Universidade do Estado do Rio de Janeiro
Centro Biomédico
Faculdade de Ciências Médicas

Bárbara Gehrke Smith

Avaliação da densidade e microarquitetura ósseas e composição corporal dos pacientes jovens com vírus da imunodeficiência humana

Rio de Janeiro
2023

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, da Universidade do Estado do Rio de Janeiro.

Orientadora: Prof.ª Dra. Maria Caroline Alves Coelho Amaral

Coorientador: Prof. Dr. Miguel Madeira

Rio de Janeiro

2023

CATALOGAÇÃO NA FONTE
UERJ/ REDE SIRIUS/ CB/A

G311 Gehrke, Bárbara
Avaliação da densidade e microarquitetura ósseas e composição corporal dos pacientes jovens com vírus da imunodeficiência humana / Bárbara Gehrke. – 2023.
123 f.

Orientadora: Prof.ª Dra. Maria Caroline Alves Coelho Amaral
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Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Universidade do Estado do Rio de Janeiro, Faculdade de Ciências Médicas.

1. HIV (Virus) – Pesquisa – Teses. 2. Fraturas Ósseas. 3. Densidade Óssea – Teses. 4. Osso e Ossos – Fisiopatologia – Teses. I. Amaral, Maria Caroline Alves Coelho. II. Madeira, Miguel. III. Universidade do Estado do Rio de Janeiro. Faculdade de Ciências Médicas. IV. Título.

CDU 616.9:616:71

Bibliotecário: Felipe Caldonazzo CRB7/7341

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Assinatura Data

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Aprovada em 25 de julho de 2023.

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Rio de Janeiro

2023

DEDICATÓRIA

Dedico esta tese ao meu pai, Ricardo Gehrke.

Pai, amigo, conselheiro, verdadeiro orgulho, inspiração e eterno fã.

A constante motivação que obtive ao longo dos anos que estive com você me tornaram a profissional e a pessoa que sou hoje. A inspiração e a força para finalizar esse projeto foram incentivadas por você mesmo sem sua presença física. Obrigada por todos os ensinamentos passados nos dezenove anos convividos, que apesar de curtos foram intensos e suficientes para transmitir as principais mensagens de vida que eu precisava para seguir em frente. Você se faz presente por sua lembrança eterna em nossos corações.

AGRADECIMENTOS

Agradeço aos participantes voluntários que dedicaram seu tempo à realização desse projeto em prol da evolução e da ciência.

Agradeço à minha mãe, Elizabeth, por estar ao meu lado ao longo dessa e de todas as jornadas que decidi seguir em minha vida, me estimulando constantemente para atingir meus objetivos e sonhos.

Agradeço ao meu marido, Roberto, por ter participado e inventivado a decisão de seguir carreira acadêmica e realizar o Doutorado. Obrigada por estar ao meu lado nos momentos mais difíceis durante esse e outros processos vividos.

Agradeço ao meu irmão, Felipe, e sua esposa, Luiza, por sempre torcerem por mim e por se mostrarem orgulhosos dos desafios superados e objetivos atingidos.

Agradeço aos familiares e amigos que estiveram ao meu lado ao longo desta jornada.

Agradeço aos técnicos, Dean e Rogério, que realizaram os exames no aparelho de densitometria. Agradeço à Ednaura que auxiliou no processo de marcação de exames.

Agradeço ao Dr Francisco Paranhos, que me ensinou a manipular o aparelho de microtomografia, além de laudar os exames.

Agradeço à Prof.ª Laura Mendonça que realizou as análises e laudos do aparelho de densitometria.

Agradeço à equipe do Ambulatório de Doenças Infecto-parasitárias do HUPE/UERJ, em especial, Dra Giovanna Ianini, Dra Valéria Ribeiro e Dra. Dirce Bonfim, por terem me acolhido e me ajudado a recrutar os pacientes.

Agradeço à equipe da Endocrinologia e Metabologia do HUPE e do Laboratório de Hormônios da Endocrinologia (Labhor), em especial ao Dr. Rogério Bosignoli e ao Andrey, que me ajudaram na coleta dos exames laboratoriais.

Agradeço à minha orientadora Professora Dra. Maria Caroline, que esteve incansavelmente ao meu lado e me estimulou durante todas as etapas do projeto. Sem dúvidas sua liderança foi essencial para a finalização desse sonho. Obrigada por confiar em mim e acreditar no meu potencial como profissional e como futura professora.

Agradeço ao meu coorientador Professor Dr. Miguel Madeira, que também me ensinou a manusear o aparelho de microtomografia, auxiliou na realização dos exames de imagem e me estimulou durante todo o processo.

Agradeço à Professora Dra. Maria Lucia Fleiuss de Farias, que participou ativamente do processo final de confecção dos artigos e se mostrou, mais uma vez, uma excelente professora e um poço de conhecimento no assunto.

"Se não fosse pela grande variabilidade entre os indivíduos, a medicina poderia muito bem ser uma ciência, não uma arte."

- *William Osler*

RESUMO

GEHRKE, Bárbara. **Avaliação da densidade e microarquitetura ósseas e composição corporal dos pacientes jovens com vírus da imunodeficiência humana.** 2023. 123 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2023.

Objetivo: Avaliar os parâmetros ósseos de densidade e microarquitetura, além da composição corporal, de pacientes jovens portadores do vírus da imunodeficiência humana (HIV) e comparar com grupo controle, à fim de entender os mecanismos fisiopatológicos envolvidos e sugerir protocolos de rastreio precoce. Metodologia: Foram recrutados 135 participantes (81 infectados e 54 controles saudáveis), de ambos os sexos, com idades entre 20 e 50 anos. Os participantes foram submetidos a questionário detalhado, análise de prontuário, exames laboratoriais e urina de 24 horas. Densitometria óssea (DXA) e tomografia computadorizada quantitativa periférica de alta resolução (HRpQCT) foram realizados para avaliar quantidade e qualidade óssea, respectivamente. Aplicação do questionário SARC-F, avaliação da força de preensão manual (FPM) com dinamômetro, Teste Up & Go (TUG) e avaliação da composição corporal foram realizados para investigar sarcopenia. O rastreio de fraturas vertebrais foi feito por morfometria vertebral (VFA) utilizando DXA. As ferramentas FRAX e NOGG foram aplicados para avaliação do risco de fraturas. Resultados: Cinquenta participantes de cada grupo completaram o estudo. Idade, sexo, etnia, peso, altura, índice de massa corporal (IMC), e consumo de álcool foram estatisticamente semelhantes entre os grupos. Densidade mineral óssea [1,177 g/cm^3 (0,971 – 1,446) versus 1,271 g/cm^3 (0,947 – 1,627), p 0,023] e Z-score [-0,5 desvios padrão (DP) (-2,1 – +2,4) versus +0,2 DP (-1,9 – +2,3), p 0,021] foram estatisticamente inferiores em coluna lombar dos participantes portadores de HIV em comparação com grupo controle. Houve parâmetros ósseos estatisticamente inferiores na HRpQCT principalmente da tíbia distal dos pacientes do grupo HIV. Encontramos uma prevalência de 12% de fraturas vertebrais morfométricas em pacientes assintomáticos do grupo HIV. Resultados de testes funcionais também mostraram inferioridade no grupo HIV com significância estatística (TUG e SARC-F) e a FPM apresentou p 0,052 com valores mais baixos para grupo HIV. Na composição corporal um maior número de participantes do grupo HIV apresentaram alterações em massa magra apendicular e massa magra apendicular corrigida para altura ao quadrado quando utilizamos pontos de corte sugeridos por protocolos mundiais bem estabelecidos para o diagnóstico de sarcopenia. Participantes do grupo HIV apresentaram maior risco de fraturas, com significância estatística, calculado através da ferramenta FRAX. Conclusões: Encontra-se bem estabelecido na literatura que indivíduos portadores de HIV desenvolvem mecanismos fisiopatológicos que reduzem massa óssea, alteram propriedades da composição corporal e apresentam maior risco de fratura, principalmente em idades mais avançadas. No nosso estudo, encontramos parâmetros inferiores e presença de fraturas em uma população jovem, a qual não é habitualmente rastreada para as complicações citadas. Desenvolvemos protocolos de rastreio para diagnóstico precoce a fim de reduzir a morbimortalidade e melhorar a qualidade de vida de indivíduos jovens com elevado risco de fraturas.

Palavras-chave: HIV. HR-pQCT. DXA. VFA. Baixa massa óssea. Fraturas por fragilidade. Composição corporal. Sarcopenia.

ABSTRACT

GEHRKE, Bárbara. *Evaluation of bone density and microarchitectural parameters and body composition of young people living with the human immunodeficiency virus.* 2023. 123 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2023.

Objective: To evaluate bone density and microarchitecture, in addition to body composition, in young patients with the human immunodeficiency virus (HIV) and compare with healthy control group, in order to understand the pathophysiological mechanisms involved and suggest early screening protocols. Methodology: We recruited 135 participants (81 infected and 54 healthy controls), of both genders, aged between 20 and 50 years. Participants were examined, questionnaires were applied, analysis of medical records, laboratory tests and 24-hour urine were completed. Dual X-ray absorptiometry (DXA) and peripheral quantitative high-resolution computed tomography (HR-pQCT) were performed to assess bone quantity and quality, respectively. Application of SARC-F quetionnaire, assessment of muscle strength with handgrip test, Test Up & Go (TUG) and body composition were performed to suspect or diagnose sarcopenia in these individuals. In order to screen morphometric vertebral fractures, vertebral fracture assessment (VFA) was performed using DXA. The tools FRAX and NOGG were applied to evaluate the risk porbability of fractures. Results: Fifty participants from each group completed the study. The median age was 40 (25–49) vs. 36.5 (22–50) for the HIV and control groups, respectively (p 0.120). Gender, race, weight, height, body mass index, and smoking were also statistically similar between groups. Bone mineral density [1.177 g/cm^3 (0.971 – 1.446) versus 1.271 g/cm^3 (0.947 – 1.627), p 0.023] and Z-score [-0.5 standard deviations (SD) (-2.1 – +2.4) versus +0.2 SD (-1.9 – +2.3), p 0.021] were statistically lower at lumbar spine of participants with HIV when compared to the control group. There were statistically lower bone parameters in the HRpQCT, mainly in the distal tibia of patients with HIV. Participants from HIV group presented higher risk of fractures, with statistical significance, calculated through FRAX tool. We found a 12% prevalence of morphometric vertebral fractures in asymptomatic individuals with HIV. Functional tests also showed statistically significant inferior results in the HIV subjects (TUG and SARC-F) and handgrip strength showed p 0.052 with lower values for the infected group. In terms of body composition, a greater number of participants in the HIV group showed changes in appendicular lean mass and appendicular lean mass adjusted for height when we used cutoff points suggested by well-established global protocols for the diagnosis of sarcopenia, although no significant differences were evidenced between groups. Conclusions: It is well established in literature that individuals with HIV develop pathophysiological mechanisms that reduce bone mass, alter body composition properties and present a higher risk of fractures, especially at older ages. In our study, we found lower bone and muscle parameters and the presence of fractures in a young population, which is not usually screened for the aforementioned complications. We have developed screening protocols for early diagnosis in order to reduce morbimortality and improve quality of life in young individuals at high-risk for fractures.

Keywords: HIV. HR-pQCT. DXA. VFA. Low bone density. Fragility fractures. Body composition. Sarcopenia.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

$1,25(OH)_2D_3$	1,25-dihidroxi vitamina D
25(OH)D	25-hidroxi vitamina D
CA	California
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CT	Computed tomography
CTX	C-terminal cross-linking telopeptide
DIP	Doenças infecto-parasitárias
DMO	Densidade mineral óssea
DP	Desvio padrão
DXA	Dual X-ray absorptiometry
EFV	Efavirenz
EUA	Estados Unidos da America
EWGSOP2	European Working Group on Sarcopenia in Older People
FCM/UERJ	Faculdade de Ciências Médicas da Universidade do Estado do Rio de Janeiro
FPM	Força de preensão palmar
FRAX	Fracture risk assessment tool
HIV	Human immunodeficiency virus
HR-pQCT	High resolution peripheral quantitative computed tomography
HUPE	Hospital Universitário Pedro Ernesto
IL	Interleucina
IMMA	Índice de massa magra apendicular
IMC	Índice de massa corporal
IP	Inibidor de protease
ISCD	International Society for Clinical Densitometry
LGSI	Low grade systemic inflammation
MMA	Massa magra apendicular
IMMA	Índice de massa magra apendicular
NOGG	National Osteoporosis Guideline Group
OC	Osteocalcina

OPG	Osteoprotegerina
PTH	Parathyroid hormone
RANKL	Receptor activator of nuclear factor kappa beta
SARC-F	Strength, assistance with walking, rise from chair, climb stairs and falls questionnaire
SHBG	Sexual hormone binding globulin
TAF	Tenofovir alafenamide
TARV	Terapia antiretroviral
TDF	Tenofovir disoproxil fumarate
TNF	Tumor necrosis factor
TUG	Timed Up and Go
UFRJ	Universidade Federal do Rio de Janeiro
VFA	Vertebral fracture assessment
WI	Wisconsin

LISTA DE SÍMBOLOS

%	Porcentagem
α	Alfa
β	Beta
κ	Kappa
dL	Decilitro
mL	Mililitro
cm	Centímetro
cm^2	Centímetros quadrados
cm^3	Centímetros cúbicos
g	Gramas
Kg	Quilogramas
&	E
m	Metros
m^2	Metros quadrados
r	Coeficiente de correlação
p	P valor

SUMÁRIO

INTRODUÇÃO

O vírus da imunodeficiência humana (HIV) tem sido um desafio para o sistema de saúde desde o início da década de 1980 (1, 2). Nos dias atuais, ainda estamos diante de incidência e prevalência consideráveis da doença. Em 2021, estimou-se que mais de 38,4 milhões de pessoas viviam com a infecção pelo HIV mundialmente (3).

A doença se tornou uma condição crônica e tratável e atualmente uma pessoa jovem que adquire o vírus apresenta uma expectativa de vida próxima ou igual à população que não apresenta a doença (4). O uso de terapia antirretroviral (TARV) não apenas reduziu a morbidade e mortalidade, mas também contribuiu à prevenção da doença (5). À despeito da disponibilidade de diversos recursos para a prevenção da transmissão do vírus, a incidência permanece alta (3). Devido à transição demográfica, com aumento da expectativa de vida (longevidade) dos pacientes HIV, osteopenia e osteoporose têm se tornado mais comuns nesse grupo populacional (6).

Osteoporose é uma desordem esquelética caracterizada por baixa massa óssea com a deterioração da microarquitetura do osso, predispondo o indivíduo a fraturas por fragilidade (7). Essa condição geralmente resulta em uma piora da qualidade de vida e aumenta a morbimortalidade, sendo observada com maior frequência na população idosa. Existem diversos mecanismos envolvidos na fisiopatologia da doença óssea (8):

a) Defeitos na microarquitetura trabecular;
b) Defeitos intrínsecos das propriedades materiais do tecido ósseo;
c) Reparo disfuncional de microdanos provenientes de atividades diárias;
d) Taxas excessivas de remodelamento ósseo.

Para a prevenção da doença óssea que progride no envelhecimento, existem recomendações bem estabelecidas na literatura, as quais devem ser instituídas ainda na infância, adolescência e no adulto jovem. Um elemento essencial para prevenir osteoporose consiste na obtenção de um pico de massa óssea adequado, o qual ocorre por volta dos 30 anos de idade (9). Os principais fatores que interferem no pico de massa óssea são etnia, gênero e fatores genéticos (hereditariedade– responsável por determinar 60 a 80% do pico de massa óssea) (10, 11). A nutrição adequada com ingesta apropriada de cálcio e exposição solar periódica para conversão da vitamina D se mostraram essenciais para a manutenção da saúde óssea a longo prazo (9). Outros aspectos importantes para o pico de massa óssea são ciclos menstruais regulares e realização de atividade física regular e balanceada (9). No período do climatério ou início da menopausa a mulher apresenta queda do estrogênio o que leva a uma perda óssea mais

acelerada devido ao processo de reabsorção do tecido ósseo que ocorre principalmente em osso trabecular (mais presente em esqueleto axial, isto é, colunas dorsal e lombar) (9). Homens também iniciam processo de redução de testosterona (andropausa) por volta dos 50 anos e sofrem perda óssea (9). A perda de um desvio padrão (DP) aumenta o risco de fraturas vertebrais em duas vezes ou risco de fratura de quadril em duas vezes meia (9).

Doenças que geram inflamação crônica no organismo, como no caso dos indivíduos com HIV ou uso de medicamentos que interferem no metabolismo ósseo podem comprometer o pico de massa óssea, elevando o risco de fraturas devido ao desenvolvimento de osteoporose grave em pacientes mais jovens (12).

Infelizmente, a grande maioria dos pacientes apenas diagnosticam osteoporose após um episódio de fratura. As fraturas são de baixo-impacto (como queda da própria altura) e ocorrem com maior frequência nas vértebras, quadril ou punho. Após a primeira fratura, o risco de uma nova fratura aumenta em 5 vezes no primeiro ano após o evento. Aproximadamente 50% dos indivíduos que sofreram fratura osteoporótica sofrerão um novo evento no futuro (13-15). As fraturas aumentam morbimortalidade e pioram a qualidade de vida do indivíduo, portanto, devem ser prevenidas e tratadas (16-18).

Nos indivíduos infectados pelo HIV, o risco de fraturas aumenta aproximadamente 10 anos mais cedo do que na população geral (19). Além disso, diversos estudos reportaram baixa massa óssea com elevado risco de osteoporose, osteopenia e osteomalacia em pacientes infectados pelo HIV, incluindo mulheres, homens, pacientes jovens e idosos e crianças verticalmente contaminadas (12, 20).

Osteopenia e osteoporose atingem aproximadamente 50% e 25% dos pacientes HIV, respectivamente (21). Fraturas ocorrem nesse grupo devido a uma combinação multifatorial de riscos para osteoporose, os quais estes pacientes podem apresentar (uso crônico de glicocorticoides, sedentarismo, uso de opióides, álcool, tabagismo, deficiência de cálcio e vitamina D e hipogonadismo) (11, 22). Além disso, diversos fatores de risco associados ao HIV, como uso de TARV, contagem de células T $CD4^{+}$, duração da doença, morbidade crônica, alterações da composição corporal, estado inflamatório crônico e reconstituição imune, podem estar envolvidos com a fisiopatologia da doença óssea nos pacientes infectados pelo HIV (21). Grandes estudos descreveram o aumento do risco de fraturas em pacientes portadores do vírus com relatos de aumento no risco de fratura podendo chegar de 4 a 5 vezes maior em população com HIV quando comparados a indivíduos não infectados (23-28).

O desequilíbrio da interface imunoesquelética está associado à osteoporose nos pacientes infectados pelo HIV. Essa interface consiste na centralização de células e citocinas

compartilhadas entre os sistemas esquelético e imune. As células B secretam osteoprotegerina (OPG), um fator anti-osteoclastogênico potente que preserva a massa óssea (29). Um estudo reportou que as células B podem ser responsáveis pela produção de 64% de OPG na medula óssea, portanto, diante da disfunção desta célula, a produção de OPG fica prejudicada, favorecendo a reabsorção óssea (30). As células B e T ativadas secretam fatores pró-osteoclastogênicos incluindo o *receptor activator of nuclear factor κB ligand* (RANKL, em português, ligante do receptor ativador do fator nuclear κB), interleucina (IL)-17A e *tumor necrosis factor* (TNF, em português, fator de necrose tumoral)-α promovendo perda óssea em estados inflamatórios crônicos (29). Existem evidências de que na população com HIV a expressão do RANKL encontra-se aumentada e que existe uma menor atividade da OPG em células imunes, o que gera uma perda óssea acelerada (31). A TARV também intensifica a perda da massa óssea durante a reversão da doença, visto que a reconstituição imune produz citocinas osteoclastogênicas (29).

Existem medicamentos no arsenal de TARVs, os quais apresentam maior influência na massa óssea e que requerem maior atenção. Um estudo que avalia os efeitos da TARV no metabolismo ósseo descreve uma perda de densidade mineral óssea (DMO), que varia entre 2 a 6% em quadril e coluna, ocorrendo nos primeiros 24 a 48 meses com posterior estabilização (32). A magnitude dessa perda óssea é comparada à que ocorre com uso de glicocorticoides ou durante o primeiro ano de transição menopausal (32). Essa perda inicial é marcada por um aumento dos marcadores de reabsorção óssea no sangue seguida do aumento compensatório de marcadores de formação óssea, configurando um estado de elevada remodelação óssea, que parece ser um dos principais mecanismos envolvidos na perda da massa óssea causada pelas TARVs (32).

Um estudo de revisão de 2016 correlacionou o *tenofovir disoproxil fumarate* (TDF, em português, fumarato de tenofovir desoproxila - inibidor de transcriptase reversa análogo de nucleotídeo) com uma piora da DMO 1 a 3% maior em comparação com o uso de outras TARVs (33). Essa piora ocorre principalmente no primeiro ano de exposição ao tratamento e mostrou-se reversível nos resultados de estudos switch (33). É descrito na literatura que o início da terapia com TDF pode ocasionar alterações osteometabólicas com interferência no metabolismo da vitamina D e consequente redução dos níveis de vitamina D ativa [1,25-dihidroxi vitamina D ($1,25(OH)_2D_3$)] (32). Pode haver também aumento dos níveis de *parathyroid hormone* (PTH, em português, paratormônio), tubulopatias com hipofosfatemia (casos raros de Síndrome de Fanconi) ou até mesmo osteomalacia. Portanto, sugerem reposição da vitamina D no início da terapia com TDF a fim de evitar repercussões osteometabólicas (33).

Outro medicamento que mostrou interferência no metabolismo ósseo dos pacientes com HIV é o efavirenz (EFV – inibidor da transciptase reserva não-análogo dos nucleosídeos) e foi descrita a diminuição dos níveis séricos de 25-hidroxi vitamina D [25(OH)D] em 2,5 a 5 ng/mL após o seu início. O mecanismo deste medicamento consiste em indução das enzimas citocromo P450 envolvidas no metabolismo da vitamina D, acelerando o catabolismo de 25(OH)D e $1,25(OH)_2D_3$ (32).

Tem descrito na literatura que a vitamina D possui efeito imunomodulatório mediado por seus receptores nas células do sistema imune inato e adaptativo. Ela aumenta a expressão de CD14 e catelicidina, as quais são moléculas envolvidas em respostas imunes inatas, além de reduzir expressão de citocinas em células T ativas, suprimindo a proliferação de células T e a produção de citocinas inflamatórias, consequentemente reduzindo o estado inflamatório (32).

Ainda faltam estudos para esclarecer os mecanismos exatos dos quais os inibidores de protease (IP) agem interferindo no metabolismo ósseo, no entanto, mostrou-se que ocorre a perda da massa óssea com o uso dessa classe de drogas (34). Dentre os IPs, as diferentes drogas mostraram efeitos distintos, no entanto, é possível concluir que elas podem ocasionar inibição de atividade osteoblástica e estímulo à formação de osteoclastos, favorecendo a reabsorção óssea (34).

A ativação imune crônica provocada pelo HIV leva a uma *low-grade sistemic inflammation* (LGSI, em português, inflamação sistêmica de baixo grau), o que pode reduzir a atividade de substâncias responsáveis pela síntese de proteínas musculares e levar a uma disfunção do tecido adiposo, com acúmulo de gordura ectópica (35). Os antirretrovirais promovem dano mitocondrial através do aumento da inflamação muscular e por ação direta, podem reduzir a expressão gênica de proteínas envolvidas na síntese proteica. Além disso, podem levar a alterações na composição corporal (35).

As primeiras drogas utilizadas no tratamento do HIV apresentavam como efeito adverso a lipodistrofia devido à redistribuição de gordura no organismo (36, 37). Inibidores da transcriptase reversa análogos de nucleosídeo – estavudina e zidovudina - foram associados a lipoatrofia, enquanto os IP ocasionaram lipohipertrofia (36, 37). *Tenofovir alafenamide* (TAF), recentemente adicionado ao arsenal de TARVs no Brasil, mostrou ter menos efeito nas massas óssea e muscular, no entanto, apresentou resultados de maior índice de massa corporal (IMC) e percentual de gordura corporal em seus usuários em comparação com pacientes que não usavam a droga (38). Alguns inibidores da integrase (raltegravir, elvitegrvir e dolutegravir) também foram associados ao aumento de peso (39) e os IP demonstraram alterações

metabólicas e ganho de gordura corporal em estudos prévios (40-42). Em contrapartida, usuários de TDF parecem apresentar menor impacto no ganho de peso em comparação com outras TARVs (43). Esse aumento de percentual de gordura corporal leva a uma LGSI que eleva o risco de resistência à insulina e diabetes, o que pode resultar em redução de força e qualidade muscular (35).

Assim, a população infectada pelo HIV é mais propensa ao desenvolvimento de sarcopenia que frequentemente ocorre em concomitância ao envelhecimento, porém nos pacientes HIV pode ser visto em idades mais precoces aumentando morbimortalidade e reduzindo performance física e qualidade de vida (44). O RANKL também se expressa na musculatura esquelética, e como dito anteriormente, apresenta maior expressão em pacientes infectados pelo HIV, o que pode resultar em disfunção e perda musculoesquelética, por inibição da diferenciação miogênica (31).

Nos últimos anos, osso e músculo são cada vez mais reconhecidos como tecidos que interagem, não apenas por conta das superfícies adjacentes ou como resultado dos efeitos mecânicos do músculo sobre a função do osso, criando-se o conceito de "unidade osso-músculo", que se comunica via sinais parácrinos e endócrinos para coordenar seu desenvolvimento e adaptar a resposta a qualquer injúria (45, 46). Portanto, temos evidência de que a sarcopenia e a osteoporose compartilham muitas vias comuns incluindo a sensibilidade para reduzir a secreção de hormônios anabólicos, atividade inflamatória aumentada com citocinas e moléculas anabólicas ou catabólicas secretadas pelo músculo esquelético ou células ósseas (7).

Atualmente os critérios utilizados para diagnóstico de sarcopenia seguem o *European Working Group on Sarcopenia in Older People* (EWGSOP2) (47). Para classificação de provável sarcopenia se avalia a força muscular por meio da força de preensão palmar (FPM) com utilização de dinamômetro, que quando abaixo dos pontos de corte definidos, revela provável sarcopenia. Sarcopenia é confirmada quando o indivíduo apresenta baixa força muscular associada a baixa massa muscular [massa magra apendicular (MMA) abaixo dos valores de referência obtidos através da composição corporal por *Dual-energy X-ray Absortiometry* (DXA, em português, absorciometria de raios-X de dupla energia). Sarcopenia severa leva em consideração alteração dos parâmetros descritos acima associados à baixa performance física (47). Os parâmetros definidos para o diagnóstico de sarcopenia levam em consideração uma população idosa, portanto, não devem ser generalizados para grupos mais jovens.

Em suma, em vista dos fatos expostos acima, o estudo proposto visa avaliar a quantidade e qualidade ósseas, através da densidade e microarquitetura ósseas, além da composição corporal e realização de testes funcionais para avaliar músculo em pacientes jovens infectados pelo HIV, a fim de identificar qual a contribuição deste vírus e dos TARVs para a deterioração da massa óssea e desenvolvimento da sarcopenia em idades mais jovens. O diagnóstico precoce e consequente tratamento objetivam evitar fraturas por fragilidade e perda de funcionalidade e mobilidade em uma população que ainda apresenta potencial laborativo prolongado. Além disso, o rastreio osteometabólico ao diagnóstico da infecção e acompanhamento no início e na escolha da TARV podem ser importantes, permitindo a reposição do colecalciferol, no intuito de evitar a piora da massa óssea. Pode-se também orientar medidas dietéticas e de atividade física visando a melhora da sarcopenia, o que contribui para uma menor perda de massa óssea ao longo da vida.

Atualmente os protocolos definidos para rastreio de doença óssea na população infectada por HIV recomendam a realização do *fracture risk assessment tool* (FRAX - ferramenta que calcula a probabilidade de fratura de quadril e fratura osteoporótica maior em 10 anos) em mulheres pré menopausa $\geq$40 anos e homens entre 40-49 anos. A realização de DXA é recomendada em mulheres pós-menopausa, homens com $\geq$50 anos, pacientes em uso crônico de glicocorticoides e/ou com elevado risco de quedas ou história prévia de fratura por fragilidade (48). Entretanto, esperarmos essa idade para fazer a primeira avaliação nos pacientes portadores do vírus pode ser catastrófico, pois permitirá a perda óssea mais acelerada do que na população geral e com um risco maior de fraturas, elevando a morbimortalidade e prejudicando a funcionalidade desses pacientes.

