista que resultava de hiperparatiroidismo secundário associado a um defeito de mineralização provavelmente em relação ao défice da vitamina D[1]. Outras alterações presentes eram a osteíte fibrosa, resultante de hiperparatiroidismo secundário predominante, e a osteomalacia em relação com a toxicidade óssea por alumínio. Nos últimos anos verificou-se um aumento da incidência de doença óssea adinâmica[1-3] que apresenta um padrão de baixa remodelação óssea. Esta nova patologia, mais frequente em doentes diabéticos e idosos, parece também associar-se a outros fatores, entre eles o uso de concentrações elevadas de cálcio no dialisante, o uso de doses elevadas de carbonato ou acetato de cálcio como quelante do fósforo e a supressão excessiva dos níveis de PTH após paratiroidectomia ou doses excessivas de vitamina D[3]. O aumento de doença adinâmica coincidiu com a modificação da terapêutica recomendada para a hiperfosfatemia nos doentes em diálise. No final dos anos 80, o uso de hidróxido de alumínio como quelante

do fósforo foi fortemente desaconselhado devido a estar associado ao desenvolvimento de osteomalacia, e foi substituído por doses elevadas de cálcio oral. No momento atual, os dois principais diagnósticos ósseos são a doença óssea adinâmica e o hiperparatiroidismo secundário[1], sendo a frequência da doença adinâmica superior à do hiperparatiroidismo secundário, tal como se comprovou num grupo de doentes portugueses submetidos a biopsia óssea[4].

A hiperfosfatemia e a hipercalcemia podem ocorrer, quer no hiperparatiroidismo secundário quer na doença adinâmica, embora por mecanismos diferentes. No hiperparatiroidismo, situação de elevada remodelação óssea, o próprio osso liberta cálcio e fósforo para a circulação. Na doença óssea adinâmica, o osso apresenta uma baixa remodelação óssea e é incapaz de incorporar cálcio e fósforo. O aumento de cálcio e de fósforo séricos não são totalmente removidos na diálise e depositam-se nos tecidos moles e nos vasos. Nas células musculares lisas das paredes das artérias identificou-se um processo ativo de diferenciação genética que conduz à transformação destas células em osteoblastos e é responsável pelo desenvolvimento das calcificações vasculares[5]. O aumento dos níves de fósforo e de cálcio são alguns dos fatores indutores desta transformação fenotípica[5].

Por proposta da iniciativa KDIGO 2006[6], a doença mineral e óssea da doença renal crónica (CKD-MBD) é atualmente definida como uma desordem sistémica que se manifesta por um ou mais dos seguintes aspetos: anomalias bioquímicas, anomalias ósseas e calcificações vasculares e de tecidos moles. O logótipo da CKD-MBD mostra que a mortalidade, a doença cardiovascular e as fraturas ósseas resultam da interação de alterações laboratoriais, alterações ósseas e calcificações vasculares. Estas são as bases fisiopatológicas da atual hipótese da existência de um elo de ligação entre a doença óssea e a doença vascular nos doentes renais crónicos. É contudo necessário demonstrar ainda que a correção destas alterações minerais e ósseas conduz a uma redução da mortalidade e da morbilidade nestes doentes.

London *et al*[7], em 2004, foram os primeiros a analisar a relação entre calcificações vasculares e dados de biopsias ósseas. Verificaram, num grupo de 58 doentes, uma associação entre calcificações vasculares avaliadas por ecografia e baixa remodelação óssea avaliada por biopsia óssea. Este estudo apresentava elevada percentagem de doentes submetidos a paratiroidectomia e doentes com depósitos de alumínio no osso.

Raggi *et al* demonstraram em 2005 que a terapêutica com cálcio, em comparação com a terapêutica de outro quelante do fósforo sem cálcio, o sevelamer, se associou a um efeito deletério no metabolismo ósseo e nos vasos[8]. Estes autores verificaram um aumento das calcificações coronárias e, simultaneamente, uma diminuição dos valores da paratormona e da fosfatase alcalina. Verificaram também que os doentes tratados com cálcio apresentaram uma diminuição da densidade mineral óssea, enquanto nos doentes tratados com sevelamer não houve alteração da densidade mineral óssea.

Num grupo de 38 doentes em hemodiálise[9] analisamos a relação entre calcificações coronárias avaliadas pelo *score* de Agatston avaliado em TAC helicoidal multicorte com dados obtidos por análise histomorfométrica de biopsias ósseas. Este estudo constituiu uma análise alargada feita num grupo de doentes que tinham participado num ensaio clínico que comparou o efeito do sevelamer e do carbonato da cálcio[4]. Os diagnósticos obtidos na biopsia óssea neste grupo de doentes foram de baixa remodelação em 50% dos doentes e de alta remodelação em 39%; apenas 11% dos doentes apresentava remodelação óssea normal. Nos nossos doentes verificamos a existência de associação entre calcificações vasculares e diminuição do volume ósseo. Em análise multivariada, verificou-se que esta associação persistia durante os primeiros anos após início de diálise, desaparecendo nos doentes deste estudo após 6 anos de diálise. Verificou-se pois uma interação entre o volume ósseo e a duração de hemodiálise. Por cada aumento de uma unidade no volume ósseo/volume total houve redução de risco de apresentar um *score* de Agatston superior a 400, de 24% nos doentes com 2 anos de diálise e de 16% nos doentes com 4 anos de diálise. Nos doentes com mais de 6 anos de diálise o aumento de volume ósseo não teve efeito benéfico nas calcificações coronárias.

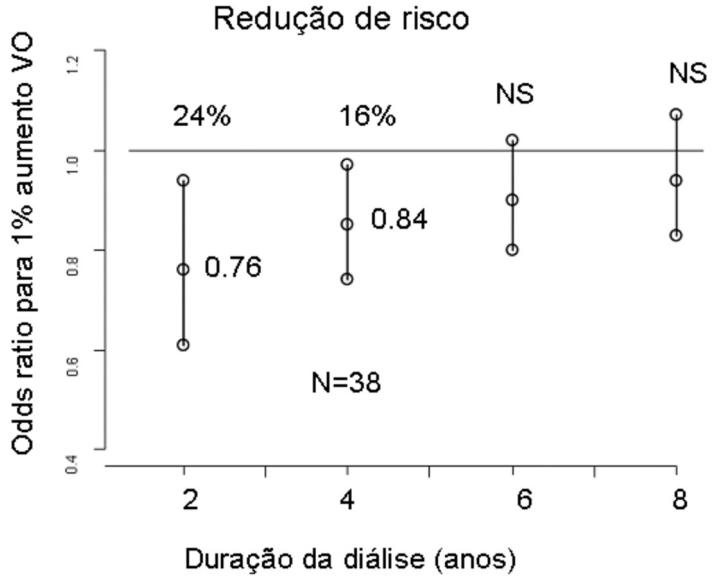

Fig. 6.1. Nos doentes com menos de 4 anos de diálise verificou-se uma redução de risco OR, *odds ratio*, de ter um *score* de Agatston > 400 por cada aumento de 1% do volume ósseo (VO)

A principal conclusão do nosso estudo é de que a diminuição do volume ósseo é um fator de risco para a presença de calcificações coronárias. Os primeiros anos de tratamento dialítico e provavelmente o período pré-diálise podem ser momentos importantes para intervenção

terapêutica. Na análise dos fatores de risco cardiovasculares tradicionais, idade, sexo, hipertensão arterial, níveis de colesterol e hábitos tabágicos, só a idade se associou às calcificações coronárias. Este artigo foi publicado na revista *Clinical Journal of American Society of Nephrology* e mereceu um comentário editorial de Gérard London[10].

Neste nosso estudo, as calcificações coronárias foram avaliadas pelo *score* de Agatston em 38 doentes. Dispúnhamos, contudo, da análise do *score* de calcificação vascular simples, assim como da análise histomorfométrica de biopsias ósseas num grupo de 50 doentes. Os dados relacionados com as calcificações coronárias foram considerados os mais relevantes e originais e foi decidido serem os únicos admitidos para publicação. Na presente dissertação consideramos ser indispensável a apresentação da relação entre o *score* de calcificação vascular simples e as biopsias ósseas. Neste grupo de 50 doentes (27 homens e 23 mulheres), com a idade média de 53±15 anos e duração média de hemodiálise de 63±54 meses, a presença de calcificações vasculares foi verificada em 30 doentes (60%), e um *score* de calcificação vascular > 3 estava presente em 19 (38%). Apenas 5 doentes eram diabéticos. Em análise multivariada por regressão binária verificamos que um *score* de calcificação vascular > 3 se associou diretamente à idade, à duração de hemodiálise e aos níveis séricos de cálcio e, inversamente, aos níveis séricos de albumina e ao volume ósseo (Tabela 6.1). Por cada aumento de uma unidade do volume ósseo houve uma redução de risco de 15% de SCVS > 3. Nos doentes com volume ósseo < 17,6%, o risco de um SCVS >3 foi 21 vezes superior, em comparação com os doentes com um SCVS ≤3 (Tabela 6.2). Este valor de corte do volume ósseo foi calculado por curvas ROC relacionando o volume ósseo com um SCVS > 3. Um volume ósseo <16%, valor de corte definido nas KDOQI *guidelines* para diagnosticar baixo volume ósseo[11] mas não ajustado à população portuguesa, associou-se a um risco 28 vezes superior de apresentar um SCVS>3 (IC 95% 2,1 a 366,7; p=0,011).

Fatores associados a um *score* de calcificação vascular simples >3

Regressão binária: Modelo 1

Variável dependente: SCVS > 3	B	OR	IC 95%	Sig.	R2
Idade	0,076	1,079	1,004 a 1,159	0,039	
Duração HD	0,023	1,023	1,005 a 1,042	0,013	
Cálcio sérico	0,169	1,183	1,012 a 1,382	0,034	54%
Albumina sérica	-0,42	0,643	0,440 a 0,938	0,022	
Volume ósseo	-0,159	0,853	0,744 a 0,977	0,022	

Tabela 6.1. Por cada diminuição de uma unidade de volume ósseo houve uma redução de risco de 15% de *score* de calcificação vascular simples SCVS > 3

Factores associados a um *score* de calcificação vascular simples >3

Regressão binária: Modelo 2

Variável dependente: SCVS > 3	B	OR	IC 95%	Sig.	R^2
Idade	0,087	1,091	1,003 a 1,187	0,043	
Duração HD	0,024	1,024	1,004 a 1,044	0,021	
Cálcio sérico	0,152	1,165	1,002 a 1,353	0,046	59%
Albumina sérica	-0,57	0,633	0,426 a 0,941	0,024	
Volume ósseo < 17.6%	3,050	21,115	2,518 a 177,096	0,005	

Tabela 6.2. Nos doentes com volume ósseo < 17,6%, o risco de ter um *score* de calcificação vascular simples >3 foi 21 vezes superior, em comparação com os doentes com um SCVS ≤3

Os estudos de London[5] mostram uma associação entre calcificações vasculares e baixa remodelação óssea. London demonstrou igualmente, noutro grupo de doentes, que a baixa remodelação óssea se associou a calcificações vasculares e a rigidez arterial na presença de tratamento com cálcio[12]. Outro estudo demonstrou que as calcificações vasculares não se desenvolvem exclusivamente em associação a baixa remodelação óssea. Barreto D, *et al* demonstraram que a progressão de calcificações coronárias se associa a doença óssea não controlada[13]. Neste estudo, num grupo de 38 doentes em diálise com calcificações coronárias na avaliação inicial, verificou-se em duas situações progressão das calcificações coronárias após um ano: nos doentes que evoluíram de uma situação de alta remodelação óssea para baixa remodelação, mas também na situação oposta, de baixa remodelação óssea para alta remodelação. Esta evolução foi independente do tipo de captador de fósforo usado, sugerindo que a alta ou a baixa remodelação óssea não controladas podem contribuir para a progressão das calcificações vasculares. Em resumo, todos estes estudos confirmam a hipótese da existência de um elo de ligação entre doença óssea e doença vascular nos doentes em diálise. As calcificações vasculares podem surgir quer em associação a baixa quer a alta remodelação óssea. O nosso estudo foi o primeiro a demonstrar que o baixo volume ósseo é um fator de risco para a presença de calcificações coronárias.

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Low Bone Volume—A Risk Factor for Coronary Calcifications in Hemodialysis Patients

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Background and objectives: There is increasing evidence that altered bone metabolism is associated with cardiovascular calcifications in patients with stage 5 chronic kidney disease on hemodialysis (HD). This study was conducted to evaluate the association between bone volume, turnover, and coronary calcifications in HD patients.

Design, setting, participants, & measurements: In a cross-sectional study, bone biopsies and multislice computed tomography were performed in 38 HD patients. Bone volume/total volume, activation frequency, and bone formation rate/bone surface were determined by histomorphometry and coronary calcifications were quantified by Agatston scores.

Results: Prevalence of low bone turnover was 50% and of low bone volume was 16%. Among the studied traditional cardiovascular risk factors, only age was found to be associated with coronary calcifications. Lower bone volume was a significant risk factor for coronary calcifications during early years of HD, whereas this effect was not observed in patients with dialysis duration >6 yr. Histomorphometric parameters of bone turnover were not associated with coronary calcifications.

Conclusions: Low bone volume is associated with increased coronary calcifications in patients on HD.

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Cardiovascular disease and stroke account for 60 to 70% of all deaths in patients with chronic kidney disease (CKD) on maintenance hemodialysis (HD) (1,2). In a study utilizing electron beam computed tomography, dialysis patients presented with higher coronary calcification scores when compared with nondialysis patients (3). In HD patients, vascular calcifications were shown to be associated with cardiovascular morbidity and mortality (4). Conspicuously, however, two prospective trials on modification of traditional cardiovascular risk factors in CKD patients could not demonstrate significant differences in cardiovascular outcomes, including death between an intensive "multiple traditional risk factor intervention" group and the standard therapy group despite significantly improved traditional risk factor control in the former (5,6). Recent evidence shows that vascular calcifications in CKD can occur early, and that deposition of calcium in the vascular wall is a complex and tightly regulated process that is akin to bone mineralization (7,8). In addition, abnormalities in bone turnover and bone volume are associated with more vascular calcifications in uremic patients (9,10).

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Taking this evidence into consideration, the international initiative, Kidney Disease: Improving Global Outcomes, recommends the inclusion of evaluation for extraskeletal calcifications into a new classification for "chronic kidney disease-mineral and bone disorder" (11). Although an association between low bone turnover and increased vascular calcification (measured by a semiquantitative vascular calcification score) was reported previously (9), the relationship between histomorphometric parameters of bone formation and bone volume and quantitative determinations of coronary calcifications by multislice computed tomography (MSCT) while controlling for the traditional cardiovascular risk factors of hypertension, gender, age, cholesterol, and tobacco use has not been investigated in HD patients. The study presented here aims at providing this information.

Materials and Methods

Study Design

This is an extension study in a cohort of patients who participated in the Sevelamer Study Group randomized trial (12). Briefly, the Sevelamer Study Group study was a 54-wk randomized open-label study to compare the effects of sevelamer hydrochloride and calcium carbonate on bone histomorphometric parameters. This extension study investigates the interaction between histologically determined parameters of bone turnover/bone volume and coronary calcifications measured by MSCT in a cross-sectional study design. The Institutional Review Boards of all participating institutions approved the protocol. The study has been conducted in adherence to the Declaration of Helsinki, and all patients provided informed consent.

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Population

Thirty-eight patients provided informed consent for performing histomorphometric analysis of bone samples and MSCT for evaluation of coronary calcifications and were enrolled in this extension study. Inclusion criteria were ages 18 yr or older, dialysis duration of at least 3 mo, mental competence, and willingness to participate in the study. All patients were required to have stable serum phosphorus of ≤8.1 mg/dl because higher levels were considered indicative of medication noncompliance. The extension study called for providing consent to undergo evaluation for coronary calcifications by MSCT, which was performed on average 3.8 ± 1.9 mo after the bone biopsy. Exclusion criteria were failed kidney transplant during the past 6 mo; pregnancy; uncontrolled systemic illnesses or organic diseases with potential influence on bone metabolism such as diabetes mellitus, active or chronic liver disease, malabsorption, malignancy, and thyroid dysfunction; history of or present treatment with pharmacologic agents known to affect bone metabolism such as bisphosphonates, fluoride, calcitonin, glucocorticoids or other immunosuppressive agents, hormone replacement therapy, and selective estrogen receptor modulators; chronic alcoholism and/or drug addiction; and allergy to tetracycline compounds.

Hypertension was defined as blood pressure ≥140/90 mmHg and/or treatment with antihypertensive medications. Tobacco use was defined as inhalative smoking of ≥1 cigarette per day.

Bone Biopsies

Anterior iliac crest bone biopsies were done after tetracycline labeling under local anesthesia and conscious sedation. The labeling schedule consisted of a 2-d oral administration of tetracycline hydrochloride (250 mg twice daily) followed by a drug-free interval of 10 d, and subsequent oral administration of demeclocycline hydrochloride (300 mg twice daily) for 4 d. Bone biopsies were performed 3 to 4 d after completing the second label. Bone samples were obtained with the one-step electrical drill technique (Straumann Medical, Waldenburg, Switzerland). Bone samples were processed undecalcified and sections were stained with the modified Masson–Goldner trichrome stain, the aurin tricarboxylic acid stain, and solochrome azurin for assessment of stainable aluminum in bone (13). Unstained sections were prepared for phase contrast and fluorescence light microscopy. Histomorphometric analysis of bone was done at standardized sites in cancellous bone using the semiautomatic method (Osteoplan II, Kontron, Munich, Germany) at 200× magnification. Bone volume/total volume (BV/TV) was calculated for assessment of mineralized bone volume, whereas activation frequency (Ac.f.) and bone formation rate/bone surface (BFR/BS) were calculated for assessment of bone turnover. All bone samples were processed and analyzed without knowledge of the clinical data. BV/TV classification was based on three categories characterized by the distribution of our data (5th, 50th, and 95th percentile). The classification of "low," "normal," and "high" bone turnover was based on our normative database (14–16). The outcome group for low bone turnover was defined as Ac.f. <0.49 yr^{-1} and/or BFR/BS <1.8 $mm^3/cm^2/yr$. The outcome group for normal bone turnover was defined as Ac.f. 0.49 to 0.72 yr^{-1} and/or BFR/BS 1.8 to 3.9 $mm^3/cm^2/yr$. The outcome group for hig bone turnover was defined as Ac.f. >0.72 yr^{-1} and/or BFR/BS >3.9 $mm^3/cm^2/yr$.

Assessment of Coronary Calcifications

Vascular calcifications were assessed by a quantitative score using MSCT. MSCT scans were performed with the four-slice technique on the model Somatom Volume Zoom (Siemens AG, Erlangen, Germany). Slices of 2.5-mm thickness were acquired under the following conditions: 120 kVp, 130 mAs, and 0.5 gantry rotation time. All images were transferred to a workstation and analyzed with calcium scoring software (HeartView CT, Siemens AG, Erlangen, Germany). Quantification of coronary calcification was performed by calculating the Agatston (Agt.) score on the basis of the maximum x-ray attenuation coefficient (measured in Hounsfield units).

Biochemical Analyses

Blood was drawn at the time of the bone biopsy after an overnight fast. The following biochemical parameters were measured: serum calcium and phosphorus by an autoanalyzer (Hitachi 747, Globe Scientific Inc.), immunoreactive parathyroid hormoneby DPC IMMULITE PTH IRMA (Diagnostics Products Corporation, Los Angeles, California; normal range 16 to 87 pg/ml; intra- and interassay coefficients of variation <7 and <9%, respectively); 25-(OH)-vitamin D by LIAISON 25-OH Vitamin D assay (Diasorin, Saluggia, Italy; normal range 25 to 100 ng/ml; intra- and interassay coefficients of variation 4.1 and 7%, respectively); and total cholesterol was measured by the Synchron LX system (Beckman Coulter, Fullerton, California; desirable range <200 mg/dl; intra- and interassay coefficients of variation both <3%).

Statistical Analyses

Descriptive statistics are presented as means, medians, minimums, maximums, and SD stratified according to Agt. score groups of <100, 100 to 400, and >400. The variables Ac.f., BFR/BS, and HD duration had right-skewed distributions and were log transformed for analysis. Boxplots were used to characterize the distributions of BV/TV, log Ac.f., and log BFR/BS for each Agt. score group. Bivariate associations were assessed using the nonparametric Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. Multivariable associations were assessed using separate ordinal (proportional odds) logistic regression analyses to evaluate the effects of BV/TV, log Ac.f., and log BFR/BS on Agt. score, adjusted for measured demographic and biologic factors. All calculations were performed using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Demographic characteristics of the study population are presented in Table 1. There were no statistically significant differences between the coronary Agt. score groups except for age. The study included only nondiabetic patients.

BV/TV was low in 6 (16%), normal in 9 (24%), and high in 23 (60%) patients. Low bone turnover was diagnosed in 19 patients (50%), normal turnover in 4 patients (11%), and high turnover in 15 patients (39%) on the basis of Ac.f. and BFR/BS. There were no disagreements regarding classification of bone turnover between Ac.f. and BFR/BS. None of the biopsies showed positive staining for aluminum or iron. In unadjusted analyses, there were no statistically significant differences between the three Agt. score groups regarding the bone histomorphometric parameters BV/TV, Ac.f., and BFR/BS (Figure 1).

Ordinal logistic regression revealed that, among the studied traditional cardiovascular risk factors, age was the only variable that predicted Agt. score groups ($P = 0.02$). Our data showed that Agt. score groups were also predicted by BV/TV ($P = 0.02$); one unit (%) increase in BV/TV in patients with the same age and on HD for ≤2 yr decreases the odds of being in the high Agt. score group (Agt. >400) by 24% [odds ratio: 0.76; 95% confidence interval: 0.61 to 0.94]. In addition, the interaction term between BV/TV and HD duration was associated

Table 1. Demographic characteristics of the study population

Characteristic	Agatston Score			
	<100	100 to 400	>400	*P*
Age (yr)				0.03[a]
N	19	8	11	
mean (SD)	45.2 (15.2)	59.8 (14.9)	57.2 (15.1)	
median (min, max)	45 (21, 74)	59 (39, 76)	57 (37, 78)	
Cholesterol (g/L)				0.44[a]
N	19	8	11	
mean (SD)	1.70 (0.3)	1.6 (0.2)	1.5 (0.2)	
median (min, max)	1.7 (1.2, 2.4)	1.6 (1.2, 2.0)	1.5 (1.2, 2.0)	
Calcium (mg/dl)				0.35[a]
N	19	8	11	
mean (SD)	96 (6.6)	94 (6.2)	98 (5.7)	
median (min, max)	95.4 (87, 111)	93.6 (85, 104)	100 (87, 105)	
Phosphorus (mg/dl)				0.08[a]
N	19	8	11	
mean (SD)	5.4 (0.9)	4.6 (0.8)	5.0 (0.7)	
median (min, max)	5.5 (3.9, 7.2)	4.8 (3.2, 5.3)	5.0 (3.8, 6.3)	
iPTH[c] (pg/ml)				0.47[a]
N	19	8	11	
mean (SD)	620 (614)	293 (167)	570 (700)	
median (min, max)	353.4 (50, 2164)	259 (155, 679)	301 (100, 2572)	
25-(OH)-vitamin D (ng/ml)				0.78[a]
N	19	8	11	
mean (SD)	21.2 (7.8)	19.5 (6.2)	20.2 (10.0)	
median (min, max)	21.5 (7.8, 37.6)	17.5 (13.5, 27.8)	14.6 (11.0, 41.4)	
Dialysis duration (mo)				0.48[a]
N	19	8	11	
mean (SD)	73.1 (56.6)	44.9 (23)	87.2 (77.2)	
median (min, max)	48.3 (21, 206)	39.5 (19, 77)	45 (23, 242)	
Gender				0.27[b]
N	19	8	11	
male (*N*, %)	9 (47.3)	3 (37.5)	8 (72.7)	
female (*N*, %)	10 (52.6)	5 (62.5)	3 (27.3)	
Tobacco use				0.88[b]
N	19	8	11	
yes (*N*, %)	4 (21.0)	1 (12.5)	3 (27.3)	
no (*N*, %)	15 (79.0)	7 (87.5)	8 (72.7)	
Hypertension				0.63[b]
N	19	8	11	
yes (*N*, %)	11 (57.9)	4 (50.0)	8 (72.7)	
no (*N*, %)	8 (42.1)	4 (50.0)	3 (27.3)	

[a]*P* value computed from Kruskal–Wallis test.
[b]*P* value computed from Fisher's exact test.
[c]iPTH, immunoreactive parathyroid hormone.

with Agt. score groups ($P < 0.05$); the effect of BV/TV on the odds of being in a specific Agt. score group decreased with increasing HD duration (Figure 2) and was no longer statistically significant in patients on HD for >6 yr (odds ratio: 0.90; 95% confidence interval: 0.80 to 1.02).

To better study the interactions between BV/TV, age, and HD duration for predicting Agt. score groups, we divided these independent variables into three categories characterized by the distribution of our data (5th, 50th, and 95th percentiles). Classification according to these percentiles corresponded to BV/TV values of 15, 24, and 37%; ages of 30, 50, and 75 yr; and HD durations of 2, 4, and 17 yr. Because ordinal logistic regression revealed that the associations between bone volume (BV/TV) and coronary calcifications were no longer statistically

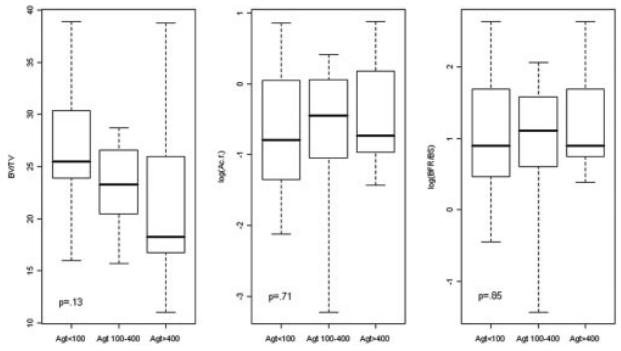

Figure 1. Distribution of values for bone volume/tissue volume (BV/TV), activation frequency (Ac.f.), and bone formation rate/bone surface (BFR/BS) among the Agatston (Agt.) score classes <100, 100 to 400, and >400. Box = median, 25 to 75%; T-bars = minimum and maximum values.