Protocolos atuais de rastreio de sarcopenia apresentam pontos de corte voltados para populações idosas, sem considerar grupos específicos como os pacientes portadores de HIV. Portanto, é preciso criar novos protocolos de rastreio para populações mais jovens que apresentam elevado risco de perda de massa e força muscular.

1. OBJETIVOS

1.1. Gerais

Avaliar os parâmetros ósseos e musculares de pacientes jovens portadores do HIV através de DXA e *high resolution peripheral quantitative computed tomography (*HR-pQCT, em português, tomografia computadorizada quantitativa periférica de alta resolução) e comparar com população de características semelhantes.

1.2. Específicos

a) Identificar presença de fraturas vertebrais morfométricas;
b) Avaliar a presença de alterações na microestrutura e densidade ósseas, composição corporal e testes funcionais musculares na população jovem com HIV.

2. MATERIAL E MÉTODOS

2.1 Tipo de pesquisa

Trata-se de um estudo clínico observacional e transversal. Os participantes foram selecionados aleatoriamente (desde que atendidos os critérios de inclusão e removidos os critérios de exclusão) nos ambulatórios de doenças infecto-parasitárias (DIP) e de medicina interna/Clínica Médica do Hospital Universitário Pedro Ernesto (HUPE) da Universidade do Estado do Rio de Janeiro (UERJ). Os participantes foram separados em 2 grupos: grupo controle e grupo com portadores de HIV.

2.2 Ética da pesquisa

O projeto foi aprovado pelo Comitê de Ética em Pesquisa do HUPE (ANEXO A), Certificado de Apresentação de Apreciação Ética sob número 29162020.1.0000.5259. Os procedimentos envolvendo os participantes foram realizados de acordo com os princípios descritos na Declaração de Helsinki. Todos os participantes assinaram o termo de consentimento livre e esclarecido (ANEXO B).

2.3 Amostra

Foram analisados um total de 135 prontuários de indivíduos elegíveis ao estudo (81 pessoas com o HIV e 54 para grupo controle), provenientes dos ambulatórios de DIP e de medicina interna/Clínica Médica HUPE/UERJ.

2.3.1 Critérios de inclusão

Os participantes poderiam ser de ambos os sexos com idade entre 20 e 50 anos. Mulheres precisavam apresentar ciclos menstruais regulares. Pacientes do grupo HIV precisavam ter diagnóstico e estar em tratamento da doença há pelo menos um ano e deveriam comparecer regularmente às consultas no ambulatório de DIP (a cada 6 meses).

2.3.2 Critérios de exclusão

Foram excluídos indivíduos portadores de doenças do metabolismo ósseo, tais como: hipoparatireoidismo, hiperparatireoidismo primário, raquitismo, osteogênese imperfeita, mieloma múltiplo, doença de Paget; assim como condições que interfiram no metabolismo ósseo, tais como: diabetes, hipertireoidismo, hiperparatireoidismo, hipercortisolismo, menopausa ou falência ovariana precoce, doenças hepáticas, reumatológicas e/ou hematológicas, síndromes disabsortivas intestinais ou história de cirurgia bariátrica, acidose tubular renal, história de câncer, hipogonadismo ou alteração da função renal de caráter agudo ou crônico correspondendo a uma estimativa de filtração glomerular menor que 60 mL/min/1.73m^2 calculada através da equação desenvolvida pela *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI, em português, Colaboração de Epidemiologia e Doença Renal Crônica). Indivíduos com nível cognitivo alterado, incapazes de compreender as etapas do estudo também foram excluídos. Gestantes foram excluídas devido à radiação emitida pelo DXA e HR-pQCT e indivíduos com peso superior a 120 quilogramas (Kg) não puderam participar devido à capacidade máxima suportada pelo aparelho de DXA. Pessoas em uso crônico de glicocorticoides, inibidores de bombas de prótons, anticonvulsivantes e thiazolidinedionas foram excluídos do estudo devido à interferência no metabolismo ósseo (49).

2.4 **Delineamento experimental**

Os participantes passaram inicialmente por processo de análise de prontuários para identificação de possíveis patologias ou uso de medicações que poderiam interferir no metabolismo ósseo. Os mesmos foram abordados em consultas de rotina agendadas nos ambulatórios de origem. Após explicação, fornecimento do termo de consentimento livre e esclarecido e assinatura do mesmo, iniciaram processo de participação no estudo. Foram realizados questionário (ANEXO C), exame físico completo, uso das calculadoras FRAX e *National Osteoporosis Guideline Group* (NOGG) para avaliação do risco de fratura, solicitação de exames laboratoriais (sangue e urina de 24 horas), aplicação de questionário SARC-F, Timed Up & Go (TUG) e avaliação da força de preensão palmar (FPM) através de dinamômetro. Em um segundo momento, participantes foram contactados através de número telefônico fornecido pelos mesmos para realização de exames de imagem [DXA e HR-pQCT]. Para a realização de DXA os participantes não poderiam estar em período menstrual ou pré-menstrual.

2.4.1 Exame físico

Foi feito exame físico com análise ectoscópica para avaliar presença de deformidades ósseas pré-existentes, mensuração de pressão arterial e aquisição de medidas antropométricas [peso, altura e IMC (peso em quilogramas/altura em metros quadrados)].

2.4.2 Avaliação do risco de fraturas

Foi feita avaliação do risco de probabilidade de fraturas em 10 anos por meio da ferramenta conhecida como FRAX, em participantes com 40 anos ou mais de ambos os sexos. Esta ferramenta encontra-se disponível em: https://abrasso.org.br/calculadora/calculadora/. A infecção pelo HIV foi considerada uma “causa secundária de osteoporose” para ajuste de risco de probabilidade. Em seguida, procedemos com o cálculo de risco de fraturas com a ferramenta da NOGG para classificar como risco baixo, moderado ou alto.

2.4.3 Análise laboratorial

Investigação bioquímica foi conduzida para excluir possíveis condições que afetam o metabolismo ósseo. Cálcio total, fósforo, magnésio, 25(OH)D, PTH e níveis séricos de albumina foram dosados para avaliar o metabolismo ósseo. Também foram dosados no sangue: hemograma completo, funções renal, tireoidiana e hepática, hormônios sexuais (níveis de testosterona total, sex hormone binding globulin [SHBG, em português, globulina ligadora dos hormônios sexuais) e estradiol em homens], hormônios luteinizante e folículo estimulante, prolactina, volume de hemossedimentação, glicose de jejum (pelo menos 8 horas), hemoglobina glicada, insulina, perfil lipídico, hepatograma completo e reserva alcalina. Marcadores de remodelação óssea, tais como: C-terminal cross-linking telopeptide, em português, C-telopeptídeo terminal do colágeno tipo 1 (ß-CTX) e osteocalcina (OC) também foram analisados. Urina de 24 horas com dosagem de calcio, fósforo, sódio e creatinina foi solicitada. O método utilizado para PTH, ß-CTX e OC foi quimioluminescência não competitiva e para 25(OH)D utilizamos quimioluminescência competitiva, para ambos os métodos utilizamos o aparelho Maglumi X8 da Snibe (China). Os participantes foram orientados a realizar jejum de 8 horas para a coleta dos exames. O diagnóstico de hipogonadismo foi baseado no cálculo da testosterona livre a partir da testosterona total, SHBG e albumina. Pacientes com valores de testosterona livre inferiores a 6,5 ng/dL foram excluídos do estudo.

2.4.4 Questionário SARC-F

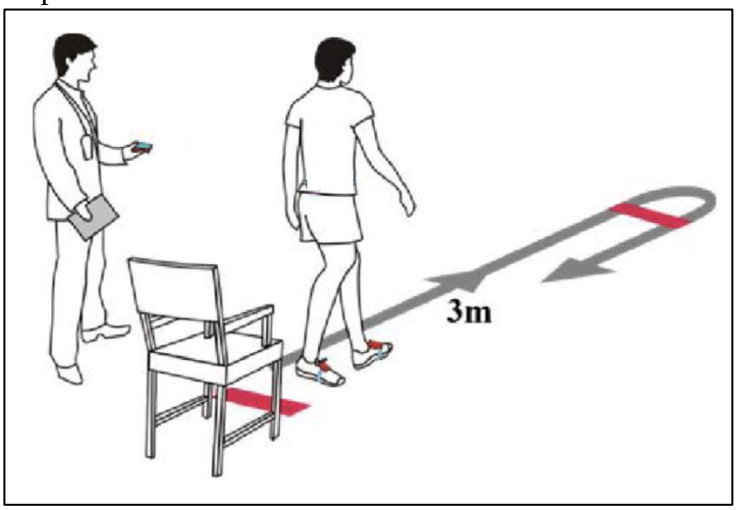
Para rastreio do risco de sarcopenia e avaliação do comprometimento funcional foi aplicado o questionário SARC-F. Participantes de ambos os grupos responderam baseados em sua percepção a respeito dos seguintes itens: levantar-se de uma cadeira ou cama, subir 10 degraus de escada, capacidade de mobilidade e locomoção entre cômodos, força muscular e número de quedas nos últimos 12 meses. Cada um desses aspectos apresentam classificações e pontuações e o participante que pontua 4 ou mais apresenta alta probabilidade de sarcopenia (47, 50).

2.4.5 Timed Up & Go (TUG)

Consiste em um teste que avalia a performance física do participante. O indivíduo foi orientado a sentar-se em uma cadeira e instruído pelo examinador a se levantar sem suporte,

em seguida caminhar a distância de 3 metros, girar 180º e retornar ao ponto de partida para sentar-se na cadeira novamente. O tempo levado para completar o movimento foi cronometrado. Tempo superior a 20 segundos é considerado alterado (51). A Figura 1 ilustra a realização do teste.

Figura 1 - Timed Up & Go



2.4.6 Força de preensão palmar (FPM)

A FPM é realizada para avaliar a força muscular. Cada participante foi orientado a exercer a capacidade de força muscular máxima no membro superior dominante utilizando o dinamômetro manual portátil (Jamar Model 1 – 70729, Asimow Engineering Company, Santa Monica, CA, EUA), de acordo com as recomendações da *American Society of Hand Therapists* (em português, Sociedade Americana de Terapêutas de Mãos) (52). Participantes foram orientados a se sentar em cadeira com posição aduzida dos ombros, cotovelos flexionados a 90º, antebraço em posição neutra e pés encostando o chão. O examinador instruiu o participante a exercer a força muscular máxima em 3 tentativas com intervalos de 2 minutos entre cada medida. A média de resultados foi adquirida para posterior análise dados. Valores ≤ 16 e ≤ 27 Kg para mulheres e homens, respectivamente, eram considerados alterados (53). A Figura 2 mostra o dinamômetro e o posicionamento adequado.

Figura 2 Dinamômetro manual Jamar e posicionamento adequado para realização da FPM

Figura 2 - Dinamômetro manual Jamar e posicionamento adequado para realização da FPM

2.4.7 DXA

Cada indivíduo foi submetido a exame de Densitometria com dupla fonte de raios-X com o aparelho GE Lunar Prodigy Advance, GE Healthcare Madison, WI, EUA. Foram avaliadas composição corporal total – massa magra, massa gorda, massa livre de gordura, percentual de gordura e densitometria óssea com análise de DMO areal nos sítios de coluna lombar (das vértebras L1-L4), fêmur proximal e rádio 33%. Resultados foram indicados em valores absolutos (g/cm^2) e DP da DMO esperada como referência para população da mesma idade (Z-score). De acordo com a *International Society for Clinical Densitometry* (ISCD, em português, Sociedade Internacional de Densitometria Clínica), indivíduos que apresentaram DMO Z-score de $\leq$ -2.0 DP foram classificados como baixa massa óssea para idade. Homens tinham menos de 50 anos e mulheres estavam na pré-menopausa; por esse motivo, T-scores não foram utilizados. O coeficiente de variação para DMO no aparelho utilizado é de 2.3% em fêmur e 1.8% em coluna lombar. Os exames de DXA foram operados pelo mesmo técnico e avaliados pela mesma médica.

Massa magra apendicular de membros superiores e inferiores foi utilizada para calcular a MMA em Kg e os valores de referência considerados alterados são < 15 Kg and < 20 Kg, para mulheres e homens, respectivamente (47, 54, 55). Em seguida, calculamos o índice de MMA (IMMA), através da razão da MMA pela estatura ao quadrado (em Kg/m^2). Foi adotado a redução do IMMA $\geq$ 2 DP abaixo da média de controles jovens com idades entre 18– 40 anos,

sendo < 5,5 Kg/m^2 e < 7,00 Kg/m^2 considerados alterados, para mulheres e homens, respectivamente (56). Utilizamos pontos de corte recomendados pela EWGSOP2 e baseados na definição operacional de Baumgartner's, a qual corresponde a > 2 DP abaixo da média de referência para controles jovens com idades entre 18 e 40 anos (Z-score). Também analisamos tecido adiposo visceral e tecido adiposo subcutâneo. Calculamos o índice de massa gorda pela razão do peso de gordura (Kg) dividido por altura ao quadrado (m^2) (57).

2.4.8 *Vertebral fracture assessment* (VFA - avaliação de fraturas vertebrais)

A pesquisa de fraturas vertebrais foi feita através do método DXA-VFA. O paciente é posicionado em decúbito lateral pelo técnico e a imagem da coluna é captada com análise das alturas anterior, medial e posterior de cada vértebra. Por meio da escala semiquantitativa de Genant foram identificadas fraturas pré-existentes.

2.4.9 HR-pQCT

Foi feita análise da densidade volumétrica e microarquitetura ósseas mediante HR-pQCT utilizando o aparelho X-treme CT (SCANCO Medical AG, Brüttisellen, Switzerland). Participantes realizaram imagem dos membros não dominantes e imobilizados. Os membros utilizados foram rádio e tibia distal. DMO volumétrica foi estimado em osso total [Tt.BMD], osso cortical [Ct.BMD] e osso trabecular [Tb.BMD]. Parâmetros microestruturais analisados foram: espessura cortical [Ct.Th: razão entre volume cortical ósseo e superfície óssea externa], número de trabéculas [Tb.N], fração de volume ósseo trabecular [BV/TV, derivado da densidade trabecular/1200 mg de hidroxiapatita], espessura trabecular [Tb.Th: (BV/TV)/Tb.N], separação trabecular [Tb.Sp: (1-BV/TV)/Tb.N], e não homogeneidade trabecular [Tb.1/N.SD, desvio padrão de 1/Tb.N (58). Em cada sítio obtivemos 104 cortes, contribuindo para a formação de imagem tridimensional (3D) com representação em direção axial. O coeficiente de variação no aparelho de HR-pQCT utilizado é de 3-5% para parâmetros microarquiteturais e 1-2% para medidas de densidade.

2.5 **Amostra Metodologia estatística**

A análise estatística foi realizada utilizando o software SPSS versão 29.0.0.0 (241) para Windows (SPSS Inc., Chicago, IL, EUA). Teste de Kolmogorov-Smirnov avaliou o padrão de distribuição das variáveis numéricas. A maior parte das variáveis não respeitou a curva de normalidade e, portanto, utilizamos testes não paramétricos. Variáveis numéricas foram expressas em mediana (mínimo e máximo) e foram analisadas pelo teste de Mann-Whitney. Variáveis categóricas foram analisadas pelo teste de Qui-quadrado de Pearson. Um valor de $p < 0.05$ foi considerado estatisticamente significativo. Correlações entre variáveis numéricas foram realizadas com o teste de Spearman. Graus de força de correlação foram baseados nos seguintes coeficientes (r): muito fraca (0.00 – 0.19), fraca (0.20 – 0.39), moderada (0.40 – 0.69), forte (0.70 – 0.89) e muito forte (0.90 – 1.00). Diferenças foram consideradas significantes quando $\alpha = 5\%$ ($p < 0.05$), afirmando que a hipóstese estudada foi significativamente distinta entre os grupos estudados.

3. RESULTADOS

3.1 **Artigo científico publicado em periódico indexado**

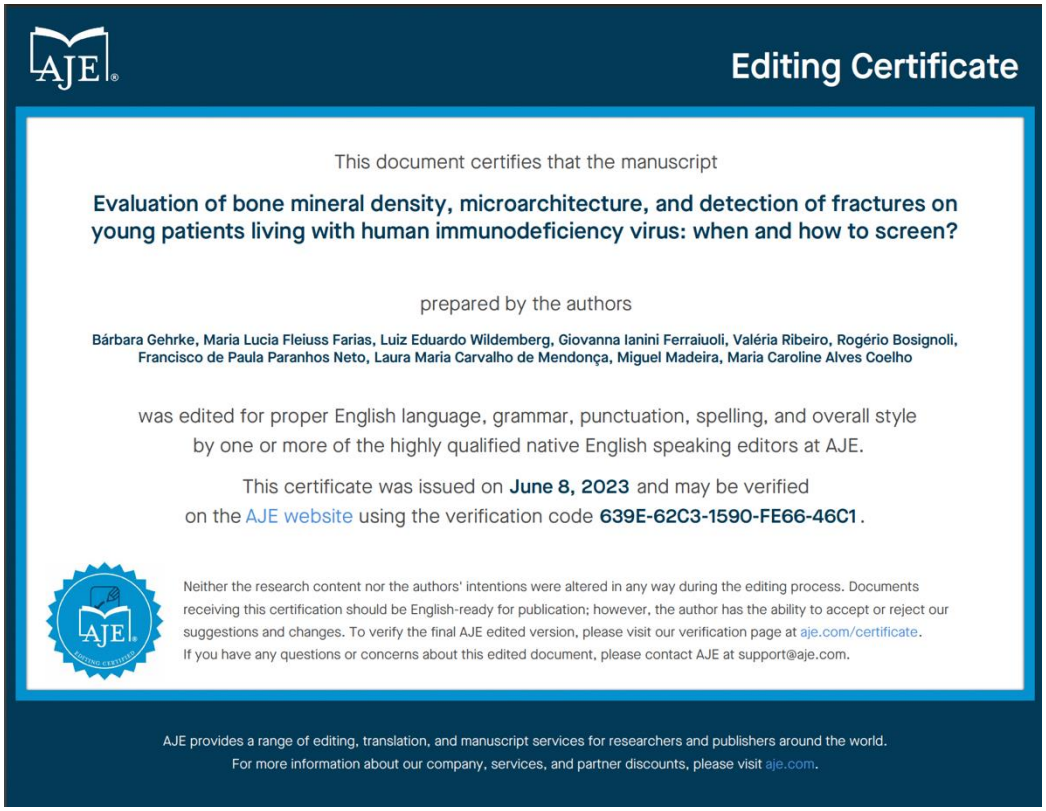
Título do artigo: "Evaluation of bone mineral density, microarchitecture, and detection of fractures on young patients living with human immunodeficiency virus: when and how to screen?”

Nome da revista: Endocrine

DOI: https://doi.org/10.1007/s12020-023-03501-9

Fator de impacto 2022: 3.7

Fator de impacto nos últimos 5 anos: 3.8



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prepared by the authors

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Endocrine
https://doi.org/10.1007/s12020-023-03501-9

ORIGINAL ARTICLE

Evaluation of bone mineral density, microarchitecture, and detection of fractures on young patients living with human immunodeficiency virus: when and how to screen?

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Received: 10 July 2023 / Accepted: 17 August 2023

Abstract

Purpose People living with the human immunodeficiency virus (PLWH) developed higher life expectancy along with chronic bone disease over the past years. Our purpose is to evaluate bone mineral density, bone microarchitecture and fractures in young PLWH and understand the disease's contribution to bone derangements and fracture risk.

Methods Eighty-one HIV-infected and 54 control young (20–50 years) male and female subjects were enrolled in this study. Methods for patient evaluation included DXA-VFA (dual energy X-rays and vertebral fracture assessment), HR-pQCT (high resolution peripheral quantitative computed tomography), biochemistry and FRAX.

Results Fifty participants from each group completed all exams. Median age was 40 (25–49) vs. 36.5 (22–50) for the HIV and control groups, respectively (p 0.120). Ethnicity, body mass index, serum phosphorus, 25-hydroxyvitamin D, PTH and CTX were similar between groups, although ALP and OC suggested higher bone turnover in PLWH. VFA identified morphometric vertebral fractures in 12% of PLWH. PLWH had lower values for lumbar spine areal BMD and Z score, volumetric BMD, trabecular bone fraction (BV/TV) and trabecular number measured at the distal tibia by HR-pQCT; as a consequence, trabecular separation and heterogeneity were higher (all $p < 0.05$). The FRAX-estimated risk for hip and major osteoporotic fractures was statistically higher in PLWH ($p < 0.001$).

Conclusion Our results confirm severe bone impairment and fractures associated with HIV in young patients. Thus, we developed a screening protocol for young PLWH to detect bone fragility, reduce skeletal disease progression and morbi-mortality, decrease fracture risk, and increase quality of life.

Keywords HR-pQCT · Bone density · DXA · VFA · Fractures · HIV

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Published online: 06 September 2023

Introduction

Human immunodeficiency virus (HIV) has been a challenge to the health system since the beginning of the 1980s [1, 2], and it was estimated that more than 38.4 million people were living with HIV infection worldwide in 2021 [3]. It has become a chronic and treatable condition, and currently, a young person who acquires the virus is expected to live almost as long as a person of the same age without it [4], which contributes to a higher incidence and prevalence of osteopenia and osteoporosis in people living with HIV (PLWH) [5].

Osteoporosis is a skeletal disorder characterized by low bone mass and deterioration of bone microarchitecture, predisposing the individual to fractures [6]. This condition usually leads to a worsening of quality of life and an increase in morbimortality, frequently observed in the elderly population. However, there is evidence of bone loss in PLWH since disease onset, and with an increase in life expectancy, they have been shown to be at risk for fractures due to severe osteoporosis at earlier ages [7, 8].

Osteopenia and osteoporosis are described in approximately 50% and 20% of PLWH, respectively [9]. PLWH are exposed to the traditional multifactorial risks for bone loss [10] and several risk factors associated with HIV, such as antiretroviral therapy (ART), $CD4^+$ T-cell counts, duration of infection and chronic illness, may be involved in the pathophysiology of bone disease in PLWH [9].

In HIV-infected patients, bone loss occurs due to several mechanisms: (a) B-cell dysfunction, increased production of proinflammatory cytokines [tumour necrosis factor-α (TNF-α), interferon-γ, interleukin (IL)-1, IL-6, IL-7, IL-17 and macrophage-colony stimulating factor (M-CSF)], enhanced osteoclast activity [11]; (b) changes in Wnt/β-catenin signalling [7, 11]; and (c) increased expression of receptor activator of nuclear factor kappa beta (RANKL) and reduced activity of osteoprotegerin (OPG) [11]. Figure 1 summarizes bone loss mechanisms in PLWH.

Currently, worldwide protocols recommend screening in PLWH following the same criteria as for the general population [12]. The aim of our study was to evaluate the bone properties of young PLWH by dual energy X-ray absorptiometry (DXA), including vertebral fracture assessment (VFA) and high-resolution peripheral quantitative computed tomography (HR-pQCT). Based on these results, we determined and proposed the most appropriate moment to screen for bone alterations in PLWH to prevent early loss and fragility fractures.

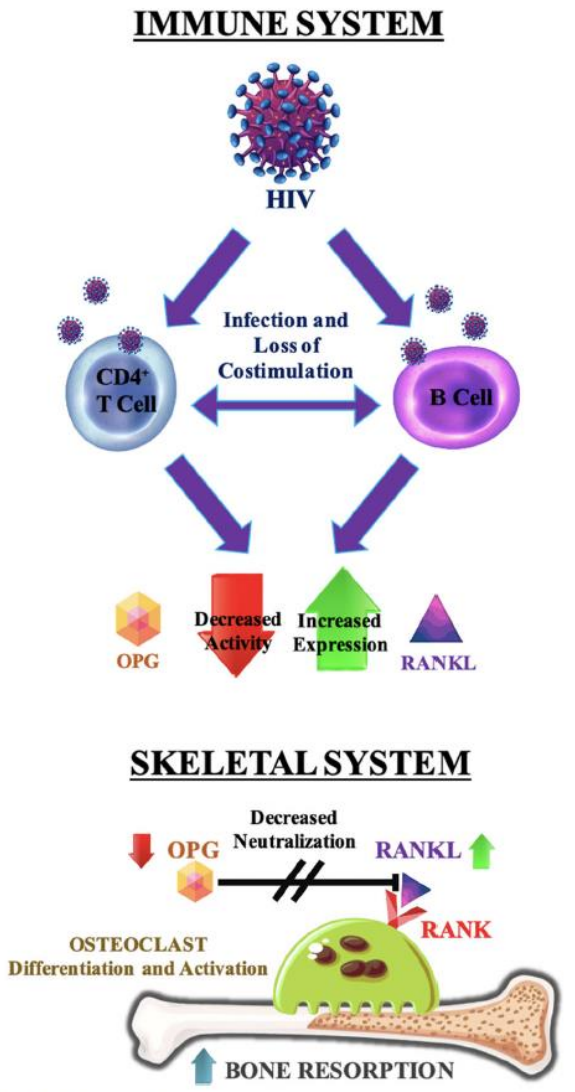

Fig. 1 Effects of Human immunodeficiency virus on immune and skeletal systems. The image describes the pathophysiology of HIV in the immune system and skeletal system. Its influence on B and T cells leads to a decreased activity of osteoprotegerin (OPG) and an increased expression of the ligand of receptor activator of nuclear factor kappa beta (RANKL), favouring differentiation and activation of osteoclasts and consequent increase in bone resorption

Methods

Study design and population

An observational and cross-sectional study was carried out from March 2020 to April 2023, and 135 participants (81 PLWH, 54 uninfected) of both sexes and aged 20–50 years were recruited. Participants were selected at random from infectious diseases and general internal medicine clinics at Hospital Universitário Pedro Ernesto (HUPE) in Rio de Janeiro. Eligible participants self-identified their race/ethnicity. PLWH and control subjects needed to be in good

health circumstances, presenting no preexisting conditions that may interfere with bone health.

Written informed consent was signed by each participant. Ethical standards were followed according to the Helsinki Declaration. The study protocol was approved by the Research Ethics Committee of HUPE (number: 29162020.1.0000.5259).

Data obtained for each subject included age, weight, and height to calculate body mass index (BMI: weight/height^2), alcohol intake (≥3 units/day), smoking habit (current smoker), comorbid conditions, current and previous ART, and cumulative duration of ART. Inclusion criteria for PLWH were diagnosis of HIV infection for at least one year, the need to attend medical appointments regularly (at least every six months), and the use of ART or not. In addition, women in both groups needed to have regular menstrual cycles. Data regarding the disease, such as viral charge, serum CD4$^+$ levels and infection duration, were obtained in PLWH.

Exclusion criteria were weight greater than 120 kilograms (Kg) due to DXA machine, menopause/premature ovarian failure, pregnancy, HIV/HCV coinfection, preexisting conditions [13] or use of medications that may interfere with bone health (such as chronic use of glucocorticoids), except ART. Subjects with a diagnosis of acquired immunodeficiency syndrome were excluded from our sample.

Biochemistry

The biochemical investigation was conducted to rule out conditions that affect bone metabolism. Calcium, phosphorus, magnesium, 25-hydroxyvitamin D [25(OH) D], parathyroid hormone (PTH) and albumin serum levels of participants were assessed to evaluate their bone status. Renal, thyroid, and hepatic functions and haemogram, sexual hormones (total testosterone, sex-hormone-binding globulin and oestradiol levels were dosed in men), prolactin, erythrocyte sedimentation volume, glucose, glycated haemoglobin, insulin, lipid profile and alkaline reserve were observed. The bone turnover markers C-terminal telopeptide of type 1 collagen (ß-CTX) and osteocalcin (OC) were also analysed. Twenty-four-hour urine with calcium, phosphorus, sodium, and creatinine dosage was requested. The method used for PTH, ß-CTX, and OC was noncompetitive chemiluminescence, and for 25(OH) D, we used competitive chemiluminescence with Maglumi X8 from Snibe (China). Values ≥ 30 ng/mL are considered adequate for PLWH [14].

Fracture risk assessment tool (FRAX)

The probability of fracture risk in ten years was assessed by FRAX (https://abrasso.org.br/calculadora/calculadora/) in all participants ≥40 years. HIV infection was considered a cause of "secondary osteoporosis" to adjust for risk probability. Patients with previous fractures were marked as "yes" at the risk tool. We used the risk calculator tool from the National Osteoporosis Guideline Group (NOGG) to classify patients as low, moderate or high risk.

Vertebral fracture assessment (VFA)

VFA was completed in the HIV group to screen for morphometric asymptomatic vertebral fractures. The semi-quantitative scale of Genant was used to identify preexisting fractures.

Areal bone density evaluation by DXA

Subjects underwent DXA using Prodigy-GE equipment (GE Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA) for areal bone mineral density (BMD) at radius 33%, femoral neck (FN), total hip (TH) and lumbar spine (L1-L4) assessment. The results are indicated as absolute values (g / cm^2) and standard deviations (SDs) from the expected mean BMD for the reference age-matched population (Z score). According to the International Society for Clinical Densitometry (ISCD), participants with a BMD *Z* score of ≤ −2.0 SD were classified as below the expected range for age. Male participants were <50 years old, and women were premenopausal; therefore, *T* scores were not used. The BMD coefficient of variation in our institution is 2.3% at the hip and 1.8% at the lumbar spine (LS). DXA scans were performed by the same operator and assessed by the same physician.

HR-pQCT

Volumetric density and bone microarchitecture were assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT) using X-treme CT (SCANCO Medical AG, Brüttisellen, Switzerland). The immobilized non-dominant distal radius and tibia of participants were imaged. Volumetric BMD was estimated for the entire bone [Tt. BMD], cortical bone [Ct. BMD] and trabecular bone [Tb.BMD]. Microstructural parameters included cortical thickness [Ct. Th: ratio of cortical bone volume to the outer bone surface], trabecular number [Tb. N], trabecular bone volume fraction [BV/TV, derived from trabecular density/1200 mg hydroxyapatite], trabecular thickness [Tb. Th: (BV/TV)/Tb. N], trabecular separation [Tb. Sp: (1-BV/TV)/Tb. N], and trabecular inhomogeneity [Tb.1/ N.SD, the standard deviation of 1/Tb. N, reflects the inhomogeneity of the network] [15]. This machine utilizes a 2-dimensional detector combined with a 0.08-mm point-focus X-ray tube, enabling the acquisition of numerous CT sections with a nominal resolution of 82 µm. At each site,

104 slices were obtained, forming a 9-mm 3-dimensional representation in the axial direction. The radiation dose is <3 μSv/measurement, comparable to DXA. The coefficient of variation for HR-pQCT in our institution is 3–5% for microarchitectural parameters and 1–2% for density measurements.

Statistical analysis

Statistical analysis was performed using SPSS version 29.0.0.0 (241) for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to evaluate the distribution pattern of numerical variables. Most variables did not follow the normality curve; therefore, we used nonparametric tests. Numerical variables are expressed as medians (minimum and maximum) and were analysed by Mann–Whitney or Student's t tests. Categorical variables were analysed by Pearson's chi-squared or Fisher's tests. Correlations between numeric variables were evaluated by Spearman tests. A p value < 0.05 was considered statistically significant.

Results

Fifty subjects in each group completed the study. Participants who did not complete all exams were excluded, as well as those who presented secondary causes for bone disease (four control subjects were excluded). All women were at menacme and presented regular menstrual cycles. Men with hypogonadism were excluded. Figure 2 shows the enrolment of PLWH.

The time since infection onset varied from 3 to 40 years, with a median value of 156 (36–480) months. Exposure to ART with tenofovir (TDF) varied from 0 to 252 months, with a median value of 96 (0–252) months. Of the 50 PLWH, 34% and 90% had a history or were currently using protease inhibitors (PIs) and/or TDF, respectively. Some PLWH had to discontinue TDF due to worsening of kidney function. Twenty-two percent of HIV-infected patients had a history of opportunistic infections. In this study, the serum CD4 count was 659.5 (65–1279) cells/mm^3.

There was no significant difference between groups with respect to sex, age, weight, height, BMI, alcohol intake and ethnicity. Three PLWH were current smokers, while there were no smokers in the control group. Dietary calcium intake varied from very low to as high as 1200 mg in the control group and 1500 mg in the HIV group (p 0.01). However, serum calcium (corrected for albumin) was lower in HIV patients ($p < 0.001$). Eighteen percent of PLWH presented high levels of PTH (>65 pg/mL) vs. 10% in the controls (highest serum levels were 102 pg/mL vs. 93.24 pg/mL, respectively), although no significant difference was found.

On the other hand, PLWH showed alterations in bone health. Serum OC and total alkaline phosphatase (ALP), although not CTX, were higher in HIV-infected, suggesting higher bone turnover. The risk of major osteoporotic and hip fractures evaluated by FRAX was higher in PLWH who also showed a 12% prevalence of morphometric vertebral fractures. Participants had no previous history of fracture or high-impact traumas.

FRAX and NOGG were calculated with and without FN BMD for participants with ≥40 years. In PLWH without FN BMD, 21 subjects (42%) presented moderate risk of fracture (indicating that they should proceed with DXA), while 3 subjects (6%) were high risk (those with vertebral fractures). When FN BMD was added to the tool, all 3 fractured patients changed to low risk, and 4 (8%) nonfractured HIV-infected patients changed from moderate to high risk. In the control group before FN BMD was added to the tool, 17 subjects (34%) were at moderate risk and none were at high risk. After including FN BMD, 2 subjects (4%) changed from moderate to high risk and the remaining became low risk.

These data are shown in Table 1.

Bone density and microstructure evaluated by DXA and HR-pQCT were also compromised in PLWH, as demonstrated in Table 2. LS BMD and Z score were significantly reduced in PLWH. Total vBMD was also lower at the distal tibia, mostly due to differences in trabecular BMD. Microstructure alterations detected in PLWH included lower bone fraction (BV/TV), trabecular number and thickness, and higher separation and heterogeneity of the trabecular network.

We studied separately the six HIV-infected that had vertebral morphometric fractures as evidenced by VFA. The four males and two females were young, 33 to 47 years old, but had long-term duration of infection and treatment, ranging from 72 to 264 months. None of them had BMD Z score values ≤−2 SD, suggesting low bone density for their age. These data are shown in Table 3. None of the fractured PLWH presented the history of significant weight loss.

Discussion

Our study identified morphometric vertebral fractures by VFA in 12% of young PLWH. With the results found, we propose a screening protocol for young PLWH. Late bone disease diagnosis might bring catastrophic consequences by increasing physical limitations and reducing working hours and functionality.

Our prevalence of vertebral fractures was higher than that encountered by Tan et al. [16], as they revealed an 8% prevalence of fractures in PLWH and none among controls. Their sample was composed of 23 HIV patients and 23

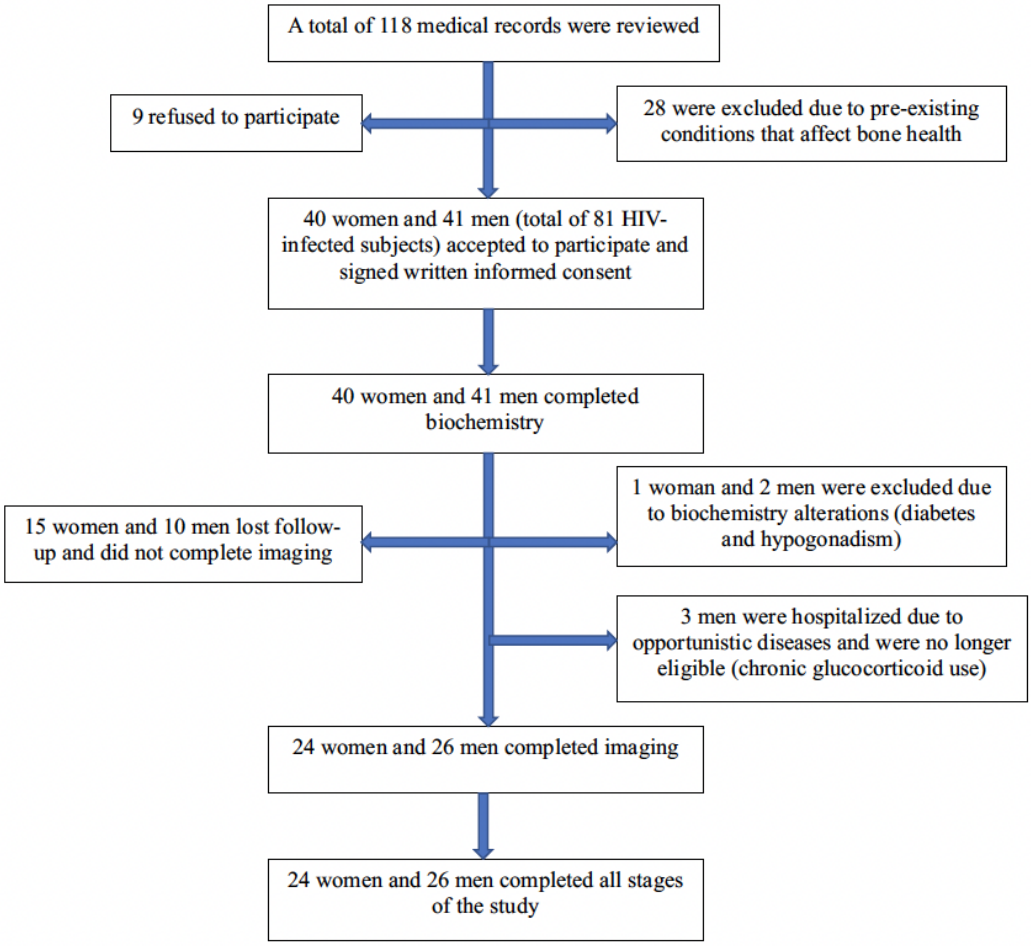

Fig. 2 Flowchart describing study design to recruit subjects for HIV-infected group

controls, predominantly men, aged between 45 and 56 years. Two studies revealed a prevalence similar to ours, with 11.1% (95% confidence interval, 4.5 to 25.0) and 12.4%, respectively [17, 18]. In the latter study, 70% of subjects who had vertebral fractures were in the non-osteoporotic range, and no significant increase in fracture prevalence was observed with BMD reduction [18]. It seems that in PLWH, DXA results are not compatible with the risk of fracture, underestimating bone fragility and fracture probability. In a prospective cohort study with 5826 PLWH, fracture rates were 1.98 to 3.69 times higher than those in the general population [19]. It is expected that fracture incidence at any site in PLWH varies from 0.1 to 8.4 per 1000 person-years, which is considered a high incidence when compared to the uninfected population [8]. A meta-analysis with >100.000 PLWH presented a higher prevalence of all fracture events (4.08% vs. 0.44%) and fragility fractures (2.66% vs. 2.19%) in affected individuals than healthy controls [20].

We diagnosed 3 male subjects aged 33 years who had vertebral fractures, probably associated with bone derangement and low peak bone mass at younger ages. They were diagnosed with HIV 6, 8 and 11 years before our study. Knowing that initial symptoms of the disease might take several years to appear, they could have been infected before or during the peak bone mass phase, which justifies higher bone fragility and fractures in early ages. The other 3 fractures occurred in patients aged ≥40 years. The oldest participant had an infection duration of 22 years, implying that the combination of ageing, low peak bone mass (disease onset at 25 years) and progressive bone loss (due to HIV and ART exposure) contributed to bone fragility and fracture.

The FRAX tool gave the risk probability of hip and major osteoporotic fractures in 10 years. Further analysis was carried out using the NOGG tool. Both FRAX and NOGG were higher when calculated without BMD in patients with fractures when compared to using FN BMD.

Table 1 Participants characteristics in HIV and control groups

	HIV group $n = 50$	Control group $n = 50$	p
Age (years)	40 (25–49)	36.5 (22–50)	0.120
Weight (Kg)	77.75 (50–103.5)	77.4 (54–110)	0.659
Height (cm)	166 (150–187)	169.5 (153–186)	0.473
BMI (Kg/cm^2)	26.9 (17.44–37.5)	27.7 (19.34–39.4)	0.459
Caucasian (%)	56	58	1.00
Gender	24 F / 26 M	25 F / 25 M	1.00
Serum calcium (mg/dL)	9.57 (8.52–10.6)	9.92 (9.04–10.66)	**<0.001**
Serum magnesium (mg/dL)	2.0 (1.7–2.4)	2.0 (1.8–2.7)	0.311
Serum phophorus (mg/dL)	3.8 (2.7–5.8)	3.9 (2.9–5.4)	0.568
25(OH) vitamin D (ng/mL)	27.7 (12.9–42.4)	27.9 (13.5–57.8)	0.936
PTH (pg/mL)	41.82 (15.87–102)	40.76 (14.45–93.24)	0.424
ß-CTX (ng/mL)	0.389 (0.175–0.897)	0.396 (0.111–0.640)	0.311
ALP (iU/L)	175 (63–494)	146 (35–350)	**0.003**
Osteocalcin (ng/mL)	20.32 (4.76–59.6)	17 (6.14–29.27)	**0.041**
Estradiol in men (pg/mL)	28.3 (3–79.2)	32 (10.3–129.6)	0.356
Total testosterone in men (ng/dL)	430 (270–820)	456 (269–811)	0.880
Free testosterone in men (ng/dL)	8.87 (6.62–13.7)	8.71 (6.64–25.77)	0.391
FRAX hip with BMD (%)	0.1 (0.0–0.6)	0.0 (0.0–0.8)	0.268
FRAX major with BMD (%)	1.9 (1.3–4.1)	1.75 (1.4–3.1)	0.077
FRAX hip without BMD (%)	0.2 (0.1–0.8)	0.1 (0.0–0.1)	**<0.001**
FRAX major without BMD (%)	2.3 (1.7–5.9)	1.6 (1.1–2.1)	**<0.001**

Variables are expressed in median values (minimum and maximum)

HIV Human immunodeficiency virus, *F* female, *M* male, *25 (OH) D* 25-hydroxi-vitamin D, *PTH* parathyroid hormone, *β-CTX* C-terminal telopeptide of type 1 collagen, *ALP* alkaline phosphatase, *FRAX* Fracture Risk Assessment Tool

Bold values show statistical significance ($p < 0.05$) of the analyzed variables

The hypothesis is that although they presented vertebral fractures, DXA results were not compatible with low bone mass; therefore, fracture risk seems to be lower than the actual risk for PLWH. The FRAX and NOGG results underestimate the real fracture risk in young PLWH who already suffered a fracture, as they do not take into consideration bone quality parameters. In PLWH who did not suffer previous fractures, both tools increased risk after considering FN BMD results.

Our study found that OC and ALP levels were higher (p 0.041 and p 0.003, respectively) in PLWH. All participants had normal hepatic parameters; therefore, we may infer that the increased levels of ALP are probably of bone origin. Although there was no statistical significance, the minimum and maximum ß-CTX serum levels of PLWH were higher than those of the control group [0.389 (0.175–0.897) vs. 0.396 (0.111–0.640), p 0.311]. These data are comparable to those found in the study by Bedimo et al. [21] and reinforce that the pathophysiology of impaired bone metabolism in PLWH is largely associated with increased bone turnover.

Furthermore, a study concluded that patients treated with ART present higher levels of OC, mainly those using TDF, when compared to those of untreated PLWH [22]. There were significantly higher serum levels of OC in PLWH, which might be explained by the fact that 90% of the group was exposed to TDF during ART treatment. Evidence shows that therapies containing PI induce increased osteoclast activity as well as a decrease in osteoblastogenesis, resulting in higher concentrations of pyridinoline and deoxypyridinoline crosslinks, resembling an increased bone turnover state. We found that longer periods of TDF exposure were inversely correlated with serum calcium, radius 33% BMD and some HR-pQCT parameters at the distal radius and tibia. In addition, therapies containing TDF mediate mitochondrial dysfunction, which causes an inflammatory state, increasing cytokine production and consequent osteoclast maturation and bone resorption [22].

There is evidence that the prevalence of vitamin D deficiency ranges widely among PLWH. Although some studies relate low vitamin D levels to longer ART duration and cumulative ART use in PLWH, we were not able to

Table 2 Results of dual energy X-ray absorptiometry (DXA) and high resolution peripheral quantitative computed tomography (HR-pQCT) of HIV and control groups

	HIV group	Control group	*p*
DXA			
LS BMD (g/cm^2)	1.177 (0.971 to 1.446)	1.271 (0.947 to 1.627)	**0.023**
LS *Z*-score (SD)	−0.55 (−2.1 to +2.4)	+0.2 (−1.9 to +2.3)	**0.021**
FN BMD (g/cm^2)	1.029 (0.822 to 1.417)	1.070 (0.774 to 1.444)	0.253
FN *Z*-score (SD)	−0.2 (−2.1 to +3.1)	+0.2 (−2.1 to +2.7)	0.275
TH BMD (g/cm^2)	1.048 (0.811 to 1.396)	1.093 (0.819 to 1.456)	0.208
TH *Z*-score (SD)	−0.2 (−2.3 to +3.2)	+0.1 (−2.4 to +2.4)	0.172
R 33% BMD (g/cm^2)	0.921 (0.779 to 1.133)	0.897 (0.504 to 1.114)	0.986
R 33% *Z*-score (SD)	−0.45 (−1.8 to +1.3)	−0.2 (−2.1 to +1.1)	0.409
HR-pQCT			
Distal radius			
Tt.BMD (mg HA/cm^3)	349.6 (220.8–519.8)	373.4 (233.5–500.2)	0.080
Ct.BMD (mg HA/cm^3)	936.3 (774.5–1034.1)	945.9 (830.5–1059.2)	0.362
Ct.Th (mm)	0.840 (0.470–1.370)	0.870 (0.470–1.360)	0.429
Tb.BMD (mg HA/cm^3)	176.4 (69–279.6)	177.9 (100.9–351.6)	0.110
BV/TV (1)	0.147 (0.057–0.233)	0.148 (0.084–0.293)	0.112
Tb.N (mm^{-1})	1.92 (1.20–2.62)	2.06 (1.50–2.59)	0.056
Tb.Th (mm)	0.072 (0.048–0.114)	0.077 (0.049–0.145)	0.184
Tb.Sp (mm)	0.442 (0.307–0.786)	0.415 (0.294–0.580)	**0.047**
Tb.1/N.SD (mm)	0.191 (0.109–0.515)	0.167 (0.111–0.341)	0.077
Distal tibia			
Tt.BMD (mg HA/cm^3)	314.3 (204.2–491.0)	345.7 (223.1–502.8)	**0.015**
Ct.BMD (mg HA/cm^3)	949.0 (841.5–1044.2)	977.1 (845.1–1037.2)	0.054
Ct.Th (mm)	1.30 (0.92–1.84)	1.32 (0.90–2.22)	0.272
Tb.BMD (mg HA/cm^3)	150.95 (84.3–291.4)	177.2 (91.7–280.5)	**0.005**
BV/TV (1)	0.126 (0.070–0.243)	0.148 (0.076–0.234)	**0.005**
Tb.N (mm^{-1})	1.72 (1.15–2.43)	1.94 (0.98–2.56)	**0.032**
Tb.Th (mm)	0.071 (0.044–0.113)	0.083 (0.045–0.111)	**0.012**
Tb.Sp (mm)	0.504 (0.329–0.798)	0.443 (0.303–930)	**0.020**
Tb.1/N.SD (mm)	0.235 (0.139–0.449)	0.194 (0.124–0.513)	**0.017**

BMD bone mineral density, *vBMD* volumetric bone mineral density, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *R 33%* Radius 33%, *Tt.BMD* vBMD of entire bone, *Ct.BMD* cortical vBMD, *Ct.Th* cortical thickness, *Tb.BMD* trabecular vBMD, *BV/TV* bone volume-to-total volume ratio, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation, *Tb.1/N.SD* inhomogeneity of trabecular network

Bold values show statistical significance ($p < 0.05$) of the analyzed variables

explain this association. Additionally, studies have considered the association between low vitamin D concentrations and disease progression, which was also not confirmed in our study. An association between lower vitamin D levels and the use of efavirenz, ritonavir, zidovudine and TDF has been described in the literature [23]. Despite similar 25(OH) D values between groups, 18% of PLWH had increased levels of PTH (>65 pg/mL) vs. 10% of the control group. PLWH presented calcium levels were statistically lower when compared to that of healthy subjects, which emphasizes the importance of maintaining 25(OH) D levels >30 ng/mL (as recommended for high-risk patients with comorbidities) to reduce bone fragility [14].

Most articles on PLWH and microarchitectural commitment study smaller samples or participants >50 years old, including postmenopausal women, which present alterations compatible with oestrogen drop [24]. Our study ruled out these adversities to understand the pathophysiology of bone disease throughout infection and ART. Table 4 shows several studies and their results.

Our study observed similar bone parameters at HR-pQCT in the distal radius in both groups, with only trabecular separation (Tb. Sp) differences, presenting higher values in PLWH than in the controls (*p* 0.047). However, several bone parameters at the distal tibia showed significant differences between groups. Yin et al. [24] studied

Table 3 Characteristics of HIV-infected participants that revealed morphometric vertebral fracture with VFA

Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)	33	33	33	40	43	47
Gender	Male	Male	Male	Female	Male	Female
BMI (Kg/h^2)	29.4	26	24	34.6	23.6	27.4
Time of infection (months)	72	132	96	252	240	264
25(OH) D (ng/mL)	32.8	27.8	26	26.8	38	18.9
Osteocalcin (ng/mL)	22.46	17,5	22.4	7.9	21	37.19
ß-CTX (ng/mL)	0.535	0.471	0.528	0.290	0.200	0.372
PTH (pg/mL)	28.79	67.56	37.47	54.74	33.91	49.09
Estradiol (pg/mL)	17.9	39.9	38.93	NA	18	NA
Total testosterone (ng/dL)	439	722	362	NA	421	NA
LS BMD (g/cm^2)	1.202	1.409	0.971	1.274	1.250	1.169
LS *Z*-score (SD)	−0.7	+0.9	−1.6	+0.4	+0.3	−0.4
TH BMD (g/cm^2)	1.122	1.261	0.982	1.052	1.169	0.865
TH *Z*-score (SD)	0.0	+0.8	−0.5	+0.3	+1.4	−0.2
FN BMD (g/cm^2)	1.143	1.330	0.994	1.036	1.125	0.956
FN *Z*-score (SD)	+0,3	+1,6	−0.2	+0.2	+1.1	−1.0
Radius 33% BMD (g/cm^2)	0.985	0.992	0.938	0.928	0.982	0.877
Radius *Z*-score (SD)	−0.2	0.0	−0.6	+0.5	+1.1	0.0
FRAX hip-fracture (%) with BMD	NA	NA	NA	0.1	0.1	0.2
FRAX major osteoporotic fracture (%) with BMD	NA	NA	NA	3.2	3.1	4.1
NOGG risk with BMD	NA	NA	NA	Low	Low	Low
FRAX hip-fracture (%) without BMD	NA	NA	NA	0.5	0.6	0.8
FRAX major osteoporotic fracture (%) without BMD	NA	NA	NA	4.0	5.0	5.9
NOGG risk without BMD	NA	NA	NA	High	High	High
Vertebral fracture location	L3	T11	T9	T9	T8	T10

VFA Vertebral Fracture Assessment, *25(OH) D* 25-hydroxi vitamin D, *PTH* parathyroid hormone, *β-CTX* C-terminal telopeptide of type 1 collagen, *BMD* bone mineral density, *LS* lumbar spine, *TH* total hip, *FN* femoral neck, *SD* standard deviation, *FRAX* Fracture Risk Assessment Tool, *NA* not applicable

postmenopausal women that showed similar results, affecting mainly the distal tibia.

In another study with younger subjects, pre-menopausal women (22 HIV and 44 controls), using HR-pQCT and DXA, showed lower trabecular density and trabecular number in HIV-infected (14.1% and 13.2%, respectively), with $p < 0.05$, and only the distal radius had lower cortical density (−3%), with statistical significance in PLWH [25]. DXA showed lower areal BMD at LS in HIV-infected women (LS *T* score −0.70 vs. −0.03, *p* 0.014), despite similar proximal femur BMD. Similarly, we found that LS BMD was statistically lower in PLWH.

A study with younger men ranging 22 years in both groups analysed changes at HR-pQCT and DXA (15 perinatal transmission, 15 adolescence-infected). DXA showed statistically significant lower BMD and *Z* scores at all sites (LS, hip, and radius). Abnormal trabecular and cortical microarchitecture results were seen in those with lower peak bone mass, predisposing to fragility fractures at early ages [26]. Another study with young male subjects (38 HIV-infected (18 perinatally infected, 20 adolescence-infected) and 20 uninfected, aged 20 to 25 years) also observed that areal BMD, *Z* scores and volumetric BMD measures were lower in PLWH. It seems that chronic immune activation and decreased osteoblast precursor numbers caused by infection contribute to lower peak bone mass [27]. These data agree with ours, as we also found that young PLWH presented compromised bone parameters and 12% had vertebral fractures.

Kazakia et al. [28]. demonstrated that there was a significantly lower bone quality at the distal tibia in PLWH compared to healthy controls (comparable to data found in our study), although DXA results presented no significant differences between groups. Similarly, a Brazilian study [29] compared male, ≥50-year-old HIV-infected ($n = 30$)

Table 4 Previous studies with HIV subjects and results

Authors	Year	Study	Sample	Age	Results
Yin, M.T. et al.	2013	*Observational and cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV + = 46 HIV− = 60 Post-menopausal women	HIV+ = 58 ± 1 years HIV− = 61 ± 1 years	Trabecular and cortical vBMD were similar at distal radius but cortical área and thickness at distal tíbia were 12% lower in HIV + ($p < 0.05$) Areal BMD *Z*-scores were lower in HIV+ at LS, TH and ultradistal radius.
Calmy, A. et al.	2013	*Observational and cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV+ = 22 HIV− = 44 Pre-menopausal women	HIV+ = 44.3 (38.9–46.4) years HIV− = 44.4 (41.3–46.1) years	Lower LS (*T*-score −0.70 vs −0.03, $p = 0.014$) at HIV+, but similar proximal femur BMD. At distal tibia, HIV+ women had 14.1 % lower trabecular density and a 13.2 % reduction in trabecular number compared to HIV− (p 0.013 and 0.029, respectively). HR-pQCT differences in distal radius were significant for cortical density (−3.0 %; p 0.029).
Yin, M.T. et al.	2014	*Observational and Cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV+ = 30 HIV− = 15 HIV+: 15 perinatal, 15 adolescence Men HIV+ more african-american (p 0.01)	HIV+ = 22.5 ± 0.3 years HIV− = 22.0 ± 0.5 years	Total and trabecular and vBMD, and cortical and trabecular thickness were between 6 and 19% lower in HIV+ than uninfected men ($p < 0.05$). aBMD *Z*-scores were 0.4–1.2 lower in HIV+ men at the LS, TH, and R ($p < 0.05$).
Biver, E. et al.	2014	*Observational and Cross-sectional* *HIV+ on ART* *HR-pQCT and DXA*	HIV+ = 28 HIV− = 112 Men	HIV+ = 64 (62–67) years HIV− = 64.6 (63.7–66.3) years	Lower areal BMD at TH (P 0.050) and ultra-distal radius (p 0.001) in HIV+. At distal radius and tibia, lower D100, Dtrab and cortical area. Also, lower trabecular number (P 0.036), higher trabecular spacing (p 0.027) and lower cortical thickness (p 0.008) at distal radius of HIV+.
Sellier, P. et al.	2016	*Observational and Cross-sectional* HIV+ on ART for at least 60 months HR-pQCT	HIV+ = 53 HIV− = 50 Men	HIV + = 49 ± 9 years HIV− = 52 ± 10 years	Lower volumetric trabecular bone density and disrupted trabecular micro-architectural parameters in HIV+.
Manavalan, J.S. et al.	2016	*Observational and Cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV+ = 38 HIV− = 20 HIV+: 18 perinatal, 20 adolescence, 20 adulthood Men	HIV+ = 22.6 ± 0.3 years HIV− = 22.3 ± 04 years	DXA-derived aBMD *Z*-scores and HRpQCT-derived vBMD measures were lower in HIV+. Impaired peak bone mass in HIV-infected precociously (perinatal).
Tan, D. et al.	2017	*Observational and Cross-sectional* HIV+ on ART DXA + HR-pQCT + VFA + TBS	HIV+ = 23 HIV− = 23 Majority male (78.3%)	HIV+ = 50 (45, 57) years HIV− = 49 (46, 56) years	Lower aBMD at the TH (median difference in *T*-score −0.25, p 0.04), but not the LS (median difference in *T*-score 0.10, p 0.68) in HIV+. Greater abnormalities in most HR-pQCT, by up to 15%, in HIV +. 2 subclinical fractures in HIV+.