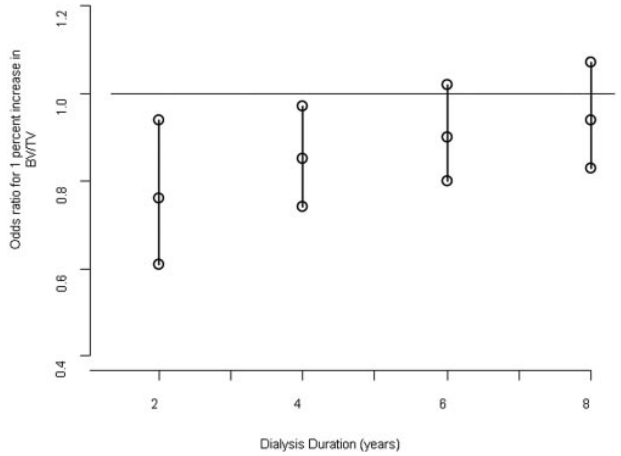

Figure 2. Effect of the interaction between BV/TV volume and hemodialysis (HD) duration on the odds of an Agt. score of >400. Odds ratio represents the change in the odds for an Agt. score >400 for each 1% increase in BV/TV. Vertical bars = 95% confidence interval.

significant after a HD duration of 6 yr, the sixth-year HD duration time point was also examined. Accordingly, Figure 3 depicts changes in the probabilities for the Agt. score group <100 (Figure 3, A through D) and for the Agt. score group >400 (Figure 3, E through H) for the three different BV/TV values (depicted on the *x*-axis) and three different ages (depicted by different lines) on the basis of the four different HD durations (Figure 3, A and E = 2 yr; Figure 3 B and F = 4 yr; Figure 3, C and G = 6 yr; and Figure 3, D and H = 17 yr). Irrespective of HD duration, increasing age carries a higher likelihood of having an Agt. score of >400 and accordingly a lower likelihood of having an Agt. score <100. This age effect was observed in each BV/TV class. However, the magnitude of this bone volume effect is conditional on HD duration; in the HD duration classes of 2 and 4 yr, lower BV/TV bears a higher likelihood of having an Agt. score >400 and, accordingly, lower likelihood of having

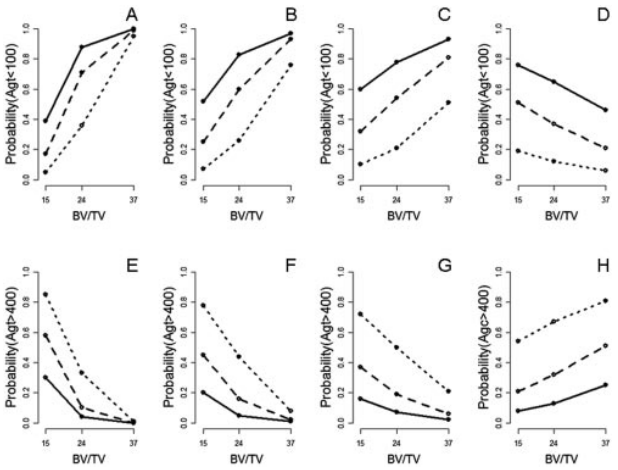

Figure 3. Changes in the probabilities for (A through D) the Agt. score group <100 and (E through H) the Agt. score group >400 for different BV/TV values and ages on the basis of four different HD durations (A and E = 2 yr, B and F = 4 yr, C and G = 6 yr, D and H = 17 yr). Solid line = 30-yr-old patient; long dashed line = 50-yr-old patient; short dashed line = 75-yr-old patient.

an Agt. score <100 irrespective of the age group (Figure 3). However, in long HD duration classes (17 yr), higher BV/TV is associated with lower likelihood of an Agt. score <100 and higher likelihood of an Agt. score >400 irrespective of age.

In the case of shorter HD duration (<6 yr), the incremental increase in the likelihood of having an Agt. score <100 is the largest for increasing BV/TV from the 5th to the 50th percentile for younger patients (30 yr), whereas older patients (75 yr) derive the highest benefit from increasing BV/TV from the 50th to the 95th percentile. It is of note that, after 2 yr of HD duration, a 30-yr-old patient with a low BV/TV has about the same probability of having an Agt. score <100 ($P = 0.39$) than a 75-yr-old patient with normal BV/TV ($P = 0.36$). Figure 3 also shows that the likelihood of having an Agt. score <100 is lower at the lowest BV/TV at the shortest HD duration than at normal or high BV/TV at the longest HD duration irrespective of age.

Ordinal logistic regression conducted with Ac.f. and BFR/BS revealed that only age was predictive of the Agt. score class of coronary arteries ($P = 0.03$). No specific interactions were found between variables of bone turnover and the studied traditional risk factors ($P > 0.05$).

Discussion

Our data confirm previous studies reporting on the effect of age on vascular calcifications in HD patients (17,18). We also confirm previous observations reporting lack of predictive value of the traditional cardiovascular risk factors of hypertension, smoking, gender, and cholesterol for cardiovascular outcomes in HD patients (5,6,18,19).

The novel aspect of our study is the investigation of the interaction between the bone volume and the bone turnover components of renal osteodystrophy (determined by histomorphometry) and coronary calcifications (determined by MSCT)

in prevalent HD patients. Low bone volume and bone loss are common findings in patients on renal replacement therapy (20,21). Our study demonstrates the importance of bone volume for predicting coronary calcifications. Specifically, it appears that lower bone volume is predictive of higher Agt. scores, reflecting higher risk of cardiovascular events (Agt. ≥100) irrespective of age. Although correlations between bone loss and progressive arterial calcifications were described in the general population, (22,23) there is little information available on their possible interaction in HD patients. Peripheral arterial calcifications assessed by plain radiographs and low bone volume were observed in an early study by Zuchelli and colleagues, but the authors did not examine a possible interrelation between bone volume and vascular calcifications and did not report on the contribution of traditional risk factors for cardiovascular calcifications (24). Similarly, assessment of bone mineral density by quantitative computed tomography in HD patients showed less coronary calcifications with higher vertebral bone mineral density (25). Although several serum markers of bone metabolism such as bone morphogenic proteins (26) and osteoprotegerin (27) were implicated in the pathogenesis of vascular calcifications, to our knowledge this is the first population-based study that describes the predictive value of histologically measured bone volume for coronary calcifications in HD patients.

An interesting finding in our study is that the effect of bone volume on Agt. scores is conditional on HD duration; if HD duration is >6 yr, the bone volume effect seems to be overridden by other risk and/or protective factors that determine coronary calcifications. In light of a 5-yr mortality rate of ≥75% for patients on dialysis (1), it appears that the bone volume effect is of great importance for most HD patients. The implications of lower bone volume for higher Agt. scores is further highlighted by our finding that 30-yr-old patients who exhibit low bone volume early in the course of HD have probabilities for low Agt. scores comparable to 75-yr-old patients with normal bone volume.

London and colleagues previously reported on the association between histologically diagnosed low bone turnover and arterial calcifications detected on plain radiographs and evaluated by semiquantitative calcifications scores in HD patients (9). Our study presented here did not find an association between bone turnover parameters and coronary calcifications measured by MSCT, although a relationship was demonstrated between histologically determined bone turnover and thoracic aorta and iliac artery calcifications in a previous publication (10). Discordance between predictors of aortic and coronary calcifications has been described in a series of publications from the large, population-based Multi-Ethnic Study of Atherosclerosis (MESA) (28–30). Accordingly, our findings supplement rather than contradict the findings of London and colleagues and highlight that studies aiming at predicting vascular calcifications have to take into account the site examined within the vascular tree, the morphology of the vascular tissue, and the localization of calcification within the vascular wall.

Limitations of our study are that patients were recruited from dialysis centers in Portugal and diabetic patients were not included. Accordingly, we cannot generalize our findings to other populations and draw inferences on a possible effect of diabetes mellitus on the observed interaction between bone volume and coronary calcifications. We are currently in the process of designing larger studies that focus on the contribution of renal osteodystrophy to coronary calcifications in different HD patient populations. On the basis of recent improvements in the sensitivity of MSCT scanners for detecting coronary calcifications, it is conceivable that the magnitude of the bone volume effect will be greater than observed in our presented here. More sensitive detection methods for coronary calcifications might also contribute to a more precise estimation of the contribution of bone turnover to coronary calcification.

In summary, our data implicate that low bone volume is a significant risk factor for coronary calcifications in HD patients, and that this risk is dependent on the patient's age and HD duration. Although statistical associations do not necessarily imply cause and effect relationships, our findings suggest that early prevention of bone loss in CKD patients might carry important implications for reducing coronary calcifications, especially because age and HD duration are not modifiable risk factors.

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Disclosures

None.

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See related editorial, "Bone–Vascular Axis in Chronic Kidney Disease: A Reality?" on pages 254–257.

Editorial

Bone–Vascular Axis in Chronic Kidney Disease: A Reality?

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Chronic kidney disease (CKD) is characterized by changes in mineral metabolism associated with alterations of its hormonal regulation and various forms of bone disease. In the past, these associations focused attention on the kidney–bone axis. The last decade has seen renewed interest on interactions among mineral metabolism disorders and extraosseous and cardiovascular calcifications observed in CKD or end-stage renal disease (ESRD). Vascular calcification is an active process similar to bone formation that implicates a variety of proteins involved in bone and mineral metabolism (1,2) and is considered part of a systemic dysfunction defined as CKD–mineral and bone disorder (3). Growing evidence linking bone with different functional and structural characteristics of the arterial tree has contributed to developing the concept of the bone–vascular axis (4).

The first observations suggesting the existence of the bone–vascular axis were the frequent associations of osteoporosis and atherosclerotic vascular calcifications observed in postmenopausal women (5–7). Longitudinal population-based studies revealed a relationship between the progression of vascular calcifications and bone demineralization, and others were identified between bone mineral density (BMD) and aortic or central artery calcifications (6), or coronary arteries in type 2 diabetes (8). The osteopenia–osteoporosis association was also linked with arterial functional indexes, such as aortic stiffness and interactions independent of calcifications, suggesting broader biologic interplay (9,10).

Relationships between bone and vascular modifications were also observed in CKD and ESRD patients. In dialysis patients, coronary artery calcification score was found to be inversely correlated with vertebral bone mass (11,12). In addition, a high systemic arterial calcification score combined with bone histomorphometry suggestive of low bone activity was observed in hemodialysis patients (13,14). Arterial stiffening and low spine BMD or calcaneal osteopenia were significantly associated in CKD and hemodialysis patients (15–17).

In the present issue of the *Journal*, Adragao *et al.* (18) provide new evidence linking altered bone metabolism to coronary calcifications in patients with stage-5 CKD on hemodialysis. In their cross-sectional study performed on 38 hemodialyzed patients, they analyzed the relationships between bone biopsy parameters (bone volume/total volume, bone formation rate/bone surface, and activation frequency) and coronary calcifications determined by multislide computer tomography. Their principal conclusion was that low bone volume is associated with more coronary calcifications, whereas histomorphometric parameters of bone turnover were independent of coronary calcifications.

Their results complete those of Barreto *et al.* (12), who found a negative correlation between coronary calcifications and trabecular bone volume or its thickness. The absence of correlations between histomorphometric indexes of bone turnover and coronary calcifications differ from reports by London *et al.* (13,14), who found an association between systemic arterial calcifications (aorta and the main peripheral arteries) and indexes of low bone turnover, but not trabecular volume. One important difference between these publications resides in the arterial territories analyzed and the relationships of the different bone changes (bone volume, osteoblasts number, or tetracycline labeling), which do not necessarily reflect the same mechanisms. Adragao *et al.* (18) studied coronary artery calcifications, whereas London *et al.* (13,14) examined the aorta and systemic arteries.

Researchers investigating the arterial system should keep in mind the marked heterogeneity of the arterial tree (19). Blood vessel formation recruits cells of different origins whose components are derived from vascular smooth muscle cells (VSMC) and pericytes. VSMC is a tissue generated by at least seven unique and nonoverlapping sources, and different vessels or even segments of the same vessel, are composed of VSMC arising from distinct progenitors that respond in origin-specific ways to different common stimuli. The observations linking arterial calcifications to bone usually concern the aorta or large conduit arteries and coronary arteries. Whereas the coronary arteries are derived from proepicardium, the aorta's origin even more complex. The ascending aorta and aortic arch originate from neural crest, the thoracic aorta (athero-resistant) is derived from somites, and splanchnic mesoderm gives rise to the abdominal aorta (athero-susceptible). The boundaries of these different segments are sharp and are associated with quite different responses to common risk factors. Arterial bed-specific susceptibilities were documented in human studies showing that despite common risk factors, each analyzed vascular segment (coronary arteries, ascending aorta and its major arteries, thoracic aorta at the renal artery level, and terminal aorta and femoral arteries) mounted its own distinct response to atherogenesis (20).

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The eventual biologic link between vascular calcifications and bone changes is certainly part of the aging process, but in many studies, these bone–vascular associations remained significant after adjustment for age, which suggests an age-independent causal relationship (5–7). The mechanisms responsible for bone–vascular interactions are not well understood.

The results of increasing numbers of experimental studies led to the recognition of similarities between bone development and mineralization and the process of arterial calcifications. The calcification process involves VSMC differentiation into osteoblast-like cells, with subsequent mineralization. This process is induced and regulated by equilibrium between factors promoting or inhibiting calcification, involving a variety of proteins that are important for bone metabolism but are also expressed in arteries (1–3). Clinical data on humans indicate that osteoporosis and vascular calcifications are influenced by several common risk factors, such as diabetes, inflammation, dyslipidemia, oxidative stress, estrogen deficiency, vitamin D and K deficiencies, and others (21–24). The roles of dyslipidemia, oxidative stress, and inflammation seem to be of importance. Oxidized lipids can paradoxically induce atherosclerosis and the differentiation of osteoblasts, with subsequent arterial wall mineralization having the opposite effect, that is, inhibiting osteoblastic differentiation into bone osteoblasts (25). Oxidized lipids are also a substrate for peroxisome proliferator-activator receptor-γ (PPARγ), which redirects the differentiation of mesenchymal progenitors from preosteoblasts to adipocytes and Cbfa1/Runx2 expression essential for osteoblastogenesis.

In osteoporosis, it is osteoprotegerin (OPG), the receptor activator of the nuclear factor-kappa B (NF-κB) ligand (RANKL) system, that has attracted the most attention (26,27). OPG-deficient mice develop osteoporosis with severe cortical and trabecular bone porosity and high fractures rates. In parallel with these bone lesions, these mice develop medial calcification of the aorta and large arteries (28). By binding RANKL, OPG inhibits osteoclastogenesis and bone resorption. OPG and RANKL are also involved in immune-induced inflammatory responses. OPG can limit local inflammatory responses, and *in vitro* OPG, produced by smooth muscle and endothelial cells, acts as an antiapoptotic factor prolonging endothelial cell survival (27). Changes of the RANKL/OPG ratio are critical to the evaluation of clinical impact, and high OPG-associated cardiovascular risk probably represents an inadequate response of OPG to increased RANKL activity (29).

Although osteoporosis–arterial calcification interactions could be observed in general populations in the absence of overt mineral metabolism disorders, in CKD/ESRD patients, the associations between vascular calcifications and bone disorders are linked to deterioration of mineral and bone metabolism caused by serum phosphate and calcium changes, and disruption of endocrine and humoral pathways, including parathyroid hormone (PTH), calcitriol, fibroblast growth factor-23 (FGF-23)/Klotho, and others (1–3). Experimental and clinical data indicate that hyperphosphatemia plays a direct role in the osteoblast-like transformation of VSMC by upregulating Cbfa1/Runx2 and osterix transcription factors (1,2,30,31). The calcification could be enhanced by the imbalance between inducers and local or systemic inhibitors of calcification, such as low fetuin-A, pyrophosphate, or osteopontin (31–33). In CKD and ESRD patients, the relationship between bone and vascular calcifications concerns several aspects of bone disorders, such as high bone turnover (secondary hyperparathyroidism), and low bone activity (adynamic bone disease), and low bone volume. In secondary hyperparathyroidism, the increased bone resorption associated with the endogenous release of phosphate and calcium could play a critical role in the induction of vascular calcification. Chronic PTH elevation upregulates RANKL, downregulates *OPG* gene expression, and raises the RANKL/OPG ratio (34). The high prevalence and extent of arterial calcification is also observed in ESRD patients with bone demineralization or low bone activity (11–14,18), a clinical situation closer to the osteoporosis–vascular calcification association seen in general populations.

In general populations and ESRD patients, relationships between bone disorders and vascular dysfunction were observed independently of calcifications, age, BP, and other confounding factors, thereby suggesting direct bone–vascular cross-talk. Bone is an active "endocrine" organ, as demonstrated by fibroblast growth factor 23 (FGF-23) regulation of the phosphate balance. FGF-23 is synthesized and released by osteocytes, which are terminally differentiated osteoblasts. Osteoblast function could be an important player in the bone–vascular axis. Lee *et al.* (35) showed that osteoblasts exert endocrine regulation of energy metabolism, with osteocalcin (OCN) playing an important role. OCN can regulate the expression of insulin genes, β-cell proliferation, and adiponectin (ADPN) release and its expression adipocytes (36). In general populations, serum OCN was positively associated with ADPN (37,38). ADPN protects arteries against hypertension, slows atherosclerosis, and activates osteoblastogenesis (39,40). An inverse relationship was found between ADPN and OCN and arterial stiffness (38). Plasma ADPN levels are low in metabolic syndrome and type 2 diabetes patients. Whether this low ADPN could account for decreased osteoblastogenesis and frequently observed adynamic bone disease in diabetic patients remains a hypothesis.

Multiple hormones involved in the endocrine regulation of adipose tissue and energy metabolism could affect bone structure, including leptin. Leptin is a powerful inhibitor of *in vivo* bone formation (41) and facilitates vascular calcification (42). In ESRD, serum leptin is elevated and is associated with low PTH (43), suggesting that leptin might diminish bone activity and promote arterial calcifications in this setting.

Bone loss may also occur as a secondary consequence of ischemia related to vascular disease. Arteries and arterioles within the bone are also subject to arteriosclerosis, and a link between compromised intraosseous circulation and consequent osteoporosis may exist (44). Intraosseous angiogenesis and bone remodeling are regulated by similar cytokines and growth factors, and interactions between bone formation–resorption and blood supply are known to occur (45). A recent study showed that in otherwise healthy women, bone-perfusion indices were lower in women with osteoporosis compared with women with osteopenia or normal BMD (46).

In conclusion, increasing numbers of articles on general populations and CKD and ESRD patients have reported significant associations among arterial pathology (atherosclerosis and arterial calcifications) and bone disorders, including osteoporosis, and high or low bone activity. The pathophysiology and biologic links between bone and arterial abnormalities suggest the existence of bone–vascular cross-talk. The nature of this communication is not well understood. It could be a consequence of (*1*) the action of common factors on bone remodeling and atherosclerosis/calcification, (*2*) the direct action of bone cells (osteoblasts and/or osteocytes) on vascular biology and structure, and/or (*3*) the compromised bone blood supply resulting from arteriosclerosis of bone vessels and reduced perfusion.

Disclosures

None.

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See related article, "Low Bone Volume—A Risk Factor for Coronary Calcifications in Hemodialysis Patients," on pages 450–455.

REVIEW ARTICLE

Port J Nephrol Hypert 2008; 22(2): 157-163

Is there a link between bone and vessel in dialysis patients?

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INTRODUCTION

Haemodialysis patients are at a very high cardiovascular risk not explained by traditional risk factors[1,2]. Conventional treatment which reduces cardiovascular risk in the general population may not be effective in dialysis patients, as shown in the 4D trial which compared atorvastatin with placebo in a group of diabetic patients on haemodialysis[3]. Atorvastatin as compared with placebo did not reduce the primary composite endpoint which included cardiovascular death or non fatal acute myocardial infarction or stroke.

These results may be partially explained by the characteristics of cardiovascular death observed in dialysis patients. Cardiovascular death is the main cause of death in dialysis patients and accounts for 48% of all deaths in dialysis patients in the USA, as shown by USRDS annual report[4]. This report states that sudden death is the main cause of cardiovascular death, responsible for 27% of all deaths, while atherosclerosis and acute myocardial infarction, situations in which statin administration is recommended, accounts for only 11% of all deaths[4].

Another possible explanation for the ineffectiveness of statins observed in the 4D trial is the type of vascular calcification that haemodialysis patients develop. Vascular calcification is another factor associated with cardiovascular mortality in dialysis patients and can onset in two different clinical situations with different treatment options[5]. There are two types of vascular calcifications: intimal and medial calcification. Intimal calcification is associated with atherosclerosis and is related to altered lipid metabolism. Medial calcification is associated with arteriolosclerosis and in chronic kidney disease (CKD) patients is associated with altered mineral metabolism[6], in a clinical setting where statins are probably not operative.

The main manifestations of intimal calcification are the result of the formation of atherosclerotic plaques with stenotic lesions and ischaemia. Medial calcification does not cause obstructive lesions but modifies the properties of the arterial wall and increases arterial stiffness. Arterial stiffness causes an increase in pulse pressure and pulse wave velocity, alterations associated with the development of left ventricular hypertrophy and decrease in coronary perfusion during diastole and which may cause myocardial ischaemia, even in the absence of stenotic lesions.

It has been demonstrated that vascular calcification in dialysis and non-dialysis patients is an active cellular process, similar to bone formation[7-9]. Vascular smooth muscle cells can differentiate into osteoblasts with different stimuli, which, in dialysis patients, may be hyperphosphataemia and hypercalcaemia[8]. Reduction of calcification inhibitors, such as fetuin-A or matrix-Gla protein, may be another factor associated with the development of calcification[10]. CKD patients experience two distinct situations associated with an increase in Ca and P levels: hyperparathyroidism, with high bone turnover where the bone itself is the source of the high levels of Ca and P and adynamic bone disease with low bone turnover. Here the bone behaves as a "frozen bone" and it is not able to capture the Ca and P that the patient receives in food or with treatment.

EVALUATION OF VASCULAR CALCIFICATIONS

KDIGO has recommended a new classification for chronic kidney disease mineral and bone disorder (CKD MBD) which for the first time includes evaluation of vascular calcifications[11]. In haemodialysis patients vascular calcifications can be evaluated by different techniques: electron beam computed tomography (EBCT)[12], multislice computed tomography (MSCT)[13], ultrasonography[5] and plain X-ray[6,14,15]. EBCT and MSCT allow a quantitative measurement and are considered the gold standard for evaluating vascular calcification but are very expensive. The use of plain X-ray for screening vascular calcifications has been suggested by KDOQI[16] and KDIGO[11]. We have developed a vascular calcification score evaluated by plain X-ray of hands and pelvis which is a predictor of cardiovascular death and cardiovascular morbidity in dialysis patients[14] (Fig. 1). This simple vascular calcification score has also been correlated with valvular calcifications[17] and arterial stiffness[18].

Plain X-ray and ultrasonography are semi-quantitative, less expensive and useful for screening for vascular calcifications. They can be used to identify patients at higher risk of a cardiovascular event. EBCT and MSCT are useful for evaluating the progression of vascular calcification and the effect of different treatments on vascular calcification progression. These two techniques do not differentiate intimal from medial calcification and the explanation for the very high scores evaluated in dialysis patients is the presence of both intimal and medial calcification.

VASCULAR CALCIFICATIONS AND HISTOMORPHOMETRIC ANALYSIS OF BONE BIOPSIES

London *et al.*[19] demonstrated an association between vascular calcifications and low bone turnover in a study evaluating 58 haemodialysis patients. 23 of these had undergone parathyroidectomy and 33 had aluminium deposits in bone. Vascular calcifications were evaluated by ultrasonography. More calcifications were associated with lower osteoblasts surface and with other

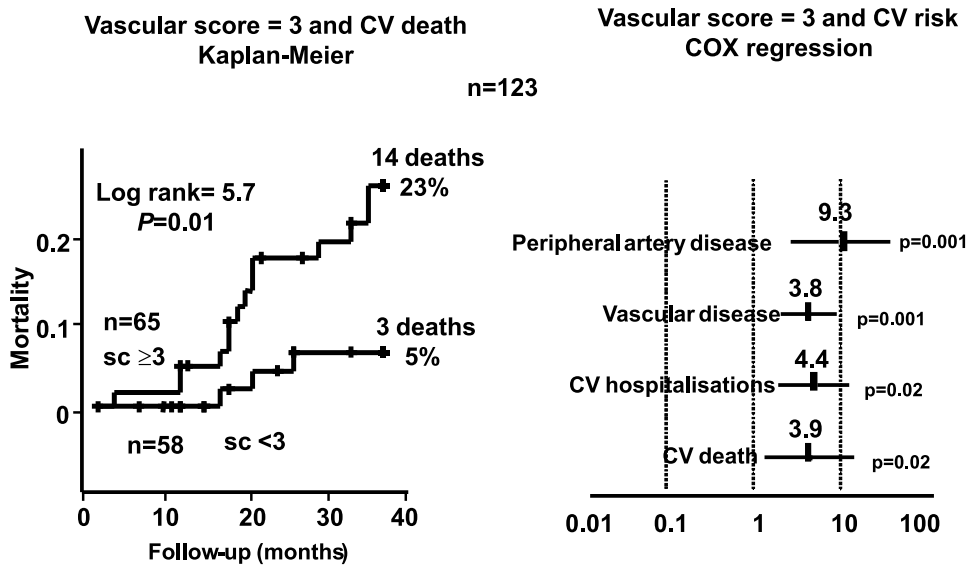

Figure 1

Results after 3 years' follow-up*

*Adragao T, Pires A, Lucas C. et al. Nephrol Dial Transplant 2004;19:1480–8

markers of low bone turnover, with lower PTH levels and with higher calcium dose. In a group of 42 Portuguese haemodialysis patients who underwent bone biopsy[20] we verified that low bone volume was associated with vascular calcifications evaluated by MSCT (Fig. 2) or by plain X-ray and with higher pulse wave velocity. Adynamic bone disease was present in 50% of patients. There were no cases of osteomalacia and no aluminium deposits in bone.

Gulay *et al*. demonstrated in a group of 224 patients that vascular calcifications evaluated by MSCT were associated with lower activation frequency evaluated in bone biopsies[21]. These studies show an association between vascular calcifications with low bone volume and with low bone turnover in dialysis patients, suggesting that patients whose bone is not able to retain calcium or phosphorus have higher vascular calcification scores. One added risk factor for the development of vascular calcifications in the setting of low bone turnover could be the administration of higher calcium doses, as verified in the London study.

VASCULAR CALCIFICATIONS AND BONE MINERAL DENSITY

An association between low bone mineral density and vascular calcifications has already been described in the general population. In post-menopausal women it was demonstrated that low bone mineral density is associated with increase in aortic calcifications[22].