Table 4 (continued)

Authors	Year	Study	Sample	Age	Results
Kazakia, G. J. et al.	2018	*Observational and Cross-sectional* HRpQCT + DXA + MRI	HIV+ = 8 HIV− = 11 Men	HIV+ = 55.5 ± 6.7 years HIV− = 61.5 ± 6.9 years	Significantly lower bone quality in HIV-infected patients compared to healthy controls. DXA areal BMD data showed no significant differences between HIV-infected patients and healthy controls.
MacDonald, H. et al.	2020	*Observational and Cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV+ = 50 (44% postmenopausal) HIV− = 50 (52% postmenopausal) Women	HIV + = 50.4 ± 1.2 years HIV− = 51.8 ± 1.2 years	For women ≥50 years ($n = 61$), TH aBMD *T*-score was lower among HIV+. Adjusted distal radius trabecular BMD and thickness and distal tibia trabecular BMD lower in HIV+ than controls ($p < 0.05$)
Foreman, S. C. et al.	2020	*Observational and Cross-sectional* HIV+ on ART HR-pQCT	HIV+ = 43 No control group Majority males (86%)	HIV+ = 57 (50–69) years	Regular exercise could help maintain or improve trabecular bone structure and bone strength while nutritional support is important for maintaining cortical bone structure. Patients on specific ART combinations such as TDF and PI may require close monitoring to assess bone loss and fracture risk.
Oliveira, F. P. et al.	2022	*Observational and Cross-sectional* HIV+ on ART HR-pQCT and DXA	HIV+ = 30 HIV− = 36 Males	Both groups > 50 years	Number of patients classified as osteoporosis (*T*-score) was higher among HIV+ when compared to controls (17.9% vs. 5.9%, *p* 0.011). HIV+ with significant alterations in cortical and trabecular bone, which were not associated with the duration of infection or ART. These differences in vBMD and bone microstructure persisted in nonosteoporotic HIV+ as compared to nonosteoporotic control subjects.

HIV Human immunodeficiency virus, *DXA* dual energy X-ray absorptiometry, *HR-pQCT* High Resolution peripheral quantitative computed tomography, *BMD* bone mineral density, *vBMD* volumetric bone mineral density, *aBMD* areal bone mineral density, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *R* radius 33%, *ART* anti-retroviral therapy, *TDF* tenofovir disoproxil fumarate, *PI* protease inhibitors, *TBS* trabecular bone score, *VFA* vertebral fracture assessment, *MRI* magnetic resonance imaging

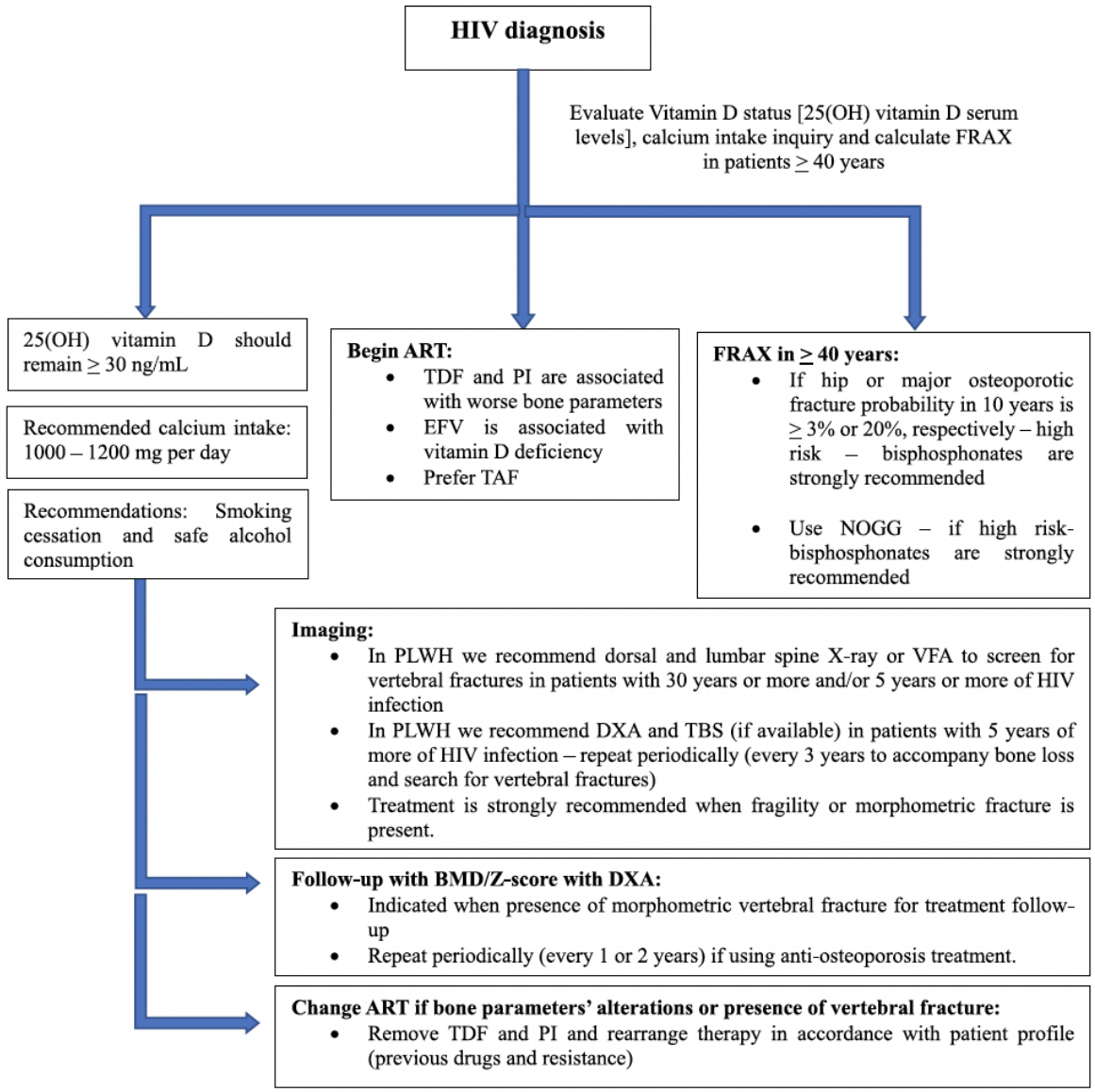

Fig. 3 Recommendations for screening of bone disease in young PLWH. Flowchart showing suggested screening protocol for bone disease in young HIV-infected patients. PLWH people living with HIV, HIV human immunodeficiency virus, 25(OH) D 25-hidroxi vitamin D, FRAX Fracture Risk Assessment Tool, ART anti-retroviral therapy, TDF tenofovir disoproxil fumarate, PI protease inhibitors, EFV efavirenz, TAF tenofovir alafenamide, NOGG National Osteoporosis Guideline Group, BMD bone mineral density, DXA dual energy X-ray absorptiometry, TBS trabecular bone score, VFA vertebral fracture assessment

and controls ($n = 36$) for DXA and HR-pQCT. Osteoporosis was more prevalent among PLWH (17.9 vs. 5.9%, p 0.011). Nevertheless, microarchitecture and quality differences were observed by HR-pQCT even in subjects who were not in the osteoporotic range and had HIV and bone derangements when compared to the controls [29].

According to Brown et al. [12], screening for bone disease in PLWH is recommended for men between 40–49 years and premenopausal women (≥40 years) using FRAX. DXA is recommended in postmenopausal women, men aged ≥50 years, patients with chronic use of glucocorticoids and/or at high-risk for falls or with previous fragility fractures. Nevertheless, we believe that an active search for vertebral fractures should be included in screening protocols, as the FRAX tool and DXA were not able to express fracture risk according to microarchitectural commitment

evidenced in young PLWH. Our suggested protocol is shown in Fig. 3 [30, 31].

HRp-QCT is available in a few research centres around the world, therefore limiting its routine use. It would have been interesting to assess bone quality by Trabecular Bone Score software with DXA to evaluate if the results correlate with what was found by HR-pQCT in PLWH. A previous study evidenced the association between lower TBS values and a higher prevalence of vertebral fractures [32].

Alendronate and zoledronate are the main drugs with proof of effectiveness and safety for osteoporosis treatment in elderly PLWH [33]. Few studies consider younger populations and there is scarce data regarding the effectiveness of therapy on fracture risk reduction [34, 35]. Also, the effectiveness and safety of denosumab (a fully human monoclonal antibody that inhibits RANKL) in PLWH remains unknown. Its use brings concern to secondary infections, although a small study with PLWH did not describe this adverse event [36].

A limitation of our study was that we did not perform VFA in healthy control subjects, as they had no complaint of dorsal symptoms or history of bone disease. We also found difficulties in identifying the start date of ART and determining the drugs used as subjects had long periods of disease with several therapeutic approaches due to drug resistance. The duration of the disease was based on its onset, although patients could have been infected much earlier, presenting few or no symptoms and delaying diagnosis. Finally, some patients had a history of therapy interruption due to poor adherence, which was also difficult to estimate. These factors interfere in bone disease progression in PLWH and must be elucidated whenever possible.

The data obtained in our study show that severe bone impairment and fragility (with consequent fractures) occur in young PLWH. New protocols are needed, particularly in young PLWH where prediction tools have not been able to quantify fracture risk adequately. We developed a screening protocol for young PLWH that includes evaluation of bone metabolism and risk factors for fragility fractures and spinal imaging to identify early low bone mass and vertebral fractures. An early detection of an elevated risk of fractures will enable preventive strategies.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by B.G., M.L.F.F., L.E.W., G.I.F., V.R., R.B., F.de P.P.N., L.M.C.de M., M.M. and M.C.A.C. The first draft of the manuscript was written by Bárbara Gehrke and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent to participate Written informed consent was obtained from all individual participants included in the study.

Ethics All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of the Hospital Universitário Pedro Ernesto from Universidade do Estado do Rio de Janeiro and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. W.C. Greene, A history of AIDS: looking back to see ahead. Eur. J. Immunol. **37**(1), S94–S102 (2007)
2. O. Sued, P. Cahn, Latin America priorities after 40 years of the beginning of the HIV pandemic. Lancet Reg. Health Am. **1**, 100024 (2021)
3. Global HIV & AIDS statistics - Fact sheet UNAIDS [Available from: (https://www.unaids.org/en/resources/fact-sheet)
4. M.T. Yin, T.T. Brown, HIV and bone complications: understudied populations and new management strategies. Curr. HIV/AIDS Rep. **13**(6), 349–58. (2016)
5. F. Finnerty, K. Walker-Bone, S. Tariq, Osteoporosis in postmenopausal women living with HIV. Maturitas **95**, 50–54 (2017)
6. J.Y. Reginster, C. Beaudart, F. Buckinx, O. Bruyere, Osteoporosis and sarcopenia: two diseases or one? Curr. Opin. Clin. Nutr. Metab. Care **19**(1), 31–36 (2016)
7. A.N. Ahmad, S.N. Ahmad, N. Ahmad, HIV infection and bone abnormalities. Open Orthop. J. **11**, 777–784 (2017)
8. M.O. Premaor, J.E. Compston, The hidden burden of fractures in people living with HIV. JBMR **2**(5), 247–256 (2018)
9. E. Negredo, A. Bonjoch, B. Clotet, Management of bone mineral density in HIV-infected patients. Expert Opin. Pharmacother. **17**(6), 845–852 (2016)
10. P. Sellier, A. Ostertag, C. Collet, H. Trout, K. Champion, S. Fernandez et al. Disrupted trabecular bone micro-architecture in middle-aged male HIV-infected treated patients. HIV Med. **17**(7), 550–556 (2016)
11. K. Titanji, Beyond antibodies: B cells and the OPG/RANK-RANKL pathway in health, Non-HIV disease and HIV-induced bone loss. Front Immunol. **8**, 1851 (2017)
12. T.T. Brown, J. Hoy, M. Borderi, G. Guaraldi, B. Renjifo, F. Vescini et al. Recommendations for evaluation and management of bone disease in HIV. Clin. Infect. Dis. **60**(8), 1242–1251 (2015)
13. E. Stein, E. Shane, Secondary osteoporosis. Endocrinol. Metab. Clin. North Am. **32**(1), 115–134 (2003).
14. C.A. Moreira, C. Ferreira, M. Madeira, B.C.C. Silva, S.S. Maeda, M.C. Batista et al. Reference values of 25-hydroxyvitamin D revisited: a position statement from the Brazilian society of endocrinology and metabolism (SBEM) and the Brazilian society of clinical pathology/laboratory medicine (SBPC). Arch. Endocrinol. Metab. **64**(4), 462–478 (2020)
15. D.E. Whittier, S.K. Boyd, A.J. Burghardt, J. Paccou, A. Ghasem-Zadeh, R. Chapurlat et al. Guidelines for the assessment of bone density and microarchitecture in vivo using high-resolution peripheral quantitative computed tomography. Osteoporos. Int. **31**(9), 1607–1627 (2020)

16. D.H. Tan, J. Raboud, L. Szadkowski, E. Szabo, H. Hu, Q. Wong et al. Novel imaging modalities for the comparison of bone microarchitecture among HIV+ patients with and without fractures: a pilot study. HIV Clin. Trials **18**(1), 28–38 (2017)
17. T. Ilha, F.V. Comim, R.M. Copes, J.E. Compston, M.O. Premaor, HIV and vertebral fractures: a systematic review and metanalysis. Sci. Rep. **8**(1), 7838 (2018)
18. L. Gazzola, A. Savoldi, F. Bai, A. Magenta, M. Dziubak, L. Pietrogrande et al. Assessment of radiological vertebral fractures in HIV-infected patients: clinical implications and predictive factors. HIV Med. **16**(9), 563–571 (2015)
19. B. Young, C.N. Dao, K. Buchacz, R. Baker, J.T. Brooks, Investigators HIVOS. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000–2006. Clin. Infect. Dis. **52**(8), 1061–1068 (2011)
20. C.J. Chang, Y.L. Chan, I. Pramukti, N.Y. Ko, T.W. Tai, People with HIV infection had lower bone mineral density and increased fracture risk: a meta-analysis. Arch. Osteoporos. **16**(1), 47 (2021)
21. R. Bedimo, J. Cutrell, S. Zhang, H. Drechsler, A. Gao, G. Brown et al. Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. AIDS **30**(4), 601–608 (2016)
22. M.C. Vlot, M.L. Grijsen, J.M. Prins, R.T. de Jongh, R. de Jonge, M. den Heijer et al. Effect of antiretroviral therapy on bone turnover and bone mineral density in men with primary HIV-1 infection. PLoS One **13**(3), e0193679 (2018)
23. E.A. Yu, S. Mehta, Vitamin D and HIV. In: Mehta S, Finkelstein JL, editors. Nutrition and HIV: epidemiological evidence to public health. New York (NY)2018. p. 153-89.
24. M.T. Yin, A. Shu, C.A. Zhang, S. Boutroy, D.J. McMahon, D.C. Ferris et al. Trabecular and cortical microarchitecture in postmenopausal HIV-infected women. Calcif. Tissue Int. **92**(6), 557–565 (2013)
25. A. Calmy, T. Chevalley, C. Delhumeau, L. Toutous-Trellu, R. Spycher-Elbes, O. Ratib et al. Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women. Osteoporos. Int **24**(6), 1843–1852 (2013)
26. M.T. Yin, E. Lund, J. Shah, C.A. Zhang, M. Foca, N. Neu et al. Lower peak bone mass and abnormal trabecular and cortical microarchitecture in young men infected with HIV early in life. AIDS **28**(3), 345–353 (2014)
27. J.S. Manavalan, S. Arpadi, S. Tharmarajah, J. Shah, C.A. Zhang, M. Foca et al. Abnormal bone acquisition with early-life HIV infection: role of immune activation and senescent osteogenic precursors. J. Bone Min. Res. **31**(11), 1988–96. (2016)
28. G.J. Kazakia, J. Carballido-Gamio, A. Lai, L. Nardo, L. Facchetti, C. Pasco et al. Trabecular bone microstructure is impaired in the proximal femur of human immunodeficiency virus-infected men with normal bone mineral density. Quant. Imaging Med Surg. **8**(1), 5–13 (2018)
29. F.P. Oliveira, L.F.C. Lima, F. de Paula Paranhos Neto, L.M.C. de Mendonca, A. Schtscherbyna, L.A.A. de Lima et al. Lower bone density and microarchitecture alterations in HIV-infected Brazilian men aged 50 years and older are associated with estradiol levels. Clin. Endocrinol. (Oxf.) **97**(1), 142–149 (2022)
30. J.P. Bilezikian, L. Bailey, P.J. Elmer, M.J. Favus, V.L.W. Go, S.R. Goldring et al. Optimal calcium intake. JAMA **272**(24), 1942–1948 (1994)
31. T. Welz, K. Childs, F. Ibrahim, M. Poulton, C.B. Taylor, C.F. Moniz et al. Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. Aids **24**(12), 1923–1928 (2010)
32. L. Ciullini, A. Pennica, G. Argento, D. Novarini, E. Teti, G. Pugliese et al. Trabecular bone score (TBS) is associated with subclinical vertebral fractures in HIV-infected patients. J. Bone Min. Metab. **36**(1), 111–118 (2018)
33. E. Negredo, A.H. Warriner, Pharmacologic approaches to the prevention and management of low bone mineral density in HIV-infected patients. Curr. Opin. HIV AIDS **11**(3), 351–357 (2016)
34. S. Ferrari, M.L. Bianchi, J.A. Eisman, A.J. Foldes, S. Adami, D.A. Wahl et al. Osteoporosis in young adults: pathophysiology, diagnosis, and management. Osteoporos. Int **23**(12), 2735–2748 (2012)
35. O. Makitie, M.C. Zillikens, Early-onset osteoporosis. Calcif. Tissue Int **110**(5), 546–61. (2022)
36. P. Makras, P. Petrikkos, A.D. Anastasilakis, A. Kolynou, A. Katsarou, O. Tsachouridou et al. Denosumab versus zoledronate for the treatment of low bone mineral density in male HIV-infected patients. Bone Rep. **15**, 101128 (2021)

3.2 **Artigo científico a ser submetido para publicaçao em periódico indexado**

Título do artigo: "The importance of muscle strength and physical performance as part of the diagnosis and management of sarcopenia in young adults living with human immunodeficiency virus”

TITLE: The importance of muscle strength and physical performance as part of the diagnosis and management of sarcopenia in young adults living with human immunodeficiency virus

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Acknowledgments: The authors have nothing to declare.

Conflict of Interest Disclosure Statement: Bárbara Gehrke, Maria Lucia Fleiuss Farias, Luiz Eduardo Wildemberg, Giovanna Ianini Ferraiuoli, Valéria Ribeiro, Rogério Bosignoli, Francisco de Paula Paranhos Neto, Laura Maria Carvalho de Mendonça, Miguel Madeira, Maria Caroline Alves Coelho declare that they have no conflict of interest.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions: All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; been involved in drafting of the manuscript or revised it critically for important intellectual content; approved the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Compliance with ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of the Hospital Universitário Pedro Ernesto from Universidade do Estado do Rio de Janeiro and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Written informed consent was obtained from all individual participants included in the study.

ABSTRACT:

Objective: To evaluate muscle functionality and performance and body composition in young people living with human immunodeficiency virus (PLWH) and define screening protocols for sarcopenia in this population.

Methods: Eighty-one HIV-infected and 54 uninfected (20 to 50 years) male and female subjects were enrolled to participate. Patient evaluation included body composition by DXA (dual energy X-rays), SARC-F questionnaire, hand grip test and timed up & go.

Results: Fifty PLWH and 50 age-gender matched controls completed the study. The median age was 40 (25–49) vs. 36.5 (22–50) for the HIV and control groups, respectively (p 0.120). Race, gender, body mass index, phosphorus and 25-hydroxyvitamin D were similar between groups. HDL-c was significantly lower in HIV-infected (p 0.006). Groups had similar body composition parameters, although more PLWH presented appendicular lean mass (ALM) and ALM adjusted to height (ALM/h^2) below reference values (18% vs 4%). SARC-F questionnaire and TUG were significantly compromised in HIV-infected when compared to controls (p 0.001 and 0.005, respectively). Hand grip test was slightly lower in PLWH than in control group (29.0 Kg (9.3 – 56.0) vs. 32.8 Kg (13.3 – 57.3); p 0.052).

Conclusion: Our results confirm that there is loss of functionality, physical performance and muscle strength in young HIV-infected. Therefore, we developed a screening protocol with new cut-off values considering a younger population of HIV-infected that are at high-risk for sarcopenia. With early diagnosis we may decrease muscle dysfunction, morbimortality, providing an increase in quality of life and working hours.

Key words: Body composition; Sarcopenia; Muscle strength; Appendicular lean mass; HIV

INTRODUCTION:

People living with human immunodeficiency virus (PLHIV), nowadays, present higher life expectancy thanks to the development of antiretroviral therapies (ART). This population have almost the same life expectancy as healthy individuals and they may develop chronic diseases, such as osteoporosis, cardiovascular disease, diabetes, and sarcopenia more frequently (1). The prevalence of sarcopenia in PLHIV is around 30% and this number is similar to the prevalence in individuals with cardiovascular disease, dementia, diabetes mellitus and respiratory disease in the general population (1, 2).

Sarcopenia consists of a generalized and progressive skeletal muscle disease that may culminate in adverse outcomes such as: reduced functionality and physical performance and higher risk of falls and fractures, therefore, increased morbimortality (3). Sarcopenia is frequent in elderly individuals, but it may be recognized in earlier stages of life, mainly in people with risk factors and comorbidities. There is evidence in literature that young PLHIV present higher prevalence of sarcopenia when compared to healthy controls, which may vary between 17.5 and 21.9% in women and men, respectively (4, 5).

The period of highest muscle strength is observed in healthy individuals during young adulthood (up to 40 years), naturally lower in women when compared to men (6). Muscle strength decline occurs around 16.6 to 40.9% in >40 years when compared to younger population (6).The diagnosis of sarcopenia is identified by low muscle strength and confirmed by low muscle quantity or quality. These two associated with low physical performance classify sarcopenia as severe (3).

There are several risk factors that contribute for the development of sarcopenia in PLHIV such as use of ART and chronic immune activation caused by virus leads to a low-grade systemic inflammation (LGSI – low grade systemic inflammation), which can reduce the activity of substances responsible for the synthesis of muscle proteins and lead to a dysfunction of the adipose tissue, with ectopic fat accumulation. Antiretrovirals promote mitochondrial damage through increased muscle inflammation and, by direct action, can reduce gene expression of proteins involved in protein synthesis. In addition, they can lead to changes in body composition (Figure 1).

There is also evidence that Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL) plays an important role in the inhibition of miogenic differentiation. PLHIV present increased expression of RANKL, favouring bone and muscle loss and dysfunction (7).

The objective of our study is to comprehend the different tools that may be used to diagnose sarcopenia in young PLHIV and to understand the best moment to start screening this condition in a population considered at high-risk.

METHODS:

Study design and population

We performed a cross-sectional study in which 135 participants (81 PLHIV and 54 uninfected) were recruited to participate. The study was carried out during the period of March 2020 and April 2023. Inclusion criteria were men and women with age between 20 and 50 years. All participants signed written informed consent and the study's ethical standards were in accordance with the Helsinki Declaration. Study protocol was approved by the Research Ethics Committee of Hospital Universitário Pedro Ernesto (HUPE/State University of Rio de Janeiro, Brazil) - number 29162020.1.0000.5259.

PLHIV were enrolled from an outpatient clinic for infectious diseases at HUPE. These participants needed to have the diagnosis of HIV infection for at least one year, could be treating with ART or not, and needed to attend medical appointments at least every six months. Data concerning infection and treatment were gathered in HIV-infected subjects. Healthy control participants were recruited from the internal medicine outpatient clinic at HUPE.

Exclusion criteria were menopause or premature ovarian failure, low testosterone levels in males, glomerular filtration rate below 60 mL/min/1.73m^2, weight superior to 120 kilograms (Kg) (due to equipment maximum capacity), pregnancy, use of medications that may interfere with muscle and bone health (chronic use of glucocorticoids, anticonvulsants, proton pump inhibitors, loop diuretics) and preexisting conditions that may affect muscle and bone metabolism (endocrine, liver, kidney, rheumatologic and hematologic diseases, tubular renal acidosis, malabsorptive intestinal syndromes or bariatric surgery, and neoplasms).

Biochemistry

Blood was collected during fast for routine tests (haemogram, glucose, glycated hemoglobin, lipid profile, urea, creatinine, alkaline reserve, hepatic function), free T4, TSH, 25 hydroxyvitamin D [25(OH) D] and sexual hormones (oestradiol, testosterone and sex hormone binding globulin (SHBG) in men). 25(OH) D was measured by competitive chemiluminescence

(Maglumi X8 from Snibe, China). Recommended levels were ≥20 ng/mL for healthy controls and ≥30 ng/mL for those at risk of fractures, which include PLHIV (8).

Physical exam

Anthropometric data was collected, such as: weight (Kg), height (m) and body mass index (BMI - calculated by the reason of weight over squared height in Kg/m^2).

Evaluation of muscle parameters and performance

As detailed below, lean mass was evaluated by dual energy X-ray absorptiometry (DXA) whereas muscle function and performance were evaluated by the SARC-F questionnaire, hand grip test and timed up & go (TUG), as recommended by the European Working Group on Sarcopenia in Older People (EWGSOP2) (3).

- *SARC-F questionnaire*

SARC-F consists of a self-reported questionnaire used for screening of sarcopenia risk. Patients that score ≥4 are likely to have sarcopenia, according to literature, in elder individuals (3, 9). In our sample, which consists of young subjects, we considered altered whenever they scored ≥1. Figure 2 shows SARC-F questionnaire.

- *Timed Up & Go (TUG)*

This test evaluates the participant's physical performance. The time taken to complete the movement is considered altered when >20 seconds for elder individuals (10). In our sample, we used a cut-off value of >10 seconds to consider the test altered.

- *Hand Grip Test*

The hand grip test evaluates muscle strength. Each participant exerted their highest muscular strength capacity with dominant arm using a handheld calibrated manual dynamometer (Jamar Model 1 – 70729, Asimow Engineering Company, Santa Monica, CA, USA), according to the recommendations of the American Association of Hand Therapists (11). Grip strength is considered weak when <26Kg for men and <16Kg for women, determined by the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project (12). Intermediate grip strength is classified between 26 – 31.9Kg in men and 16 – 19.9Kg in women (12). In our sample, we considered altered muscle strength when <32 Kg for men and <20 Kg for women.

Body composition by DXA

The evaluation of lean mass was performed by DXA with the objective of determining muscle quantity. Lean mass measured at upper and lower limb was used to calculate appendicular lean mass (ALM) in Kg. ALM was then adjusted to height by calculating the reason of ALM and squared height (Kg/m^2) (13). We used cut-offs suggested by the EWGSOP2 and based on Baumgartner's operational definition, which correspond to > 2 standard deviations (SD) below the mean reference values for young controls with ages between 18 and 40 years (Z score) , in which abnormal results for appendicular lean mass index (ALMI = ALM/height^2) are <5.5 Kg/m^2 and <7.00Kg/m^2 and ALM are <15 Kg and <20 Kg, for women and men, respectively (3, 14, 15). We also analyzed: fat, lean and tissue mass in grams (g); fat region and fat tissue (%); visceral adipose tissue and subcutaneous adipose tissue area, volume, and mass (cm^2, cm^3 and g, respectively). Fat mass index (FMI) was calculated by the reason of weight (Kg) over squared height (m^2) (16).

Diagnosis of sarcopenia

According to the recommendations of EWGSOP2, the criterion used to classify as probable sarcopenia was low muscle strength (altered hand grip test) and diagnosis of sarcopenia is confirmed by low muscle quantity (low appendicular lean mass by DXA). The condition is considered severe when both criteria are achieved in association with low physical performance (altered TUG) (3).

Statistical analysis

We used SPSS version 29.0.0.0 (241) for Windows (SPSS Inc., Chicago, IL, USA). Non-parametric tests were used as the Kolmogorov-Smirnov test revealed that most numerical variables didn't respect normality curve. The study compared results between healthy subjects and PLHIV. Chi-squared test or Fisher's test were used to compare categoric variables. Numerical variables used Mann-Whitney or Student t tests. Correlations between numeric variables were evaluated by Pearson or Spearman tests. Strength degrees of correlation were based on correlation coefficient (r): very weak (0.00 – 0.19), weak (0.20 – 0.39), moderate (0.40 – 0.69), strong (0.70 – 0.89) and very strong (0.90 – 1.00). Differences were considered statistically significant when α=5% ($p < 0.05$).

RESULTS:

Medical records of 135 young PLHIV on current treatment at our institution were evaluated. After signed consent, they were submitted to laboratory tests, in which abnormalities leaded to exclusion (four controls were excluded due to hypogonadism or diabetes, 6 PLHIV were excluded due to chronic glucocorticoid use/hospitalization, hypogonadism or diabetes, while 25 PLHIV lost follow-up) and scheduled for physical function tests and DXA. Fifty PLWH and 50 age-gender matched controls completed the study with SARC-F questionnaire, TUG, hand grip test and body composition.

PLHIV consisted of 24 women and 26 men, median age 40 years (25-49). Duration of HIV infection was estimated as 156 (36-480) months, exposure to tenofovir as 96 (0 – 252) months, and serum CD4+ was 659.5 (65 – 1279) cell/mm^3.

Groups were similar in respect to age, race, gender, BMI, serum phosphorus and vitamin D levels. The only metabolic difference was a lower HDL-cholesterol (HDL-c) in HIV-patients. Median BMI was compatible with overweight in both groups. However, PLHIV performed worse on physical tests, as shown in Table 1.

Table 2 presents body composition results obtained with DXA. All analyzed parameters were similar between groups either those related to fat mass distribution and visceral fat, or those related to muscle mass. There was a high prevalence of overweight and obesity in both groups according to BMI, 38% of PLHIV and 60% of controls were overweight, while obesity was diagnosed in 24% of PLHIV (14% grade 1 and 10% grade 2) and 20% of controls (14% grade 1 and 6% grade 2); only one male patient was underweight. In PLHIV FMI revealed 1 female with mild fat deficit, 32% had normal values, 40% excess fat, 18% were obese class 1 and 8% obese class 2. In control group FMI was classified as 31.25% normal, 47.9% excess fat, 16.6% obese class 1 and 4% obese class 2.

Table 3 shows the alterations of muscle function tests, ALM and ALM/h^2 in both groups. SARC-F questionnaire was considered altered when the participant scored 1 or more and revealed statistical significance in between groups; only 3 HIV-infected women scored 4 in SARC-F. In HIV-infected group 8 subjects scored 1, 6 scored 2, 1 scored 3 and 3 scored 4 (considered high-risk for sarcopenia). Among control subjects 4 scored 1 and 1 scored 2. TUG duration was considered inadequate when >10 seconds and was significantly higher in PLHIV. None of the participants had TUG result superior to 20 seconds, however 32% of HIV-infected patients vs 6% of control subjects had TUG test >10 seconds. Despite similar total lean mass,

the proportion of PLWH (18%) with ALM below sex-specific normal range was higher than in control group (4%) (with ALM and/or ALM/h^2 below reference values).

Five (10%) of PLWH presented intermediate grip strength while seven (14%) evidenced weak grip strength. Therefore, 14% of our HIV-infected sample presented probable sarcopenia. It is important to highlight that these cut-off points take into consideration an elder population as reference. Thus, if we use a less rigid criterion and expand our cut-off to <32 Kg in men <20 Kg in women, 24% of PLHIV evidenced altered results and were classified as probable sarcopenia. Eight percent of PLHIV confirmed sarcopenia with low muscle strength (weak or intermediate hand grip) and low muscle quantity (ALM and/or ALM/h^2 below reference values), while 2% by the more rigid criteria had sarcopenia (weak hand grip plus low muscle mass). In control group 2% presented weak grip strength classifying as probable sarcopenia. Considering a less rigid criterion, 4% had intermediate grip strength, adding up 6% of control subjects with probable sarcopenia. None of the control subjects confirmed diagnosis of sarcopenia with concomitant low muscle quantity by both criteria (more or less rigid). The lowest value of muscle strength for women in HIV group was 9.3 Kg vs. 13.3 Kg for a woman in control group.

The population was studied for statistical correlations for all variables analyzed in the study. The parameters that showed significant correlations are exposed in Table 4.

DISCUSSION:

Considering that frequently used definitions for diagnosis of sarcopenia are mainly for elderly populations, the results found are substantial for a younger group. We believe that the use of functional muscle tests to screen for muscle disorders and progressive loss of functionality in young PLHIV are of extreme importance. The fact that in literature we only dispose of cut-off values that contemplate older populations, highlight the need of new protocols with individualized cut-off values focused on younger individuals. Also, more studies are needed to understand the role of ALM and ALM/h^2 in body composition assessment of young HIV-infected. Our study is the first to suggest a screening protocol for young PLWH considering individualized cut-off values, according to the results found.

SARC-F questionnaire was applied to screen sarcopenia and data evidenced p 0.001, considering altered results whenever subjects score $\geq$1, with higher results for PLHIV. This

underscores that PLWH may develop physical limitations and higher morbidity when compared to healthy individuals with similar age. However, of twelve (24%) participants from HIV-infected group that presented altered results for hand grip strength, only 5 (10%) had at least one concomitant mobility or physical functionality complaint while answering the questionnaire. For this reason, SARC-F questionnaire might not be the best tool for initial screening of sarcopenia in young PLHIV, as it may underestimate diagnosis considering its focus on elderly individuals. This tool is intended to identify people at risk for sarcopenia and PLHIV are already considered at high-risk(17).

Also, altered TUG results with predominance in PLWH, considering >10 seconds cut-off value, evidence a higher compromise of mobility and physical performance in young PLHIV (p 0.005). When comparing the number of subjects with TUG >10 seconds in each group, statistical significance was maintained (p 0.002).

In reference to muscle strength evaluation, considering the higher cut-off values, probable sarcopenia was statistically higher in PLHIV when compared to controls (p 0.023). EWGSOP2 highlight that low muscle strength as a determinant of sarcopenia diagnosis has gained force recently, outweighing the importance of muscle mass at diagnosis (3). Also, they concluded that although sarcopenia is associated with decreased muscle quantity and quality, muscle strength is better than the quantification of muscle mass to predict undesirable outcomes (3).

Results from this study confirm decreased functionality and mobility in young PLHIV, which may be related to lower appendicular lean mass. Although median values of body composition parameters did not differ between PLHIV and controls, the proportion of PLWH with ALM and ALM/h2 below reference values was higher than that found in controls, although not statistically significant. Higher BMI, serum CD4 values and proportion of individuals in ART are factors that may attenuate the difference of muscle mass in PLHIV when compared to controls (2).

Therefore, we suggest that simple and unexpensive tools, such as SARC-F questionnaire to screen for functionality and mobility commitment (cut-off $\geq$1), TUG to estimate physical performance (cut-off value >10 seconds) and hand grip test to estimate muscle strength (cut-off values <32 Kg and <20 Kg for men and women, respectively) must be used in young PLHIV since disease onset to diagnose and treat sarcopenia, avoiding unwanted outcomes such as higher morbidity and progressive loss of functionality at younger ages (3).

Several studies have shown the association of PLHIV and ART with low muscle mass, sarcopenia and fat redistribution (5, 18-21). A systematic review and metanalysis of 2022

concluded that the prevalence of sarcopenia in 18 years or older (mean ages from 33.0 to 62.1 and from 24.2 to 60 in PLWH and healthy controls, respectively) is 30.3% for low muscle mass only and 4.5% for low muscle mass and strength in PLHIV (2). The age ranges from the latter study also considered individuals over 50 years, therefore, a larger number of individuals with low muscle mass was found when compared to our study (30.3% vs. 18%). Also, using the same criteria, low muscle mass and strength were identified in 2% of our HIV sample against 4.5% in this metanalysis that considers a higher age range.

Konishi et al. investigated sarcopenia in Japanese PLHIV (male subjects >60 years) and found that 46% had hidden obesity (BMI is adequate but the body fat percentage is higher than reference values), 10.3% had pre sarcopenia (low muscle mass) and 16.1% had sarcopenia (low muscle mass and low muscle strength or decreased physical function) (23). In our study, 18% evidenced pre sarcopenia, 2% had sarcopenia and 8% revealed hidden obesity (while no participant in control group had hidden obesity, even though BMI was similar between groups). These differences found between Konishi et al. and our study might be explained by the differing age ranges and race. They described that subjects using tenofovir alafenamide (TAF) had significantly higher skeletal muscle mass, BMI, body fat mass, and skeletal muscle mass index when compared to those not using TAF (23). This ART seems to be an alternative to preserve bone and muscle health in PLHIV, although higher BMI and fat mass were observed. On the other hand, treatments with TDF present a smaller impact in weight gain (24). During our study none of the patients were receiving TAF. Cases of renal tubulopathy with hypophosphatemia have been described in PLHIV using TDF (25-27). In our sample none of the HIV-infected presented hypophosphatemia.

Considering that both groups were overweight, the hypothesis of protective effect of oestrogen on bone and muscle tissues might justify the absence of significant differences between groups in body composition parameters. Fat tissue is one of the main sources of aromatase, responsible for the synthesis of oestrogen from androgen precursors. Oestrogen plays an important role in homeostasis of bone and muscle tissues and exerts protective action on bone (28).

We found that a longer duration of HIV-infection is related to fat accumulation and muscle loss, as well as increase in time at TUG (reduced physical performance) and decrease in muscle strength. Higher $CD4^+$ values were associated to higher grip strength, which means that immunocompetent PLHIV were able to maintain muscle strength. Also, longer exposure to TDF was correlated to muscle loss and fat accumulation. Although correlations were statistically significant, most coefficients were weak. Nevertheless, we may infer that longer

periods of HIV-infection, ART exposure and immunodeficiency must draw attention to unfavorable outcomes related to sarcopenia and these findings are compatible with literature (17).

Nowadays the available options to treat sarcopenia are mainly non-pharmacological (29). The measures that present significant evidence are adequate nutrition and practice of resistance exercises. Nutritional approach must include adequate protein intake according to weight, antioxidant substances intake and vitamin D supplementation (29). Further studies are needed to elucidate proper and consistent recommendations regarding selective androgenic receptor modulators, anabolic/androgenic steroids, growth hormone and other therapies (29).

Literature suggests that vitamin D status must always be assessed [recommended levels $\geq$ 30ng/dL for HIV-infected (8)], as well as and protein intake [1.0 – 1.2 g/Kg body weight per day divided in several meals (30)] to provide adequate recommendations as preventive measures. Also, healthcare professionals must incentive the practice of $\geq$150 minutes per week of physical activity divided in aerobic and resistance exercises (31). These measures may prevent not only sarcopenia and osteoporosis, but also weight gain and metabolic disorders in PLWH. Physical activity plays an important role in the preservation of muscle strength and physical functionality according to literature (17, 29).

A limitation of our study was that we did not manage assess muscle quality. Magnetic resonance imaging and computed tomography have been used to determine muscle attenuation and fat infiltration, but their use have been limited to research purposes as no cut-off values have been defined for the diagnosis of sarcopenia (16, 32). Also, we did not evaluate protein intake, which is an important determinant of muscle health.

We conclude that sarcopenia is still underdiagnosed in young PLHIV, which are considered at high-risk. New cut-off values for muscle function tests were suggested to reach a higher number of patients with functionality and mobility commitment and that might benefit from sarcopenia treatment. Factors such as: duration of disease, exposure to ART and immunodeficiency seem to be important determinants of muscle dysfunction and require attention. If not treated, sarcopenia may culminate in dependence, reduced physical performance, higher morbimortality, reduction of working capacity and of quality of life. Thus, we draw attention to the necessity of screening since disease onset, covering every aspect of this individual's treatment to provide long-lasting health.

REFERENCES:

1. Pacifico J, Geerlings MAJ, Reijnierse EM, Phassouliotis C, Lim WK, Maier AB. Prevalence of sarcopenia as a comorbid disease: A systematic review and meta-analysis. Exp Gerontol. 2020;131:110801.

2. Guimaraes NS, Raposo MA, Greco D, Tupinambas U, Premaor MO. People Living With HIV, Lean Mass, and Sarcopenia: A Systematic Review and Meta-Analysis. J Clin Densitom. 2022;25(1):113-23.

3. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(4):601.

4. Dutta D, Garga UC, Gadpayle AK, Bansal R, Anand A, Gaurav K, et al. Occurrence & predictors of osteoporosis & impact of body composition alterations on bone mineral health in asymptomatic pre-menopausal women with HIV infection. Indian J Med Res. 2018;147(5):484-95.

5. Buehring B, Kirchner E, Sun Z, Calabrese L. The frequency of low muscle mass and its overlap with low bone mineral density and lipodystrophy in individuals with HIV--a pilot study using DXA total body composition analysis. J Clin Densitom. 2012;15(2):224-32.

6. Dodds RM, Syddall HE, Cooper R, Benzeval M, Deary IJ, Dennison EM, et al. Grip strength across the life course: normative data from twelve British studies. PLoS One. 2014;9(12):e113637.

7. Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL inhibition improves muscle strength and insulin sensitivity and restores bone mass. J Clin Invest. 2019;129(8):3214-23.

8. Moreira CA, Ferreira C, Madeira M, Silva BCC, Maeda SS, Batista MC, et al. Reference values of 25-hydroxyvitamin D revisited: a position statement from the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC). Arch Endocrinol Metab. 2020;64(4):462-78.

9. Malmstrom TK, Miller DK, Simonsick EM, Ferrucci L, Morley JE. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. J Cachexia Sarcopenia Muscle. 2016;7(1):28-36.

10. Bischoff HA, Stahelin HB, Monsch AU, Iversen MD, Weyh A, von Dechend M, et al. Identifying a cut-off point for normal mobility: a comparison of the timed 'up and go' test in community-dwelling and institutionalised elderly women. Age Ageing. 2003;32(3):315-20.

11. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age Ageing. 2011;40(4):423-9.

12. Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. J Gerontol A Biol Sci Med Sci. 2014;69(5):547-58.

13. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-adjusted models in assessing sarcopenia. Korean J Intern Med. 2016;31(4):643-50.

14. Gould H, Brennan SL, Kotowicz MA, Nicholson GC, Pasco JA. Total and appendicular lean mass reference ranges for Australian men and women: the Geelong osteoporosis study. Calcif Tissue Int. 2014;94(4):363-72.

15. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147(8):755-63.

16. Maeda SS, Albergaria BH, Szejnfeld VL, Lazaretti-Castro M, Arantes HP, Ushida M, et al. Official Position of the Brazilian Association of Bone Assessment and Metabolism (ABRASSO) on the evaluation of body composition by densitometry-part II (clinical aspects): interpretation, reporting, and special situations. Adv Rheumatol. 2022;62(1):11.

17. Conde-Higuera P, Garduno-Garcia JJ, Cruz-Jentoft AJ, Pena-Ordonez GG, Huitron-Bravo GG. Sarcopenia in people living with HIV. A review. AIDS Rev. 2022;24(4):166-72.

18. Mialich MS, Dos Santos AP, da Silva BR, de Paula FJ, Jordao AA, Navarro AM. Relationship Between Adiposity Indices, Lipodystrophy, and Sarcopenia in HIV-Positive Individuals With and Without Lipodystrophy. J Clin Densitom. 2017;20(1):73-81.

19. Erlandson KM, Allshouse AA, Jankowski CM, MaWhinney S, Kohrt WM, Campbell TB. Functional impairment is associated with low bone and muscle mass among persons aging with HIV infection. J Acquir Immune Defic Syndr. 2013;63(2):209-15.

20. Moreno S, Miralles C, Negredo E, Domingo P, Estrada V, Gutierrez F, et al. Disorders of body fat distribution in HIV-1-infected patients. AIDS Rev. 2009;11(3):126-34.

21. Dos-Santos-Quaresma MVL, Lima-Ribeiro SM. Sarcopenia in Persons Living with HIV under Antiretroviral Therapy: Literature Review. AIDS Rev. 2022;24(1):1-15.

22. Almeida TS, Cortez AF, Cruz MRD, Almeida VP. Predictors of sarcopenia in young hospitalized patients living with HIV. Braz J Infect Dis. 2021;25(2):101574.

23. Konishi K, Nakagawa H, Asaoka T, Kasamatsu Y, Goto T, Shirano M. Sarcopenia among people living with HIV and the effect of antiretroviral therapy on body composition. Medicine (Baltimore). 2022;101(42):e31349.

24. Sax PE, Erlandson KM, Lake JE, McComsey GA, Orkin C, Esser S, et al. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. Clin Infect Dis. 2020;71(6):1379-89.

25. Badiou S, De Boever CM, Terrier N, Baillat V, Cristol JP, Reynes J. Is tenofovir involved in hypophosphatemia and decrease of tubular phosphate reabsorption in HIV-positive adults? J Infect. 2006;52(5):335-8.

26. Kichloo A, Chugh SS, Gupta S, Panday J, Goldar GE. Tenofovir and Severe Symptomatic Hypophosphatemia. J Investig Med High Impact Case Rep. 2019;7:2324709619848796.

27. Fioroti CEA, Distenhreft JIQ, Paulino BB, Lacchine K, Ramos DR, Seguro AC, et al. Tenofovir-induced renal and bone toxicity: report of two cases and literature review. Rev Inst Med Trop Sao Paulo. 2022;64:e10.

28. Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. J Musculoskelet Neuronal Interact. 2020;20(3):372-81.

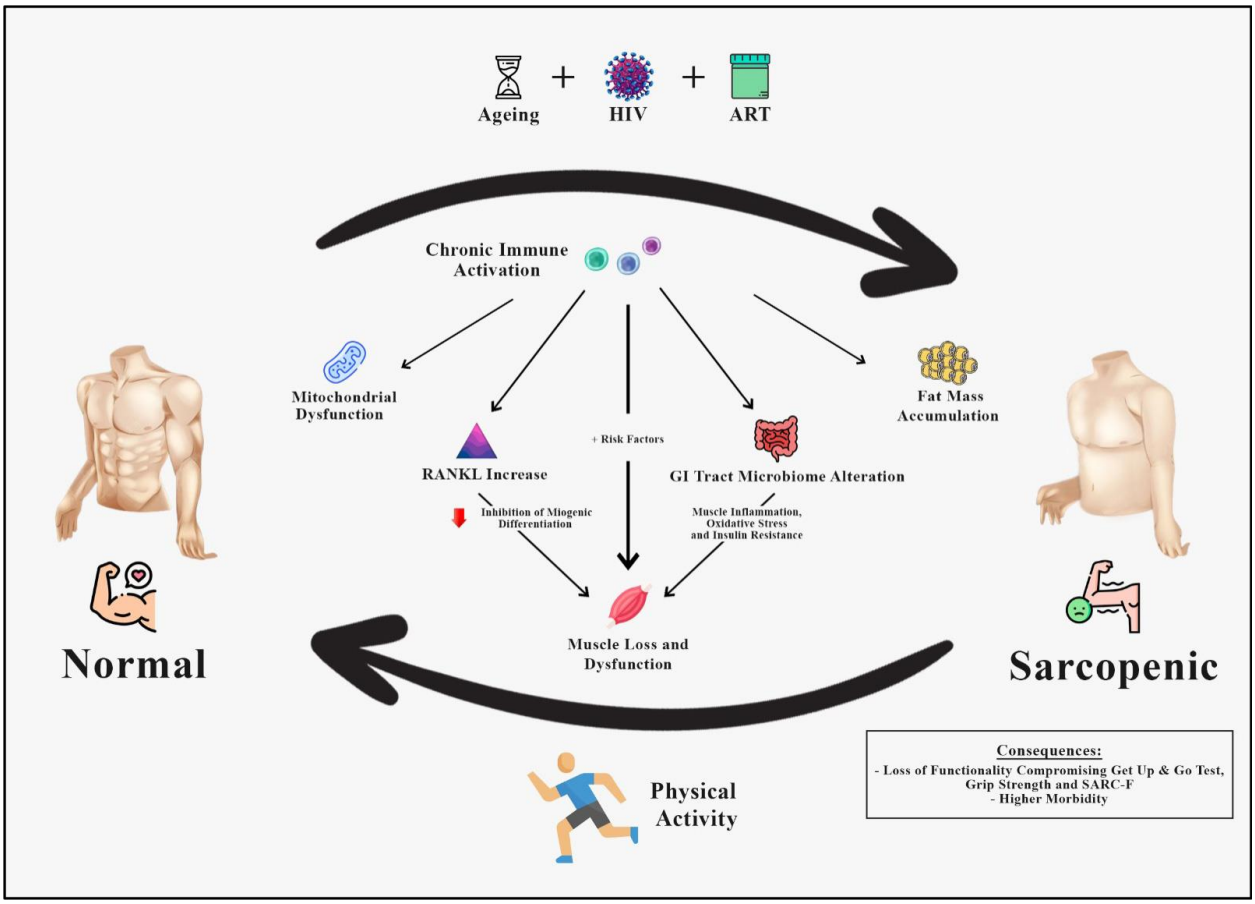
29. Cho MR, Lee S, Song SK. A Review of Sarcopenia Pathophysiology, Diagnosis, Treatment and Future Direction. J Korean Med Sci. 2022;37(18):e146.

30. Robinson SM, Reginster JY, Rizzoli R, Shaw SC, Kanis JA, Bautmans I, et al. Does nutrition play a role in the prevention and management of sarcopenia? Clin Nutr. 2018;37(4):1121-32.

31. Organization WH. World report on ageing and health: World Health Organization; 2015.

32. Albano D, Messina C, Vitale J, Sconfienza LM. Imaging of sarcopenia: old evidence and new insights. Eur Radiol. 2020;30(4):2199-208.

Figure 1 Pathophysiological mechanisms responsible for sarcopenia development in patients living with HIV



The image shows multifactorial processes involved in sarcopenia development in people living with human immunodeficiency virus (PLWH). The process of ageing by itself is known to contribute to muscle loss and decreased functionality. Ageing allied to HIV infection and anti-retroviral therapy (ART) enhances the mechanisms that lead to sarcopenia. PLWH present chronic immune activation and inflammation, which contribute to mitochondrial dysfunction, increased expression of Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL), gastrointestinal (GI) tract microbiome alteration, fat redistribution and muscle loss and dysfunction. Risk factors such as sedentarism, drug abuse, sociodemographic factors combined with duration of disease and ART exposure intensifies muscle loss and dysfunction. Also, microbiome alteration and accumulation of fat tissue predispose to muscle inflammation, oxidative stress, and insulin resistance, increasing diabetes and cardiovascular disease risk. Consequences of sarcopenia in PLWH are loss of functionality, higher morbimortality and decrease in life quality in earlier ages. The practice of physical activity may help slow down the development and progression of sarcopenia.

Figure 2 SARC-F questionnaire

Component:	Question	Score
Muscle strength	How much difficulty do you have in lifting and carrying 10 pounds (approximately 4.5 Kg)?	None = 0 Some = 1 A lot or unable = 2
Walking assistance/dependence	How much difficulty do you have walking across a room?	None = 0 Some = 1 A lot or unable without help = 2
Rising from chair	How much difficulty do you have transferring from a chair or bed?	None = 0 Some = 1 A lot or unable without help = 2
Climbing stairs	How much difficulty do you have climbing 10 stairs?	None = 0 Some = 1 A lot or unable = 2
Number of falls	How many times have you fallen in the past 12 months (1 year)?	None = 0 1 – 3 falls = 1 4 or more falls = 2

Score $\geq$ 4 predicts higher risk of sarcopenia and further investigation is recommended.

Table 1 Participants characteristics and tests for sarcopenia

	HIV group n = 50	**Control group n = 50**	**p**
Gender	24 F / 26 M	25 F / 25 M	1.00
Race (% Caucasian)	56	58	1.00
Age (years)	40 (25 – 49)	36.5 (22 – 50)	0.120
Weight (Kg)	76.7 (50.1 – 102.6)	76.7 (54.4 – 109.8)	0.448
Height (cm)	166 (150 – 187)	169.5 (153 – 186)	0.473
BMI (Kg/cm^2)	26.9 (17.44 – 37.5)	27.7 (19.34 – 39.4)	0.459
Biochemistry			
Serum phosphorus (mg/dL)	3.8 (2.7 – 5.8)	3.9 (2.9 – 5.4)	0.568
25(OH) vitamin D (ng/mL)	27.7 (12.9 – 42.4)	27.9 (13.5 – 57.8)	0.936
25(OH)D below 30 ng/mL (%)	58	56	1.000
HDLc (mg/dL)	44.5 (23 – 124)	53 (19 – 99)	**0.006**
LDLc (mg/dL)	110.8 (56 – 177.2)	113 (76.6 – 202)	0.146
Triglycerides (mg/dL)	103 (37 – 405)	81 (21 – 276)	0.189
Muscle Function Tests			
Test Get Up & Go (seconds)	8.91 (5.70 – 16.26)	8.23 (5.57 – 10.95)	**0.005**
Grip test (Kg)	29.0 (9.3 – 56.0)	32.8 (13.3 – 57.3)	0.052
SARC-F (score)	0 (0 – 4)	0 (0 – 2)	**0.001**

Variables are expressed in median values (minimum and maximum).

HIV: Human immunodeficiency virus; F: female; M: male; 25 (OH) D: 25-hydroxi-vitamin D; HDLc: high density lipoprotein; LDLc: low density lipoprotein; ß-CTX: C-terminal telopeptide of type 1 collagen

Table 2 Body composition evaluated by DXA

	HIV group **n = 50**	**Control group** **n = 50**	**p**
Total mass (Kg)	76.7 (50.1 – 102.6)	76.7 (54.4 – 109.8)	0.448
Total tissue mass (g)	74252.5 (47901 – 100127)	74321 (52588 – 107069)	0.448
Total lean mass (g)	47569 (33215 – 69460)	50085 (33777 – 71506)	0.234
Total fat free mass (g)	50233.5 (35302 – 72931)	52580 (35588 – 75869)	0.236
ALM (Kg)	22.55 (13.8 – 32.8)	24.3 (13.5 – 35.4)	0.198
ALM/h² (Kg/m^2)	7.95 (5.51 – 10.63)	8.42 (5.36 – 10.82)	0.137
ALM/BMI ($Kg/Kg/m^2$)	0.79 (0.494 – 1.217)	0.83 (0.531 – 1.238)	0.477
Total fat mass (g)	24140.5 (11923 – 53610)	26030 (9362 – 54687)	0.845
Total fat region (%)	32.75 (18.5 – 52.3)	33.5 (14.6 – 49.8)	0.767
Total fat tissue (%)	34 (19.3 – 53.5)	34.8 (15.2 – 51.1)	0.751
VAT volume (cm^3)	600 (103 – 2368)	622 (2 – 1995)	0.374
VAT mass (g)	566 (97 – 2234)	586.5 (2 – 1882)	0.374
VAT area (cm^2)	71 (14 – 257)	69.5 (0 – 235)	0.372
SAT volume (cm^3)	1401 (51 – 3579)	1492 (152 – 3737)	0.836
SAT mass (g)	1322 (48 – 3376)	1408 (144 – 3525)	0.836
SAT area (cm^2)	168 (6 – 430)	165 (16 – 401)	0.803

G: grams; Kg: kilograms; VAT: Visceral adipose tissue (intra-abdominal fat); SAT: Subcutaneous adipose tissue; ALM: Appendicular lean mass; ALM/h^2: Appendicular lean mass adjusted for height squared; ALM/BMI: Appendicular lean mass adjusted for body mass index; cm^2: squared centimeters; cm^3: cubic centimeters

Table 3 Analysis of alterations in each group divided by gender

	HIV male	HIV female	Control male	Control female	p
Alteration SARC-F score ≥1	6	12	0	5	**0.004**
Alteration TUG test ≥10 seconds	6	10	2	1	**0.002**
Intermediate grip strength 16 – 19.9Kg for women and 26 – 31.9 Kg for men	4	1	2	0	0.436
Weak grip strength <16 Kg for women and <26 Kg for men	5	2	0	1	0.059
Probable sarcopenia <20 Kg for women and <32 Kg for men	9	3	2	1	0.023
Sarcopenia (low muscle strength <20 Kg and <32 Kg for women and men, respectively) + low muscle mass)	3	1	0	0	0.117
ALM <15 Kg for women and <20 Kg for men	3	4	0	2	0.160
ALM/h^2 <5.5 Kg/m^2 for women and <7.00 Kg/m^2 for men	3	2	0	1	0.204

HIV: human immunodeficiency virus; TUG: Test Get Up & Go; Kg: kilograms; ALM: appendicular lean mass, ALM/h^2: ALM adjusted for height

Table 4 Correlations found in our study

	Time of infection	**$CD4^+$ cell count**	**Exposure to TDF**
TUG (seconds)	p 0.029; r 0.309	---	---
Total fat tissue (%)	p 0.005; r 0.393	---	p 0.015; r 0.343
Total fat region (%)	p 0.006; r 0.386	---	p 0.018; r 0.334
VAT area (cm^2)	p 0.034; r 0.309	---	---
Hand grip test (Kg)	p 0.037; r -0.276	p 0.030; r 0.307	---
Total lean mass (g)	p 0.002; r -0.420	---	p 0.026; r -0.315
ALM (Kg)	p <0.001; r -0.452	---	p 0.028; r -0.310
ALM/h^2 (Kg/m^2)	p 0.045; r -0.285	---	---

TUG: Test Up & Go; VAT: Visceral adipose tissue (intra-abdominal fat); cm^2: squared centimeters; Kg: kilograms; G: grams; ALM: Appendicular lean mass; ALM/h^2: Appendicular lean mass adjusted for squared height

4. **DISCUSSÃO**

Os dados do nosso estudo, no qual avaliamos a densidade e microarquitetura ósseas, além da composição corporal e testes funcionais que fazem parte do espectro diagnóstico da sarcopenia, demonstraram que pacientes jovens que vivem com o HIV apresentam comprometimento da qualidade óssea e da funcionalidade muscular. Grande parte dos estudos previamente publicados avaliam populações idosas portadoras do vírus, confirmando que há piora dos parâmetros ósseos e musculares com o envelhecimento (6, 19, 38, 59-63). Até a presente data, esse é o estudo com maior número de pacientes HIV e controle, de ambos os sexos, que avaliou uma população jovem e sem comorbidades que possam interferir nas condições estudadas. A partir dos dados obtidos, fomos os primeiros a sugerir novos protocolos de rastreio para diagnóstico precoce de doenças óssea e muscular em jovens com HIV, visto que os protocolos atuais recomendam rastreio apenas em idades mais avançadas e com ferramentas que podem subestimar o risco de fraturas e de desfechos musculares.