Few studies correlating bone mineral density with vascular calcifications have been performed in dialysis patients. Taal *et al.*[23] verified that osteopenia and osteoporosis evaluated by DEXA were associated with decreased survival in dialysis patients. Raggi *et al.*[24] showed that lower bone mineral density evaluated in lumbar spine by quantitative computed tomography was associated with higher pulse wave velocity. In a group of 70 Portuguese peritoneal dialysis patients we verified that low bone mineral density evaluated by DEXA at the femoral neck, but not at the lumbar spine, was associated with more vascular calcifications evaluated by plain X-ray, with higher pulse wave velocity and with peripheral artery disease[25] (Fig. 3).

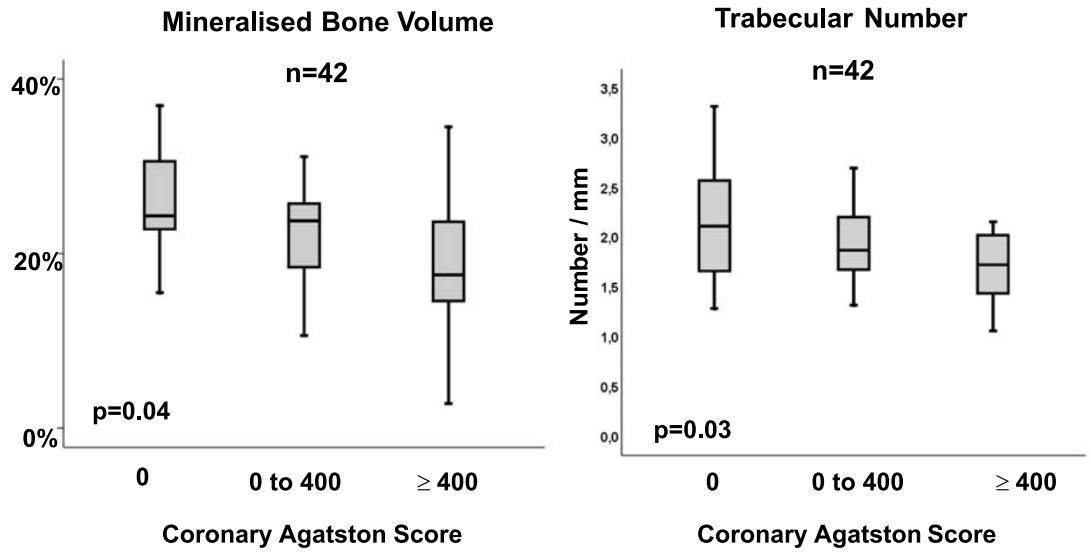

Figure 2

Vascular Calcifications and Bone Biopsies*

*Adragao T, Ferreira A, Frazao J, et al. Nephrol Dial Transplant 2006; 21(4), iv 292 (abstract)

Patients with osteopenia or osteoporosis had higher prevalence of peripheral artery disease (37%) than patients with normal bone mineral density (7%). These associations were adjusted for age, gender, diabetes, haemodialysis duration, systolic pressure and CaxP product. The absence of correlation of bone mineral density evaluated at the lumbar spine with vascular calcifications and arterial stiffness is probably explained by the presence of vascular calcifications in the aorta which may affect the correct measurement of bone mineral density.

The pathogenesis of the association between low bone mineral density parameters and vascular calcifications is not yet known and may be explained by a cause-effect relationship or by a common aetiological factor affecting both the bone and the vessel. Increase in calcium and phosphorus are some of the factors that induce vascular calcification[8]. Either hyperparathyroidism or adynamic bone disease may be associated with osteoporosis[26] and may be the cause of hyperphosphataemia and hypercalcaemia. Oestrogen deficiency is associated with osteoporosis[27] and oestrogen receptors have been identified in vascular smooth muscle cells[28] and in osteoblasts[29].

CARDIOVASCULAR RISK FACTORS AND VITAMIN D DEFICIENCY

In several observational studies, treatment with active vitamin D has been associated with reduced mortality[30-32]. This effect was independent of PTH, Ca and P levels[31], suggesting a pleiotropic effect of vitamin D, beyond the control of hyperparathyroidism. London *et al.* demonstrated that vitamin D deficiency in dialysis patients was associated with vascular calcification and arterial stiffness[33]. Vitamin D deficiency may also be associated with development of cardiac ventricular hypertrophy, as demonstrated in Vitamin D receptor knock-out mice[34].

One possible explanation for this hypothetical beneficial effect of vitamin D is given by the discovery that 1,25-vitD suppresses renin gene expression[35] and by the association between vitamin D deficiency and activation of the renin angiotensin system[34]. Activation of vitamin D receptor has also been associated with reduction of vascular calcifications[36]. In a group of 48 Portuguese haemodialysis patients we verified that vitamin D deficiency was independently associated with vascular calcifications evaluated either by plain X-ray or by MSCT. Vitamin D deficiency was also associated with aortic augmentation

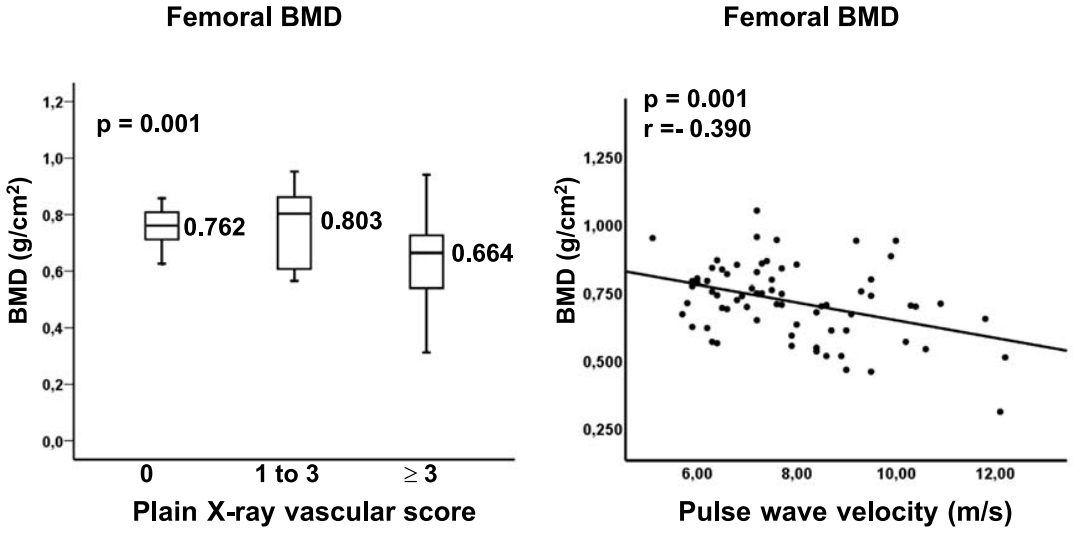

Figure 3

Vascular Calcifications, Arterial Stiffness and Bone Mineral Density*

*Adragao T, Branco P, Birne R. J Am Soc Nephrol 2006;17:272A TH-PO779 (abstract)

index, a marker of arterial stiffness and with left ventricular mass index (LVMI) evaluated by M Mode echocardiography[37] (Fig. 4). This association was adjusted for age, mean arterial pressure, haemoglobin and cholesterol levels.

The association of 25-vitD deficiency with LVMI increase that we verified in our patients is an important finding that goes towards explaining the reduction of mortality associated with treatment with active vitamin D. Left ventricular hypertrophy is associated with arrhythmic risk and with sudden death[38,39] and sudden death is the main cause of cardiovascular death in the dialysis population[4].

WILL TREATMENT OF BONE DISEASE REDUCE CARDIOVASCULAR RISK IN DIALYSIS PATIENTS?

If it is true that there is a link between bone disease and cardiovascular disease, it is necessary to demonstrate that the management of mineral and bone disorder is associated with a reduction in cardiovascular risk. There is already some evidence demonstrating this effect. The TTG[40] and RIND[41] trials have evaluated the progression of coronary calcifications in patients with hyperphosphataemia treated with sevelamer or calcium salts. The TTG evaluated prevalent patients and the RIND evaluated incident patients. The TTG showed that patients treated with calcium showed a 25% increase in the coronary Agatston score while coronary calcification did not increase in patients treated with sevelamer. In the RIND trial patients treated with calcium had an 11-fold increase in median coronary calcification score as compared with patients treated with sevelamer.

Both studies also demonstrated that calcium and sevelamer had a similar effect on the reduction of phosphorus levels, showing that it is not enough to reduce phosphorus levels to avoid progression of vascular calcifications. In terms of biochemical parameters, the main difference was that in patients treated with calcium there was also an increase in Ca levels and a decrease in PTH levels.

Raggi *et al.*[42] evaluated the effect of phosphate binders on bone mineral density in a group of 200 haemodialysis patients. Vascular calcifications and bone mineral density were assessed at baseline and at the end of treatment by either sevelamer or

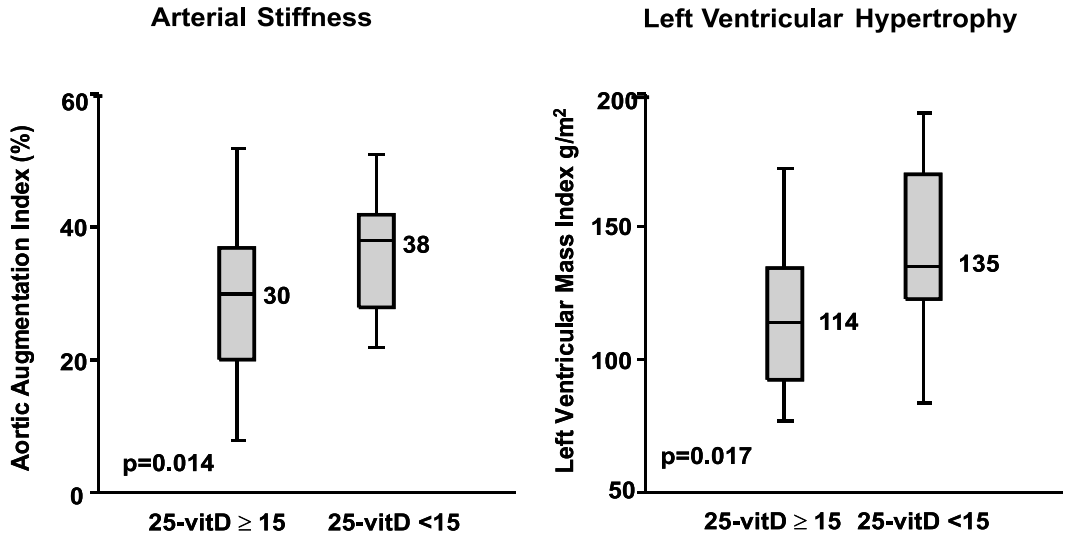

Figure 4

Vitamin D deficiency and cardiovascular risk factors*

*AdragaoT, Ferreira A, Frazão J. Port J Nephrol Hypert 2008;22:66 (abstract)

calcium salts. Patients treated with calcium salts showed an increase in coronary calcifications and a decrease in trabecular bone density, in association with higher Ca levels, lower PTH levels and lower total and bone-specific alkaline-phosphatase levels. This effect of calcium treatment on bone density was confirmed in the Asmus *et al.*[43] study, showing that treatment of 72 haemodialysis patients with calcium carbonate was associated with a decrease in bone density as compared with sevelamer.

The follow-up study of the RIND trial[44] showed that in incident patients treatment with calcium was associated with lower survival than sevelamer. The DCOR trial[45], a multicentre, open-label study, compared the effect on mortality of sevelamer *versus* calcium salts in 2100 prevalent haemodialysis patients. All-cause and cardiovascular mortality were not statistically different between the two treatment groups. This study could only demonstrate that treatment with sevelamer reduced mortality in patients over 65 years of age, and in patients treated for more than 2 years.

There are many reasons which could explain the different outcomes of the DCOR and RIND trials. The former evaluated prevalent patients while the latter was performed in patients new to dialysis. The mean follow-up was shorter in the DCOR trial: 20 months in DCOR *versus* 44 months in RIND. In the DCOR trial, the number of previewed cardiovascular events necessary to demonstrate a difference between the two treatment groups was not reached. Annual mortality rate in the DCOR trial was lower than the annual mortality rate reported in USRDS[4]: 15.02% in the sevelamer group and 16.15% in the calcium binders group. Vascular calcification was not evaluated in DCOR but prevalent patients may have a higher vascular calcification score than incident patients. For instance, baseline coronary calcification score was higher in prevalent patients in the TTG trial, where median score was 641 Hounsfield units, than in incident patients in the RIND trial with a median score of 473 Hounsfield units. These results underline the need to begin treatment at an earlier phase in haemodialysis patients.

In non-randomised studies, treatment with active vitamin D has been associated with a reduction of mortality in dialysis patients. In a small cohort of haemodialysis patients with secondary hyperparathyroidism, treatment with calcitriol was associated with regression of left ventricular hypertrophy[46]. This result may be the consequence of a cardioprotective effect of vitamin D or could be associated with control of hyperparathyroidism. It is necessary to demonstrate if correction of 25-vitD deficiency with or without treatment with active vitamin D contributes to a reduced cardiovascular risk in dialysis patients.

CONCLUSIONS

Vascular calcifications are the result of a complex balance between calcification inducers and inhibitors and are associated with cardiovascular risk in dialysis patients.

Vascular calcifications are associated with low bone turnover, low bone volume and low bone density. Management of bone and mineral disorders in chronic kidney disease may be associated with cardiovascular risk reduction. Plain X-ray can be used for screening vascular calcifications in dialysis patients and the evaluation of the simple vascular calcification score identifies patients at higher cardiovascular risk. This information is an important aid in choosing the most suitable treatment for these patients.

Presence of vascular calcifications, old age, diabetes mellitus, haemodialysis vintage, previous parathyroidectomy and adynamic bone disease are the most frequent factors associated with development or progression of vascular calcifications. In these patients it is important to avoid positive calcium balance, hyperphosphataemia and oversuppression of PTH. Vitamin D deficiency has also been associated with vascular calcifications, arterial stiffness and left ventricular hypertrophy and it is necessary to demonstrate if treatment with vitamin D is associated with a reduced cardiovascular risk.

Understanding the mechanisms linking bone disease to cardiovascular disease is opening up a new treatment field that might reduce the high cardiovascular risk of dialysis patients.

Conflict of interest statement. Dr Adragão has received research grants from Amgen and Genzyme and has received lecture fees from Amgen, Genzyme and Abbott.

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Treatment of hyperphosphatemia with sevelamer hydrochloride in dialysis patients: effects on vascular calcification, bone and a close look into the survival data

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In chronic kidney disease patients, bone and mineral abnormalities have a major impact on morbidity and mortality. Hyperphosphatemia has been associated with increased mortality and with the development of cardiovascular calcification, an independent predictor of mortality. Vascular calcifications have been associated with low bone turnover, low bone volume and lower activation frequency. In dialysis patients, the treatment of hyperphospathemia with calcium based compounds, when compared with sevelamer, is associated with more frequent episodes of hypercalcemia, suppression of intact parathyroid hormone and with progression of coronary calcifications. In the presence of adynamic bone disease, calcium load has a significantly higher impact on aortic calcifications and stiffening. A randomized, prospective, open label study, evaluated patients with bone biopsies at the beginning and after 1 year treatment period with sevelamer hydrochloride or calcium carbonate. Sevelamer treatment resulted in no statistically significant changes in bone turnover or mineralization compared with calcium carbonate, but bone formation rate increased and trabecular architecture improved only with sevelamer. In incident dialysis patients, treatment with sevelamer has been associated with better survival, while in prevalent patients a clear benefit could only be demonstrated in older patients and in patients treated for more than 2 years. In conclusion, the treatment of hyperphosphatemia with sevelamer hydrochloride, a non-calcium and non-metal containing phosphate binder, is associated with a beneficial effect on vascular calcification progression, bone disease and most likely with a survival benefit in some hemodialysis patients populations.

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The last few years have seen major developments in the management of bone and mineral disorders associated with chronic kidney disease (CKD). Acknowledgment of the fact that these bone and mineral abnormalities in CKD patients have a major impact on morbidity and mortality drove nephrologists' attention to the importance of controlling these alterations. In addition, new compounds have been developed for the control of hyperphosphatemia and for the treatment of secondary hyperparathyroidism, raising many questions toward the use of non-calcium-containing phosphate binders.

Until recently, the only phosphate binders available were aluminum or calcium-based compounds. These compounds were efficacious but were also associated with significant side effects. The use of aluminum-containing phosphate binders is associated with bone disease as well as hematologic and central nervous system toxicity, whereas the use of calcium-containing phosphate binders is associated with increased risk of hypercalcemia and cardiovascular calcification.[1–4] It is now known that serum calcium levels are not accurate in predicting the calcium balance and burden. The excessive amount of calcium ingested from diet and calcium-containing binders has been associated with cardiovascular calcifications, even in the presence of normal calcium serum levels.[1–4] The non-calcium, non-metal-containing, and non-absorbed phosphate binder, sevelamer hydrochloride, has provided an effective way to bind phosphorus in the gut without the risks of hypercalcemia, soft tissue or vascular calcifications, or heavy metal accumulation.

EFFECTS ON VASCULAR CALCIFICATION

In recent years, it has become clear that the presence of elevated serum phosphorus levels in CKD stage 5 patients is positively associated with increased mortality.[5,6] Hyperphosphatemia and elevated calcium-phosphorus product (Ca X P) are associated with cardiovascular calcification.[1,2] Cardiovascular calcification is an independent predictor of mortality. Several authors have now reported a strong positive association between the presence and extent of vascular calcification and both cardiovascular and all-cause mortality.[3,7,8] Raggi *et al.*[9] reported that previous myocardial

infarction, angina, and known coronary artery disease were all more common in CKD stage 5 patients with extensive calcification.

Most stage 5 CKD patients present with hyperphosphatemia. The clinical outcomes of this mineral abnormality include secondary hyperparathyroidism with consequent renal bone disease, extra-osseous calcification, and increased mortality.

Therapeutic strategies to control phosphorus levels include dietary restrictions, dialysis, and the use of phosphate-binding agents. Reduction of phosphate intake in the diet is often difficult and is also limited by the associated protein restriction, as all proteins contain phosphate. Phosphate is also difficult to remove by dialysis. Increased dialysis time or frequency may be effective, but it is often difficult to implement because of logistic problems and poor patient acceptance.

Sevelamer hydrochloride has been widely studied and shown to be effective in reducing phosphorus levels and Ca X P without causing hypercalcemia and soft tissue calcification in stage 5 CKD population on hemodialysis, with the added benefit of cholesterol reduction (total and low-density lipoprotein cholesterol). This compound is well tolerated with few side effects, the more frequently reported ones being diarrhea, constipation, dyspepsia, nausea, and vomiting.

Chertow *et al.*[4] reported the results of a randomized parallel design clinical trial comparing sevelamer with calcium-based phosphorus binders in 200 hemodialysis patients. Sevelamer and calcium-based compounds provided similar control of serum phosphorus and Ca X P. Adherence to the prescribed dose of binder in the sevelamer and calcium-containing binder groups was similar: 86 vs 80%, respectively. The group treated with sevelamer received an average binder dose of 6.5 ± 2.9 g per day and the group treated with calcium-based binders, 4.3 ± 1.9 g per day (4.6 and 3.9 g per day of calcium acetate or calcium carbonate, respectively). The calcium-based group had more frequent episodes of hypercalcemia when compared with the sevelamer group: 43 and 17%, respectively. Suppression of intact parathyroid hormone secretion below the 150–300 pg/ml range was more common at the end of the study in the calcium-based binders group, 57 vs 30%, despite the protocol-specified reduction or cessation of vitamin D for intact parathyroid hormone below 150 pg/ml. Twelve percent of patients in the calcium group required rescue therapy with aluminum-containing binders for a calcium-phosphorus product above 72 $mg^2/100ml^2$, compared with 4% of patients in the sevelamer group. Total and low-density lipoprotein cholesterol decreased significantly in the sevelamer-treated group compared with a non-change in the calcium binders group. It is relevant that, the electron beam computed tomography (EBCT) performed at the beginning of the study detected a prevalence of coronary artery calcification of 83% and aortic calcification of 80% of the study patients. There was significant progression of the coronary artery and aortic calcification EBCT score, at weeks 26 and 52, in the calcium-containing binders-treated group, despite the use of an average dose of calcium-containing binders of only 4.3 ± 1.9 g per day, which corresponds to values of elemental calcium under the Kidney Disease Outcomes Quality Initiative recommendations. There was no significant progression in the sevelamer-treated group.

The high prevalence of vascular calcification seen in the dialysis population in the Chertow study[4] has been confirmed by other reports and is of major concern because of the positive association between the presence and severity of calcification and mortality in this population. There is also some evidence that most of the patients develop vascular calcifications while on hemodialysis treatment. In fact, a report from Spiegel *et al.*[10] revealed that only 34% of patients with advanced CKD starting dialysis had coronary artery calcifications scores that placed them above the 90th percentile for age and sex. In the same patient population initiating dialysis, 109 patients underwent baseline and at least one additional measurement of coronary artery calcification.[11] At baseline, 37% of the patients who underwent treatment with sevelamer and 31% of the patients who underwent treatment with calcium-based binders had no evidence of calcification. The authors report that no patients with a zero coronary calcium score progressed to a coronary artery calcium score >30, using EBCT, in an 18-month period of time. Patients already having a coronary artery calcium score >30 at baseline progressed during the time of the study in both arms. The patients treated with calcium-based binders showed a more rapid and severe progression when compared with those receiving sevelamer. It can be noted that, during this study, all the patients were maintained on dialysis with a calcium dialysate concentration of 2.5 mEq/l. The authors conclude that patients new to dialysis, with no evidence of coronary calcification, showed little evidence of disease development over a period of 18 months independent of the phosphate binder used. Patients with even little evidence of coronary calcification progress with both binders; however, the group treated with calcium-based binders have a much more severe progression when compared with the patients treated with sevelamer.[11] Without any doubt, this study confirms the importance of the Kidney Disease Outcomes Quality Initiative clinical practice guidelines for bone metabolism and disease in CKD, recommending that calcium-based binders should be avoided in patients with evidence of severe calcification.[12]

'Kidney Disease: Improving Global Outcomes' has recommended a new classification for CKD mineral and bone disorder that includes for the first time the evaluation of vascular calcifications.[13] In hemodialysis patients, vascular calcifications may be evaluated by different techniques: EBCT,[14] multislice computed tomography (MSCT),[15] ultrasonography,[7] and plain X-ray.[3,8,16] EBCT and MSCT allow a quantitative measurement and are considered the gold standard for evaluating vascular calcification, but are very expensive. The utilization of plain X-ray for screening

vascular calcifications has been suggested by Kidney Disease Outcomes Quality Initiative[12] and 'Kidney Disease: Improving Global Outcomes'.[13] We have developed a vascular calcification score evaluated in plain X-ray of hands and pelvis (Figure 1), which was a predictor of cardiovascular death and cardiovascular morbidity in dialysis patients (Figure 2).[8] This simple vascular calcification score has been also correlated with valvular calcifications[17] and with arterial stiffness.[18]

Plain X-ray and ultrasonography are semiquantitative, less expensive, and useful for screening the presence of vascular calcifications. They can be used to identify patients at higher risk of a cardiovascular event. EBCT and MSCT are useful for evaluating the progression of vascular calcification and the effect of different treatments on progression of vascular calcification. These two techniques do not differentiate intimal from medial calcification, and the explanation for the very high scores evaluated in dialysis patients is the presence of both types of calcification.

Figure 1 | Pelvis score evaluates the presence of vascular calcifications in iliac and femoral arteries. Hands score evaluates the presence of vascular calcification in the radial and digital arteries. Calcification score is the sum of the presence (1) or absence (0) of vascular calcifications in each section. In this example pelvis score $(1+1+1+1)=4$ and hands score $(1+1+1+1)=4$. Total score is 8.

EFFECTS ON BONE

In a randomized prospective, open label study, 119 hemodialysis patients were evaluated with bone biopsies performed at the beginning and after a 1-year treatment period, to compare the effects of sevelamer hydrochloride and calcium carbonate on bone.[19] Biopsy-proven adynamic bone disease was the most frequent bone abnormality at baseline (59%). The serum phosphorus levels were similarly controlled in both groups, although the serum calcium level was consistently lower and intact parathyroid hormone higher in patients treated with sevelamer. Compared with baseline values, there were no changes in mineralization lag time or measures of bone turnover after 1 year of treatment with both sevelamer and calcium carbonate. Bone formation rate per bone surface increased significantly from baseline only in the sevelamer-treated patients. In addition, of those with abnormal microarchitecture at baseline (that is, trabecular separation), 7 of 10 in the sevelamer group normalized after 1 year compared with 0 of 3 in the calcium group. In summary, this study showed that sevelamer treatment resulted in no statistically significant changes in bone turnover or mineralization compared with calcium carbonate, but bone formation rate increased and trabecular architecture improved only with sevelamer.

In a group of 42 hemodialysis patients submitted to bone biopsies,[20] we have verified that low bone volume was associated with vascular calcifications evaluated by MSCT or by plain X-ray and with higher pulse wave velocity. Adynamic bone disease was present in 50% of patients. There were no cases of osteomalacia and no aluminum deposits in bone.

London *et al.*[21] showed the existence of an association between vascular calcifications and low bone turnover. In this study, 58 hemodialysis patients were evaluated; 23 patients had been submitted to parathyroidectomy and 33 patients had aluminum deposits in bone. Vascular calcifications were evaluated by ultrasonography. More calcifications were associated with lower osteoblast surface and with other

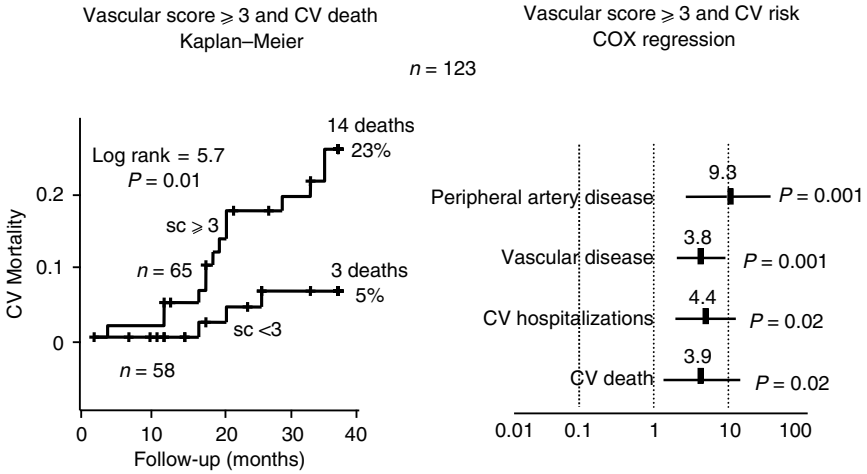

Figure 2 | Calcification score from plain X-ray of hands and pelvis was a predictor of cardiovascular death and cardiovascular morbidity in dialysis patients. After 3 years of follow-up vascular score ⩾3 was associated with higher cardiovascular mortality and morbidity.[8]

markers of low bone turnover, with lower parathyroid hormone levels and with higher calcium dose. Asci *et al.*[22] showed, in a group of 224 patients, that vascular calcifications evaluated by MSCT were associated with lower activation frequency evaluated in bone biopsies.