A doença óssea no portador de HIV passou a chamar atenção principalmente devido ao envelhecimento dessa população, que tem sido possível desde a descoberta das TARVs (5). A terapia visando negativação da carga viral e aumento da contagem de células T $CD4^+$, trouxe repercussões clínicas com alterações metabólicas crônicas devido aos efeitos adversos das drogas (64). Alguns efeitos adversos descritos são dislipidemia, diabetes, toxicidade renal e óssea, além de redistribuição de gordura e aumento do risco cardiovascular (65-69).

A inflamação crônica de baixo grau causada pelo vírus e pelos efeitos adversos exercidos pelas TARVs mostraram ser fatores importantes para a piora da massa óssea (35). Além disso, a soma de fatores inerentes ao envelhecimento como a menopausa e andropausa (com queda dos níveis de estrogênio nas mulheres e testosterona nos homens), intensifica a reabsorção óssea já presente devido ao vírus e à TARV (70-74).

No entanto, ao estudarmos uma amostra de pacientes com mediana de idade de 40 anos (25 – 49) no grupo HIV e 36,5 anos (22 – 50) no grupo controle, excluindo-se mulheres com ciclos menstruais irregulares (ou menopausa), homens com hipogonadismo, usuários de medicamentos ou portadores de comorbidades que interferem no metabolismo ósseo, retiramos os vieses de perda óssea ocasionada por questões que não envolvem o vírus. Os portadores de HIV apresentavam mediana de 13 anos de doença e eram, em sua maioria, imunocompetentes (mediana da contagem de células T $CD4^+$ foi de 659,5 (65-1279) células/mm^3. Mesmo excluindo os possíveis fatores confundidores, encontramos piores parâmetros ósseos de DMO areal em coluna lombar (BMD e Z-score, com p 0,023 e 0,021, respectivamente) e da

microarquitetura óssea através de HR-pQCT (em tíbia distal, principalmente), com significância estatística. Esses achados foram semelhantes a estudos previamente publicados (75-78). Devemos ressaltar que apesar do fato de que apenas 2 pacientes no grupo HIV apresentaram Z-score $\leq$-2,0 na densitometria óssea, o comprometimento da qualidade óssea nos parâmetros de HR-pQCT foi significativo no grupo HIV em comparação com grupo controle. Esses dados chamam atenção à hipótese de que a densitometria óssea pode não ser o melhor exame para rastreio da doença óssea nessa população, visto que subestima o risco de fraturas por fragilidade por não avaliar parâmetros relacionados à qualidade do osso.

Pacientes com diagnóstico na infância e adolescência podem apresentar maior prejuízo da massa óssea em fases mais precoces. Isso ocorre pelo fato de que eles adquiriram a doença durante período de formação óssea, o que explica a aquisição de um pico de massa óssea inferior. Estudos prévios em adultos jovens, os quais adquiriram o vírus por transmissão vertical ou na adolescência, mostram que existe prejuízo do pico de massa óssea nos pacientes jovens com HIV e isso se confirma em nosso estudo (76, 79). Uma paciente com Z-score -2,1 em coluna lombar tinha 32 anos no momento do estudo e apresentava duração da infecção de 23 anos (diagnóstico aos 9 anos de idade, com provável comprometimento do pico de massa óssea). O outro paciente que apresentou Z-score do colo do fêmur e fêmur total alterados (-2,3 e -2,1, respectivamente) tinha 26 anos com duração de infecção de 8 anos (diagnóstico aos 18 anos).

Um achado de extrema importância e que guiou grande parte da confecção do nosso protocolo de rastreio de doença óssea no paciente HIV jovem foi a prevalência de 12% de fraturas vertebrais morfométricas após realização de rastreio com DXA-VFA. Estudos prévios descrevem aumento da prevalência de fraturas nessa população (80-83). Chamamos atenção ao fato de que os protocolos atuais de rastreio não indicam avaliação por imagem da coluna vertebral com busca ativa para fraturas vertebrais assintomáticas. O achado de fratura vertebral é de extrema importância, uma vez que muda a classificação de risco desse paciente e indica início de terapia farmacológica anti-osteoporótica (84, 85). Uma vez iniciada terapia para tratamento da osteoporose, o DXA é indicado para acompanhamento e avaliação da eficácia no tratamento.

Observamos que os pacientes com HIV apresentaram maior risco de fratura apenas pelo fato de terem a infecção e selecionarmos “osteoporose secundária” na ferramenta para cálculo do FRAX. Ao preenchermos o campo de DMO em colo do fêmur, os pacientes que apresentavam fraturas vertebrais deixaram de ter alto risco por apresentarem densitometria óssea normal. Como descrito acima, apesar de não apresentarem alteração na DMO, a qualidade

óssea mostrou-se alterada de forma significativa, o que torna o FRAX uma ferramenta que pode subestimar o real risco de fratura ao acrescentarmos DMO do colo do fêmur em pacientes já fraturados. Naqueles sem fraturas, ao adicionar o dado de DMO do colo do fêmur, 4 mudaram de risco moderado para alto risco, e nenhum desses apresentavam fratura prévia. Sendo assim, o FRAX parece ser uma ferramenta útil para cálculo do risco de fraturas em pacientes com HIV ≥40 anos que não apresentam fratura pré existente.

No nosso estudo não encontramos diferença significativa entre os grupos em relação ao marcador de reabsorção óssea (ß-CTX). Apesar disso, OC e fosfatase alcalina foram maiores no grupo com HIV (p 0,041 e 0,003, respectivamente), o que indica um maior remodelamento ósseo nesse grupo. Estudos prévios descrevem que o aumento da remodelação óssea parece ser um dos principais mecanismos fisiopatológicos que explica a piora da massa óssea nessa população (86, 87).

Apesar dos resultados de 25(OH)D e PTH serem semelhantes entre os grupos estudados, mais pacientes no grupo HIV apresentaram PTH acima do valor de referência. Além disso, os valores de cálcio sérico corrigido para albumina foram significativamente menores no grupo HIV em comparação com os controles (p <0,001). Levando em consideração as recomendações para populações de alto risco para osteoporose, sugerimos a manutenção dos níveis séricos de 25(OH)D ≥30 ng/mL e recomendamos ingesta satisfatória de cálcio entre 1000 e 1200mg ao dia, no intuito de reduzir a progressão da doença óssea no paciente com HIV (88, 89).

No protocolo sugerido após análise dos dados do nosso estudo, recomendamos a realização de imagem do esqueleto axial (coluna torácia e lombar) em posições ântero-posterior e perfil através de radiografia à partir dos 30 anos de idade e/ou nos pacientes com duração de infecção ≥5 anos. Recomendamos também o cálculo de risco por meio das calculadoras FRAX e NOGG, que quando classificados como alto risco (probabilidade de fratura osteoporótica maior >20% e fratura de quadril >3% em 10 anos), o uso de bisfosfonato é fortemente recomendado. A realização de densitometria óssea foi recomendada após 5 anos do diagnóstico e deve ser repetida a cada 3 anos para avaliação de perda da massa óssea. Também recomendamos DXA para acompanhamento dos casos de alto risco pelo FRAX/NOGG e naqueles com fratura pré existente, os quais devem realizar tratamento farmacológico anti-osteoporose (repetir a cada 1 a 2 anos). O protocolo também preconiza reavaliação da TARV com preferência para drogas com menos efeitos sobre o metabolismo ósseo (retirada de TDF, e considerar troca de IP e EFV) e recomenda que o paciente cesse tabagismo e faça consumo seguro de álcool.

Ainda que diagnostiquemos e tratemos a doença óssea no HIV, não podemos desconsiderar os aspectos musculares e a interferência da sarcopenia ou da baixa força muscular como contribuintes para desfechos indesejáveis no paciente jovem com HIV. Está bem estabelecida na literatura a relação entre osteoporose e sarcopenia (45, 90, 91).

No nosso estudo, avaliamos diversos parâmetros do espectro de diagnóstico da sarcopenia. Atualmente, o EWGSOP2 (47) recomenda iniciar o rastreio com o questionário SARC-F (para identificação dos casos suspeitos), seguido de avaliação da força muscular (classificando como provável sarcopenia os casos com baixa força muscular) e confirmando o diagnóstico com a realização da composição corporal para obtenção da massa magra apendicular. Por ultimo, é feito o teste de performance física classificando o indivíduo com sarcopenia severa, se alterado. Realizamos todas as etapas sugeridas com SARC-F, FPM, DXA e TUG.

Observamos em nosso estudo que houve heterogeneidade nos resultados e que o questionário SARC-F não parece ser um bom rastreio para população jovem com HIV, visto que esses indivíduos ainda apresentam certa mobilidade e funcionalidade em comparação com população mais idosa, para a qual esses protocolos foram criados. Encontramos resultados inferiores, com significância estatística, no TUG e SARC-F (quando consideramos pontos de corte de >10 segundos e qualquer pontuação $\geq$1 no questionário, respectivamente). Esses resultados alterados evidenciam que indivíduos infectados apresentam maior comprometimento de mobilidade, performance física e, consequentemente, maior morbidade. Força de preensão palmar mostrou valores discretamente inferiores no grupo com HIV com p 0,052. Apesar de não encontrarmos dados da composição corporal significativamente menores na população jovem com HIV em comparação com grupo controle, mais pacientes infectados apresentaram valores inferiores à normalidade no que tange MMA e IMMA. Dessa forma, chamamos atenção ao fato de que precisamos de protocolos atualizados voltados para populações mais jovens com pontos de corte individualizados, visando maior sensibilidade no rastreio e objetivando diagnóstico precoce e tratamento.

Encontramos prevalência de provável sarcopenia e sarcopenia confirmada de 14% e 2%, respectivamente, no grupo HIV, enquanto que no grupo controle 2% tinham provável sarcopenia e nenhum confirmou sarcopenia. A maioria dos estudos que avaliam sarcopenia em população portadora do vírus leva em consideração pacientes mais idosos (5, 38, 59, 92).

Um estudo realizado em população de pacientes jovens com HIV (com idade semelhante ao nosso estudo) avaliou indivíduos com IMC dentro da normalidade, porém imunodeficientes, e que encontravam-se hospitalizados por período curto. O diagnóstico de

sarcopenia apresentou prevalência maior do que a encontrada em nosso estudo, mas reforçamos que se trata de um grupo com características distintas do nosso (93).

É importante ressaltar que nossa amostra apresentou predominância de sobrepeso, com mediana de IMC de 26,9 (17,44 – 37,5) versus 27,7 (19,34 – 39,4) no grupo HIV e controle, respectivamente. Dessa forma, acreditamos que pode existir um componente protetor do estrogênio presente nos tecidos muscular e ósseo. No tecido adiposo ocorre a aromatização do androgênio em estrogênio e existem evidências de que o estrogênio possui papel importante na homeostase dos tecidos muscular e ósseo (94).

Uma alteração metabólica encontrada no nosso estudo foi níveis estatisticamente inferiores de lipoproteínas de alta densidade (HDL), com p 0,006, e esse achado é descrito na literatura (95, 96). As TARVs mais antigas parecem contribuir de forma mais importante para essa alteração (inibidores da transcriptase reversa análogos dos nucleosídeos – zidovudina e estavudina e inibidores da protease) e, nos dias atuais, dispomos de drogas com menos efeitos no perfil lipídico (inibidores da integrase – raltegravir e dolutegravir) (68, 97).

Baseado nos resultados encontrados em nosso estudo, listamos recomendações para o diagnóstico e rastreio de sarcopenia na população jovem com HIV. Para aplicação do SARC-F, recomendamos investigar mais a fundo o paciente que apresentar $\geq$1 score após responder o questionário, considerando que as perguntas são voltadas para uma população idosa e que jovens deveriam exercer os elementos questionados sem grandes dificuldades. Também recomendamos ampliar os valores de corte da FPM para <32 Kg e <20 Kg em homens e mulheres, respectivamente, para conferir maior sensibilidade ao teste e abranger maior número de pacientes jovens com comprometimento da força muscular. Para o TUG sugerimos o ponto de corte de >10 segundos para considerar o teste como alterado, tendo em vista que indivíduos jovens não devem utilizar o mesmo ponto de corte utilizado para idosos no intuito de avaliar a performance física. Sugerimos a realização destes testes desde o diagnóstico. Por último, é recomendado na literatura a ingesta proteica de 1,0 a 1,2 g/Kg de peso por dia associada à prática regular de exercícios de pelo menos 150 minutos semanais (97-99).

CONCLUSÃO

Podemos concluir através do nosso estudo, que o paciente jovem portador do HIV apresenta desordens importantes na DMO e microarquitetura óssea, que aumentam o risco do surgimento de fraturas por fragilidade. Além disso, observamos comprometimento da mobilidade e da funcionalidade/força muscular desses pacientes em comparação com grupo controle, ressaltando a importância de estudo funcional muscular em pacientes mais jovens. Os protocolos sugeridos visam realização de diagnóstico precoce para reduzir morbimortalidade, aumentar capacidade e tempo laborativos, permitir maior funcionalidade muscular e performance física com consequente aumento na qualidade de vida em grupo populacional que se encontra com crescente aumento da expectativa de vida.

REFERÊNCIAS

1. Greene WC. A history of AIDS: looking back to see ahead. Eur J Immunol. 2007;37 Suppl 1:S94-102.

2. Sued O, Cahn P. Latin America Priorities after 40 years of the beginning of the HIV pandemic. Lancet Reg Health Am. 2021;1:100024.

3. Global HIV & AIDS statistics - Fact sheet UNAIDS [Available from: (https://www.unaids.org/en/resources/fact-sheet).

4. Yin MT, Brown TT. HIV and Bone Complications: Understudied Populations and New Management Strategies. Curr HIV/AIDS Rep. 2016;13(6):349-58.

5. Echeverria P, Bonjoch A, Puig J, Estany C, Ornelas A, Clotet B, et al. High Prevalence of Sarcopenia in HIV-Infected Individuals. Biomed Res Int. 2018;2018:5074923.

6. Finnerty F, Walker-Bone K, Tariq S. Osteoporosis in postmenopausal women living with HIV. Maturitas. 2017;95:50-4.

7. Reginster JY, Beaudart C, Buckinx F, Bruyere O. Osteoporosis and sarcopenia: two diseases or one? Curr Opin Clin Nutr Metab Care. 2016;19(1):31-6.

8. Armas LA, Recker RR. Pathophysiology of osteoporosis: new mechanistic insights. Endocrinol Metab Clin North Am. 2012;41(3):475-86.

9. Lane JM, Russell L, Khan SN. Osteoporosis. Clin Orthop Relat Res. 2000(372):139-50.

10. Zhu X, Zheng H. Factors influencing peak bone mass gain. Front Med. 2021;15(1):53-69.

11. Vigano A, Mora S. Adverse effects of antiretroviral therapy: focus on bone density. Expert Opin Drug Saf. 2004;3(3):199-208.

12. Ahmad AN, Ahmad SN, Ahmad N. HIV Infection and Bone Abnormalities. Open Orthop J. 2017;11:777-84.

13. Johnell O, Kanis JA, Oden A, Sernbo I, Redlund-Johnell I, Petterson C, et al. Fracture risk following an osteoporotic fracture. Osteoporos Int. 2004;15(3):175-9.

14. Lawrence TM, Wenn R, Boulton CT, Moran CG. Age-specific incidence of first and second fractures of the hip. J Bone Joint Surg Br. 2010;92(2):258-61.

15. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004;35(2):375-82.

16. Sattui SE, Saag KG. Fracture mortality: associations with epidemiology and osteoporosis treatment. Nat Rev Endocrinol. 2014;10(10):592-602.

17. Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporos Int. 2005;16 Suppl 2:S3-7.

18. Boonen S, Singer AJ. Osteoporosis management: impact of fracture type on cost and quality of life in patients at risk for fracture I. Curr Med Res Opin. 2008;24(6):1781-8.

19. Biver E. Osteoporosis and HIV Infection. Calcif Tissue Int. 2022;110(5):624-40.

20. Bruera D, Luna N, David DO, Bergoglio LM, Zamudio J. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. AIDS. 2003;17(13):1917-23.

21. Negredo E, Bonjoch A, Clotet B. Management of bone mineral density in HIV-infected patients. Expert Opin Pharmacother. 2016;17(6):845-52.

22. Sellier P, Ostertag A, Collet C, Trout H, Champion K, Fernandez S, et al. Disrupted trabecular bone micro-architecture in middle-aged male HIV-infected treated patients. HIV Med. 2016;17(7):550-6.

23. Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. J Clin Endocrinol Metab. 2008;93(9):3499-504.

24. Young B, Dao CN, Buchacz K, Baker R, Brooks JT, Investigators HIVOS. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000-2006. Clin Infect Dis. 2011;52(8):1061-8.

25. Womack JA, Goulet JL, Gibert C, Brandt C, Chang CC, Gulanski B, et al. Increased risk of fragility fractures among HIV infected compared to uninfected male veterans. PLoS One. 2011;6(2):e17217.

26. Sharma A, Shi Q, Hoover DR, Anastos K, Tien PC, Young MA, et al. Increased Fracture Incidence in Middle-Aged HIV-Infected and HIV-Uninfected Women: Updated Results From the Women's Interagency HIV Study. J Acquir Immune Defic Syndr. 2015;70(1):54-61.

27. Guerri-Fernandez R, Vestergaard P, Carbonell C, Knobel H, Aviles FF, Castro AS, et al. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. J Bone Miner Res. 2013;28(6):1259-63.

28. Prieto-Alhambra D, Guerri-Fernandez R, De Vries F, Lalmohamed A, Bazelier M, Starup-Linde J, et al. HIV infection and its association with an excess risk of clinical fractures: a nationwide case-control study. J Acquir Immune Defic Syndr. 2014;66(1):90-5.

29. Weitzmann MN. Bone and the Immune System. Toxicol Pathol. 2017;45(7):911-24.

30. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008;473(2):139-46.

31. Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL inhibition improves muscle strength and insulin sensitivity and restores bone mass. J Clin Invest. 2019;129(8):3214-23.

32. Overton ET, Chan ES, Brown TT, Tebas P, McComsey GA, Melbourne KM, et al. Vitamin D and Calcium Attenuate Bone Loss With Antiretroviral Therapy Initiation: A Randomized Trial. Ann Intern Med. 2015;162(12):815-24.

33. Grant PM, Cotter AG. Tenofovir and bone health. Curr Opin HIV AIDS. 2016;11(3):326-32.

34. Moran CA, Weitzmann MN, Ofotokun I. The protease inhibitors and HIV-associated bone loss. Curr Opin HIV AIDS. 2016;11(3):333-42.

35. Dos-Santos-Quaresma MVL, Lima-Ribeiro SM. Sarcopenia in Persons Living with HIV under Antiretroviral Therapy: Literature Review. AIDS Rev. 2022;24(1):1-15.

36. Premaor MO, Compston JE. The Hidden Burden of Fractures in People Living With HIV. JBMR Plus. 2018;2(5):247-56.

37. Guzman N, Vijayan V. HIV-associated Lipodystrophy. StatPearls. Treasure Island (FL)2023.

38. Konishi K, Nakagawa H, Asaoka T, Kasamatsu Y, Goto T, Shirano M. Sarcopenia among people living with HIV and the effect of antiretroviral therapy on body composition. Medicine (Baltimore). 2022;101(42):e31349.

39. Eckard AR, McComsey GA. Weight gain and integrase inhibitors. Curr Opin Infect Dis. 2020;33(1):10-9.

40. Silva M, Skolnik PR, Gorbach SL, Spiegelman D, Wilson IB, Fernandez-DiFranco MG, et al. The effect of protease inhibitors on weight and body composition in HIV-infected patients. AIDS. 1998;12(13):1645-51.

41. Mulligan K, Grunfeld C, Tai VW, Algren H, Pang M, Chernoff DN, et al. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. J Acquir Immune Defic Syndr. 2000;23(1):35-43.

42. Richmond SR, Carper MJ, Lei X, Zhang S, Yarasheski KE, Ramanadham S. HIV-protease inhibitors suppress skeletal muscle fatty acid oxidation by reducing CD36 and CPT1 fatty acid transporters. Biochim Biophys Acta. 2010;1801(5):559-66.

43. Sax PE, Erlandson KM, Lake JE, McComsey GA, Orkin C, Esser S, et al. Weight Gain Following Initiation of Antiretroviral Therapy: Risk Factors in Randomized Comparative Clinical Trials. Clin Infect Dis. 2020;71(6):1379-89.

44. Pinto Neto LF, Sales MC, Scaramussa ES, da Paz CJ, Morelato RL. Human immunodeficiency virus infection and its association with sarcopenia. Braz J Infect Dis. 2016;20(1):99-102.

45. Clynes MA, Gregson CL, Bruyere O, Cooper C, Dennison EM. Osteosarcopenia: where osteoporosis and sarcopenia collide. Rheumatology (Oxford). 2021;60(2):529-37.

46. Tagliaferri C, Wittrant Y, Davicco MJ, Walrand S, Coxam V. Muscle and bone, two interconnected tissues. Ageing Res Rev. 2015;21:55-70.

47. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(4):601.

48. Brown TT, Hoy J, Borderi M, Guaraldi G, Renjifo B, Vescini F, et al. Recommendations for evaluation and management of bone disease in HIV. Clin Infect Dis. 2015;60(8):1242-51.

49. Vestergaard P. Drugs Causing Bone Loss. Handb Exp Pharmacol. 2020;262:475-97.

50. Malmstrom TK, Miller DK, Simonsick EM, Ferrucci L, Morley JE. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. J Cachexia Sarcopenia Muscle. 2016;7(1):28-36.

51. Bischoff HA, Stahelin HB, Monsch AU, Iversen MD, Weyh A, von Dechend M, et al. Identifying a cut-off point for normal mobility: a comparison of the timed 'up and go' test in community-dwelling and institutionalised elderly women. Age Ageing. 2003;32(3):315-20.

52. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age Ageing. 2011;40(4):423-9.

53. Dodds RM, Syddall HE, Cooper R, Benzeval M, Deary IJ, Dennison EM, et al. Grip strength across the life course: normative data from twelve British studies. PLoS One. 2014;9(12):e113637.

54. Gould H, Brennan SL, Kotowicz MA, Nicholson GC, Pasco JA. Total and appendicular lean mass reference ranges for Australian men and women: the Geelong osteoporosis study. Calcif Tissue Int. 2014;94(4):363-72.

55. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147(8):755-63.

56. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-adjusted models in assessing sarcopenia. Korean J Intern Med. 2016;31(4):643-50.

57. Maeda SS, Albergaria BH, Szejnfeld VL, Lazaretti-Castro M, Arantes HP, Ushida M, et al. Official Position of the Brazilian Association of Bone Assessment and Metabolism (ABRASSO) on the evaluation of body composition by densitometry-part II (clinical aspects): interpretation, reporting, and special situations. Adv Rheumatol. 2022;62(1):11.

58. Whittier DE, Boyd SK, Burghardt AJ, Paccou J, Ghasem-Zadeh A, Chapurlat R, et al. Guidelines for the assessment of bone density and microarchitecture in vivo using high-resolution peripheral quantitative computed tomography. Osteoporos Int. 2020;31(9):1607-27.

59. de Almeida LL, Ilha T, de Carvalho JAM, Stein C, Caeran G, Comim FV, et al. Sarcopenia and Its Association with Vertebral Fractures in People Living with HIV. Calcif Tissue Int. 2020;107(3):249-56.

60. Barbu EC, Moroti-Constantinescu VR, Lazar M, Chitu CE, Olariu CM, Bojinca M, et al. Body Composition Changes in Men with Hiv/Hcv Coinfection, Hiv Monoinfection, and Hcv Monoinfection. Acta Endocrinol (Buchar). 2022;18(4):442-51.

61. Oursler KK, Iranmanesh A, Jain C, Birkett KL, Briggs BC, Garner DC, et al. Short Communication: Low Muscle Mass Is Associated with Osteoporosis in Older Adults Living with HIV. AIDS Res Hum Retroviruses. 2020;36(4):300-2.

62. Dutta D, Garga UC, Gadpayle AK, Bansal R, Anand A, Gaurav K, et al. Occurrence & predictors of osteoporosis & impact of body composition alterations on bone mineral health in asymptomatic pre-menopausal women with HIV infection. Indian J Med Res. 2018;147(5):484-95.

63. Guimaraes NS, Raposo MA, Greco D, Tupinambas U, Premaor MO. People Living With HIV, Lean Mass, and Sarcopenia: A Systematic Review and Meta-Analysis. J Clin Densitom. 2022;25(1):113-23.

64. Shibuyama S, Gevorkyan A, Yoo U, Tim S, Dzhangiryan K, Scott JD. Understanding and avoiding antiretroviral adverse events. Curr Pharm Des. 2006;12(9):1075-90.

65. Dupont E, Yombi JC. Antiretroviral therapy and weight gain in antiretroviral treatment-experienced HIV patients: A review. AIDS Rev. 2023;25(1):54-64.

66. Anuurad E, Semrad A, Berglund L. Human immunodeficiency virus and highly active antiretroviral therapy-associated metabolic disorders and risk factors for cardiovascular disease. Metab Syndr Relat Disord. 2009;7(5):401-10.

67. Vos AG, Venter WDF. Cardiovascular toxicity of contemporary antiretroviral therapy. Curr Opin HIV AIDS. 2021;16(6):286-91.

68. Maggi P, Di Biagio A, Rusconi S, Cicalini S, D'Abbraccio M, d'Ettorre G, et al. Cardiovascular risk and dyslipidemia among persons living with HIV: a review. BMC Infect Dis. 2017;17(1):551.

69. Calza L. Renal toxicity associated with antiretroviral therapy. HIV Clin Trials. 2012;13(4):189-211.

70. Cortes YI, Yin MT, Reame NK. Bone Density and Fractures in HIV-infected Postmenopausal Women: A Systematic Review. J Assoc Nurses AIDS Care. 2015;26(4):387-98.

71. Gomes DC, Valadares AL, Amaral E, Ferreira Nde O, Pinto-Neto AM, Baccaro LF, et al. Association between HIV infection and bone mineral density in climacteric women. Arch Osteoporos. 2015;10:33.

72. Cezarino PYA, Simoes RDS, Baracat EC, Soares Junior JM. Are women living with HIV prone to osteoporosis in postmenopause? A systematic review. Rev Assoc Med Bras (1992). 2018;64(5):469-73.

73. Oliveira FP, Lima LFC, de Paula Paranhos Neto F, de Mendonca LMC, Schtscherbyna A, de Lima LAA, et al. Lower bone density and microarchitecture alterations in HIV-infected Brazilian men aged 50 years and older are associated with estradiol levels. Clin Endocrinol (Oxf). 2022;97(1):142-9.

74. Biver E, Calmy A, Delhumeau C, Durosier C, Zawadynski S, Rizzoli R. Microstructural alterations of trabecular and cortical bone in long-term HIV-infected elderly men on successful antiretroviral therapy. AIDS. 2014;28(16):2417-27.

75. Yin MT, Shu A, Zhang CA, Boutroy S, McMahon DJ, Ferris DC, et al. Trabecular and cortical microarchitecture in postmenopausal HIV-infected women. Calcif Tissue Int. 2013;92(6):557-65.

76. Yin MT, Lund E, Shah J, Zhang CA, Foca M, Neu N, et al. Lower peak bone mass and abnormal trabecular and cortical microarchitecture in young men infected with HIV early in life. AIDS. 2014;28(3):345-53.

77. Kazakia GJ, Carballido-Gamio J, Lai A, Nardo L, Facchetti L, Pasco C, et al. Trabecular bone microstructure is impaired in the proximal femur of human immunodeficiency virus-infected men with normal bone mineral density. Quant Imaging Med Surg. 2018;8(1):5-13.

78. Calmy A, Chevalley T, Delhumeau C, Toutous-Trellu L, Spycher-Elbes R, Ratib O, et al. Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women. Osteoporos Int. 2013;24(6):1843-52.

79. Manavalan JS, Arpadi S, Tharmarajah S, Shah J, Zhang CA, Foca M, et al. Abnormal Bone Acquisition With Early-Life HIV Infection: Role of Immune Activation and Senescent Osteogenic Precursors. J Bone Miner Res. 2016;31(11):1988-96.

80. Mallon PW. Aging with HIV: osteoporosis and fractures. Curr Opin HIV AIDS. 2014;9(4):428-35.

81. Shiau S, Broun EC, Arpadi SM, Yin MT. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. AIDS. 2013;27(12):1949-57.