A recent study was performed to assess the impact of bone activity on the relationships between the dosage of calcium-containing binders and aortic stiffness or abdominal aorta calcification score.[23] A significant interaction was found between the dosage of calcium-containing phosphate binders and bone activity such that the calcium load had a significantly higher impact on aortic calcifications and stiffening in the presence of adynamic bone disease.

The data presented suggest that sevelamer treatment has a beneficial effect on bone, with an increase in the bone formation rate and an improvement in the trabecular architecture. There is an association between vascular calcifications and low bone volume and with low bone turnover in dialysis patients. Finally, in dialysis patients with adynamic bone, calcium load has a greater influence on aortic calcifications and stiffening. Patients whose bone is not able to retain calcium or phosphorus have higher vascular calcification scores. One added risk factor for the development of vascular calcifications in the setting of low bone turnover is the administration of calcium-containing phosphate binders.

A CLOSE LOOK INTO THE SURVIVAL DATA

The promise of a survival benefit with the use of sevelamer hydrochloride has been evaluated in two randomized prospective, controlled studies with interesting results that generate some controversy and certainly have not completely solved the issue.

The first was the 'Dialysis Clinical Outcomes Revisited (DCOR)' study.[24] This 3-year trial involving more than 2100 patients compared the difference in mortality and morbidity outcomes for patients receiving sevelamer hydrochloride and those receiving calcium-containing phosphate binders. This was the largest outcomes study ever conducted in the hemodialysis population. This study showed that the patients treated with sevelamer hydrochloride experienced a reduction of 7% in the risk of death from all causes when compared with the patients treated with calcium-based phosphate binders, which was statistically not significant ($P = 0.3$). The patients aged 65 years or more (a predefined analysis) were 23% less likely to die when treated with sevelamer hydrochloride, as compared with treatment with calcium-based binders. In addition, patients treated with sevelamer hydrochloride for more than 2 years had a 34% reduction of the mortality risk for all causes compared with those treated with the calcium-containing binders.

The second study was the 'Renagel in New to Dialysis Patients.'[25] This was a randomized controlled, prospective, open label study with 127 patients incident to dialysis, assigned to 18 months treatment with sevelamer hydrochloride or calcium-containing phosphate binders, to assess coronary artery calcification progression. Mortality was a predetermined secondary end point.[25] Twenty-three deaths in the calcium-containing phosphate binders group and 11 deaths in the sevelamer hydrochloride-assigned patients occurred during the median 44 months of follow-up time after randomization, a significantly lower mortality for patients treated with sevelamer hydrochloride. The survival benefit observed with sevelamer hydrochloride treatment persisted after full multivariate adjustment.

It is very important to analyze the reasons for the differences observed in the outcomes of these two trials. The DCOR trial evaluated prevalent patients probably with a more important burden of calcification, whereas 'Renagel in New to Dialysis Patients' trial was performed in patients new to dialysis. It is probably very difficult to reverse already existing vascular calcifications. The DCOR trial has been criticized for the short follow-up time of less than 2 years. The median follow-up was shorter in the DCOR trial compared with the 'Renagel in New to Dialysis Patients' trial, 19 vs 44 months, respectively. The short follow-up time in the DCOR trial did not allow the differences in mortality to appear. In fact, for the patients followed for more than 2 years, the difference in mortality became significant. In the DCOR trial, the number of previewed cardiovascular events necessary to demonstrate a difference between the two treatment groups was not reached. The annual mortality rate in the DCOR trial was inferior to the annual mortality rate reported in United States Renal Data System.[26]

The results of these two studies strongly suggest that the use of sevelamer as a phosphate binder decreases mortality in incident and in elderly hemodialysis patients and reinforces the importance of earlier initiation of treatment with sevelamer hydrochloride in hemodialysis patients.

A final comment is on the systematic review of the clinical efficacy and safety of sevelamer hydrochloride in dialysis patients published by Tonelli *et al.*[27] In the mortality analysis, the authors included five studies (Table 1), with only one of them having mortality as the primary end point.[25] The other three studies included (Table 1) in the mortality analysis involved a small number of patients, had a short follow-up, and mortality was not an end point. These studies[4,28,29] were not powered in terms of follow-up time, number of patients, and end points to evaluate mortality. In our view, it is impossible to withdraw any mortality information in studies with 42 patients and a 5-month follow-up, or a crossover study with 20 patients and a total follow-up of 18 weeks. The Chertow study's[4] primary end point was vascular calcification; mortality was not even an end point and received 24% weight in the analysis. Regarding the 'Renagel in New to Dialysis Patients' study, with a long follow-up for the secondary end point mortality and evidence of survival benefit in the sevelamer-treated group, the weight attributed was only 4.26%.

In our opinion, the available data on mortality benefit with sevelamer hydrochloride treatment from two randomized prospective controlled trials constitute a very positive

Table 1 | Summary of the studies used for mortality analysis

Author	Number of patients	Follow-up	Primary end points
Block *et al.*[25]	127	18 months for the primary end point (calcifications) and 44 months for the secondary end point (mortality)	Progression of calcifications
Chertow *et al.*[4]	200	52 weeks	Progression of calcifications
Sadek *et al.*[28]	42	5 months	Biochemical parameters
Shaheen *et al.*[29]	20	Crossover study 18 weeks	Biochemical parameters
Suki *et al.*[24]	2103	22 months	Mortality

fact that is certainly innovative in the nephrology field. We are not aware of any other pharmacological intervention in dialysis patients with such ground in terms of hard outcome data.

In a recent editorial,[30] the authors state that 'to cultivate a balanced approach to understanding results generated by meta-analysis of data from small trials it is important to accept the limitations implicit in this method.' Meta-analysis only generates hypotheses and certainly should be carefully interpreted. One should always keep in mind that well-designed, randomized controlled trials are the strong bases for evidence-based medicine.

There is mounting evidence from basic science,[31] observational studies,[32] and randomized trials with surrogate end points such as cardiovascular calcification[4,11] and mortality[25] that calcium can be toxic for dialysis patients. With this level of information, the nephrology community should be asking what level of scientific evidence is needed to convince us to discontinue, or at least to be extremely cautious with the use of calcium-containing phosphate binders, a potentially harmful therapy.

DISCLOSURE

João M Frazão has received consulting and lecture fees from Amgen and Genzyme. Teresa Adragão has declared no financial interests.

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CAPÍTULO 7

EXISTE UM ELO DE LIGAÇÃO ENTRE O OSSO E O VASO NOS DOENTES EM DIÁLISE? (2.ª PARTE)

Densidade mineral óssea, histomorfometria óssea e calcificações vasculares

RELAÇÃO ENTRE DENSIDADE MINERAL ÓSSEA E HISTOMORFOMETRIA ÓSSEA

Em 2001, a osteoporose foi definida pelo *National Institute of Health* (NIH) como uma patologia óssea caracterizada pela redução da força óssea predispondo para um aumento do risco de fraturas[1]. A força óssea depende fundamentalmente de dois fatores: da densidade mineral óssea e da qualidade óssea[1]. Por sua vez, a qualidade óssea resulta da remodelação, da mineralização e da arquitetura ósseas. Nos indivíduos da população geral, a densidade mineral óssea contribui em 70% para a força óssea[1] e a baixa densidade mineral óssea relaciona-se com o risco de fraturas. A Organização Mundial de Saúde define osteoporose baseando-se em valores de densidade mineral óssea inferiores a 2,5 desvio-padrão do valor médio de mulheres jovens caucasianas (T-*score* ≤ -2,5 DP) e define osteopenia para valores de T-*score* inferiores ou iguais a -1,5 DP[2]. Os indivíduos da população geral com osteopenia ou osteoporose têm um risco de fraturas ósseas, respectivamente, de 4 e de 8 vezes superior ao risco de fraturas dos indivíduos com densidade mineral óssea normal[3]. Os doentes em diálise apresentam um risco de fraturas 4,4 vezes superior ao da população geral[4]. Pela compreensão dos diferentes fatores que contribuem para a força óssea, e conhecendo as diversas alterações ósseas que se desenvolvem no doente renal crónico, é fácil compreender que as alterações da densidade mineral óssea não podem ser as únicas a explicar o risco de fraturas destes doentes. Ao contrário do que se verifica nos indivíduos da população geral[5], já foi demonstrado que a densidade mineral óssea, nos doentes renais em diálise, não permite identificar o risco de fraturas[6,7]. Diferentes estudos têm apresentado resultados contraditórios na análise da relação entre os dados de biopsias ósseas e os resultados da avaliação da densidade mineral óssea por DXA (*dual energy X-ray absorptiometry*), mostrando que a baixa densidade mineral óssea pode associar-se tanto a baixa remodelação como a alta remodelação óssea. Gerakis *et al*[8] mostraram, num grupo de 62 doentes em diálise, que a baixa densidade mineral óssea se associou a marcadores bioquímicos e

histológicos de hiperparatiroidismo secundário. Lobão R *et al*[9] verificaram, num grupo de 40 doentes renais crónicos nos estadios 3 e 4, e com baixa densidade mineral óssea, que o diagnóstico histológico predominante foi a doença óssea adinâmica e a osteomalacia. Estes autores não encontraram qualquer relação entre volume ósseo e densidade mineral óssea. As *guidelines* KDIGO 2009[10] não aconselham a avaliação da densidade mineral óssea nos doentes em diálise por este método não predizer nem o risco de fraturas nem o tipo de osteodistrofia renal nesta população. Foi contudo encontrada uma associação entre a densidade mineral óssea avaliada no antebraço com o risco de fraturas[11] e os valores de paratormona[12]. Os resultados medidos nos corpos vertebrais refletem a avaliação do osso trabecular, enquanto os resultados medidos no colo do fémur ou no antebraço avaliam fundamentalmente o osso cortical. A biopsia óssea usada no diagnóstico de osteodistrofia renal avalia geralmente apenas o osso trabecular, mas é possível também avaliar o osso cortical. Num grupo de 46 doentes portugueses em diálise, avaliados simultaneamente por DXA e por análise histomorfométrica de biopsias ósseas, encontramos uma relação inversa entre os valores de densidade mineral óssea obtidos por DXA e os marcadores bioquímicos e histológicos de hiperparatiroidismo secundário[13] (fosfatase alcalina óssea, PTH intacta, número osteoblastos/cm, taxa de formação óssea e frequência de ativação óssea). Não encontramos qualquer relação entre volume ósseo trabecular e densidade mineral óssea. Numa análise posterior a este mesmo grupo de doentes, avaliamos a associação entra a densidade mineral óssea avaliada por DXA e o volume ósseo cortical e trabecular avaliados por biopsia óssea. Ajustando para a idade e duração de hemodiálise, verificámos que a densidade mineral óssea avaliada no colo do fémur, mas não na coluna lombar, se correlacionava de forma muito significativa com a porosidade cortical mas não com o volume ósseo trabecular (Tabela 7.1). Fomos os primeiros a descrever esta associação e este estudo foi publicado na *Osteoporosis International*[14]. Este achado inovador do nosso estudo foi mencionado pela ERBP (*European Renal Best Practice)* no comentário feito à posição da KDIGO que considera ser reduzida a utilidade da densidade mineral óssea nos doentes em diálise[15]

Variáveis preditoras de porosidade cortical (regessão linear)

Variável dependente: porosidade cortical	B	SE	Sig.
DMO femoral	-157,49	52,36	0,005
Idade > 50 anos	-8,7	2,18	<0,001
Duração de HD	-25,44	8,40	0,005

Tabela 7.1. A densidade mineral óssea (DMO) femoral, a idade superior a 50 anos e a duração da hemodiálise (HD) correlacionaram-se negativamente com a porosidade cortical avaliada nas biopsias ósseas

Já foi demonstrado que o hiperparatiroidismo secundário pode ter efeitos opostos no osso trabecular e no osso cortical[16,17], e este dado pode explicar os diferentes resultados obtidos pela DXA consoante o local estudado e a ausência de correlação entre os dados da densidade mineral óssea obtida por DXA e o risco de fraturas. Nos indivíduos do sexo masculino da população geral, não existe correlação entre as fraturas osteoporóticas vertebrais e os resultados da densidade mineral óssea. Ostertag A *et al* mostraram que as fraturas vertebrais no sexo masculino se associaram à porosidade cortical avaliada em biopsia óssea[18].

A utilidade diagnóstica da DXA nos doentes renais crónicos ainda não está demonstrada. Na nossa opinião, o termo "osteoporose" deve ser usado com cuidado nos doentes renais crónicos, pois não existe uniformidade neste diagnóstico utilizando estes dois métodos de diagnóstico: a biopsia óssea e a DXA. É mais correto falar de baixa densidade mineral óssea quando se utiliza a DXA e de baixo volume ósseo trabecular, porosidade, ou menor espessura cortical quando se utiliza a biopsia óssea. O nosso estudo atribui pela primeira vez à DXA um papel no diagnóstico de porosidade cortical nos doentes em diálise. A utilidade da avaliação diferencial da densidade mineral óssea cortical e trabecular necessita de ser confirmada em estudos prospetivos. É necessário avaliar se a baixa densidade mineral óssea femoral avaliada em doentes renais crónicos, que no nosso estudo se associou à porosidade cortical, se associa a maior risco de fraturas nesta população.

Relação entre densidade mineral óssea e calcificações vasculares

A associação entre baixa densidade mineral óssea e calcificações vasculares já foi demonstrada em inúmeros estudos na população geral[19-22]. Os mecanismos responsáveis por esta associação não estão bem esclarecidos, podendo ser explicados por um fator comum ou por uma relação causal entre a doença óssea e a doença vascular. A dislipidemia pode contribuir simultaneamente para a formação de placas ateroscleróticas e para a osteoporose[23]. Já foi demonstrado *in vitro* que os produtos de oxidação dos lípidos são capazes de promover uma diferenciação osteogénica de células musculares lisas humanas[24] mas também têm um efeito inibitório na diferenciação de pré-osteoblastos[25]. Esta dupla ação da dislipidemia poderia explicar a associação, inicialmente considerada paradoxal, entre calcificações arteriais e osteoporose. Contradizendo estes achados *in vitro*, o estudo observacional PERF[26], que avaliou 1176 mulheres dos 60 aos 85 anos, demonstrou a existência de uma associação entre doença arterial periférica e osteoporose, mas não conseguiu evidenciar uma relação independente entre a dislipidemia e as alterações da densidade mineral óssea. Uma das hipóteses destes autores é a de que a dislipidemia não tem um efeito modulador direto mas indireto na densidade mineral óssea, através das lesões que provoca nos vasos nutritivos do osso.

A associação entre densidade mineral óssea e calcificações vasculares, amplamente estudada nos indivíduos idosos da população geral, tem sido pouco investigada nos doentes em diálise. O grupo de trabalho de imagiologia das KDIGO, International Controversies Conference em 2006[27] propôs o estudo da relação entre densidade mineral óssea e calcificações vasculares nos doentes em diálise. Até ao momento, poucos estudos tinham avaliado esta associação. Taal *et al* mostraram que a osteoporose e a osteopenia, num grupo de 88 doentes em diálise, se associaram a maior mortalidade[28]. Braun *et al* mostraram a existência de uma relação inversa entre calcificações cardíacas e massa óssea avaliadas por tomografia computorizada de feixes de eletrões[29]. Num grupo de 110 doentes em diálise, Raggi P *et al*[30] demonstraram a existência de uma relação inversa entre a velocidade de onda de pulso carotidofemoral e a densidade mineral óssea vertebral avaliada por tomografia computorizada quantitativa. Não encontraram relação entre a velocidade de onda de pulso e a densidade mineral óssea avaliada por DXA na coluna lombar. Nos doentes sem calcificações vasculares, verificaram a existência de uma correlação entre os resultados obtidos por DXA e por tomografia computorizada. Na presença de calcificações vasculares, esta correlação desaparecia, e a hipótese apresentada por estes autores foi a de que a presença de calcificações vasculares na aorta, muito prevalentes nos doentes em diálise, falsearia os resultados da DXA. Num grupo de 70 doentes portugueses em diálise peritoneal[31], demonstramos a existência de uma associação

Densidade Mineral Óssea no Colo do Fémur

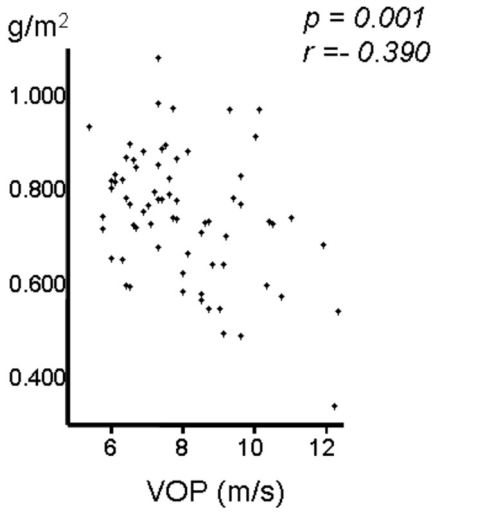

Fig. 7.1. A velocidade de onda de pulso (VOP) correlacionou-se inversamente com a densidade mineral óssea avaliada no colo do fémur

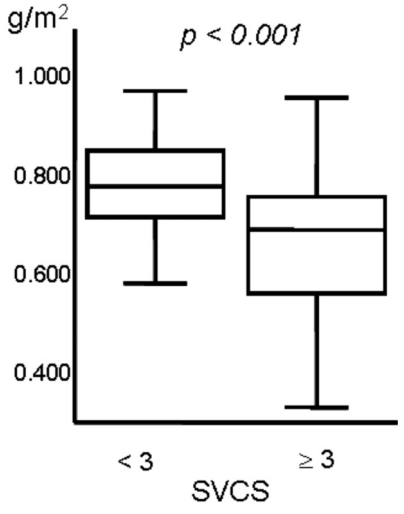

Fig. 7.2. Um *score* de calcificação vascular simples (SCVS) > 3 associou-se a menor densidade mineral óssea avaliada no colo do fémur, em comparação com um SCVS<3 (teste *T* de *Student*)

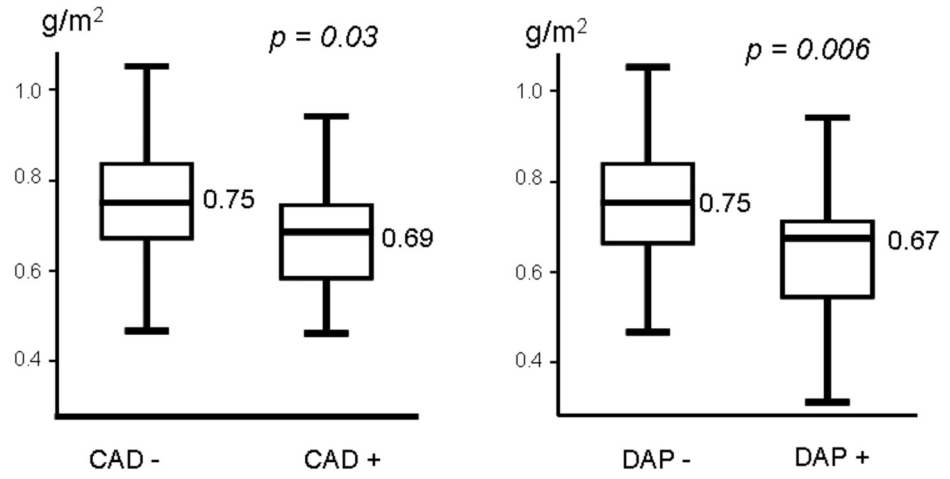

Fig.7.3. Associação inversa entre a densidade mineral óssea avaliada no colo do fémur e a presença de doença coronária (CAD+) e doença arterial periférica (DAP+) (teste *T* de *Student*)

inversa entre a densidade mineral óssea avaliada por DXA no colo do fémur e a velocidade de onda de pulso carotidofemoral avaliada através do Complior (Artech Medical, Pantin, France) (Fig.7.1), com calcificações vasculares avaliadas pelo *score* simples de calcificação (Fig 7.2) e com a presença de doença coronária e doença arterial periférica (Fig.7.3).

Estas correlações não se verificaram com a densidade mineral óssea avaliada na coluna lombar. Em análise multivariada confirmou-se a associação independente e inversa entre a densidade mineral óssea avaliada no colo do fémur e a velocidade de onda de pulso, com um SCVS >3 e com a presença de doença arterial periférica[31]. Verificamos que os doentes com calcificações vasculares apresentavam um T-*score* lombar superior quando comparados com os doentes sem calcificações vasculares, enquanto o T-*score* femoral era semelhante nos doentes com e sem calcificações vasculares. Este dado sugere que as calcificações vasculares contribuem para os valores mais elevados do T-*score* lombar e está de acordo com os achados de Raggi *et al*[30]. O nosso estudo foi o primeiro a demonstrar, numa população de doentes no estadio 5D, uma associação independente e inversa entre a densidade mineral óssea e as calcificações vasculares avaliadas por RX simples e com doença arterial periférica. Em conclusão, demonstramos com este estudo a existência de uma associação entre valores mais baixos de densidade mineral óssea femoral e calcificações vasculares avaliadas por RX simples, com rigidez arterial e com doença arterial periférica. Os mecanismos para esta associação não estão ainda identificados, mas estes dados reforçam a hipótese da existência de um elo de ligação entre a doença óssea e a doença vascular nos doentes em diálise.

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Bone Mineral Density, Vascular Calcifications, and Arterial Stiffness in Peritoneal Dialysis Patients

The objective of this study was to evaluate the correlation of bone mineral density (BMD), evaluated by DXA, with vascular calcifications, arterial stiffness, and vascular disease in patients on peritoneal dialysis. Vascular calcifications were evaluated by vascular calcification score on plain x ray, and arterial stiffness was measured by pulse wave velocity using the Complior device (Artech Medical, Pantin, France). Adjusting for multiple factors, lower BMD at the femoral neck, but not at the lumbar spine, was associated with higher pulse wave velocity ($p = 0.037$), higher vascular calcification score ($p = 0.013$), and peripheral artery disease ($p = 0.006$). These data reinforce the hypothesis of the existence of a link between bone disease and cardiovascular disease in dialysis patients.

Perit Dial Int **2008; 28:668–672** **www.PDIConnect.com**

KEY WORDS: Bone mineral density; vascular calcifications; arterial stiffness.

In dialysis patients there is increasing evidence of the existence of a link between bone disease and cardiovascular disease. Low bone mineral density (BMD) in the general population is associated with atherosclerotic calcifications (1). The relationship between BMD values and vascular calcifications is one of the issues recommended for clinical research by the imaging work group of the KDIGO International Controversies Conference (2). The objective of this study was to evaluate, in a group of

peritoneal dialysis patients, the relationship of BMD with vascular calcifications, pulse pressure, pulse wave velocity (PWV), and cardiovascular disease.

PATIENTS

This study was a cross-sectional analysis of a cohort of peritoneal dialysis patients. Seventy patients (37 males, 33 females) without previous parathyroidectomy were evaluated after signing an informed consent form. Mean age was 52 ± 14 years and mean dialysis duration was 46 ± 28 months; 17 patients were diabetic. During the 6 months preceding BMD evaluation, 29 and 33 patients were treated, respectively, with calcium carbonate (1.2 ± 0.45 g/day) and calcitriol (1.6 ± 1.2 µg/week). During this period, dialysate calcium concentrations of 1.25 and 1.75 mmol/L were used, respectively, in 40 and 30 patients. No patient had been previously treated with bisphosphonates or cinacalcet. Coronary artery disease, peripheral artery disease (PAD), and cerebral vascular disease were diagnosed, respectively, in 20, 18, and 8 patients, based on clinical manifestations and diagnostic tests.

METHODS

Bone mineral density was evaluated by dual-energy x-ray absorptiometry (DXA) using the Hologic QDR Discovery scanner (Hologic, Bedford, Massachusetts, USA) in the lumbar spine and the femoral neck, using NHANES III as the database reference (3). DXA parameters are not standardized for dialysis patients and we used the WHO cutoff of a T-score > –1 SD, which defines normal BMD. According to the Osteoporosis Work Group (4), a Z-score ≤ –1 SD, which is adjusted to age, could be more appropriate to diagnose low BMD in dialysis patients. During the first month after BMD evaluation, vascular calcifications were evaluated on plain x rays of pelvis and hands using a method previously described (5), with the final score ranging from 0 to 8. Arterial stiffness was evaluated by pulse pressure (pulse pressure = systolic blood pressure – diastolic blood pressure) and by carotid–femoral PWV using a noninvasive automated device (Complior; Artech Medical, Pantin, France). Serum levels of the following biochemical parameters were evaluated every month and time averaged for the 6 months preceding the DXA evaluation: calcium, phosphorus, calcium–phosphate product, albumin, alkaline phosphatase, and C-reactive protein. Total intact parathyroid hormone was evaluated every 3 months by immunochemiluminescence using a second-generation assay.

Statistical Analysis: Data are presented as frequencies for categorical variables and as mean values with SD for continuous variables. Univariate analysis was performed by independent samples t-test, chi-square, Fisher's exact test, paired-samples t-tests, and Pearson correlation coefficient. The association of DXA parameters with cardiovascular factors was evaluated by linear and logistic regression models adjusting for age, gender, diabetes, and body mass index. Absence of colinearity was checked in all models. Vascular calcification score (VCS) was evaluated as a categorical dependent variable (VCS ≥ 3) based on our previous studies showing an association between VCS ≥ 3 and higher cardiovascular mortality (5). Statistical analyses were performed using the SPSS system 14.0 (SPSS Inc., Chicago, Illinois, USA). A p value < 0.05 was considered statistically significant.

RESULTS

Vascular calcifications were present in 43 patients. A T-score ≤ –1 SD was observed at the lumbar spine in 39 patients and at the femoral neck in 43 patients. A Z-score ≤ –1 SD was present at the lumbar spine in 21 patients and at the femoral neck in 24 patients.

In univariate analysis, lower femoral T-score (Table 1) was associated with higher pulse pressure, higher PWV, higher prevalence of vascular calcification, and higher prevalence of PAD. Lower femoral BMD was associated with higher VCS and higher PWV (Figure 1). Lower femoral BMD was also associated with presence of coronary artery disease (0.66 ± 0.15 vs 0.74 ± 0.13 g/cm^2, p = 0.03) and PAD (0.64 ± 0.14 vs 0.75 ± 0.13 g/cm^2, p = 0.006) (Figure 1). Patients with vascular calcifications had a higher lumbar T-score than patients without calcifications (–1.1 ± 1.5 vs –1.7 ± 1.6 SD, p = 0.02). In multivariate analysis, BMD evaluated at the femoral neck was negatively associated with VCS ≥ 3, PAD (Table 2), and PWV (Table 3), adjusting for age, gender, diabetes, and body mass index. In similar models, a lower femoral Z-score, but not T-score, was associated with VCS ≥ 3 [odds ratio (OR) = 0.5, p = 0.030] and PAD (OR = 0.44, p = 0.016).

DISCUSSION

In this group of peritoneal dialysis patients, we have verified that low BMD at the femoral neck, but not at the lumbar spine, is independently associated with VCS on plain x ray and with arterial stiffness. The association of lower bone mineral mass or density with vascular calcifications or PWV has already been demonstrated in nonrenal and dialysis patients (1,6,7) but, for the first

TABLE 1
Cardiovascular Characteristics According to T-Score

	All patients (*n*=70)	T-score in lumbar spine > –1 SD (*n*=31)	T-score in lumbar spine ≤ –1 SD (*n*=39)	T-score in femoral neck > –1 SD (*n*=27)	T-score in femoral neck ≤ –1 SD (*n*=43)
PP (mmHg)	57.9±16.8	55.8±14.4	59.5±18.5	52.8±13.2	61.0±18.2[a]
PWV (m/s)	7.8±1.6	7.6±1.6	8.1±1.6	7.4±1.5	8.2±1.6[a]
VCS≥3	29 (41%)	12 (39%)	17 (44%)	7 (26%)	22 (51%)[b]
PAD	18 (26%)	5 (16%)	13 (3%)	2 (7%)	16 (37%)[c]

PP = pulse pressure; PWV = pulse wave velocity; VCS = vascular calcification score; PAD = peripheral artery disease; SD = standard deviation.
[a] $p < 0.05$: comparison between groups by t-test.
[b] $p < 0.05$: comparison between groups by chi-square.
[c] $p < 0.01$: comparison between groups by Fisher's exact test.

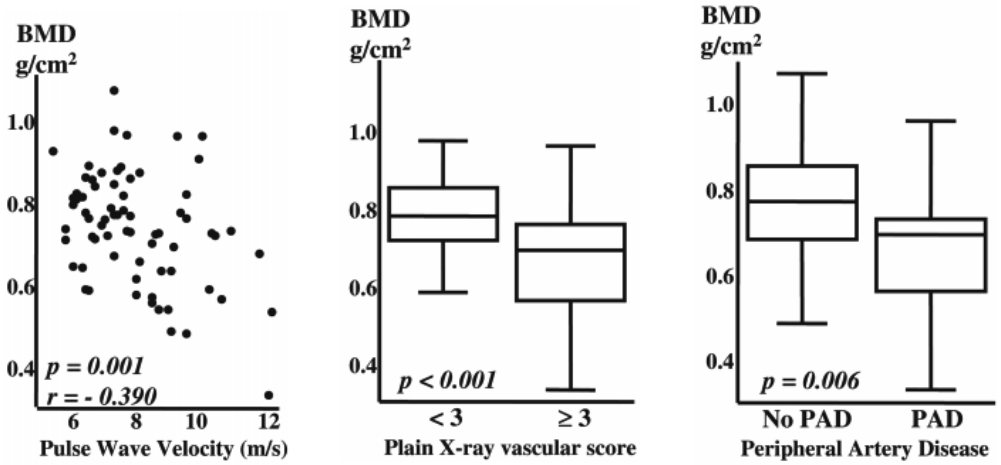

Figure 1 — Lower bone mineral density (BMD) at femoral neck is associated with higher pulse wave velocity, with more calcifications, and with peripheral artery disease (PAD).

time, an association between low BMD and plain x-ray vascular calcifications and vascular disease is shown in dialysis patients. Evaluation of vascular calcifications is now required to classify the mineral and bone disorder seen in chronic kidney disease (2), and plain x ray may be a simple and inexpensive method to do so. One explanation for the association of lower BMD with PWV or vascular disease is their common relationship with vascular calcifications (6,8). The absence of association between lumbar BMD and arterial stiffness or vascular calcifications present in our study has already been described in dialysis patients (7) and may be explained by the presence of vascular calcifications in the aorta possibly contributing to a higher BMD value (7). This can also explain why, in our study, patients with calcifications had a higher lumbar T-score compared with patients without calcifications. The pathogenesis of the association between low BMD parameters and vascular calcifications is not known yet and might be explained by a common etiologic factor. Both hyperparathyroidism and adynamic bone disease may be associated with low bone volume (9) and may also be associated with hyperphosphatemia and hypercalcemia, which are inducers of vascular calcifications (10). Low bone turnover has also been associated with vascular calcifications (11). This study presents, however, some limitations: Vascular score has the advantage of simplicity and low cost but it is a semiquantitative evaluation, and the cross-sectional analysis allows only identification of associations between the variables.

In conclusion, in this group of patients, lower values of bone mineral density, evaluated by DXA, at the femoral neck but not at the lumbar spine were associated with more calcifications, arterial stiffness, and peripheral

TABLE 2
Factors Associated with Vascular Calcifications and Peripheral Artery Disease (PAD)

Dependent variable	Independent variable	B	OR	95% CI	*p* Value	R^2
VCS≥3[a]						0.45
	Age	0.11	1.12	1.05 to 1.20	0.001	
	Diabetes	2.58	13.13	1.96 to 87.73	0.008	
	Phosphorus	0.76	2.13	1.16 to 3.93	0.015	
	BMD (femoral neck)	–8.83	0.00	0.00 to 0.15	0.013	
PAD[b]						0.29
	Diabetes	2.11	8.24	1.65 to 41.14	0.010	
	Phosphorus	0.74	2.09	1.07 to 4.10	0.031	
	BMD (femoral neck)	–8.82	0.00	0.00 to 0.08	0.006	

VCS = vascular calcification score; BMD = bone mineral density; OR = odds ratio; CI = confidence interval.
[a] Adjusted for gender and body mass index.
[b] Adjusted for age, gender, and body mass index.

TABLE 3
Factors Associated with Pulse Wave Velocity (PWV)

Dependent variable	Independent variable	B	95% CI	*p* Value	R^2
PWV[a]					0.58
	Age	0.07	0.05 to 0.09	<0.001	
	SBP	0.02	0.00 to 0.03	0.020	
	Ca×P	0.03	0.00 to 0.05	0.028	
	BMD (femoral neck)	–2.52	–4.87 to –0.16	0.037	

SBP = systolic blood pressure; Ca×P = calcium–phosphate product; BMD = bone mineral density; CI = confidence interval.
[a] Adjusted for gender, diabetes, and body mass index.

artery disease. The mechanisms for this association are not yet identified but these findings reinforce the hypothesis of the existence of a link between bone disease and vascular disease in dialysis patients.

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ORIGINAL ARTICLE

Femoral bone mineral density reflects histologically determined cortical bone volume in hemodialysis patients

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Abstract
Summary We evaluated the associations between dual energy X-ray absorptiometry (DXA) and histologically determined cancellous and cortical bone volume by controlling for vascular calcifications and demographic variables in hemodialysis (HD) patients. Femoral bone mineral density (f-BMD) was associated with cortical porosity.
Introduction Assessment of bone mass in chronic kidney disease patients is of clinical importance because of the association between low bone volume, fractures, and vascular calcifications. DXA is used for noninvasive assessment of bone mass whereby vertebral results reflect mainly cancellous bone and femoral results reflect mainly cortical bone. Bone histology allows direct measurements of cancellous and cortical bone volume. The present study evaluates the association between DXA and histologically determined cancellous and cortical bone volumes in HD patients.
Methods In 38 HD patients, DXA was performed for assessment of bone mass, anterior iliac crest bone biopsies for bone volume, and multislice computed tomography for vascular calcifications.
Results While lumbar bone mineral density (l-BMD) by DXA was not associated with histologically measured cancellous bone volume, coronary Agatson score showed a borderline statistically significant association (P=0.055). When controlled for age and dialysis duration, f-BMD by DXA was associated with cortical porosity determined by histology (P=0.005).
Conclusions The usefulness of l-BMD for predicting bone volume is limited most probably because of interference by soft tissue calcifications. In contrast, f-BMD shows significant association with cortical porosity.

Keywords Bone biopsy · Bone mineral density · Bone volume · Chronic kidney disease · Cortical porosity · Vascular calcifications

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Introduction

Low bone mass is a common complication of chronic kidney disease (CKD), and the most prominent clinical complications of low bone mass are fractures. Compared to the general population, the incidence of fractures was reported to be 3.6–9.8 times higher for stage 5 CKD patients on dialysis (CKD-5 patients) [1]. In the Dialysis

Outcomes and Practice Patterns Study, the authors found a 7% increased risk of hip fracture per year of dialysis treatment [2]. Most recently, low bone mass has also been shown to be associated with increased cardiovascular calcifications in non-CKD and CKD patients [3, 4]. In light of the high mortality associated with fractures and cardiovascular disease in CKD [5, 6], noninvasive methods for assessing bone mass in this patient population is of great clinical importance.

Dual energy X-ray absorptiometry (DXA) is the most widely used tool for assessment of bone mass in the general population [7, 8]. However, DXA measures not only the mineral content of bone but also of the surrounding soft tissue [9, 10] limiting the interpretation of results [11]. CKD-5 patients represent a unique population particularly prone to developing soft tissue and vascular calcifications [12, 13]. Therefore, overestimation of bone mineral density (BMD) by anterioposterior (AP) lumbar DXA represents a great problem in these patients; this limitation should be less pronounced but not excluded in femoral DXA measurements [14]. Overall, studies evaluating the role of BMD determination by DXA for assessment of fracture risk in CKD-5 patients report conflicting results [15–18].

In order to guide appropriate therapeutic interventions, characterization of the relationship between DXA measurements and histologically determined bone volume in CKD-5 patients is desirable. In an early study, Lindergard and colleagues could not find a correlation between BMD measured at the radius (consisting primarily of cortical bone) and histologically determined bone volume at the iliac crest of CKD-5 patients (cancellous bone) [19]. In contrast, a more recent study in CKD-5 patients reported that hip and spine T-scores were associated with histologically determined bone volume at the iliac crest [20]. In light of these conflicting results, we evaluated the associations between BMD measured by DXA at the spine and femur and histologically determined bone volume in cancellous bone (bone volume/tissue volume) and in cortical bone (cortical width and cortical porosity) in CKD-5 patients. Moreover, vascular calcifications were assessed by multislice computed tomography (MSCT) and adjusted for in statistical analyses.

Materials and methods

Study design

This study investigates the association between BMD measured by DXA at the hip and spine and histologically determined parameters of bone volume in cancellous and cortical bone in a cross-sectional study design. In addition, vascular calcifications were measured by multislice computed tomography at the thoracic aorta and the coronary and iliac arteries and were adjusted for in the analyses. The protocol was approved by the Institutional Review Boards of participating institutions. The study has been conducted in adherence to the Declaration of Helsinki, and all patients provided informed consent.

Patients

Thirty-eight white stage 5 CKD patients on hemodialysis were recruited from 11 medical centers in Portugal. All patients provided informed consent for performing DXA measurements, bone biopsies, and multislice computed tomography. DXA measurements were performed at time of bone biopsy and patients underwent multislice computed tomography on average 3.8±1.9 months after the bone biopsy.

Inclusion criteria These are age 18 years or older, dialysis duration of at least 3 months, mental competence, and willingness to participate in the study.

Exclusion criteria These are kidney transplant; pregnancy; uncontrolled systemic illnesses or organic diseases with potential influence on bone metabolism such as diabetes mellitus, active or chronic liver disease, malabsorption, malignancy, and thyroid dysfunction; history of or present treatment with bisphosphonates, fluoride, calcitonin, glucocorticoids, or other immunosuppressive agents, hormone replacement therapy, and selective estrogen receptor modulators; and chronic alcoholism and/or drug addiction.

Bone biopsies

Anterior iliac crest bone biopsies were performed under local anesthesia and conscious sedation. Bone samples were obtained with the one-step electrical drill technique (Straumann Medical, Waldenburg, Switzerland). Bone samples were processed undecalcified and cut avoiding cracks or overlaps of bone tissue. Sections were stained with the modified Masson–Goldner trichrome stain [21], the aurin tricarboxylic acid stain [22], and solochrome azurin [23], assessment of stainable aluminum, and modified Gomori stain for detection of iron [24]. Unstained sections were prepared for phase contrast and fluorescent light microscopy. Histomorphometric analysis of bone was done at standardized sites in cancellous (×200 magnification) and cortical bone (×80 magnification) using the semi-automatic method (Osteoplan II, Kontron, Munich, Germany). For cancellous bone, the volume of bone trabecules (BV) and total tissue volume (TV) were traced and bone volume/total volume (BV/TV) was calculated for assessment of mineralized trabecular bone volume. For cortical bone, cortical width was measured; cortical porosity was determined by tracing the total cortex and all Haversian canals and

computing the ratio between canal area over total cortical tissue area.

Results were compared to our normative database consisting of histomorphometric results of age- and gender-matched healthy individuals [25, 26]. "Low" cancellous bone volume was defined as BV/TV <16.8%, "normal" as BV/TV between 16.8% and 22.9%, and "high" as BV/TV >22.9%. Cortical width was considered "low" for values <0.52 mm, "normal" for values between 0.52 and 1.65 mm, and "high" for values >1.65 mm. Cortical porosity was classified "low" for values <1.9%, "normal" for values between 1.9% and 12%, and "high" for values >12%. All bone samples were processed and analyzed without knowledge of the clinical data at the Bone Diagnostic and Research Laboratory, University of Kentucky, Lexington, KY, USA.

Bone mineral density

DXA was performed by the same operator on the same Hologic QDR Discovery scanner according to the manufacturer's recommendations for patient positioning, scan protocols, and scan analysis. Measurements of the spine and hip were obtained from the AP projection. For AP lumbar spine, lumbar vertebrae 1 to 4 were measured and BMD results were analyzed for mean measurements of L1–L4. For proximal femur scans, BMD was measured at the femoral neck. The coefficients of variation for these BMD measurements are AP spine 1.2% and femur 0.9%.

Assessment of vascular calcifications

Vascular calcifications were assessed at the thoracic aorta and coronary and iliac arteries by a quantitative score using MSCT. MSCT scans were performed on the model Somatom Volume Zoom (Siemens AG, Erlangen, Germany). Slices of 2.5 mm thickness were acquired under the following conditions: 120 kVp, 130 mAs, and 0.5 gantry rotation time. All images were transferred to a workstation and analyzed with calcium scoring software (HeartView CT, Siemens AG, Erlangen, Germany). Quantification of vascular calcifications was performed by calculating the Agatston score based on the maximum X-ray attenuation coefficient (measured in Hounsfield units) [27].

Biochemical measurements

Blood was drawn at the time of the bone biopsy after an overnight fast. The following biochemical parameters were measured: serum calcium and phosphorus by an autoanalyzer (Hitachi 747, Globe Scientific Inc, USA), intact parathyroid hormone (iPTH) by DPC IMMULITE® PTH IRMA from Diagnostics Products Corporation (Los Angeles, CA, USA; normal range 16–87 pg/ml; intra- and interassay coefficients of variation <7% and <9%), and 25-(OH)-vitamin D by LIAISON® 25-OH Vitamin D assay (Diasorin, Saluggia, Italy; normal range 25–100 ng/ml; intra- and interassay coefficients of variation 4.1% and 7%).

Statistical analysis

Descriptive statistics are presented as means, medians, minimums, maximums, and standard deviations (SDs). The variables iPTH and hemodialysis (HD) duration were log-transformed for analysis. Boxplots were used to characterize the distributions of BV/TV, cortical width, and cortical porosity. Bivariate associations were assessed using scatter plots, nonparametric Spearman rank correlations, locally weighted regression, and generalized additive models to characterize the associations between femoral/lumbar BMD and cortical porosity, cortical width, and BV/TV. Linear regression analyses were performed to evaluate possible relationships while controlling for relevant measured correlates. All calculations were performed using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study population are presented in Table 1. All patients were receiving phosphate binder therapy (58% sevelamer hydrochloride, 42% calcium acetate) and 19 patients (50%) were treated with active vitamin D analogs. None of the patients were treated with a calcimimetic agent. There were no clinically symptomatic fractures prior and during the study.

In cancellous bone, BV/TV was low in 16%, normal in 24%, and high in 60% patients. In cortical bone, cortical porosity was low in 3%, normal 40%, and high in 57%, and cortical width was low in 8%, normal 89%, and high in 3%. None of the biopsies showed positive staining for aluminum or iron.

DXA results are reported as "measured bone mineral density" (in grams per centimeter squared). The correlation between lumbar and femoral BMD measurements was $r=0.49$ ($P=0.01$).

Femoral bone mineral density

Unadjusted analysis of the association between femoral BMD and cortical porosity revealed a correlation coefficient $r=-0.20$ ($P=0.24$). When adjusted for different Agatston score groups, no statistically significant effects of the different vascular calcifications sites were found. In the final model, a forward predictor selection routine

Table 1 Characteristics of the study population

	Mean (SD)	Median (min–max)
Age (years)	45.2 (15.2)	45 (21–74)
Gender		
Male (*N*, %)	20 (52.6)	
Female (*N*, %)	18 (47.4)	
Dialysis duration (months)	73.1 (56.6)	48.3 (21–206)
Calcium (mg/dl)	96 (6.6)	95.4 (87–111)
Phosphorus (mg/dl)	5.4 (0.9)	5.5 (3.9–7.2)
iPTH (pg/ml)	620 (614)	353.4 (50–2,164)
VIT D25 (ng/ml)	21.2 (7.8)	21.5 (7.8–37.6)
Agatston scores		
Coronary arteries	958.3 (1,888.4)	99.25 (0.0–6,726.0)
Thoracic aorta	1,391.0 (3,026.9)	3.2 (0.0–12,576.2)
Iliac arteries	2,916.0 (5,430.6)	848.2 (0.0–28,670.0)

iPTH intact parathyroid hormone, *vit D25* 25(OH)-hydroxy vitamin D

identified femoral BMD (point estimate −157.49; P=0.005), age >50 years (point estimate −8.37; P=0.001), and HD duration (point estimate −25.44; P=0.005) as being associated with cortical porosity (Table 2). Further statistical analysis recognized a statistically significant interaction between femoral BMD and HD duration (point estimate 32.48; P=0.01): independent of age group, cortical porosity increased as femoral BMD decreased, and this increase in cortical porosity was more rapid for patients with shorter HD duration (Fig. 1).

Unadjusted correlation analysis of femoral BMD and cancellous bone volume (BV/TV) yielded r=−0.06 (P=0.73). While age, gender, and HD duration were not predictive of cancellous bone volume (BV/TV), the model containing femoral BMD and coronary Agatston score >100 revealed a statistically significant negative association (point estimate −4.93; P=0.03); this association was, however, lost when the model was adjusted for age and gender.

When examining a possible association between femoral BMD and cortical width, unadjusted (r=0.004; P=0.98) and adjusted analyses (models containing age, gender, HD duration, and vascular calcifications) did not yield any significant predictors.

Table 2 Predictor variables of cortical porosity (from linear regression analysis)

	Estimate	Standard error	*P* value
fBMD	−157.49	52.36	0.005
Age >50 years	−8.37	2.18	0.0005
HD	−25.44	8.40	0.005
fBMD/HD	32.48	12.09	0.01

fBMD femoral bone mineral density, *HD* hemodialysis duration, *fBMD/HD* interaction term between femoral bone mineral density and hemodialysis duration

Lumbar bone mineral density

In unadjusted analysis, the correlation between lumbar BMD and cancellous bone volume (BV/TV) was not statistically significant (r=−0.1; P=0.53) and remained statistically nonsignificant when the model was adjusted for gender, age, and HD duration (P>0.05 for all variables). Similar results were found for the correlations between lumbar DXA and cortical width/porosity (r=−0.09, P=0.60; r=−0.15, P=0.39, respectively) and adjusted models (gender, age, HD duration, all variables P>0.05).

In the next analytical step, the model was adjusted for Agatston scores measured at the thoracic aorta, coronary artery, or iliac artery. For this purpose, patients were classified according to an Agatston score cutoff of 100 (≤100 versus >100). Regression modeling revealed that

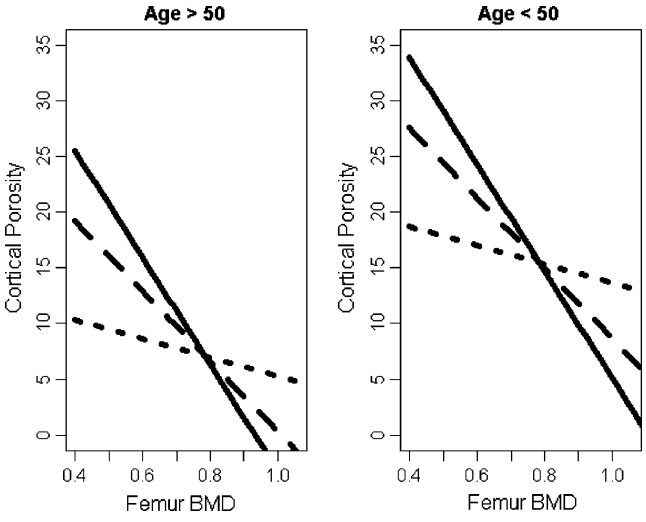

Fig. 1 Estimated mean cortical porosity based on age group and HD duration. The *three lines* in each plot correspond to patients with dialysis durations equal to the 25th (*solid line*), 50th (*dashed line*), and 75th (*dotted line*) empirical percentiles

only coronary Agatston score >100 showed borderline significant negative association with lower cancellous bone volume (BV/TV) (point estimate −4.14; $P=0.055$) but this association was lost when the model was also adjusted for gender and age.

Conclusions

The National Institutes of Health consensus conference on "Osteoporosis prevention, diagnosis and therapy" defines osteoporosis as a systemic disease of impaired bone strength [28]. Clinically and in specific populations, the diagnosis "osteoporosis" is defined on the basis of standardized BMD levels (T-score≤−2.5 SD) determined by DXA at the spine, hip, or forearm [29]. In the case of patients suffering from CKD, the use of this traditional approach to diagnose osteoporosis is problematic since all forms of renal bone disease may be accompanied by low BMD [30, 31], and erroneously high BMD measurements due to vascular/soft tissue calcifications—which are commonly encountered pathologic findings in CKD patients—are known problems of anterioposterior DXA projections [32]. Accordingly, it has been proposed that, currently, the only way to establish the diagnosis of osteoporosis in patients with stage 5 CKD is the histomorphometrical finding of low bone volume [33]. Based on these observations, we selected not to classify our patients according to specific scores but to evaluate the association between measured bone mineral density (expressed in grams per centimeter squared) at the lumbar and femoral sites and histomorphometric determinants of cancellous bone volume (BV/TV) and cortical bone volume (cortical width and cortical porosity). Furthermore, we adjusted our models for Agatston scores measured by multislice computed tomography.

Our study results corroborate previous observations regarding lack of associations between histologically determined cancellous bone volume (BV/TV) and BMD measurements reported in CKD patients (creatinine clearance 10–78 ml/min) [34] and expand those findings to stage 5 CKD patients on hemodialysis. The usefulness of iliac crest bone biopsies for assessment of bone changes in the femur has been reported in patients requiring total hip replacement [35]. Although there is paucity of data regarding the association between cortical bone and fractures in CKD patients, the role of increased cortical porosity and decreased cortical thickness in femoral neck fractures has been described in the general population [36–38]. A novel finding of our study is the statistically highly significant association between cortical porosity and femoral BMD in stage 5 CKD patients that persisted after statistical adjustments. Since cortical changes of the femoral neck contribute to the risk of hip fractures [38] and fractures of the axial skeleton are highly prevalent in stage 5 CKD patients [39], our findings suggest a possible clinical role of femoral DXA measurements for identifying stage 5 CKD patients on hemodialysis at risk for fracture. The stronger association between femoral BMD and cortical porosity at shorter HD durations implies that additional factors present after longer HD vintage such as more extensive soft tissue calcifications will limit the value of femoral BMD for assessing cortical porosity as dialysis vintage progresses.

We would like to acknowledge the following limitations of our study: Goal of our study was not the comprehensive evaluation of all risk factors for hip fractures in stage 5 CKD patients but to investigate the role of DXA measurements for assessing histological parameters of cancellous (BV/TV) and cortical bone volume (cortical porosity and cortical width). In order to limit the influence of potentially confounding variables of bone metabolism and vascular calcifications, we imposed strict inclusion and exclusion criteria for study participation; accordingly, our findings call for larger population-based studies to validate a possible role of femoral BMD measurements by DXA including hip structural analysis for assessing bone fracture risk not only in stage 5 CKD patients on different dialysis modalities but also to include patients suffering from different levels of CKD as well as CKD patients with previous fractures. Future large age-matched population-based studies will also need to answer the question on differences in bone volume between healthy and CKD patients.

In summary, our data suggest a role for femoral DXA measurements to assess cortical porosity in stage 5 CKD patients on hemodialysis. Anterioposterior lumbar measurements by DXA do not yield information useful for assessment of cancellous bone volume (BV/TV) that has been shown to be associated with coronary calcifications [4]. In light of the high morbidity and mortality of stage 5 CKD patients, future prospective clinical studies will need to further characterize the role of this relatively inexpensive and widely available clinical tool (DXA) for assessment of clinical outcomes such as fractures.

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Conflicts of interest None.