82. Guerri-Fernandez R, Villar-Garcia J, Diez-Perez A, Prieto-Alhambra D. HIV infection, bone metabolism, and fractures. Arq Bras Endocrinol Metabol. 2014;58(5):478-83.

83. Ilha T, Comim FV, Copes RM, Compston JE, Premaor MO. HIV and Vertebral Fractures: a Systematic Review and Metanalysis. Sci Rep. 2018;8(1):7838.

84. Miller PD. Management of severe osteoporosis. Expert Opin Pharmacother. 2016;17(4):473-88.

85. Anam AK, Insogna K. Update on Osteoporosis Screening and Management. Med Clin North Am. 2021;105(6):1117-34.

86. Bedimo R, Cutrell J, Zhang S, Drechsler H, Gao A, Brown G, et al. Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. AIDS. 2016;30(4):601-8.

87. Slama L, Reddy S, Phair J, Palella FJ, Jr., Brown TT, Multicenter ACSg. Changes in bone turnover markers with HIV seroconversion and ART initiation. J Antimicrob Chemother. 2017;72(5):1456-61.

88. Moreira CA, Ferreira C, Madeira M, Silva BCC, Maeda SS, Batista MC, et al. Reference values of 25-hydroxyvitamin D revisited: a position statement from the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC). Arch Endocrinol Metab. 2020;64(4):462-78.

89. Bilezikian JP, Bailey L, Elmer PJ, Favus MJ, Go VLW, Goldring SR, et al. Optimal Calcium Intake. JAMA. 1994;272(24):1942-8.

90. Hew FL, Chan SP, Yeap SS. Sarcopenia and vertebral fracture. Osteoporos Sarcopenia. 2018;4(4):151.

91. Reiss J, Iglseder B, Alzner R, Mayr-Pirker B, Pirich C, Kassmann H, et al. Sarcopenia and osteoporosis are interrelated in geriatric inpatients. Z Gerontol Geriatr. 2019;52(7):688-93.

92. Hawkins KL, Zhang L, Ng DK, Althoff KN, Palella FJ, Jr., Kingsley LA, et al. Abdominal obesity, sarcopenia, and osteoporosis are associated with frailty in men living with and without HIV. AIDS. 2018;32(10):1257-66.

93. Almeida TS, Cortez AF, Cruz MRD, Almeida VP. Predictors of sarcopenia in young hospitalized patients living with HIV. Braz J Infect Dis. 2021;25(2):101574.

94. Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. J Musculoskelet Neuronal Interact. 2020;20(3):372-81.

95. Funderburg NT, Mehta NN. Lipid Abnormalities and Inflammation in HIV Inflection. Curr HIV/AIDS Rep. 2016;13(4):218-25.

96. Kuti MA, Adesina OA, Awolude OA, Ogunbosi BO, Fayemiwo SA, Akinyemi JO, et al. Dyslipidemia in ART-Naive HIV-Infected Persons in Nigeria--Implications for Care. J Int Assoc Provid AIDS Care. 2015;14(4):355-9.

97. Lagathu C, Bereziat V, Gorwood J, Fellahi S, Bastard JP, Vigouroux C, et al. Metabolic complications affecting adipose tissue, lipid and glucose metabolism associated with HIV antiretroviral treatment. Expert Opin Drug Saf. 2019;18(9):829-40.

98. Angulo J, El Assar M, Alvarez-Bustos A, Rodriguez-Manas L. Physical activity and exercise: Strategies to manage frailty. Redox Biol. 2020;35:101513.

99. Organization WH. World report on ageing and health: World Health Organization; 2015.

ANEXO A – Aprovação do Comitê de Ética

UERJ - HOSPITAL UNIVERSITÁRIO PEDRO ERNESTO/ UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da densidade e microarquitetura ósseas e composição corporal dos pacientes com vírus da Imunodeficiência Humana

Pesquisador: BARBARA GEHRKE

Área Temática:

Versão: 1

CAAE: 29162020.1.0000.5259

Instituição Proponente: Hospital Universitário Pedro Ernesto

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.960.495

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1491783.pdf	12/01/2020 11:09:15		Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_completo.docx	12/01/2020 11:08:21	BARBARA GEHRKE	Aceito
Outros	declaracao_confidencialidade.pdf	12/01/2020 11:06:48	BARBARA GEHRKE	Aceito
Folha de Rosto	FOLHA_DE_ROSTO.pdf	12/01/2020 10:59:07	BARBARA GEHRKE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_pacientes_sem_HIV.doc	20/12/2019 17:23:33	BARBARA GEHRKE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_pacientes_HIV.doc	20/12/2019 17:23:15	BARBARA GEHRKE	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 08 de Abril de 2020

Assinado por:
WILLE OIGMAN
(Coordenador(a))

ANEXO B – Termo de consentimento Livre e Esclarecido para HIV e controle

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado(a) a participar, como voluntário(a), do estudo intitulado "Avaliação da microarquitetura óssea e composição corporal dos pacientes com vírus da Imunodeficiência Humana" conduzido por Dra. Bárbara Gehrke, sob orientação da Prof. Dra. Maria Caroline Alves Coelho Amaral. Este estudo visa avaliar as diferenças na massa óssea, na composição corporal e presença ou não de fraturas em pacientes com HIV e comparar com a população sem o vírus. O objetivo deste estudo é identificar o período ideal para rastreio desta população quanto a doenças de diminuição da massa óssea e muscular, visando prevenção e melhora da qualidade de vida.

Serão selecionados pacientes com o vírus da imunodeficiência humana (HIV) entre 20 e 50 anos, e com peso inferior a 120kg. Sua participação não é obrigatória. A qualquer momento, você poderá desistir de participar e retirar seu consentimento. Sua recusa, desistência ou retirada de consentimento não acarretará prejuízo.

Neste estudo, será realizada Densitometria Óssea para avaliação da massa óssea e da presença de fraturas e Tomografia dos ossos, os quais apresentam risco irrelevante quanto a radiação emitida, com níveis seguros à saúde do paciente. Além disso haverá coleta de sangue e de urina de 24 horas, que não apresentam risco adicional para o paciente, podendo apenas levar a um incômodo no local da coleta e dor. Por último, será realizado teste de força muscular o que pode ocasionar dor muscular após o esforço. Sua participação nesta pesquisa consistirá em realização de Densitometria, Tomografia, exames laboratoriais e de urina e avaliação do caminhar e da força muscular. Será realizado o primeiro contato com o participante através de consulta no Hospital Universitário Pedro Ernesto com a pesquisadora Bárbara Gehrke com preenchimento de questionário, análise de prontuário e de exames prévios à pesquisa. O contato do pesquisador será disponibilizado para que o participante tenha uma fonte de tirada de dúvidas e contatos referentes ao estudo. Informamos que essa pesquisa não gera custos ao participante, exceto de transporte, para realização das consultas e exames.

Os dados obtidos por meio desta pesquisa serão confidenciais e não serão divulgados em nível individual, visando assegurar o sigilo de sua participação.

O pesquisador responsável se compromete a tornar públicos nos meios acadêmicos e científicos os resultados obtidos de forma consolidada sem qualquer identificação de indivíduos participantes.

Rubrica do participante Rubrica do pesquisador

Caso você concorde em participar desta pesquisa, assine ao final deste documento, que possui duas vias, sendo uma delas sua, e a outra, do pesquisador responsável / coordenador da pesquisa. Seguem os telefones e o endereço institucional do pesquisador responsável e do Comitê de Ética em Pesquisa – CEP, onde você poderá tirar suas dúvidas sobre o projeto e sua participação nele, agora ou a qualquer momento.

Contatos do pesquisador responsável: Bárbara Gehrke, pós-graduanda, com endereço institucional Boulevard 28 de Setembro, 77, com email barbara_gehrke@yahoo.com.br e telefone celular (21) 981012123 / (21) 966855665.

Contatos do CEP HUPE: cep-hupe@uerj.br ; telefone (21) 28688253; segunda a sexta das 09:00 a 12:00 e 13:00 às 17:00. Boulevard 28 de Setembro, 77 – Vila Isabel – Rio de Janeiro, RJ.

Caso você tenha dificuldade em entrar em contato com o pesquisador responsável, comunique o fato à Comissão de Ética em Pesquisa da UERJ: Rua São Francisco Xavier, 524, sala 3018, bloco E, 3º andar, - Maracanã - Rio de Janeiro, RJ, e-mail: etica@uerj.br - Telefone: (021) 2334-2180. O CEP COEP é responsável por garantir a proteção dos participantes de pesquisa e funciona às segundas, quartas e sextas-feiras, de 10h às 12h e 14h às 16h.

Declaro que entendi os objetivos, riscos e benefícios de minha participação na pesquisa, e que concordo em participar.

Rio de Janeiro, _____ de _____ de_____.

Nome do(a) participante: _____

Assinatura: _____

Nome do(a) pesquisador: _____

Assinatura: _____

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado(a) a participar, como voluntário(a), do estudo intitulado "Avaliação da microarquitetura óssea e composição corporal dos pacientes com vírus da Imunodeficiência Humana" conduzido por Dra. Bárbara Gehrke, sob orientação da Prof. Dra. Maria Caroline Alves Coelho Amaral. Este estudo visa avaliar as diferenças na massa óssea, na composição corporal e presença ou não de fraturas em pacientes com HIV e comparar com a população sem o vírus. O objetivo deste estudo é identificar o período ideal para rastreio desta população quanto a doenças de diminuição da massa óssea e muscular, visando prevenção e melhora da qualidade de vida.

Serão selecionados pacientes saudáveis entre 20 e 50 anos, e com peso inferior a 120kg. Sua participação não é obrigatória. A qualquer momento, você poderá desistir de participar e retirar seu consentimento. Sua recusa, desistência ou retirada de consentimento não acarretará prejuízo.

Neste estudo, será realizada Densitometria Óssea para avaliação da massa óssea e da presença de fraturas e Tomografia dos ossos, os quais apresentam risco irrelevante quanto a radiação emitida, com níveis seguros à saúde do paciente. Além disso haverá coleta de sangue e de urina de 24 horas, que não apresentam risco adicional para o paciente, podendo apenas levar a um incômodo no local da coleta e dor. Por último, será realizado teste de força muscular o que pode ocasionar dor muscular após o esforço. Sua participação nesta pesquisa consistirá em realização de Densitometria, Tomografia, exames laboratoriais e de urina e avaliação do caminhar e da força muscular. Será realizado o primeiro contato com o participante através de consulta no Hospital Universitário Pedro Ernesto com a pesquisadora Bárbara Gehrke com preenchimento de questionário, análise de prontuário e de exames prévios à pesquisa. O contato do pesquisador será disponibilizado para que o participante tenha uma fonte de tirada de dúvidas e contatos referentes ao estudo. Informamos que essa pesquisa não gera custos ao participante, exceto de transporte, para realização das consultas e exames.

Os dados obtidos por meio desta pesquisa serão confidenciais e não serão divulgados em nível individual, visando assegurar o sigilo de sua participação.

O pesquisador responsável se compromete a tornar públicos nos meios acadêmicos e científicos os resultados obtidos de forma consolidada sem qualquer identificação de indivíduos participantes.

Rubrica do participante Rubrica do pesquisador

Caso você concorde em participar desta pesquisa, assine ao final deste documento, que possui duas vias, sendo uma delas sua, e a outra, do pesquisador responsável / coordenador da pesquisa. Seguem os telefones e o endereço institucional do pesquisador responsável e do Comitê de Ética em Pesquisa – CEP, onde você poderá tirar suas dúvidas sobre o projeto e sua participação nele, agora ou a qualquer momento.

Contatos do pesquisador responsável: Bárbara Gehrke, pós graduanda, com endereço institucional Boulevard 28 de Setembro, 77, com email barbara_gehrke@yahoo.com.br e telefone celular (21) 981012123 / (21) 966855665

Contatos do CEP HUPE: cep-hupe@uerj.br ; telefone (21) 28688253; segunda a sexta das 09:00 a 12:00 e 13:00 às 17:00. Boulevard 28 de Setembro, 77 – Vila Isabel – Rio de Janeiro, RJ.

Caso você tenha dificuldade em entrar em contato com o pesquisador responsável, comunique o fato à Comissão de Ética em Pesquisa da UERJ: Rua São Francisco Xavier, 524, sala 3018, bloco E, 3º andar, - Maracanã - Rio de Janeiro, RJ, e-mail: etica@uerj.br - Telefone: (021) 2334-2180. O CEP COEP é responsável por garantir a proteção dos participantes de pesquisa e funciona às segundas, quartas e sextas-feiras, de 10h às 12h e 14h às 16h.

Declaro que entendi os objetivos, riscos e benefícios de minha participação na pesquisa, e que concordo em participar.

Rio de Janeiro, _____ de _____ de_____.

Nome do(a) participante: _____

Assinatura: _____

Nome do(a) pesquisador: _____

Assinatura: _____

ANEXO C – Questionário

PROJETO DOUTORADO BÁRBARA GEHRKE Data: _____ / ____ / _____

Nome: _____

GRUPO: CONTROLE ☐ HIV ☐

Prontuário: _____

Data de nascimento _____/_____/_____ Idade: ____

Sexo: fem ☐ masc ☐ Caucasiano ☐ Não caucasiano ☐

Endereço: _____CEP: _____

Telefones: _____

Profissão/Escolaridade: _____

Medicamentos (EXCLUIR GLICOCORTICOIDES)

Especificar doses e tempo de uso:_____

_____

História Patológica Pregressa

_____

_____

_____

História Familiar

Fraturas pais (quadril): □ sim □ não Osteoporose (pais): □ sim □ não

_____

_____

História Fisiológica

Masc: Pubarca ____ anos **Fem**: Menarca ___ anos Pubarca: ___ anos Ciclos: _____ G__ P__ A__

História Social

Hábitos de alimentação (infância/adolescência/idade adulta):

_____

Ingesta de cálcio - _____copos de leite (300 mg) + _____ iogurte (300 mg) + _____ fatias de queijo (150 mg) + outros (300 mg): _____ mg/dia

Ingesta de suplementos de Cálcio ou Vitamina D? _____

Etilismo (dose e frequência):_____

Tabagismo (maços/ano): _____

Drogas ilícitas (tipo e frequência): _____

Atividade física: (tipo e frequência)_____

Exame Físico

Peso: _____ Altura: _____ IMC: _____ PA: _____ x _____ mmHg FC:_____ bpm

Mucosas: _____

Fácies: □ típica □ atípica: _____ Dentes (estado geral): _____

Pescoço: _____

Tórax/mamas (principalmente p osteoporose em homens): _____

Ap. CV:_____ Ap.Resp_____

ABD: _____ MMII: _____

Marcha: _____

Força Muscular: □ simétrica nos 4 membros □ assimétrica (descrever): _____

Risco de Queda? □ não □ sim

□ número de quedas último ano: _____ □ visão deficiente □ dificuldade de deambulação

□ fraqueza muscular □ falta de equilíbrio □ hipotensão ortostática □ neuropatia periférica

□ outros: _____

Sinais de Fratura: □ perda de altura (4 cm) □ deformidade torácica □ superposição da costela e pelve

□ protusão abdominal □ cifose

Exames Laboratoriais Data: _____ /_____ /_____ Laboratório: _____

Cálcio	(vr:	a	⇉
Proteínas totais	(vr:	a	⇉
Albumina	(vr:	a	⇉
Ca Corrigido	(vr:	a	⇉
Magnésio	(vr:	a	⇉
Fósforo	(vr:	a	⇉
PTH	(vr:	a	⇉
25-OHD	(vr:	a	⇉
CTX	(vr:	a	⇉
Osteocalcina	(vr:	a	⇉

Creatinina	(vr:	a	⇉
Ureia	(vr:	a	⇉
TSH	(vr:	a	⇉
T4L	(vr:	a	⇉
HbA1C	(vr:	a	⇉
Glicemia	(vr:	a	⇉
TGO	(vr:	a	⇉
TGP	(vr:	a	⇉
FA	(vr:	a	⇉
GGT	(vr:	a	⇉
Testo total (homens)	(vr:	a	⇉
SHBG	(vr:	a	⇉
Estradiol	(vr:	a	⇉
LH	(vr:	a	⇉
FSH	(vr:	a	⇉
Prolactina			
Ca Ur 24h	(vr:	a	⇉
Cr Ur 24h	(vr:	a	⇉
Na Ur 24h	(vr:	a	⇉
P Ur 24h	(vr:	a	⇉
Volume urinário	(vr:	a	⇉
Gasometria venosa ou reserva alcalina	(vr:	a	⇉
Hb	(vr:	a	⇉
Ht	(vr:	a	⇉
VCM	(vr:	a	⇉
HCM	(vr:	a	⇉
Leuco	(vr:	a	⇉
Plaquetas	(vr:	a	⇉
VHS	(vr:	a	⇉
Insulina	(vr:	a	⇉
Lipidograma	(vr:	a	)

FRAX: Quadril: _____ **NOGG (FRAX sem densitometria):** Quadril: _____

Fraturas maiores: _____ Fraturas maiores: _____

Tomografia Periférica Quantitativa de Alta Resolução Data: _____/_____/_____

HR-pQCT distal radius		**HR-pQCT distal tibia**	
Dtotal (mg HA/ccm)		Dtotal (mg HA/ccm)	
Ct.vBMD (mg HA/ccm)		Ct.vBMD (mg HA/ccm)	
Ct.Th (mm)		Ct.Th (mm)	
Tb.vBMD (mg HA/ccm)		Tb.vBMD (mg HA/ccm)	
BV/TV		BV/TV	
Tb.N (1/mm)		Tb.N (1/mm)	
Tb.Th (mm)		Tb.Th (mm)	
Tb.Sp (mm)		Tb.Sp (mm)	
tTb.1/N.SD (mm)		tTb.1/N.SD (mm)	

Densitometria óssea Data: _____/_____/_____

Sítio	BMD (g/cm^2)	T-score	Z-score
Coluna (L__-L__)			
L1			
L2			
L3			
L4			
Fêmur Total			
Colo do Fêmur			
Rádio (33%)			
VFA			

Composição Corporal Data: _____/_____/_____

Parâmetros	**Resultados**
Percentual de Gordura (%)	
Massa gorda (g)	
Massa magra (g)	
Massa livre de gordura (g)	
ALM (Kg)	
ALM/IMC (Kg/Kg/m^2)	
ALM/h^2 (Kg/m^2)	

Data: _____/_____/_____

Teste "get up and go" :_____

Dinamômetro manual: _____

Questionário SARC-F:

1) Força → O quanto de dificuldade você tem para levantar e carregar 4,5kg?

   0=nenhuma; 1=alguma; 2= muita ou incapaz

2) Ajuda para caminhar → O quanto de dificuldade você tem para atravessar um cômodo?

   0=nenhuma; 1=alguma; 2=muita, uso apoios ou incapaz

3) Levantar da cadeira → O quanto de dificuldade você tem para levanter de uma cama ou cadeira?

   0=nenhuma; 1=alguma; 2=muita ou não consegue sem ajuda

4) Subir escadas → O quanto de dificuldade você tem para subir um lance de escadas de 10 degraus?

   0=nenhuma; 1=alguma; 2=muita ou não consegue

5) Quedas → Quantas vezes você caiu no último ano?

   0=nenhuma; 1=1 a 3 quedas; 2=4 ou mais quedas

PACIENTES HIV

Ano diagnóstico: _____ / **Duração da doença:** _____

Carga viral: Detectável → número de cópias:_____ / log _____

Indetectável → abaixo de quantas cópias? _____

CD4: _____ **CD4/CD8:** _____

Tenofovir: SIM ☐ NÃO ☐ Tempo (meses): _____

Nadir CD4: _____

Doenças oportunistas: SIM ☐ NÃO ☐
Quais? _____

Tempo total em TARV (meses): _____

Especificar quais TARV e o tempo de cada (meses): _____

ANEXO D – Artigo publicado – Riedel's Thyroiditis as a part of the IgG4 Systemic Disease: A case Report

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CASE REPORT PEER REVIEWED | OPEN ACCESS

Riedel's thyroiditis as a part of the IgG4 systemic disease: A case report

Thamyres Marques, Bárbara Gehrke, Francinne Machado Ribeiro, Marise Machado, Sérgio de Oliveira Romano, Maria Caroline Alves Coelho

ABSTRACT

Introduction: Riedel's thyroiditis (RT) is a fibro-inflammatory process that can affect the thyroid gland alone or might extend to adjacent tissues. This condition may evolve to multifocal systemic fibrosclerosis in 30–40% of the patients. Riedel's thyroiditis is a rare disease that is usually treated by surgery as it consists of a hardened, adhered goiter with local invasion that resembles a malignant lesion. This thyroid disease is considered the most associated to immunoglobulin G4-related disease (IgG4-RD). IgG4-RD is characterized by a dense lymphoplasmacytic infiltrate with numerous IgG4 positive plasma cells present in other organs besides the thyroid.

Case Report: The report refers to a 53-year-old female Brazilian patient. The patient had a medical history of hypothyroidism and also showed increase of the cervical volume since the beginning of 2018. A surgical treatment was proposed in 2019 and histological aspects suggested the diagnosis of RT. After further analysis of the material obtained in biopsy, the microscopy assessment revealed lymphoplasmacytic inflammation with high fibrosis suggesting IgG4 related sclerotic disease.

Conclusion: This case highlights the importance of investigating IgG4-RD in patients with RT as it seems to be a new clinical entity. The diagnosis is a challenge and the combination of histopathological features/ immunohistochemical staining with clinical, laboratorial, and radiological findings is extremely important.

Keywords: Immunoglobulin G4-related disease, Retroperitoneal fibrosis, Sclerosis, Thyroiditis

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Received: 22 August 2020
Accepted: 18 December 2020
Published: 05 February 2021

Thamyres Marques and Bárbara Gehrke contributed equally to the work

How to cite this article

Marques T, Gehrke B, Ribeiro FM, Machado M, Romano SO, Coelho MCA. Riedel's thyroiditis as a part of the IgG4 systemic disease: A case report Int J Case Rep Images 2021;12:101194Z01TM2021.

Article ID: 101194Z01TM2021

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doi: 10.5348/101194Z01TM2021CR

INTRODUCTION

Riedel's thyroiditis (RT) is characterized by a fibrotic and inflammatory process that spreads to tissues that surround the thyroid gland. It is an uncommon condition with prevalence of 1 in each 100,000 people, most frequent in females between 30 and 50 years [1]. According to Rotondi et al., Berhard Riedel first described RT in 1896, but only in 2010 Dalgren suggested the histologic diagnostic criteria, which included: fibro-inflammatory process involving all or a portion of the thyroid gland; presence of fibrous extension to thyroid capsule and adjacent tissues; inflammatory

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cells infiltration (without giant cells), lymphoid follicles, oncocytes or granulomas; presence of occlusive phlebitis and absence of a neoplasm [2].

Studies demonstrate that RT seems to be the thyroid condition most associated with immunoglobulin G4-related disease (IgG4-RD), that is characterized by dense lymphoplasmacytic infiltrate with numerous IgG4 positive plasma cells, and propensity to form tumefactive lesions and storiform fibrosis [2]. The diagnosis of this disease is a challenge due to the heterogeneity of its clinical presentation and variable diagnostic criteria.

CASE REPORT

A 53-year-old woman, smoker (25 packs/year), carrier of hypothyroidism (diagnosed in 2015 with positive antibodies against thyroperoxidase), complaining of hoarseness, dysphagia and increase of cervical volume. Computed tomography (CT) of the cervical region (Figure 1) demonstrated a mass at the left base of the neck, heterogeneous, extending into the mediastinum, measuring 7.0×5.0×5.0 cm, encasing the common carotid artery and associated with thrombosis of the internal jugular vein at the same side. Magnetic resonance imaging (MRI) of cervical region showed a mass with isointense signal in T1 and hyperintense in T2, insinuating into the thoracic introit at the same side for approximately 2.7 cm, surrounding vascular structures, measuring 7.0×4.0×4.5 cm. The patient was treated by surgery and histological aspects suggested the diagnosis of Riedel's thyroiditis: fibroblastic proliferation with inflammatory lymphoplasmacytic component replacing normal thyroid tissue and extending to striated muscle, with the presence of obliterating angiitis associated. Then, prednisone was initiated at dose of 20 mg. Abdominal CT and blood samples were requested due to possible association with IgG4-RD. No changes in bone metabolism, hepatic function, or IgG4 titer (Table 1) were observed.

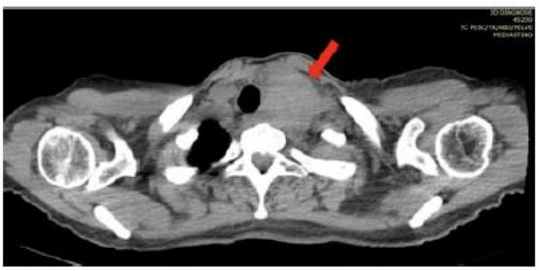

Figure 1: Computed tomography of the cervical region mass at the left base of the neck, heterogeneous, extending into the mediastinum, measuring 7.0×5.0×5.0 cm (red arrow).

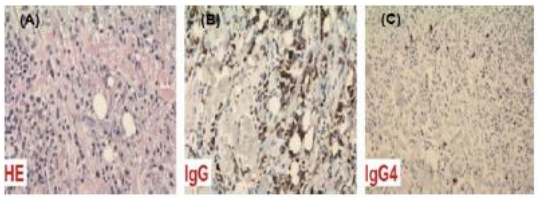

Figure 2: Immunohistochemistry of the mass at the left base of the neck immunostaining for immunoglobulin G (IgG) and IgG4 in IgG4-related thyroiditis. (A) Thyroid tissue with hematoxylin and eosin staining, (B) IgG immunostaining, and (C) IgG4 immunostaining. Both ratio $IgG4^+/IgG^+$ ratio in tissue was <10%.

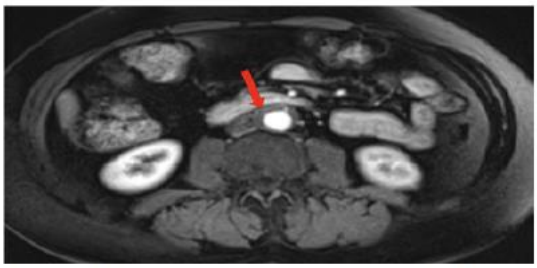

Figure 3: Magnetic resonance angiography of abdominal aorta contrast-absorbing periaortic tissue, about 0.7 cm thick, asymmetric, mainly on the anterior wall, located near the infrarenal aorta, at the level of the origin of the inferior mesenteric artery (red arrow).

Abdominal CT evidenced aorta with preserved caliber, presenting a thickening of parietal tissue, highlighting contrast from underneath the kidneys extending down to the common iliac artery to the left, suggesting retroperitoneal fibrosis. Computed tomography of cervical region eight months after prednisone evidenced lesion at topography of the thyroid gland to the left, measuring 6.7×4.2×3.1 cm. After further analysis of the material obtained through biopsy, microscopy examination revealed atrophic thyroid parenchyma and intense infiltrated lymphoplasmacytic inflammation with extensive fibrosis that extends up to muscular structures surrounding the thyroid, associated with obliterating angiitis, and also involving the parathyroid glands, suggesting IgG4 related sclerotic disease. IgG4

Table 1: Immunoglobulin results

Immunoglobulins (mg/dL)	IgG1 405–1011 range (mg/dL)	IgG2 168–786 range (mg/dL)	IgG3 11–85 range (mg/dL)	IgG4 3–201 range (mg/dL)	Total IgG (mg/dL)
Result	790	269	38	32	1230

Serum levels of immunoglobulin (Ig); Immunoglobulins (Ig); Immunoglobulin G1 (IgG1); Immunoglobulin G2 (IgG2); Immunoglobulin G3 (IgG3); Immunoglobulin G4 (IgG4).

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immunostaining with the ratio $IgG4^+/IgG^+$ ratio in tissue <10% (Figure 2).

Magnetic resonance angiography of thoracic and abdominal aorta was requested in order to visualize the retroperitoneal fibrosis and to define the best therapeutic approach for RT associated to IgG4-RD. The exam demonstrated enhancing of periaortic tissue, approximately 0.7 cm thick, asymmetric, mainly on the anterior wall, located near the infrarenal aorta, suggestive of periaortitis (Figure 3). Unfortunately, glucocorticoid dosage could not be optimized due to diabetes onset after eight months of prednisone and patient refusal. At the moment patient is undergoing evaluation to initiate Rituximab therapy.

DISCUSSION

IgG4-related disease is a new clinical entity, immune-mediated, characterized by lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells that can affect various organs, including the thyroid. It is an underdiagnosed disease with current prevalence of 6/100,000, peak incidence between 50 and 70 years of age and male predominance [2]. Riedel's thyroiditis seems to be the thyroid disease with highest association to IgG4-RD, although the real prevalence of this association remains unknown [3].