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CAPÍTULO 8

QUE MÉTODOS UTILIZAR PARA AVALIAR A CALCIFICAÇÃO VASCULAR?[1-3]

Diversos estudos em doentes renais crónicos têm utilizado diferentes métodos para diagnosticar calcificações vasculares. Braun *et al*[4], em 1996, foram os primeiros a mostrar que os doentes em diálise apresentam um *score* de Agatston muito superior ao da população geral. Blacher *et al*[5], em 2001, demonstraram que calcificações vasculares avaliadas por ecografia em grandes vasos são preditoras de mortalidade nos doentes em diálise. Em 2003, Moe S *et al*[6] verificaram que a tomografia axial computorizada multicorte pode ser utilizada em vez da tomografia de feixe de eletrões para medir o *score* de Agatston nos doentes em diálise. Em 2003, London *et al*[7] usaram, pela primeira vez, um RX simples do abdómen para avaliar calcificações vasculares e demonstraram que calcificações vasculares avaliadas por este método se associavam a maior risco de mortalidade nos doentes em diálise. Em Fevereiro de 2004 foi aceite para publicação e em Março de 2004 foi publicado *on-line* o nosso estudo[8], que descrevia um método original e semiquantitativo para avaliação de calcificações vasculares nos doentes em diálise usando RX simples da bacia e das mãos. Demonstramos nesse estudo que este *score*, de utilização muito simples, é preditor de mortalidade cardiovascular, de internamentos cardiovasculares e de doença coronária e arterial periférica nos doentes em diálise.

Avaliação quantitativa das calcificações vasculares: *score* de Agatston

O *score* de Agatston permite a avaliação quantitativa das calcificações vasculares. Este método baseia-se no coeficiente de atenuação máximo dos raios X, medido por unidades hounsfield. Braun *et al*[4] demonstraram que o valor médio do *score* de calcificação nos doentes em diálise era 10 vezes superior a doentes da população geral com doença coronária provável ou documentada. O significado clínico do *score* de Agatston é, contudo, diferente nos doentes com ou sem doença renal crónica. Na população geral está bem demonstrado que o *score* de calcificação de Agatston se relaciona com a carga aterosclerótica e que um valor superior a 400 é preditor de eventos cardiovasculares ou de necessidade de procedimentos coronários nos 2 a 5 anos após a determinação deste *score*. A localização ou o grau de calcificação podem

não se relacionar com o local da estenose, mas, quanto maior o *score*, maior a probabilidade de estenose em qualquer local, nas artérias coronárias [9].

Os doentes em diálise podem, contudo, ter *score*s de Agatston muito elevados sem se verificar estenose coronária, e isso foi demonstrado por Haydar *et al*, que compararam coronariografias com a avaliação de calcificações coronárias pelo *score* de Agatston num grupo de 46 doentes em diálise[10]. Esta discrepância entre os dados da angiografia e os dados da quantificação da calcificação coronária que se verifica nos doentes em diálise pode ser explicada pela existência simultânea de calcificações da íntima e de calcificações não estenosantes da média. Numa análise anatomopatológica das artérias coronárias, comparando indivíduos com e sem doença renal, demonstrou-se de forma inequívoca que os doentes renais crónicos apresentam uma maior quantidade de cálcio, quer na íntima quer na média, e maior intensidade de marcadores inflamatórios quando comparados com doentes não renais[11]. Por outro lado, nos doentes em diálise, valores de *score* de Agatston superiores ou iguais a 400[12] ou a 200[13] relacionam-se com a mortalidade cardiovascular, sendo esta associação consistente com todos os restantes estudos que demonstram que as calcificações vasculares, qualquer que seja o local, se associam a aumento de mortalidade.

Atualmente, a avaliação do *score* de Agatston é feita geralmente por tomografia axial computorizada multicorte, pois esta tecnologia tem múltiplas utilizações e está amplamente distribuída, bastando uma especificação do seu *software* para poder medir este *score*.

Ecografia e ecocardiografia

Blacher *et al*[5] usaram a ecografia de grandes artérias para avaliar as calcificações vasculares em quatro territórios vasculares: aorta, carótidas, artérias ilíacas e femorais. Este estudo, realizado em 2001, foi o primeiro a demonstrar que as calcificações vasculares se associam a maior risco de morte. As calcificações valvulares avaliadas por ecocardiografia são preditoras de mortalidade nos doentes em diálise[14-16] e a iniciativa KDIGO 2009[17] reconhece que a ecocardiografia pode ser utilizada para o diagnóstico de calcificações valvulares, de modo a identificar os doentes com maior risco cardiovascular. Num grupo de 123 doentes em diálise, verificamos, como previamente demonstrado, que a calcificação valvular aórtica ou mitral se associa a um risco aumentado de morte de causa global ou cardiovascular. Verificamos que as calcificações vasculares avaliadas por RX simples são preditoras de calcificações valvulares diagnosticadas por ecocardiografia[18]. Por cada aumento de uma unidade no *score* de calcificação vascular simples, verificou-se um aumento de 46% de risco de ter simultaneamente calcificação da válvula aórtica e de 66% de ter calcificação da válvula mitral. A presença de calcificações valvulares apresenta um alto valor preditivo positivo e um baixo valor preditivo negativo para

indicar presença de calcificações vasculares. Isto significa que, no caso de haver calcificações valvulares, existe elevada probabilidades de também haver calcificações vasculares. No caso de não haver calcificações valvulares, a probabilidade de não haver calcificações vasculares é baixa. O ecocardiograma não substitui a utilização do RX simples para identificar o risco cardiovascular associado às calcificações.

RX simples

London G *et al* foram os primeiros a demonstrar que calcificações vasculares avaliadas em RX simples são preditoras de mortalidade nos doentes em diálise[7]. Baseando-se numa metodologia previamente descrita por Letho[19], diferenciaram calcificação da camada média e da íntima pelas imagens radiológicas. A calcificação da média é linear e regular e apresenta frequentemente um traçado duplo em linha de comboio. A calcificação da íntima é irregular e descontínua. Este importante estudo demonstrou que as calcificações da camada média não são achados radiológicos, mas têm significado clínico. Neste estudo, os autores demonstraram que as calcificações da média e da íntima foram preditores independentes de mortalidade. O nosso estudo[8] foi publicado alguns meses após o estudo de London e foi o primeiro a usar um *score* semiquantitativo para avaliar as calcificações vasculares. As calcificações são avaliadas em setores definidos nos RX das mãos ou da bacia. Os limites do *score* são de 0 e 8. Posteriormente, mais estudos foram publicados mostrando a associação entre calcificações vasculares avaliadas por RX simples e mortalidade. A presença de calcificações na aorta abdominal[20] ou a presença de calcificações no acesso vascular [21] associaram-se a maior risco de morte, O *score* da calcificação aortoabdominal descrito por Kauppila L[22], que avalia as calcificações na aorta abdominal de L1 a L4, usando um RX simples de perfil, é preditor de mortalidade na população geral[23] e, nos doentes em diálise, apresenta uma boa correlação com o *score* coronário de Agatston[24]. Os resultados preliminares do estudo CORD[25] mostram que o *score* de Kauppila se associa à idade, à duração da diálise e à doença vascular prévia. Em comunicação apresentada no Congresso da EDTA, em 2008, Honkanen E *et al* mostraram que um *score* de Kauppila superior ou igual a 7 se associa a maior risco de mortalidade.

Comparamos o *score* simples de calcificação com o *score* de calcificação na aorta abdominal, ou *score* de Kauppila, na avaliação do índice tornozelo-braço num grupo de 219 doentes em hemodiálise[26]. Os valores preditivos positivo e negativo de um *score* vascular simples superior a 3 identificar um *score* de Kauppila superior ou igual a 6 foram, respectivamente, 79% e 85%. Ambos os métodos se associaram a maior risco de doença arterial obliterativa, definida por índice tornozelo-braço, inferior a 0,9, apresentando uma área semelhante sob a curva. Só as calcificações

detetadas no RX das mãos e nas artérias colaterais da bacia se associaram a maior risco de índice tornozelo-braço superior a 1,3. O padrão radiológico mais comum das calcificações vasculares destas artérias colaterais ou distais é linear. Esta associação sugere uma eventual contribuição de calcificações da camada média arterial para o aumento do índice tornozelo-braço.

Várias técnicas não invasivas permitem fazer o rastreio de calcificações vasculares: técnicas de tomografia axial computorizada para avaliação do *score* de calcificação coronário, ecografia de grandes vasos, ecocardiografia para avaliação das calcificações valvulares aórtica e mitral e RX simples para avaliar diferentes territórios arteriais. A tomografia axial computorizada multicorte ou a tomografia computorizada de feixe de eletrões são consideradas técnicas *gold-standard* para avaliação das calcificações coronárias ou aórticas. São técnicas quantitativas dispendiosas que têm sido usadas em vários ensaios clínicos para avaliação da progressão das calcificações coronárias. Estas técnicas não distinguem a calcificação da íntima da da média e apresentam valores muito elevados nos doentes renais crónicos, devido à elevada prevalência de calcificações da média nestes doentes[11]. Os elevados *score*s de calcificação diagnosticados por estes métodos associam-se a maior risco cardiovascular, mas podem não se relacionar com doença coronária obstrutiva. O RX simples, a ecografia dos grandes vasos e a ecocardiografia são métodos simples e baratos mais indicados para o rastreio das calcificações vasculares nos doentes em diálise. A iniciativa KDIGO 2009[17] reconhece a utilidade do RX simples e da ecocardiografia para fazer o rastreio das calcificações vasculares. Na escolha do melhor método a usar nos nossos doentes para este fim devemos, contudo, ter em conta que o ecocardiograma subavalia o risco cardiovascular relacionado com as calcificações.

Qual a utilidade de avaliar as calcificações vasculares nos doentes renais crónicos?

A mortalidade cardiovascular é a principal causa de morte nos doentes em diálise. Até ao momento atual, muito poucas intervenções terapêuticas se associaram a uma redução da mortalidade, e isto verificou-se apenas em ensaios clínicos com reduzido número de participantes. O candesartan associou-se à redução de eventos cardiovasculares[27], o carvedilol reduziu a mortalidade em doentes com cardiomiopatia[28] e o sevelamer reduziu a mortalidade num grupo de 127 doentes em diálise com hiperfosfatemia[12].

A presença de calcificações vasculares é um sinal de alerta para um elevado risco cardiovascular nos doentes renais crónicos. Nestes doentes justifica-se a intervenção terapêutica em todos os fatores associados ao desenvolvimento e progressão das calcificações vasculares, sendo a correção das alterações do metabolismo fosfocálcico um dos campos de atuação.

Esperamos que o progressivo conhecimento dos múltiplos mecanismos fisiopatológicos na base do desenvolvimento das calcificações vasculares permitam alargar o campo de intervenção terapêutica nos nossos doentes. Será sempre necessário confirmar se este tipo de intervenção terapêutica se associa à redução da morbilidade e da mortalidade nestes doentes.

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Cardiovascular risk in dialysis patients: an X-ray vision on vascular calcifications

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In dialysis patients, there is an association between vascular calcifications and mortality. Hyperphosphatemia and calcium overload are associated with development of vascular calcifications, especially in the presence of low bone turnover. Different plain X-ray methods are now available to evaluate vascular calcifications in dialysis patients. The presence of vascular calcifications is an alert sign for increased cardiovascular risk, and this information is important for choosing the most suitable treatment for dialysis patients.

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Introduction

In recent years, observational studies in dialysis patients have verified the existence of an association between vascular calcifications and mortality.[1–5] The abnormal mineral metabolism, a consequence of chronic kidney disease (CKD), has been associated with development of vascular calcifications. In an *in vitro* model using smooth muscle cells from the human aorta, it was demonstrated that high levels of phosphorus, calcium, or both induce vascular calcification[6] by an active intracellular process that transforms vascular smooth muscle cells into osteoblast-like cells. Vascular calcifications are associated with low bone turnover,[7] and the hypothesis of the existence of a link between bone disease and cardiovascular disease has been raised. Kidney Disease: Improving Global Outcomes (KDIGO) has recommended a new classification for chronic kidney disease mineral and bone disorder (CKD-MBD) that includes the presence of vascular calcifications.

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Methods to evaluate vascular calcifications

Braun *et al.*,[8] in 1996, used electron beam computed tomography to evaluate coronary calcifications and showed that hemodialysis patients had a very high coronary Agatston score when compared with nonrenal patients. Blacher *et al.*,[1] in 2001, demonstrated for the first time that the presence of vascular calcifications in hemodialysis patients, evaluated by ultrasonography in large arteries, was associated with increased mortality. In 2003, London *et al.*[2] showed that vascular calcifications evaluated in plain X-ray were associated with all-cause and cardiovascular mortality in dialysis patients. After these initial observations, other studies[3,5] confirmed the association between vascular calcifications, evaluated with plain X-ray, and mortality in the CKD stage 5 hemodialysis population. We developed a simple vascular calcification score, using the presence of vascular calcification, in a plain X-ray of the pelvis and hands[3] (Figure 1). This simple score was an independent predictor of cardiovascular death, cardiovascular hospitalizations, and vascular disease in dialysis patients. The Kauppila score, using the presence of vascular calcification in a plain X-ray of the lateral abdominal aorta, previously associated with cardiovascular death in the general population, has been recently validated for evaluating mortality risk in hemodialysis patients (E. Honkanen *et al.*, 2008; EDTA Congress; Stockholm, Sweden; abstr.). Even a simpler evaluation of vascular calcifications in the abdominal aorta, identifying only the presence or absence of calcifications,[5] was also a predictor of mortality in dialysis patients. Schlieper *et al.*[9] (this issue) now publish an interesting observation showing that the presence of vascular calcification of the hemodialysis vascular access, evaluated by plain X-ray, is also an independent predictor of mortality.

Electron beam computed tomography and multislice computed tomography are considered the gold standard for quantitative evaluation of vascular calcifications, but they are expensive and not widely available. In the setting of clinical trials performed with CKD patients, these quantitative techniques have been useful to evaluate the effect of different treatment options on vascular calcification progression. However, the meaning of the coronary Agatston score is different in dialysis patients as compared with the general population. In the general population, the coronary Agatston score is correlated with the atherosclerotic calcium load and is a predictor of cardiovascular events. In dialysis patients, there is no precise correlation between calcium score and coronary angiography. The Agatston score does not differentiate intimal from medial calcification, and hemodialysis patients can have a very high coronary Agatston score, because both intimal and medial calcifications may be present in coronary arteries. Medial calcifications contribute to aortic stiffness and to decreased coronary perfusion during diastole without causing coronary stenotic lesions. For that reason, a high coronary Agatston score cannot be used to identify dialysis patients who will benefit from a coronary angioplasty.

Figure 1 | Simple vascular calcification score evaluation. Calcification score is the sum of the presence (1) or absence (0) of vascular calcifications in each section. Pelvis score (1+1+1+1) = 4 and hands score (1+1+1+1) = 4. Total score is 8.

Vascular calcification evaluated in the vascular access

Schlieper *et al.*[9] evaluated calcification of arteriovenous fistulas or synthetic grafts with plain X-ray and verified that calcification of the vascular access was an independent predictor of mortality. The authors conclude that this is a cost-effective and easy-to-perform method to identify dialysis patients at increased mortality risk. Calcification of the vascular access was present in 23% of patients and was associated with male gender, diabetes, and dialysis vintage. The authors have verified that the presence of calcifications in the vascular access was highly correlated with the presence of vascular calcifications of iliac and femoral arteries, suggesting that there is a common pathogenic process affecting these different sites of the vasculature. This common pathogenic process could explain why vascular calcifications, independently of their localization, are associated with mortality risk. The presence of calcification in the vascular access may have, however, a different meaning from that observed in other arteries. Intimal calcification is associated with atherosclerotic plaques, and medial calcification is associated with arteriolosclerosis. In hemodialysis patients it has already been demonstrated[2] that these two types of vascular calcifications may coexist in the same patient and in the same vessel and that they are independent predictors of cardiovascular and all-cause mortality. Calcification of the vascular access seems to be predominantly composed of medial calcification.[10] Most probably, a method to evaluate vascular calcifications that includes these two types of vascular calcification, intimal and medial calcification[2,3,5] (E. Honkanen *et al.*, 2008; EDTA Congress; Stockholm, Sweden; abstr.), should be more appropriate for evaluating the global mortality risk. In our opinion, there are other limitations in the choice of this method to evaluate vascular calcifications. One limitation is the exclusion of all patients who do not have a vascular access, such as peritoneal dialysis patients and patients without vascular conditions to receive an access. Also, local hemodynamic factors, unrelated to cardiovascular risk, may be associated with development of calcifications of the vascular access. The presence of calcification in the vascular access might have an interesting advantage of identifying calcium deposition occurring after initiation of dialysis. However, the identification of this phenomenon at such a late stage might not be so helpful to guide a therapeutic intervention. An earlier identification of vascular calcifications, in predialysis or in incident patients, is probably a more intelligent and useful approach. Such patients constitute a population with elevated risk of rapid calcification progression and certainly deserve a more aggressive therapeutic approach.

What is the preferable vascular calcification screening method?

A method adequate for screening vascular calcifications should be inexpensive and simple to interpret. Any of the plain X-ray methods mentioned here for evaluation of vascular calcifications can be easily interpreted by the attending physician. Another important quality for choosing a screening method is a correct balance between sensitivity and specificity. Which of the methods using plain X-ray for evaluation of vascular calcification is the best? For a definitive answer to this question, comparative studies in the same population will be required and certainly welcome.

In our opinion, all of the methods described here that recognize the presence of vascular calcification with plain X-ray of different vascular territories have been demonstrated to be very useful in the evaluation of CKD population mortality risk. Certainly, the final choice of the method to be used in a specific institution should be based on the experience and preference of the nephrology attending physicians.

What is the importance of screening vascular calcifications in dialysis patients?

The identification of dialysis patients with calcified vessels and the evaluation of vascular calcification extension could be very useful for cardiovascular risk stratification and therapeutic guidance. Vascular calcifications most likely are multifactorial and result from a complex balance between inducers and inhibitors. However, at this stage of knowledge, we can only interfere in a very small number of those factors — mainly, alterations of mineral metabolism. Several studies have already demonstrated that vascular calcifications in dialysis patients may progress or remain stable depending on the control of the mineral metabolism alterations, such as hyperphosphatemia. Another important aspect besides the control of hyperphosphatemia per se is the choice of the phosphate-binding agent. One should avoid the use of calcium-containing phosphate binders in patients with calcified vessels.

London *et al.* demonstrated an association between vascular calcifications and low bone turnover.[7] The same group[11] found a significant interaction between dosage of calcium-containing phosphate binders and bone activity such that calcium load had a significantly higher impact on aortic calcifications and stiffening in the presence of adynamic bone disease. Low-bone-turnover status should be prevented, when possible, avoiding parathyroid hormone

oversuppression, and calcium-containing binders should be avoided in patients with such a condition.[11]

In summary, vascular calcifications are associated with increased mortality in dialysis patients. Different methods can be used to identify vascular calcifications in these patients. Plain X-ray evaluation of vascular calcifications is inexpensive and simple to interpret and should be used for the screening of vascular calcifications. Nephrologists have now at their disposal different plain X-ray methods to evaluate vascular calcifications in their CKD patients. The presence of vascular calcifications in CKD patients constitutes an important alert sign for an increased cardiovascular risk. This information is important and should serve to guide nephrologists in the design of more appropriate and aggressive therapeutic strategies to control mineral metabolism in their patients.

DISCLOSURE

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Review

Evaluation of vascular calcifications in CKD patients

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ABSTRACT: In observational studies on dialysis patients, there has been a consistent association between vascular calcifications and mortality. Hyperphosphatemia and calcium treatment are some of the factors associated with development of vascular calcifications, especially in the presence of low bone turnover disease. Several non-invasive imaging techniques have been used to screen for the presence of vascular calcifications: plain X-Ray, echocardiography, ultrasonography, and computed tomography. Presence of vascular calcifications is a warning sign for increased cardiovascular risk and this information may be relevant for choosing the most suitable treatment for dialysis patients. (Int J Artif Organs 2009; 32: 81-6)

KEY WORDS: Vascular calcifications, Imagiology, Chronic kidney disease

INTRODUCTION

High cardiovascular risk in chronic kidney disease patients is not fully explained by traditional risk factors. The Framingham risk score, based on age, gender, diabetes, hypertension, lipid profile and smoking habits, fails to predict cardiovascular events in dialysis (1) and pre-dialysis (2) patients and several non-traditional risk factors are commonly considered to contribute to the high cardiovascular risk in this population.

In the last few years a new explanation has been added to the list of the already numerous cardiovascular risk factors of chronic kidney disease patients. The association of cardiovascular calcifications with mortality, verified in multiple observational studies, is considered to be a consistent finding and has been classified as grade B evidence in recent KDIGO recommendations. In experimental studies, it is demonstrated that an altered mineral metabolism has a direct causal effect on vascular calcifications. In a model using vascular smooth muscle cells from the human aorta, Giachelli (3) demonstrated that hyperphosphatemia and hypercalcemia were inducers of vascular calcification by activation of intracellular Cbfa1, which led to the transformation of the vascular smooth muscle cell into an osteoblast.

Hypercalcemia and hyperphosphatemia are commonly seen in dialysis patients under two opposing conditions. In secondary hyperparathyroidism, a situation with high bone turnover, the bone itself contributes to the high levels of phosphorus and calcium. In adynamic bone disease, a condition with low bone turnover, the bone behaves as if it were “frozen” and is unable to capture extraosseus calcium and phosphorus derived mainly from food and, in the case of calcium, from treatment as well.

These observations have led to the hypothesis that a link exists between bone disease and vascular disease in dialysis patients. In the KDIGO position paper published in 2006 , the presence of soft tissue and of vascular calcifications have been included in the definition of chronic kidney disease mineral and bone disorder (CKD-MBD) (4).

Types of vascular calcification

Vascular calcifications present two different histological types: intimal and medial calcification. Thus far, intimal and medial calcifications have been considered to be two distinct entities, with different clinical presentations and prognoses. The possibility that these two entities may constitute a continuum of vascular pathology in CKD patients has been hypothesized (5). However, the fact that media calcification is detected earlier in the course of CKD, and in the absence of lipid and cholesterol deposition, challenges this hypothesis (6). Intimal calcification is related to dyslipidemia and accompanies the progression

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of atherosclerotic plaque. Medial calcification is associated with arteriolosclerosis, and develops mainly in diabetic, chronic kidney disease, and elderly patients. Clinical manifestations of intimal calcification are related to the presence of atherosclerotic plaques that obstruct the arteries by rupture and /or thrombosis. Medial calcification does not cause arterial obstruction and was once considered a radiological finding with no clinical consequences. Today we know that medial calcification modifies the properties of the arterial wall and is one of the factors that contributes to arterial stiffness. Arterial stiffness in manifested by an increase in pulse wave velocity and in pulse pressure. Due to the loss of distensibility in the aorta, there is an earlier reflection of the aortic wave that finds the aortic valve still opened. The consequence is an increase in the systolic and a decrease in the diastolic pressure. Systolic pressure increase contributes to the development of left ventricular hypertrophy. Decrease in diastolic pressure may compromise the coronary perfusion that is mainly performed during diastole. Medial calcification may then be associated with symptomatic coronary disease in the absence of obstructive coronary artery disease.

Methods to diagnose vascular calcifications: quantitative evaluation of coronary calcifications

Electron Beam Computed Tomography (EBCT) and Multislice Computed Tomography (MSCT)

Braun et al (7) in 1996 used Electron Beam Computed Tomography (EBCT) to evaluate coronary calcifications in dialysis patients. Quantification of calcification is performed by calculating the Agatston score. This method is based on the maximum X-ray attenuation coefficient, measured in Hounsfield units, and the area of calcification is multiplied by the density scores. Analysis of 49 hemodialysis patients demonstrated that the mean cardiac calcification score was more than 10-fold higher in hemodialysis patients than in 102 non-dialysis control patients with documented or suspected cardiovascular disease. The meaning of the Agatston score may be different in renal and non-renal patients. In non-renal patients the amount of coronary calcium is related to the overall atherosclerotic plaque burden and is an effective predictor of coronary artery disease. It is well established that individuals with Agatston scores above 400 have an increased occurrence of coronary procedures (bypass, stent placement and angioplasty) and of cardiac events (myocardial infarction and cardiac death) within the 2 to 5 years after the test. The extent and site of calcification may not correspond to the site of stenosis but the greater the amount of calcification, the greater the likelihood of obstructive disease somewhere in the coronary arteries. In general population, several studies have shown a good correlation between calcium scores and coronary stenosis (8).

Dialysis patients, however, can have very high coronary Agatston scores without coronary stenosis. Haydar et al (9) compared coronary angiographies with coronary Agatston scores obtained by EBCT in a group of 46 hemodialysis patients. A higher coronary Agatston score was associated with a higher number of diseased vessels, however, eleven patients with no occlusive coronary disease in the angiography had a median coronary Agatston score of 619, which, in the general population, is associated with coronary stenosis. Three- or four-vessel disease in twenty dialysis patients was associated with a median coronary Agatston score of 3748, a score rarely seen in the general population. Since this method does not discriminate intimal from medial calcification, these very high calcification scores in dialysis patients may be explained by the probable occurrence of both types of vascular calcification in coronary arteries.

The existence of a different morphology of coronary lesions in CKD and non-CKD patients has been demonstrated in an autopsy study (10). CKD patients show heavy calcification of the media, with higher calcium content in the media and in the intima and with more frequent presence and greater intensity of inflammation markers. In dialysis patients, there is, however, a good correlation between coronary calcifications and cardiovascular risk. Coronary calcifications have been related with cardiovascular events (11) and with mortality (12, 13). Nevertheless, the clinical significance of Agatston score cut-off values in the general population for the diagnosis of occlusive coronary disease cannot be applied to dialysis patients. Likewise, coronary calcification has also been a predictor of mortality in dialysis patients but in association with lower coronary Agatston score values than those described in association with coronary occlusive disease (9). In prevalent patients a coronary Agatston score greater than 200 was associated with all-

Fig. 1 - *Simple vascular calcification score evaluation. Calcification score is the sum of the presence (1) or absence (0) of vascular calcifications in each section. Pelvis score (1+1+1+1) = 4 and hands score (1+1+1+1) = 4. Total score is 8.*

cause death (12), and in incident patients, a coronary Agatston score greater than 400 was associated with lower survival (13). Therefore, the association of coronary calcifications with mortality is consistent with all the other observational studies showing that the presence of vascular calcifications, independently of their location, is predictive of mortality.

Currently, evaluation of coronary calcifications with EBCT has been substituted by multislice computed tomography (MSCT). Application of EBCT is limited to the assessment of coronary calcification, while MSCT has multiple uses and with software adjustments may also evaluate coronary calcification. This technique is widely available and is now an alternative for quantitative evaluation of coronary calcification. It has been demonstrated that MSCT is a viable technique for the evaluation of coronary arteries and aortic vascular calcification in CKD patients (14).

Ultrasonography and echocardiography

Ultrasonography was the method used by Blacher (15) to evaluate vascular calcifications in four arterial territories in dialysis patients: the carotid, aorta, iliac and femoral arteries. This study, performed in 2001, was the first to demonstrate that vascular calcifications were associated with increased mortality in dialysis patients and it showed that the higher the number of territories affected, the lower the survival.

Calcification of heart valves can be evaluated by echocardiography; this type of calcification was associated with lower survival in peritoneal dialysis patients (16). In a group of 127 hemodialysis patients we have also verified that valvular calcification is a predictor of all-cause and cardiovascular death. The simple vascular calcification score evaluated in plain X-ray of pelvis and hands (17), described in the next section, was associated with valvular calcification, raising the hypothesis of a common pathogenesis for valvular and vascular calcification in this population (18).

Plain X-ray

In 2003 London et al (19) were the first to demonstrate that vascular calcifications evaluated by plain X-ray were associated with all-cause and cardiovascular mortality. These authors differentiated medial from intimal calcification in plain X-ray films. Medial calcification is linear and regular, presenting a railroad track type, while intimal calcification is patchy and irregular. This study demonstrated that medial calcification is not a radiological finding but has an important clinical signification. These two types of calcification were sometimes found in the same patient and in the same vessel and were independent predictors of mortality. Intimal calcification was associated with older age and higher LDL-cholesterol levels. Medial calcification was associated with hemodialysis vintage and diabetes. Calcium carbonate dose and phosphorus levels were associated with both types of calcification.

Other studies (17, 20) have confirmed the association between vascular calcifications, evaluated with plain X-ray, and mortality in the CKD stage-5 hemodialysis population. We developed a simple vascular calcification score, evaluated in a plain X-ray of the pelvis and hands (17) (Fig. 1). This simple score was an independent pre-

dictor of cardiovascular death, cardiovascular hospitalizations and vascular disease in dialysis patients and was an independent predictor of arterial stiffness evaluated by pulse wave velocity and pulse pressure (21).

The Kauppila score evaluates the presence of vascular calcifications in the anterior and posterior wall of the abdominal aorta using a lateral plain X-ray of the lumbar vertebral segments from L1 to L4. This score has been associated with cardiovascular death in a subgroup of participants of the Framingham Heart Study (22). This score has also been tested in dialysis patients. It presents a good correlation with the coronary Agatston score and demonstrates high sensitivity and specificity in predicting a high coronary Agatston score (23). The preliminary results of the CORD study, evaluating outcomes in relation with the Kauppila score, have shown that abdominal aortic calcification is associated with age, dialysis duration and previous cardiovascular disease (24). The plain X-ray score and Kauppila scores are semi-quantitative evaluations of vascular calcifications. Simpler evaluations of vascular calcifications, assessing only the presence or absence of vascular calcifications have also been associated with mortality, such as the identification by plain X-ray of vascular calcifications in the abdominal aorta (20) or in the hemodialysis vascular access (25).

CONCLUSIONS

What is the best non-invasive method to diagnose vascular calcifications?

Several non-invasive imaging techniques have been used to screen for the presence of vascular calcifications: plain X-ray to evaluate different arterial territories, echocardiography for assessment of valvular calcification; two-dimensional ultrasound for calcification of carotid arteries, femoral arteries and aorta; and computed tomography technologies.

Existing studies demonstrate that vascular calcifications, independently of their location, are predictors of mortality in CKD patients. EBCT and MSCT are considered to be the gold standard for evaluating coronary calcifications. They allow a quantitative assessment of vascular calcifications and have been used in several randomized clinical trials for evaluating progression of calcification. These methods do not differentiate intimal from medial calcification, and high coronary scores in dialysis patients, though related with higher cardiovascular risk, may not be related with coronary obstructive disease. The clinical implications of this score in dialysis patients are not identical to those for the general population.

Plain X-Ray, ultrasonography and echocardiography are less expensive and, for that reason, more convenient for screening vascular calcifications. Plain X-ray methods have the advantage of greater simplicity and the possibility of being easily interpreted by the attending physician. The simple vascular calcification score and Kauppila score perform a semi-quantitative evaluation of vascular calcification with cut-off values that allow the identification of patients with a higher cardiovascular risk. A cardiovascular calcification index using the Kauppila score and valvular calcification evaluated by echocardiography have been demonstrated to be associated with coronary calcification in hemodialysis patients evaluated by EBCT (26). Simpler methods to evaluate vascular calcifications seem to be an attractive option but the final choice of the method to employ would depend on the available tests and on the preference of the nephrologist.

Is it useful to screen vascular calcifications in dialysis patients?

Cardiovascular death is the main cause of mortality in CKD patients and, at present, very few therapeutic interventions have shown success in the reduction of all-cause or cardiovascular mortality in this population. Candesartan (27), which has been associated with reduction of cardiovascular events in dialysis patients, carvedilol (28) in patients with dilated cardiomyopathy, and sevelamer (29), used to control phosphorus levels in incident patients, are some of the few treatments that have been associated with increased survival in dialysis patients. In CKD patients, the presence of vascular calcifications is a marker of increased cardiovascular risk and this association, based on observational studies, is classified as grade B evidence by the recent KDIGO recommendations. Vascular calcifications are multifactorial and result from a complex balance between inducers and inhibitors. At the present stage of knowledge we can only intervene in a very small number of these factors, mainly, in the correction of the mineral metabolism. Several studies have already demonstrated that vascular calcifications in dialysis patients may progress or remain stable depending on the control of the mineral metabolism and on the type of phosphate binder (30-32). London et al demon-

strated an association between vascular calcifications and low bone turnover (33) and found a significant interaction between calcium-containing phosphate binders and aortic calcification and stiffness in the presence of adynamic bone disease (34).

Identification of vascular calcifications in CKD patients is included in the classification of CKD-MBD. This information may be used for selecting the most suitable control of the mineral metabolism, such as intensive control of hyperphosphatemia, suitable choice of phosphate binder and prevention of low bone turnover status, for instance, by avoidance of calcium-containing binders or of PTH oversupression. In predialysis (35) and dialysis patients it has been demonstrated that, vascular calcifications are progressive and for this reason it seems logical to begin evaluation of vascular calcifications at an early stage, possibly in predialysis patients.

In conclusion, different methods can be used for screening vascular calcifications. Plain-X-ray is a simple and inexpensive method to perform this evaluation. Considering the poor results of therapeutic interventions on cardiovascular mortality in CKD patients, the available evidence justifies the more frequent use of this simple evaluation in these patients. This information may be used to detect cardiovascular risk and to guide therapeutic intervention in chronic kidney disease patients.

Conflict of interest statement
Teresa Adragao has received research grants from Genzyme; lecture fees from Amgen, Genzyme, Abbot and Novartis; and consultancy fees from Genzyme and Abbot.

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CAPÍTULO 9

COMENTÁRIOS FINAIS

O *score* de calcificação vascular simples avaliado em RX simples da bacia e das mãos revelou-se, como ficou demonstrado com os estudos descritos, um método simples para identificação do risco cardiovascular. Este método de avaliação das calcificações vasculares também foi usado por outros grupos nacionais e internacionais, o que permitiu confirmar a facilidade e a reprodutibilidade da sua aplicação.

Utilização do *score* de calcificação vascular simples em estudos publicados por outros grupos.

1. Matias P *et al*[1] demonstraram, num grupo de 223 doentes em hemodiálise, que os níveis de 25-hidroxvitamina D3 se correlacionaram inversamente com a presença de diabetes *mellitus*, com os níveis séricos de albumina e de BNP (*brain natriuretic peptide*) e com um *score* de calcificação vascular simples superior a 3 (Tabela. 9.1).

Dependent variable	Independent variables	β	95% CI	*P*	R^2
[25(OH)D3]	Diabetes mellitus	-0.44	-0.23 to -0.01	<0.001	0.532
	Albumin	0.41	1.24 to 2.68	0.003	
	Log_{10} BNP	-0.37	-0.24 to -0.02	0.005	
	PP > 65 mmHg	-0.36	-0.41 to -0.05	0.006	
	SVCS ≥3	-0.39	-0.28 to -0.04	0.002	

BNP. brain natriuretic peptide: PP. pulse pressure: SVCS. simple vascular calcification score.

Tabela 9.1. Preditores dos níveis séricos de 25(OH) vitamina D por regressão linear. O *score* de calcificação vascular simples ≥ 3 (SVCS, *simple vascular calcification* s*core*) apresentou uma associação inversa com os níveis séricos de 25(OH) vitamina D

Referência: Matias PJ *et al.* 25-hydroxyvitamin D3, arterial calcifications and cardiovascular risk markers in haemodialysis patients. Nephrol Dial Transplant 2009; 24(2):611-618.

2. Este mesmo grupo demonstrou noutro estudo[2] que os doentes submetidos a amputação apresentavam um *score* de calcificação vascular mais elevado (Fig. 9.1). As calcificações vasculares foram avaliadas pelo *score* de calcificação vascular simples, e um *score* de calcificação mais elevado foi um preditor independente de amputação (OR 2,01; IC 1,41-3.01; p=0,01).

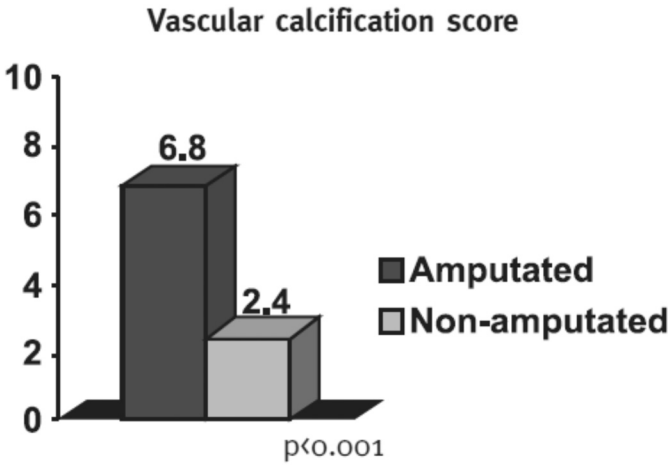

Fig. 9.1. O *score* de calcificação vascular foi mais elevado nos doentes submetidos a amputação

Referência: Matias P, *et al.* Factors associated with lower-extremity amputations in haemodialysis patients. Port J Nephrol Hypert 2008; 22(1): 31-36.

3. Wei T *et al* avaliaram, num grupo de 47 doentes em hemodiálise, a relação entre o sRANKL (*soluble receptor activator of nuclear factor kB ligand*) e os eventos cardiovasculares[3]. Neste estudo, os preditores de eventos cardiovasculares foram os níveis

Factors of cardiovascular events determined by multivariate regression analysis

Factors	Standardized coefficients, β	t	p value	95% CI
sRANKL, pmol/l	-0.322	-2.215	0.035	-2.948 ~ -0.115
Calcification score	0.584	4.087	0.000	0.051 ~0.152
Phosphate, mmol/l	0.732	2.935	0.007	0.105 ~0.588
Ca × P, $mmol^2/l^2$	-0.658	-2.818	0.009	- 0.284 ~0.045

Tabela 9.2. O score de calcificação constituído pelo SCVS (0-8) + calcificações na aorta abdominal (0-2) foi um dos preditores de eventos cardiovasculares

Referência: Wei T *et al.* Relationship of sRANKL level and vascular calcification s*core* to cardiovascular events in maintenance hemodialysis patients. Blood Purif 2009; 28:342-345.

de sRANKL, os níveis de fósforo sérico e as calcificações vasculares (Tabela 9.2). As calcificações vasculares foram avaliadas por um *score* composto pelo *score* de calcificação vascular simples (de 0 a 8), ao qual foi somado um *score* de calcificação aórtico (0 a 2), sendo o *score* final 0 a 10. Wei T *et al* foram o primeiro grupo internacional a incluir o *score* de calcificação vascular simples na avaliação de calcificações vasculares.

4. Schlieper G *et al*[4] avaliaram os fatores associados à presença de calcificações vasculares usando o *score* de calcificação vascular simples avaliado no RX simples da bacia e das mãos e um *score* de calcificação que associava as calcificações vasculares da bacia e das mãos às calcificações das fístulas arteriovenosas, calcificações valvulares cardíacas e calcificações carotídeas (Tabelas 9.3 e 9.4).
   Estes autores verificaram que a idade, o sexo masculino, a duração da diálise, um valor mais baixo de Kt/V, o produto fosfocálcico, os hábitos tabágicos e a proteína C reactiva de alta sensibilidade foram preditores independentes das calcificações vasculares. Ambos os *score*s se relacionaram com o aumento da velocidade de onda de pulso e foram ambos preditores de mortalidade. Segundo estes autores, a avaliação do *score* combinado não mostrou vantagens em relação ao *score* de calcificação vascular simples e, sob um ponto de vista prático, o *score* de calcificação vascular simples que utiliza apenas um RX da bacia e das mãos pode representar um método mais fácil para avaliação dos doentes.

Multivariate analysis of risk factors for vascular calcifications (ordinal logistic regression, Adragão *score*)

	Score 0 (n=89)	Score 1-2 (n=45)	Score 3-8 (n=54)	OR	95% CI	P
Age years	57 ± 10	61 ± 10	61 ± 11	1.08	1.04-1.11	<0.001
Male gender	36 (40%)	24 (53%)	40 (74%)	2.75	1.41-5.38	0.003
Smoking	17 (19%)	22 (42%)	22 (42%)	2.32	1.16-4.65	0.017
Dialysis vintage, years	5.74 ± 4.43	7.54 ± 4.41	7.69 ± 4.83	1.13	1.03-1.23	0.006
$Ca \times PO_4$ product, $mmol^2/l^2$	3.55 ± 1.02	3.76 ±1.02	3.96 ± 1.14	1.75	1.29-2.37	0.0003
Kt/V	1.33 ± 0.18	1.25 ± 0.13	1.23 ± 0.18	0.095	0.01-0.64	0.015
Anti-HCV antibody	16 (18%)	17 (38%)	18 (33%)	1.39	0.59-3.31	0.454
hsCRP, mg/l	7.71 ±18.9	7.88 ± 10.68	14.31 ± 20.43	1.01	1.00-1.03	0.096
Total cholesterol, mmol/l	5.35 ± 1.27	4.94 ± 1.04	5.01 ± 0.98	0.99	0.75-1.30	0.930

Tabela 9.3. Preditores do *score* de calcificação vascular simples

Referência: Schlieper *et al.* Risk factors for cardiovascular calcifications in non-diabetic Caucasian haemodialysis patients. Kidney Blood Pres Res 2009;32(3):161-168.

Multivariate analysis of risk factors for cardiovascular calcifications (ordinal logistic regression, composite *score*)

	Score 0-2 (n = 84)	Score 3-5 (n = 42)	Score 6-15 (n = 48)	OR	95% CI	p
Age years	56.75 ± 10.03	61.07 ± 9.90	60.81 ± 11.23	1.06	1.02-1.09	0.002
Male gender	35 (42%)	19 (45%)	37 (77%)	2.32	1.19-4.52	0.014
Dialysis vintage, years	5.84 ± 4.13	7.36 ± 5.29	7.66 ± 4.55	1.13	1.04-1.24	0.005
hsCRP, mg/l	6.18 ± 8.16	6.21 ± 9.79	12.85 ± 17.23	1.04	1.01-1.07	0.012
Kt/V	1.34 ± 0.18	1.27 ± 0.16	1.23 ± 0.19	0.11	0.02-0.70	0.019
Smoking	20 (24%)	12 (29%)	20 (43%)	1.84	0.89-3.83	0.097
Anti-HCV antibody	16 (19%)	15 (36%)	15 (31%)	1.25	0.52-3.02	0.614

Tabela 9.4. Preditores de um *score* composto de calcificação vascular (SCVS+calcificação da fístula AV + calcificação valvular cardíaca + calcificações carotídeas)

Referência: Schlieper *et al.* Risk factors for cardiovascular calcifications in non-diabetic Caucasian haemodialysis patients. Kidney Blood Pres Res 2009;32(3):161-168.

5. As calcificações da córnea e da conjuntiva são calcificações extravasculares relativamente frequentes nos doentes em diálise. Seyahi N *et al*[5] foram os primeiros a mostrar uma associação entre este tipo de calcificação extravascular e as calcificações vasculares. O SCVS foi o método escolhido para avaliar as calcificações vasculares. No grupo de doentes com mais calcificações conjuntivais e corneanas (CCC) verifica-se maior percentagem de doentes com calcificações vasculares avaliadas no RX simples da bacia e das mãos (Tabela 9.5).

Clinical and biochemical data. BMD results, and vascular calcification frequency of patients

	All Patients (N = 63)	Low CCC Score (n = 17)	High CCC Score (n = 16)	p
Men/women	30/33	7/10	8/8	NS
Age (y)	43.5 ± 11.7	37.9 ± 14.8	46.31 ± 14.3	NS
Peritoneal dialysis/hemodialysis	44/19	10/7	12/4	NS
RRT duration (mo)	54.5 ± 39.9	21.1 ± 5.1	42.2 ± 10.6	0.003
Parathyroidectomy	5 (7.9)	1 (5.9)	1 (6.3)	NS
Calcium (mg/dL)	9.22 ± 0.56	9.08 ± 0.38	9.39 ± 0.6	NS
Phosphorus (mg/dL)	5.38 ± 1.30	5.17 ± 1.04	5.96 ± 1.58	NS
Ca × P (mg^2/dL^2)	48.47 ± 12.41	44.76 ± 10.36	56.11 ± 17.44	0.021
ALP (U/L)	161.0 ± 116.7	133.1 ± 65.7	156.5 ± 96.5	NS
PTH (pg/mL)	310.8 ± 257.4	292.2 ± 272.5	320.6 ± 243.4	NS

(Continua)

(Continuação)

	All Patients (N = 63)	Low CCC Score (n = 17)	High CCC Score (n = 16)	p
Albumin (g/dL)	3.75 ± 0.46	3.62 ± 0.61	3.88 ± 0.36	NS
CRP (mg/dL)	14.31 ± 22.40	13.09 ± 17.95	13.14 ± 22.71	NS
Lumbar BMD (g/cm^2)	1.01 ± 0.19	1.02 ± 0.19	1.02 ± 0.23	NS
Femoral BMD (g/cm^2)	0.78 ± 0.13	0.81 ± 0.16	0.79 ± 0.09	NS
Active vitamin D (µg/d)	0.36 ± 0.34	0.37 ± 0.32	0.28 ± 0.20	NS
Elemental calcium (g/d)	0.84 ± 0.41	0.71 ±0.42	0.82 ± 0.39	NS
Elemental aluminium (g/d)	0.42 ± 0.32	0.52 ± 0.41	0.47 ± 0.28	NS
Presence of vascular calcification	21 (33.3)	1 (5.6)	9 (56.3)	002
Pelvis	16 (27.0)	1 (5.6)	7 (43.8)	017
Hands	9 (14.3)	0 (0.0)	3 (18.8)	NS

Tabela 9.5. Associação de calcificações conjuntivais corneanas (CCC) às calcificações vasculares

Referência: Seyahi N *et al.* Association of conjunctival and corneal calcification with vascular calcification in dialysis patients. Am J Kidney Dis 2005 45(3):550-556

6. Jung JY *et al* [6], num grupo de 69 doentes submetidos a diálise peritoneal, analisaram os fatores associados à rigidez arterial. Verificaram que a fetuína-A e a PTH se correlacionaram inversamente e que a pressão arterial média se correlacionou diretamente com a velocidade de onda de pulso. A fetuína-A é um inibidor da calcificação vascular e estes autores demonstraram que um *score* de calcificação vascular simples superior

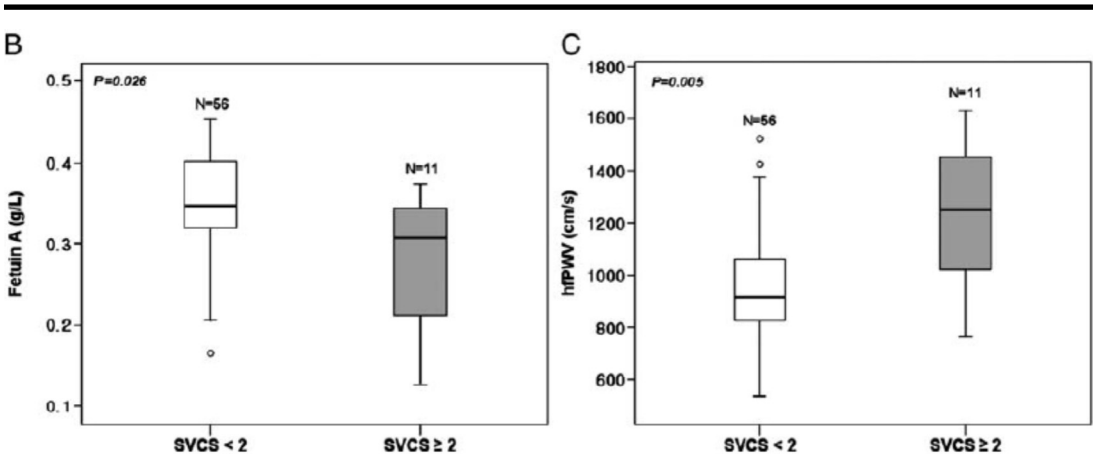

Fig. 9.2. Um *score* de calcificação vascular simples ≥ 2 (SVCS, simples vascular calcification *score*) associou-se a valores mais baixos de fetuína-A e mais elevados de velocidade de onda de pulso

Referência: Jung JY *et al.* Factors associated with aortic stiffness and its change over time in peritoneal dialysis patients. Nephrol Dial Transplant 2010; 25. [Epub ahead of print.]

a 2 se associou a valores mais baixos de fetuína-A e a valores mais elevados de velocidade de onda de pulso (Fig. 9.2).

7. Este mesmo grupo[7] verificou a constância desta associação inversa entre o *score* de calcificação vascular simples e os níveis de fetuína-A num grupo de 249 doentes em diálise peritoneal (Tabela 9.6).

Parameter		r	P
Dependent variable : Fetuin – A			
SVCS		- 0.468	<0.001
hfPWV		- 0.338	<0.001
Age		- 0.327	<0.001
Albumin		0.284	0.001
		Mean ± SD	**P**
Diabetes	Yes	0.32 ± 0.08	0.002
	No	0.35 ± 0.07	
ASVD history	Yes	0.30 ± 0.08	0.007
	No	0.35 ± 0.07	

SVCS: Simple vascular calcification score. r : Pearson´s correlation coefficient. hfPWV: heart-to-femoral pulse wave velocity.SD: standard deviation ASVD: Atherosclerotic vascular disease

Tabela 9.6. O *score* de calcificação vascular simples (SVCS, *simple vascular calcification* score) foi um dos fatores que apresentou uma correlação negativa com os níveis séricos de fetuína-A

Referência: Jung JY *et al.* Association of AHSG gene polymorphisms and aortic stiffness in peritoneal dialysis patients. Am J Nephrol 2010;31(6):510-517.

8. Kim SC *et al* [8] analisaram, num grupo de 93 doentes em diálise peritoneal, as relações entre os níveis de PTH, as calcificações vasculares avaliadas pelo *score* de calcificação vascular simples e pelo *score* coronário de Agatston e a rigidez arterial avaliada pela velocidade de onda de pulso. Verificaram que os doentes com valores mais baixos de PTH (<150 pg/mL) apresentavam mais calcificações (SCVS ≥ 3). A presença de diabetes *mellitus* e os valores mais baixos de PTH foram preditores independentes de calcificações vasculares (Tabela 9.7). Este grupo mostrou ainda que um SCVS > 1 foi preditor de um *score* de calcificação coronário de Agatston > 400 (Tabela 9.8).

Binary logistic regression analysis of risk factors associated with vascular calcification score of ≥ 3 (forward method)			
Variable	**OR**	**95% CI**	**p value**
DM	31.3	6.3-155.8	<0.001
PTH group (inter)	1.00	–	–
PTH group (low)	5.75	1.27-25.9	0.023
PTH group (high)	4.19	0.85-20.7	0.078

OR, Odds ratio; CI, confidence interval; DM, diabetes mellitus; PTII, parathyroid hormone. Excluded: age, cholesterol, HbAlc, and albumin

Tabela 9.7. A presença de diabetes *mellitus* e valores mais baixos de PTH foram preditores de um SCVS ≥ 3

Referência: Kim SC *et al*. Low iPTH can predict vascular and coronary calcifications in patients undergoing peritoneal dialysis. Nephron Clin Pract 2010 6,117(2):c113-c119. [Epub ahead of print.]

Binary logistic regression analysis of factors associated with a CAC score of ≥ 400 (forward method)			
Variables	**OR**	**95% CI**	**p value**
SVCS ≥ 1	76.0	6.00-962.3	0.001

OR, Odds ratio; SVCS, simple vascular calcification score. Excludes variables; diabetes mellitus, age, body mass index, HbAlc, and diastolic blood pressure.

Tabela 9.8. Um *score* de calcificação vascular simples > 1 (SVCS, *simple vascular calcification* score) foi um preditor de um *score* de calcificação coronária de Agatston > 400 (CAC, *coronary artery calcification*)

Referência: Kim SC *et al.* Low iPTH can predict vascular and coronary calcifications in patients undergoing peritoneal dialysis. Nephron Clin Pract 2010; 6,117(2):c113-c119. [Epub ahead of print.]

9. Garcia-Cantón *et al*[9] avaliaram as calcificações vasculares num grupo de 210 doentes renais crónicos não em diálise (estadios 4 e 5), utilizando o *score* de calcificação vascular

25-Hydroxyvitamin D	SVCS (Adragão)	Kauppila
Deficiency (<15 ng/mL)	Mean 4.8 ± 2 / median 6	Mean 12 ± 7 / median 13
Insufficiency (15-30 ng/mL)	Mean 3.7 ± 3 / median 4	Mean 9.6 ± 7 / median 9
Normal (>30 ng/mL)	Mean 2.3 ± 2 / median 2	Mean 6.3 ± 6 / median 3

SVCS, **s***imple vascular calcification score.*

Referência: García-Cantón C, Bosch E, Ramírez A, et al. Vascular calcification and 25-hydroxyvitamin D levels in non-dialysis patients with chronic kidney disease stages 4 and 5. Nephrol Dial Transplant 2010; 18. [Epub ahead of print].

Tabela 9.9. Níveis séricos de 25-hidroxi-vitamina D e calcificações vasculares em doentes não em diálise

simples descrito por nós e o *score* de Kauppila[10] avaliado na aorta abdominal; 120 doentes (57%) apresentaram um SCVS>3; 114 doentes (54%) apresentaram um *score* de Kauppila >7. Só 18,5% desta população apresentava níveis séricos adequados de 25-hidroxivitamina D. Os níveis séricos baixos de 25-hidroxivitamina D foram preditores independentes de calcificações vasculares avaliadas pelos dois métodos.

Binary logistic regression with SVCS			
	Variables entering the equation		
	OR	**95% CI**	**Significance**
Age	1.058	1.021-1.096	0.002
Diabetes	5.229	2.290-11.940	0.0001
CVD	2.957	1.343-6.511	0.007
25(OH)D	0.954	0.917-0.993	0.020

SVCS, *simple vascular calcification score*; CVD, *cardiovascular disease.*

Tabela 9.10. Preditores do s*core* de calcificação vascular simples

Referência: García-Cantón C, Bosch E, Ramírez A, *et al*. Vascular calcification and 25-hydroxyvitamin D levels in non-dialysis patients with chronic kidney disease stages 4 and 5. Nephrol Dial Transplant. 2010; 18. [Epub ahead of print.]

Binary logistic regression with Kauppila			
	Variables entering the equation		
	OR	**95% CI**	**Significance**
Age	1.103	1.061-1.146	0.0001
CVD	2.609	1.249-5.450	0.011
25(OH)D	0.953	0.918-0.992	0.017

CVD, *cardiovascular disease*.

Tabela 9.11. Preditores de *score* de calcificação de Kauppila.

Referência: García-Cantón C, Bosch E, Ramírez A, *et al*. Vascular calcification and 25-hydroxyvitamin D levels in non-dialysis patients with chronic kidney disease stages 4 and 5. Nephrol Dial Transplant. 2010; 18. [Epub ahead of print.]

Este estudo reveste-se de particular importância, pois é o primeiro a avaliar as calcificações vasculares utilizando RX simples num grupo de doentes renais crónicos não em diálise, revelando, nesta população, que a prevalência das calcificações vasculares é elevada. Tal

como foi demonstrado numa população em diálise[1], também nesta população se verificou uma associação inversa entre o *score* de calcificação vascular simples e os níveis de hidroxivitamina D. O *score* de calcificação vascular simples mostrou ser um instrumento útil com resultados semelhantes aos obtidos com o *score* de Kauppila.

A utilização do *score* de calcificação vascular simples para avaliação das calcificações vasculares nos doentes em diálise foi já sugerida por duas sociedades internacionais de nefrologia: pela Sociedade Espanhola de Nefrologia em *SEN guidelines. Recommendations of the Spanish society of nephrology for managing bone-mineral metabolic alterations in chronic renal disease patients*[11] e pelo grupo de Estudos do Metabolismo Mineral e Ósseo da Sociedade Uruguaia de Nefrologia, em *Recomendaciones para el manejo de las alteraciones del metabolismo mineral y oseo de la enfermedad renal crónica en estadio* 5[12]. O estudo OSERCE II observacional, de iniciativa da Sociedade Espanhola de Nefrologia, com início em 2009, pretende avaliar nos doentes renais crónicos nos estadios 3 a 5 alterações do metabolismo mineral e ósseo, entre outras. Prevê a inclusão de 1500 doentes. Um dos parâmetros a analisar neste estudo é a prevalência de calcificações vasculares. Os métodos escolhidos para diagnóstico das calcificações vasculares foram dois *scores* avaliados em RX simples: o *score* de calcificação da aorta abdominal descrito por Kauppila L[10] e o *score* de calcificação vascular simples descrito por nós[13].

Nos diferentes estudos efectuados por nós, verificamos que as calcificações vasculares avaliadas por este método nos doentes renais crónicos foram preditoras de maior risco de mortalidade cardiovascular[13], de mortalidade de causa global[14], de maior risco de doença coronária[13] e de doença arterial periférica[13]. O *score* de calcificação vascular simples foi ainda um preditor independente de rigidez arterial [14] avaliada por velocidade de onda de pulso ou por pressão de pulso. Este *score* de calcificação associou-se inversamente à densidade mineral óssea do colo do fémur, avaliada por *dual energy X-ray absorptiometry* (DXA)[15]. Em estudos efetuados por outros grupos foi demonstrado que as calcificações vasculares avaliadas por este método são preditoras de maior risco de eventos cardiovasculares[3], de amputações dos membros inferiores[2], de velocidade de onda de pulso[4,6], de calcificações corneanas e conjuntivais[5] e de calcificações coronárias[8]. Também foi demonstrada uma associação inversa entre o *score* de calcificação vascular simples e os níveis de PTH [8], com os níveis de 25(OH)vitamina D[1,9] e com os níveis de fetuína A[6,7]. O *score* de calcificação vascular simples mostrou ser um instrumento útil para a avaliação das calcificações vasculares também em doentes renais crónicos não em diálise[9].

Todos estes estudos realizados por diferentes grupos, que utilizaram o *score* de calcificação vascular simples na sua metodologia, comprovam a facilidade de utilização deste *score,* e a concordância de resultados comprova a sua reprodutibilidade e utilidade na avaliação dos doentes renais crónicos.

Comparação entre o *score* de calcificação vascular simples e o *score* de Kauppila.

As *guidelines* KDIGO 2009 sugerem que o conhecimento da presença de calcificações vasculares pode ser usado para orientar o tratamento da doença mineral e óssea nos doentes renais crónicos e sugerem a avaliação de calcificações vasculares na aorta abdominal[10]. Este *score* da aorta abdominal avaliado em RX simples foi comparado com o *score* coronário de Agatston. Bellasi *et al*[16] demonstraram que um *score* Kauppila superior ou igual a 7 apresentava uma elevada sensibilidade e especificidade para diagnosticar um *score* de Agatston superior a 400 ou 1000. O *score* coronário de Agatston é considerada a técnica *gold-standard* para a avaliação das calcificações coronárias e aórticas[17]. Esta será provavelmente a razão pela qual as *guidelines* KDIGO 2009 sugerem a utilização do *score* de calcificação na aorta abdominal avaliado em RX simples como alternativa ao *score* de Agatston. É contudo necessário demonstrar que as calcificações coronárias nos doentes renais crónicos são o paradigma do fenómeno de calcificação vascular sistémica nesta população.

Tivemos já a oportunidade de comparar o *score* de calcificação vascular simples com o *score* de calcificação na aorta abdominal, ao estudarmos a doença arterial periférica num grupo de 219 doentes em hemodiálise[18]. Verificamos que ambos os *score*s de calcificação são preditores de um índice tornozelo-braço <0,9, apresentando uma semelhante área sob a curva. O *score* de calcificação vascular simples superior a 3 teve uma sensibilidade de 73% e uma especificidade de 86% para identificar um *score* de calcificação aorticoabdominal superior a 6. O *score* de calcificação vascular simples permite ainda identificar calcificações em artérias colaterais e distais, sendo este tipo de calcificações preditoras de um índice tornozelo-braço >1,3. O padrão típico da calcificação vascular neste território arterial (artérias colaterais e distais) é linear, correspondendo à calcificação da camada média da parede arterial descrita por London[19]. A calcificação da íntima e da média são preditores independentes de mortalidade nos doentes em diálise[19]. Ao contrário do *score* de calcificação vascular simples, o *score* de calcificação aorticoabdominal não identifica este tipo de calcificação da média. Futuros estudos poderão avaliar a vantagem clínica de distinguir os dois tipos de calcificação vascular nestes doentes.

García-Cantón *et al* também utilizaram os dois métodos, o *score* de calcificação vascular simples e o *score* Kauppila, na avaliação das calcificações vasculares na mesma população[9]. Os resultados obtidos pelos dois métodos foram muito semelhantes. Kim *et al* verificaram que um *score* de calcificação vascular simples foi um preditor independente de um *score* coronário de Agatston > 400[8].

Calcificações vasculares em doentes renais crónicos: questões ainda em avaliação

Tal como outros, defendemos a hipótese da existência de um elo de ligação entre as alterações do metabolismo mineral e ósseo e a doença cardiovascular na doença renal crónica. As calcificações vasculares são um dos elementos desta associação e resultam da transformação fenotípica das células musculares lisas da parede dos vasos. A hipercalcemia e a hiperfosfatemia são alguns dos fatores responsáveis por este processo celular ativo[20], estabelecendo o elo de ligação entre as alterações minerais e as calcificações vasculares. Barreto D *et al* demonstraram que a progressão das calcificações vasculares se associam as alterações do metabolismo ósseo[21]. Em situações de baixa ou de alta remodelação óssea, o osso é incapaz de exercer a sua função de tampão que permite a manutenção do cálcio sérico nos limites adequados e, no caso de se verificar hipercalcemia, o depósito extraósseo de cálcio é obrigatório[22,23]. London *et al* foram os primeiros a demonstrar, num grupo de doentes em hemodiálise, associação entre calcificações vasculares e baixa remodelação óssea diagnosticada por biopsia óssea[24].O nosso grupo foi o primeiro a demonstrar, também em doentes em hemodiálise, uma associação entre calcificações vasculares e baixo volume ósseo diagnosticado por biopsia óssea[25]. Fomos igualmente os primeiros a verificar, num grupo de doentes em diálise peritoneal, uma associação entre calcificações vasculares e a baixa densidade mineral óssea avaliada no colo do fémur[15]. A importância deste achado foi demonstrada pela associação, descrita por nós, também pela primeira vez, entre a densidade mineral óssea avaliada no colo do fémur e a porosidade cortical medida em biopsia óssea[26]. Os nossos diferentes trabalhos reforçam assim esta hipótese da existência de um elo de ligação entre o osso e o vaso na doença renal crónica.

Existem ainda algumas questões sem resposta definitiva. Serão as calcificações da camada média e da íntima da parede arterial duas entidades diferentes ou uma diferente manifestação da mesma patologia? No doente renal crónico, as calcificações podem surgir em diversos tipos de artérias, desde as artérias elásticas às artérias musculares, mas nem todas as artérias desenvolvem aterosclerose. A aorta torácica e as artérias dos membros superiores, por exemplo, parecem resistentes ao processo aterosclerótico. Haimovici H demonstrou, com estudos de transposição de artérias em cães, que a aorta torácica, independentemente do local para onde era transposta, se mantinha resistente à aterosclerose, enquanto o inverso se verificava com a aorta abdominal [27].

McCullough PA *et al* defendem a hipótese de que a calcificação aterosclerótica e a calcificação da média são um contínuo da patologia vascular na doença renal crónica e que a esclerose de Monckberg, que corresponde à calcificação da média, corresponde a um processo de aterosclerose avançada[28]. Esta hipótese foi revogada por Amann K, demonstrando que

estes dois tipos de calcificação são distintos sob os pontos de vista histológico, fisiopatológico e clínico, e que a calcificação da média pode preceder a calcificação da íntima[29]. A calcifilaxis é um tipo muito particular de calcificação da camada média que afeta as arteríolas da pele e é um processo independente da aterosclerose.

A possibilidade de estabilização ou regressão das calcificações vasculares é outra questão amplamente debatida. Já se demonstrou que um captador de fósforo à base de cálcio pode aumentar a progressão das calcificações coronárias[30-32], mas este achado não foi universal[33,34]. A patologia óssea, impedindo o osso de se comportar como tampão para o cálcio, é uma das explicações para a progressão das calcificações vasculares[22,23]. O cinacalcet, agente calcimimético usado no tratamento do hiperparatiroidismo secundário, também pode afetar a progressão das calcificações. Os resultados do estudo ADVANCE foram apresentados na sessão "Late Breaking Trials" durante o XLVVII ERA-EDTA Congress 2010, em Munique. Este estudo comparou, num grupo de doentes em hemodiálise, o efeito da terapêutica conjunta de cinacalcet e dose baixa de vitamina D com a terapêutica com doses flexíveis de vitamina D e demonstrou uma redução significativa da progressão das calcificações na válvula aórtica no grupo tratado com cinacalcet. A progressão das calcificações coronárias, o parâmetro de eficácia primário, não atingiu diferença com significado estatístico entre os dois grupos.

A semelhança entre a remodelação óssea e a calcificação vascular está na base de inúmeros estudos que avaliaram o efeito dos bifosfonatos na progressão das calcificações vasculares[35]. Os bifosfonatos inibem a calcificação vascular em modelos animais de insuficiência renal crónica. Este efeito também se verificou em quatro estudos envolvendo doentes em hemodiálise[36-39], mas o número de doentes avaliados nestes estudos foi pequeno e só dois deles randomizados. A normalização da função renal obtida com a transplantação renal é outro fator que pode influenciar a progressão das calcificações vasculares. Até agora, o efeito da transplantação renal na progressão das calcificações vasculares foi analisado apenas em alguns estudos que envolveram um pequeno número de doentes. Verificou-se uma estabilização das calcificações coronárias em dois destes estudos[40,41] e a progressão das calcificações noutro[42]. Em doentes renais crónicos não em diálise já foi demonstrado que existe progressão acelerada das calcificações coronárias, em comparação com indivíduos com função renal normal[43], e a alteração da função renal após a transplantação renal pode explicar essa progressão. O tiossulfato de sódio, antídoto do cianeto e usado em casos de toxicidade da cisplatina, tem sido também aplicado com sucesso no tratamento de casos de arteriolopatia urémica cálcica, também denominada calcifilaxis. Esta patologia traduz-se histologicamente por calcificação da camada média de arteríolas do tecido celular subcutâneo com fibrose endovascular e trombose. Este agente antioxidante consegue sequestrar iões cálcio e formar compostos de cálcio muito solúveis, impedindo a sua precipitação[44].

Em resumo, o desenvolvimento e a progressão das calcificações vasculares nos doentes renais crónicos são processos complexos para os quais contribuem inúmeros fatores indutores e inibidores, muito provavelmente em associação com o estado da remodelação óssea e da capacidade ou incapacidade de o osso se comportar como tampão para o cálcio. O diagnóstico das calcificações vasculares pode ser um instrumento útil na orientação terapêutica destes doentes. Várias técnicas não invasivas permitem fazer o rastreio de calcificações vasculares, mas, independentemente do método usado, pensamos que o importante é que as calcificações vasculares sejam diagnosticadas nos doentes renais crónicos.

Congratulamo-nos com o facto de dois dos nossos estudos[13,14] terem sido incluídos nas referências das *guidelines* KDIGO 2009 nas tabelas da prevalência das calcificações vasculares (KDIGO 2009: Tabela suplementar 10, Fig. 3.6) e da associação entre calcificações vasculares e mortalidade (KDIGO 2009: Tabela suplementar 12, Fig. 3.7). A inclusão destes nossos dois estudos nas referências destas *guidelines* que utilizaram o exigente sistema GRADE (*grades of recommendation, assessment, development, and evaluation*) na classificação e seleção dos estudos valida o interesse científico destes dois trabalhos.

A presença de calcificações vasculares identifica os doentes com mais elevado risco cardiovascular. Concordamos com a sugestão das *guidelines* KDIGO 2009, que considera que a presença de calcificações vasculares nos doentes renais crónicos nos estádios 3 a 5 é uma informação que pode ser usada para orientar o tratamento da doença mineral e óssea nestes doentes. É necessário contudo ainda analisar se a ausência de desenvolvimento ou de progressão das calcificações vasculares se associa a uma redução da morbilidade ou da mortalidade nestes doentes. É necessário também avaliar se uma atuação diagnóstica e terapêutica nos estadios mais precoces da doença renal crónica poderá ter efeito na redução da progressão da doença óssea e do risco cardiovascular nestes doentes.

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CAPÍTULO 10

RESUMO DA DISSERTAÇÃO

A presente dissertação para tese de doutoramento apresenta o desenvolvimento e a validação de um método simples e original para o diagnóstico de calcificações vasculares em doentes em diálise, utilizando um *score* semiquantitativo criado por nós e obtido em RX simples da bacia e das mãos, denominado *score* de calcificação vascular simples. Demonstramos que este *score* vascular simples é preditor de risco cardiovascular nos doentes em diálise. O *score* de calcificação vascular simples associou-se ainda à baixa densidade mineral óssea avaliada por *dual energy X-ray absortiometry* (DXA) no colo do fémur. Verificamos igualmente que, em doentes em diálise, as calcificações coronárias quantificadas pelo *score* de Agatston e o *score* de calcificação vascular simples se associaram a um menor volume ósseo avaliado em biopsias ósseas. Estes trabalhos corroboram a hipótese da existência de um elo de ligação entre a doença óssea e a doença vascular nos doentes em diálise, e um dos elementos que contribuem para este elo de ligação podem ser as calcificações vasculares.

Este *score* de calcificação vascular simples avalia calcificações em artérias de grande, médio e pequeno calibre, e inclui os dois padrões radiológicos de calcificação: calcificação linear, associada à calcificação da camada média da parede arterial, e calcificação irregular, associada à calcificação da camada íntima arterial[1]. Nos diferentes trabalhos por nós publicados demonstramos que as calcificações vasculares avaliadas por este método simples e barato permitem a identificação de indivíduos com elevado risco cardiovascular. Este *score* vascular associa-se a maior risco de mortalidade cardiovascular[2], de mortalidade de causa global[3], de internamentos cardiovasculares[2], de doença cardiovascular[2], de doença arterial periférica[2,4], de calcificações valvulares[5] e de rigidez arterial[3].

As *guidelines* KDIGO (*Kidney disease: improving global outcomes*), publicadas em 2009, sugerem que os doentes renais crónicos nos estadios 3 a 5, com calcificações vasculares e valvulares, devem ser considerados como apresentando o mais elevado risco cardiovascular[6]. A elevada mortalidade dos doentes renais crónicos não é totalmente explicada pelos fatores de risco tradicionais[7]. A organização KDIGO defende, desde 2006, a hipótese da existência de um elo de ligação entre a doença óssea e a doença vascular[8]. Esta ligação pode ser explicada pelas alterações do metabolismo mineral e ósseo e pela sua interação com as calcificações vasculares. Verificamos, nos nossos trabalhos, uma associação entre calcificações vasculares

e doença óssea. O baixo volume ósseo diagnosticado por análise histomorfométrica de biopsias ósseas foi preditor de maior risco de calcificações vasculares avaliadas pelo *score* de calcificação vascular simples (dados apresentados nesta dissertação, no capítulo 6) e pelo *score* coronário de Agatston num grupo de doentes em diálise[9]. A contribuição original deste artigo[9] foi considerada merecedora de um editorial feito pelo Dr. Gérard London[10], investigador líder na área da calcificação vascular dos doentes renais crónicos e actual Presidente da EDTA (*European Dialysis and Transplantation Association*). Fomos também os primeiros a descrever uma associação independente e inversa entre a densidade mineral avaliada no colo do fémur por DXA (*dual energy X-ray absortiometry*) com calcificações vasculares avaliadas pelo *score* de calcificação vascular simples, com rigidez arterial avaliada por velocidade de onda de pulso carotidofemoral e com doença arterial periférica diagnosticada por critérios clínicos[11]. Fomos igualmente os primeiros a mostrar uma correlação significativa entre a densidade mineral óssea avaliada por DXA no colo do fémur, mas não na coluna lombar, com a espessura cortical avaliada por análise histomorfométrica em biopsia óssea[12]. O nosso estudo atribui pela primeira vez à DXA um papel no diagnóstico de porosidade cortical nos doentes em diálise. A utilidade da avaliação diferencial da densidade mineral óssea cortical e trabecular necessita ainda de ser confirmada em estudos prospectivos. Este achado inovador do nosso estudo foi mencionado pela ERBP (*European Renal Best Practice*) no comentário feito à posição da KDIGO que considera ser reduzida a utilidade da densidade mineral óssea nos doentes em diálise[13].

Dois dos trabalhos incluídos nesta dissertação foram referenciados nas *guidelines* KDIGO 2009 para avaliar a prevalência das calcificações vasculares (KDIGO 2009: Tabela suplementar 10, Fig. 3.6) e para validar a associação entre calcificações vasculares e mortalidade cardiovascular (KDIGO 2009: Tabela suplementar 12, Fig. 3.7)[6]. A inclusão destes nossos dois estudos nas referências destas *guidelines,* que utilizaram o exigente sistema GRADE (*Grades of recommendation, assessment, development, and evaluation*) na classificação e selecção dos estudos, valida o interesse científico dos nossos trabalhos.

O diagnóstico de calcificações vasculares tem um interesse prático para os doentes renais crónicos. A presença de calcificações vasculares é um sinal de alerta para a existência de um elevado risco cardiovascular, e esta informação pode ser utilizada para modificar a terapêutica nestes doentes[6]. Diferentes métodos podem ser usados para diagnosticar calcificações vasculares nos doentes em diálise[14,15]. O *score* de calcificação vascular simples tem a vantagem da simplicidade e de poder ser facilmente interpretado pelo nefrologista, sem necessidade de um radiologista. A reprodutibilidade deste *score* já foi demonstrada por diferentes grupos em estudos nacionais e internacionais[16-24]. Nestes estudos foi demonstrado que as calcificações vasculares avaliadas pelo método criado por nós são preditoras de maior risco de eventos cardiovasculares[16], de amputações dos membros inferiores[17], de velocidade de onda de pulso[18,19], de calcificações

corneanas e conjuntivais[20] e de calcificações coronárias[21]. Também foi demonstrada uma associação inversa entre o *score* de calcificação vascular simples com os níveis séricos de PTH[21], com os níveis de 25(OH)vitamina D [22,23] e com os níveis de fetuína A[19,24].

Todos estes estudos, realizados por diferentes grupos, que utilizaram o *score* de calcificação vascular simples na sua metodologia, comprovam a facilidade de utilização deste *score* e a concordância de resultados atestam a sua reprodutibilidade e a utilidade na avaliação dos doentes renais crónicos.

Abstract

This thesis presents the development and validation of a simple and original method to identify vascular calcifications in dialysis patients, using a semi-quantitative score that we have created and that is obtained in plain X-ray of pelvis and hands. This score was named in different publications as "simple vascular calcification score".

We have demonstrated that this score is a predictor of higher cardiovascular risk in dialysis patients. The simple vascular calcification score was also associated with lower mineral bone density evaluated by DXA in femoral neck. In hemodialysis patients coronary calcifications evaluated by the coronary Agatston score and by the simple vascular calcification score were associated with lower bone volume analysed in bone biopsies. These studies corroborate the hypothesis of the existence of a link between bone disease and vascular disease in dialysis patients and one of the elements of this link may be vascular calcifications.

This simple vascular calcification score identifies calcifications in large, medium and small calibre arteries and includes the two radiological patterns of arterial calcification: linear calcification which has been associated with the calcification of the media layer of the arterial wall and irregular and patchy calcification which has been associated with the calcification of the intima layer of the arterial wall[1]. In the several studies that we have published we have demonstrated that vascular calcifications evaluated by this simple and inexpensive method allow the identification of patients with high cardiovascular risk. This simple vascular calcification score is an independent predictor of cardiovascular mortality[2], all-cause mortality[3], cardiovascular hospitalizations[2], cardiovascular disease[2], peripheral artery disease[2,4], valvular calcifications[5] and arterial stiffness[3].

KDIGO (Kidney Disease: Improving Global Outcomes) guidelines published in 2009 suggest that chronic kidney disease patients in stages 3 to 5, with vascular and valvular calcifications should be considered to be at the highest cardiovascular risk[6]. The high mortality of chronic kidney disease patients is not completely explained by the traditional risk factors[7] and KDIGO group supports, since 2006, the hypothesis of the existence of a link between bone disease and vascular disease[8].

This link may be explained by the alterations of the bone and mineral metabolism and their interaction with development and progression of vascular calcifications. We have also verified in our studies the existence of an association between vascular calcifications and bone disease. Low bone volume diagnosed by histomorphometric analysis of bone biopsies, in a group of dialysis patients, was independently associated with the simple vascular calcification score (data presented in this thesis, chapter 6) and with coronary calcifications evaluated by the Agatston score[9]. The original contribution of this article published in CJASN[9] deserved a commentary in an Editorial written by Prof. Gérard London[10] leader investigator in this area and current EDTA (European Dialysis and Transplantation Association) President. We were also the first group to describe an independent and inverse association between bone mineral density evaluated in the femoral neck by DXA (dual energy X-ray absortiometry) with vascular calcifications evaluated by the simple vascular calcification score, with arterial stiffness evaluated by carotid-femoral pulse wave velocity and with peripheral artery disease diagnosed by clinical criteria[11]. We were also the first group to demonstrate a significant correlation between bone mineral density evaluated by DXA in femoral neck but not in lumbar spine, with cortical thickness evaluated by histomorphometric analysis of bone biopsy[12]. Our study has attributed to DXA, for the first time, a role in the diagnosis of cortical porosity in dialysis patients. The clinical utility of the differential evaluation of bone mineral density in cortical or trabecular bone needs, however, to be confirmed in prospective studies. This original finding of our study was mentioned by ERBP (European Renal Best Practice) commenting the KDIGO position in relation with the reduced utility of bone mineral density evaluation in dialysis patients[13].

Two of the studies included in this thesis have been integrated in a group of studies selected as references by the KDIGO guidelines published in 2009 to evaluate the prevalence of vascular calcifications in CKD patients (KDIGO 2009: Supplementary Table 10, Fig. 3.6) and to corroborate the association between vascular calcifications and cardiovascular mortality (KDIGO 2009: Supplementary Table 12, Fig. 3.7)[6]. The inclusion of both studies as references in the KDIGO guidelines that have used the exigent GRADE system (Grades of Recommendation, Assessment, Development, and Evaluation) in the classification and selection of studies, validates the scientific value of our studies.

The diagnosis of vascular calcifications has a practical interest for chronic kidney disease patients. The presence of vascular calcifications is an alert sign to the existence of a high cardiovascular risk and this information may be used to modify the treatment of these patients[6]. Different methods may be used to detect the presence of vascular calcifications in dialysis patients[14,15]. The simple vascular calcification score has the advantage of being simple, inexpensive and easily evaluated by the Nephrologist without the need for a Radiologist interpretation. The reproducibility of this method has already been demonstrated by other groups in national and international studies[16-24]. It was demonstrated in those studies that

vascular calcifications evaluated by the method created by us, predict higher risk of cardiovascular events[16], higher risk of lower limbs amputations[17], higher pulse wave velocity[18,19], corneal and conjuntival calcifications [20] and coronary calcifications[21]. A negative association between the simple vascular calcification score and PTH levels[21], 25(OH) vitamin D levels[22,23] and Fetuin A levels[19,24] has also been demonstrated. All these studies performed by different groups that have used the simple vascular calcification score in their methods demonstrate that this score is simple, useful and reproducible in the evaluation of chronic kidney disease patients.

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