Riedel's thyroiditis can be associated with primary sclerosing cholangitis, orbital and mediastinal fibrosis, or with retroperitoneal fibrosis as described in this case report. Riedel's thyroiditis can occur at the same time of the retroperitoneal fibrosis or years later, and when associated with RT there is a higher prevalence in females [2].

Riedel's thyroiditis is suspected in the presence of fixed and hard neck mass. It can commonly manifest with obstructive symptoms such as dysphagia, hoarseness, and dyspnea due to involvement of structures adjacent to the thyroid gland. In addition, it may present symptoms of hypothyroidism or hypoparathyroidism [4]. In our case, similar to what is evidenced in literature, RT caused pressure symptoms due to an increase of the thyroid gland (dysphagia and hoarseness). Although hypothyroidism may be found in 1/3 of patients with RT, the diagnosis of hypothyroidism was prior to the onset of RT symptoms in our patient. Several cases of Hashimoto's thyroiditis have been reported previously or concomitantly to RT. Usually the thyroid function is normal, although 90% of cases antibody titers against thyroglobulin (Tg-Ab) and thyroperoxidase (TPO-Ab) are elevated [5, 6]. Hypoparathyroidism may be a presenting symptom, because of its growth and compression, independent of surgical complications. However, our patient had normal laboratory levels of calcium and phosphorus.

There is a possible association of RT with smoking, which could be linked to a more aggressive course of the disease. Tobacco use may influence the immune response by activating fibrosis-related genes expression especially connective tissue growth factor, growth factor-β1, and interleukin-1β [7].

Imaging tests are not usually specific for the disease, but they can support the diagnosis. Ultrasound may present with low echogenicity of the thyroid gland, while CT shows a hypodense, infiltrative mass which enhances after contrast and MRI shows hypointensity in T1 and T2 images. In our patient, MRI showed expansive mass with isointense signal in T1 and hyperintense in T2, which differs from literature. Similar to previous reports, MRI has not added any new information compared to ultrasonography or CT. These findings explain why the diagnosis is not confirmed by imaging tests and usually requires a histopathological analysis to rule out malignant tumor [8, 9].

Anaplastic carcinoma, papillary carcinoma, lymphoma, fibrotic Hashimoto's thyroiditis, and sarcoma of the thyroid are considered differential diagnosis of RT, as they may present clinical similarities, such as compressive symptoms due to gland growth [10–12]. Histological features of diffuse sclerosing papillary thyroid cancer may have resemblance to RT and should be ruled out [13].

The pathogenesis of the connection between RT and IgG4-RD is not yet known. In healthy subjects, IgG4 represents less than 5% of the total IgG and it seems to play a minor role in the IgG4-RD, since they are generally considered anti-inflammatory. There is no clear evidence if IgG4 participates in the pathogenesis or if it is a consequence of the process. Recent data defend the idea that an increase in IgG4 levels should be interpreted as an effort to reduce inflammation mediated by a given antigen [3].

The diagnosis of IgG4-RD is a challenge and for the definitive diagnosis the combination of histopathological features/immunohistochemical stain with clinical, laboratorial, and radiological findings suggestive of the disease is necessary. The laboratorial diagnosis is marked by an increase in IgG4 (normally over 135 mg/dL) blood levels and radiological findings consist of the involvement of other organs [2, 3].

The histopathological features are divided into major and minor criteria. For the diagnosis it is necessary 2 of the 3 major criteria: infiltrate rich with IgG4 cells; storiform aspect fibrosis and obliterative phlebitis. The minor criteria: phlebitis without obliteration and an increase in eosinophil levels alone are neither sensitive nor specific for the disease.

IgG4 immunostaining is an essential test for the pathological diagnosis of IgG4-RD. There is no cutoff for the number of $IgG4^+$ plasma cells in the tissue to characterize the disease. This number appears to be tissue specific and depends on the amount of fibrosis at diagnosis. On biopsy specimens over 10 numbers of $IgG4^+$ plasma cells per high-power field (hpf) is suggestive of IgG4-RD [14, 15].

However, several inflammatory lesions, lymphoma, and malignances may increase the number of $IgG4^+$ plasma cells per hpf unrelated to IgG4-RD [16, 17]. In this case the $IgG4^+/IgG^+$ ratio seems to be better than the isolated $IgG4^+$ dosage. Some authors suggest a 40% cutoff in the $IgG4^+/IgG^+$ ratio in any organ to document the disease in case of patients with histopathological features and a compatible clinical picture [18, 19]. Nevertheless, 40% of patients with biopsy-proven disease may have normal IgG4 serum levels [20].

The patient had retroperitoneal fibrosis on imaging and met two major histopathological criteria, which strongly suggested IgG4-RD. Yet, she had normal serum dosage of IgG4 and the ratio IgG4+/IgG+ ratio in tissue was < 10% at immunohistochemical. These results may be due to sampling artifact, use of corticosteroid during a long period (eight months) before the exam or progression to a fibrotic stage. Thus, in the absence of a more specific biomarker, the diagnosis is based primarily on the morphological appearance on biopsy. Tissue IgG4 count and $IgG4^+/IgG^+$ ratio seems to be of secondary importance [21].

Regarding treatment, there are no clinical trials due to the rare number of cases involving this disease. However, according to literature, the use of glucocorticoids constitutes the base of the initial treatment, but there is no consensus about dosage, ranging from 15 to 100 mg per day. The response to therapy is evidenced by reduction of lesion dimensions, with consequent relief of compressive symptoms [22, 23]. Our patient did not show reduction of cervical mass with 20 mg of prednisone and unfortunately this dose could not be increased due to the development of secondary diabetes. For this reason, another therapy had to be considered. The increment of fibrosis evidenced in late disease in comparison to early disease (with predominance of inflammation) could explain the non-response to glucocorticoids therapy [23–25].

Patients who do not present an acceptable response to corticosteroids or those who cannot use this medication for any other reason, may use tamoxifen as a second line treatment. It is a selective estrogen receptor modulator that stimulates tumor growth factor beta (TGF-β) which works as a powerful growth inhibitor, usually administrated around 10–20 mg two times daily [26]. Rituximab and micofenolate are immunosuppressive agents that may also be considered when treating RT associated to IgG4-GD. Both have anti-fibrotic features and are used in systemic fibrosis that do not respond to the drugs named above [27, 28].

The prognosis of RT is extremely variable, from mild to rapidly progressive forms and tracheal compression is the major cause of mortality (around 10%). Surgery may be considered in order to alleviate compressive symptoms and to rule out malignity, once it is very difficult to differentiate fibrotic tissue from normal thyroid gland during procedure [9].

CONCLUSION

Riedel's thyroiditis is a rare condition, with prevalence higher in women and characterized by the appearance of goiter with compression of adjacent structures due to invasion of extra-thyroid tissues. Riedel's thyroiditis seems to be the thyroid disease that is most associated to IgG4-RD, and for this reason it is important to actively investigate this association and accomplish a biopsy with histopathology analysis and laboratory evaluation. Though it is an uncommon disease and with limited cases reported in literature, a considerable amount of patients respond well to drug therapy, being surgery preferred only to relief compressive symptoms.

ETHICS COMMITTEE

The case report was approved by the ethics committee of the State University of Rio de Janeiro under the number of CAAE 34725020.0.0000.5259.

REFERENCES

1. Hay ID. Thyroiditis: A clinical update. Mayo Clin Proc 1985;60(12):836–43.
2. Rotondi M, Carbone A, Coperchini F, Fonte R, Chiovato L. Diagnosis of endocrine disease: IgG4-related thyroid autoimmune disease. Eur J Endocrinol 2019;180(5):R175–83.
3. Kottahachchi D, Topliss DJ. Immunoglobulin G4-related thyroid diseases. Eur Thyroid J 2016;5(4):231–9.
4. Marín F, Araujo R, Páramo C, Lucas T, Salto L. Riedel's thyroiditis associated with hypothyroidism and hypoparathyroidism. Postgrad Med J 1989;65(764):381–3.
5. Graham A, Gilliland IC. Riedel's thyroiditis. Br Med J 1959;2(5146):225–6.
6. Zelmanovitz F, Zelmanovitz T, Beck M, Cerski CT, Schmid H, Czepielewski MA. Riedel's thyroiditis associated with high titers of antimicrosomal and antithyroglobulin antibodies and hypothyroidism. J Endocrinol Invest 1994;17(9):733–7.
7. Kau HC, Wu SB, Tsai CC, Liu CJL, Wei YH. Cigarette smoke extract-induced oxidative stress and fibrosis-related genes expression in orbital fibroblasts from patients with Graves' ophthalmopathy. Oxid Med Cell Longev 2016;2016:4676289.
8. Ozgen A, Cila A. Riedel's thyroiditis in multifocal fibrosclerosis: CT and MR imaging findings. AJNR Am J Neuroradiol 2000;21(2):320–1.
9. Fatourechi MM, Hay ID, McIver B, Sebo TJ, Fatourechi V. Invasive fibrous thyroiditis (Riedel thyroiditis): The Mayo Clinic experience, 1976–2008. Thyroid 2011;21(7):765–72.
10. Torres-Montaner A, Beltrán M, Romero de la Osa A, Oliva H. Sarcoma of the thyroid region mimicking Riedel's thyroiditis. J Clin Pathol 2001;54(7):570–2.
11. Won YS, Lee HH, Lee YS, et al. A case of Riedel's thyroiditis associated with benign nodule: Mimic of

anaplastic transformation. Int J Surg 2008;6(6):e24–7.
12. Canos JC, Serrano A, Matias-Guiu X. Paucicellular variant of anaplastic thyroid carcinoma: Report of two cases. Endocr Pathol 2001;12(2):157–61.
13. Chung J, Lee SK, Gong G, et al. Sclerosing Mucoepidermoid carcinoma with eosinophilia of the thyroid glands: A case report with clinical manifestation of recurrent neck mass. J Korean Med Sci 1999;14(3):338–41.
14. Lindstrom KM, Cousar JB, Lopes MBS. IgG4-related meningeal disease: Clinico-pathological features and proposal for diagnostic criteria. Acta Neuropathol 2010;120(6):765–76.
15. Saeki T, Nishi S, Imai N, et al. Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. Kidney Int 2010;78(10):1016–23.
16. Shimizu I, Nasu K, Sato K, et al. Lymphadenopathy of IgG4-related sclerosing disease: Three case reports and review of literature. Int J Hematol 2010;92(5):751–6.
17. Witkiewicz AK, Kennedy EP, Kennyon L, Yeo CJ, Hruban RH. Synchronous autoimmune pancreatitis and infiltrating pancreatic ductal adenocarcinoma: Case report and review of the literature. Hum Pathol 2008;39(10):1548–51.
18. Chen G, Cheuk W, Chan JKC. IgG4-related sclerosing disease: A critical appraisal of an evolving clinicopathologic entity. [Article in Chinese]. Zhonghua Bing Li Xue Za Zhi 2010;39(12):851–68.
19. Sato Y, Kojima M, Takata K, et al. Systemic IgG4-related lymphadenopathy: A clinical and pathologic comparison to multicentric Castleman's disease. Mod Pathol 2009;22(4):589–99.
20. Sah RP, Chari ST. Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis. Curr Opin Rheumatol 2011;23(1):108–13.
21. Deshpande V, Zen Y, Chan JK, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol 2012;25(9):1181–92.
22. Bozkirli E, Bakiner OS, Ersozlu Bozkirli ED, et al. Serum Immunoglobulin G4 levels are elevated in patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf) 2015;83(6):962–7.
23. Vaidya B, Harris PE, Barrett P, Kendall-Taylor P. Corticosteroid therapy in Riedel's thyroiditis. Postgrad Med J 1997;73(866):817–9.
24. Bagnasco M, Passalacqua G, Pronzato C, Albano M, Torre G, Scordamaglia A. Fibrous invasive (Riedel's) thyroiditis with critical response to steroid treatment. J Endocrinol Invest 1995;18(4):305–7.
25. Thomson JA, Jackson IM, Duguid WP. The effect of steroid therapy on Riedel's thyroiditis. Scott Med J 1968;13(1):13–6.
26. Butta A, MacLennan K, Flanders KC, et al. Induction of transforming growth factor beta 1 in human breast cancer in vivo following tamoxifen treatment. Cancer Res 1992;52(15):4261–4.
27. Levy JM, Hasney CP, Friedlander PL, Kandil E, Occhipinti EA, Kahn MJ. Combined mycophenolate mofetil and prednisone therapy in tamoxifen- and prednisone-resistant Reidel's thyroiditis. Thyroid 2010;20(1):105–7.
28. Carruthers MN, Topazian MD, Khosroshahi A, et al. Rituximab for IgG4-related disease: A prospective, open-label trial. Ann Rheum Dis 2015;74(6):1171–7.

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Author Contributions

Thamyres Marques – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Bárbara Gehrke – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Francinne Machado Ribeiro – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Guarantor of Submission
The corresponding author is the guarantor of submission.

Source of Support
None.

Consent Statement
Written informed consent was obtained from the patient for publication of this article.

Conflict of Interest
Authors declare no conflict of interest.

Data Availability
All relevant data are within the paper and its Supporting Information files.

ANEXO E – Artigo publicado – Vitamin D status and prevalence of hypovitaminosis D in different genders throughout life stages: A Brazilian cross-sectional study

CLINICS

ORIGINAL ARTICLE

Vitamin D status and prevalence of hypovitaminosis D in different genders throughout life stages: A Brazilian cross-sectional study

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Leão LMCSM, Rodrigues BC, Dias PTP, Gehrke B, Souza TSP, Hirose CK, et al. Vitamin D status and prevalence of hypovitaminosis D in different genders throughout life stages: A Brazilian cross-sectional study. Clinics (Sao Paulo). 2021;76:e2571

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OBJECTIVES: To evaluate the mean concentration of 25-hydroxivitamin D [25(OH) D] and prevalence of hypovitaminosis D in individuals residing in Rio de Janeiro, Brazil.

METHODS: The data of 80,000 consecutive individuals who had 25(OH) D measurements performed by electrochemiluminescence between 1/2/2018 and 2/5/2018 were selected. Patients who reported the use of therapies/supplements were excluded. Levels of 25(OH) D $\geqslant$20 ng/mL (ages $<$60 years) and $\geqslant$30 ng/mL (ages $\geqslant$60 years) were considered adequate.

RESULTS: We analyzed the data of 24,074 individuals (1–95 years old, 64.7% female). Descriptive curves showed that, in both sexes, the mean values of 25(OH) D decreased from the first years of life until adolescence, then slightly increased, and then tended to stabilize during adulthood. Levels of 25(OH) D $<$20 ng/mL were observed in 6% of girls *versus* 3.6% of boys and in 13.6% of adolescent girls *versus* 12.6% of adolescent boys and 11% of adults. The percentage of seniors with serum levels of 25(OH) D $<$20 ng/mL was 13.6% in women and 12.7% in men; 53.2% of women and 50.6% of men had levels $<$30 ng/mL.

CONCLUSIONS: Mean 25(OH) D values were higher in children and lower in adolescents and women. Approximately 90% of non-seniors and presumably healthy residents of the urban metropolitan region of Rio de Janeiro presented satisfactory levels of 25(OH) D during the summer months; however, in over half of the elderly, the serum concentrations of 25(OH) D were inadequate. Therefore, strategies for the prevention of hypovitaminosis D should be considered in the senior population.

KEYWORDS: 25-Hydroxivitamin D; Vitamin D Deficiency; Hypovitaminosis D Prevalence; Rio De Janeiro; Brazil.

INTRODUCTION

Vitamin D is a fat-soluble hormone that is present in two main forms (ergocalciferol and cholecalciferol); while it may be obtained through diet, it is essentially available by the action of type B ultraviolet rays on 7-dehydrocholesterol, present in the human epidermis (1). The endogen production of cholecalciferol depends on genetic factors, body compositions, rates of physical activity, and the use of sunscreen; its production is significantly higher in regions with greater latitudes, during the summer months, and around noon (2–4). It is known that the major role of vitamin D is related to the homeostasis of calcium/phosphorus and promotion of bone mineralization; however, studies conducted in the last decade suggested that vitamin deficiency may contribute to the pathogenesis of autoimmune diseases, cancer, insulin resistance, diabetes mellitus, dyslipidemia, arterial hypertension, metabolic syndrome, chronic inflammation, endothelial dysfunction, and cardiovascular disease (5–11). These findings have significantly increased interest in evaluating the serum concentration of 25 hydroxivitamin D [25(OH) D]; therefore, 25(OH) D is currently one of the most important hormones in clinical practice worldwide. Although recent studies have demonstrated that bioavailable 25(OH) D (not attached to binding protein), free 25(OH) D (not attached to binding protein/albumin), and 25(OH)

No potential conflict of interest was reported.

Received for publication on November 7, 2020. **Accepted for publication on** February 17, 2021

DOI: 10.6061/clinics/2021/e2571

D/24-25 dihydroxyvitamin D are promising diagnostic tools, the consensus continues to recommend that vitamin D status should be evaluated by measuring 25(OH) D with competitive tests (radioimmunoassay, chemiluminescence, and electrochemiluminescence) or, preferably, by high performance liquid chromatography with ultraviolet detection or together with tandem mass spectrometry (12). Independent of sex or age range, serum 25(OH) D concentrations <20 ng/mL (50 nmol/L) and <30 ng/mL (75 nmol/L) for many years were the standard to diagnose deficiency and insufficiency of vitamin D, respectively (13). Nevertheless, these cutoffs were elaborated based on Caucasian populations and no longer represented a consensus. Currently, most specialists consider that serum 25(OH) D values ⩾20 ng/dL are adequate for maintaining bone health and calcium homeostasis in children and adults (14–16). However, some recognize that levels >30 ng/mL are required for the preservation of bone mineralization in specific groups, including teenagers (13), and for obtaining benefits unrelated to bone metabolism (15). The reference values used currently in Brazil to classify the levels of 25(OH) D as adequate were published by the Brazilian Clinical Pathology Society and Laboratory Medicine (BCPS/LM) and the Brazilian Endocrinology and Metabolism Society (BEMS) in 2014, which were updated in 2018 (17). Values >20 ng/mL are desirable for the healthy population up to 59 years of age, and values >30 ng/mL are recommended for the elderly (aged ⩾60 years). Values >30 ng/mL are also recommended for individuals classified into certain risk groups, including pregnant women, patients with osteometabolic diseases such as sarcopenia, chronic kidney disease, diabetes mellitus, cancer, and for patients who are using glucocorticoids, anticonvulsants, and antiretroviral therapies (18). Despite the large variability of methods used for measuring and several different cutoffs, in 2007, it was estimated that approximately 1 billion people around the world presented inadequate levels of serum 25(OH) D (6). A systematic review published in 2014 analyzed 103 articles that were selected between 2003 and 2013 and concluded that vitamin D deficiency is global and mainly affects women in the Middle East. However, the authors warned that only a small number of studies were performed in children and adolescents, and that these were performed in specific populations, mainly in South America and Africa (19). In addition, in a search performed in PubMed in 11/11/2019 using the term "Vitamin D," we identified 850 publications concerning Brazilian studies, in which only 15 estimated the prevalence of hypovitaminosis D. With a total sample of 3.338 individuals evaluated through different methodologies, the percentage of hypovitaminosis D encountered was between 8.2% and 85.7% of the Brazilian population (20–34). Considering that knowledge of vitamin D status in specific populations is important for the development of public healthcare policies associated with the prevention and treatment of hypovitaminosis, we performed the present study to evaluate the secondary data of a large sample of men and women, who were presumably healthy and of all ages, to evaluate the distribution of 25(OH) D (vitamin D profile) and prevalence of hypovitaminosis D in individuals who live in the metropolitan area of the city of Rio de Janeiro.

PATIENTS AND METHODS

Following approval from the Research Ethics Committee of the University Hospital of the State University of Rio de Janeiro (UERJ) (registration N° 04191018.3.0000.5259), a retrospective study was conducted with the cooperation of the Endocrinology Division of the UERJ Medical School and Diagnósticos da America (DASA) diagnostics company.

From DASA's laboratory information system, two members of DASA mined de-identified data from 80,000 consecutive patients who had 25(OH) D measurements run by DASA's central laboratory, which is situated in Rio de Janeiro, from 1/2/2018 to 2/5/2018. Patients with chronological ages <1 year and who reported the use of therapies and/or vitamin supplements at the time of blood withdrawal were excluded, reducing our sample size to 24,483 patients.

For the analysis that required chronological age, 59 patients were excluded because of lack of data.

The analysis of 25(OH) D was performed using electrochemiluminescence (ADVIA Centaur, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The total variation coefficient and interassay variation coefficients were up to 12% and 8%, respectively. In accordance with the BCPS/LM/BEMS guidelines, we considered adequate levels of 25(OH) D >20 ng/mL and >30 ng/mL in individuals up to 59 years old and in those aged ⩾60 years, respectively.

The statistical analyses of the study were performed using the statistical software 'R' (R' Foundation for Statistical Computing, Vienna, Austria; URL: http://www.R-project.org/). Although the results of the analysis were not significantly affected, for better graphical visualization, we excluded extreme values from the Chauvent test. For this reason, the general sample included 24,074 patients. The skewness, kurtosis, and Shapiro-Welk tests were used to evaluate the normality of the distribution of the 25(OH) D levels in the studied population, and Bartlett and Kruskal-Wallis tests were used to evaluate the homogeneity and significance of the variables, respectively, when considering different genders and age ranges. As the hypothesis of equality for the distinct age subgroups was rejected by the Bartlett and Kruskal-Wallis tests, the Bonferroni test was then applied to identify homogeneous age groups in terms of serum levels of 25(OH) D. The A Chi-square test was used to estimate if the proportion of individuals with hypovitaminosis D differed significantly between the groups. The criterion for the determination of significance adopted in this study was 5%.

RESULTS

Levels of 25(OH) D in 24,074 individuals aged 1–95 years (64.7% female) were analyzed.

Skewness, kurtosis, and Shapiro-Welk tests demonstrated that the serum levels of 25(OH) D in the studied population were slightly different from a normal distribution (Figure 1).

Descriptive curves showed that, in both sexes, the mean values of 25(OH) D decreased from the first years of life to adolescence, then slightly increased, and then tended to stabilize during adulthood (Figure 2).

The levels of 25(OH) D were higher in men at all life stages. The proportion of women with 25(OH) D levels <20 ng/mL was significantly higher than that of men in the patients aged <60 years ($p<0.001$). This difference was not significant for those aged ⩾60 years ($p=0.1588$) (Figure 2).

The Bartlett test showed that there was no homogeneity in the variances when considering gender and age ranges, and the Kruskal-Wallis test indicated that, with this significant difference, the data analysis should consider the levels of 25(OH) D separately.

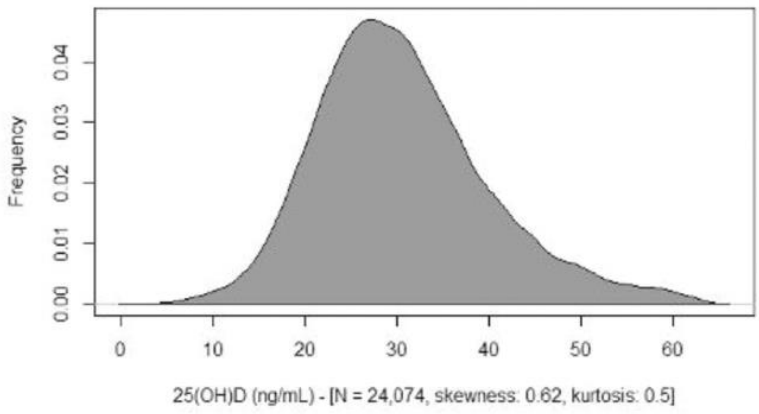

Figure 1 - Density plot for 25-hydroxyvitamin D values (ng/mL).

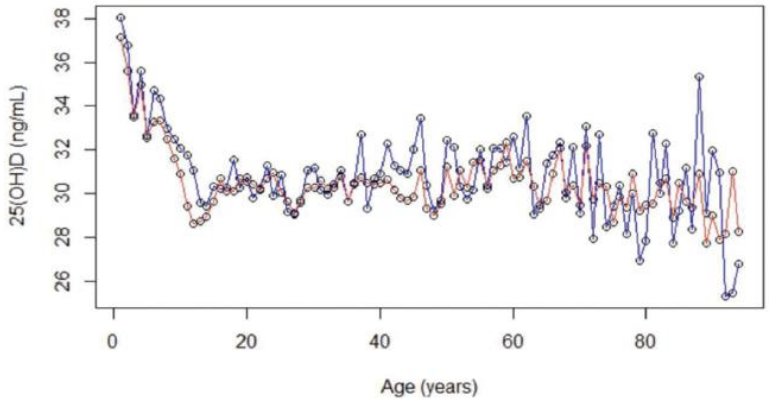

Figure 2 - Mean values of 25-hydroxyvitamin D (ng/mL) according to sex and age. Female: red line; Male: blue line.

- 25(OH) D per age: Bartlett's K-squared=227.68, df=93, p=2.697e-13.
- 25(OH) D per sex: Bartlett's K-squared=13.117, df=1, p=0.0002927.
- 25(OH) D per age: Kruskal-Wallis K-squared=581.99, df=93, p<2.2e-16.
- 25(OH) D per sex: Kruskal-Wallis K-squared=80.502, df=1, p<2.2e-16.

The Bonferroni test identified homogenous groups for 25(OH) D levels, which were significantly different from each other (Table 1).

Based on the analysis of mean values of 25(OH) D, according to sex and to conventionally established age subgroups (Table 2), we observed a statistically significant reduction in 25(OH) D levels in the age range of 1–11 years compared with the age range of 12–18 years, in both sexes ($p<0.001$). An increase in 25(OH) D levels was also observed in the age range of 12–18 years compared with the range of 19–59 years, which was statistically significant for women ($p<0.001$). A non-significant difference in the mean 25(OH) D levels was found when comparing the age ranges of those aged 60–95 years with those aged 19–59 years.

As also shown in Table 2, 6% of female and 3.6% of male children aged <12 years presented inadequate serum levels of vitamin D (25(OH) D <20 ng/mL). This percentage increased to 13.4% and 12.6%, respectively, in female and male adolescents, and was approximately 11% in adults of both sexes <60 years of age.

The percentage of seniors with serum levels of 25(OH) D that were <20 ng/mL was 13.6% in women and 12.7% in men; however, when considering the cutoffs established by

Table 1 - Homogenous age groups by sex for the levels of 25-hydroxivitamin D [the Bonferroni test was used to separate groups with statistically different means ($p \leqslant 0.05$)].

	n	Mean values	SD	1st Quartile	Median	3rd Quartile
Female subjects						
Group 1: Ages 1–4 years	501	34.3491	8.726559	28.4	32.9	39.7
Group 2: Ages 5–10 years	1,198	31.48205	7.981029	25.8	30.5	36.3
Group 3: Ages 11–15 years	1,087	27.91766	8.450635	22.4	26.7	32.4
Group 4: Ages 16–53 years	9,614	30.04439	9.271459	23.6	28.8	30.0
Group 5: Ages 54–95 years	3,139	30.36008	9.77338	24.2	29.6	35.8
Total	15,539					
Male subjects						
Group 1: Ages 1–4 years	510	35.71765	9.155856	29.2	35.0	41.5
Group 2: Ages 5–11 years	1.433	32.93936	8.683551	26.7	31.8	38.0
Group 3: Ages 12–95 years	6.533	30.63023	9.676679	23.9	29.6	35.9
Total	8,476					

n: sample size; SD: standard deviation.

Table 2 - Means, medians, and hypovitaminosis D percentages by sex and age group.

	Mean	SD	Median	25th Quartile	75th Quartile	%
From 1 to 11 (years old)						
Female (n=1928)	31.7	8.4	30.7	25.8	36.8	
25(OH) D <20 ng/mL	17.5	2.3	18.1	16.5	19.3	6.0
Male (n=1943)	33.7	8.9	32.4	27.2	39.0	
25(OH) D <20 ng/mL	17.8	2.0	18.3	17.3	19.2	3.6
From 12 to 18 (years old)						
Female (n=1429)	28.9	8.8	27.8	23.0	33.7	
25(OH) D <20 ng/mL	16.7	2.8	17.6	15.2	18.7	13.4
Male (n=1092)	30.3	9.8	29.2	23.6	35.6	
25(OH) D <20 ng/mL	16.2	3.3	17.4	14.2	18.9	12.6
From 19 to 59 (years old)						
Female (n=9918)	30.2	9.3	28.8	23.7	35.4	
25(OH) D <20 ng/mL	16.8	2.7	17.5	15.6	18.9	11.3
Male (n=4409)	30.7	9.6	29.6	24.1	35.9	
25(OH) D <20 ng/mL	16.7	2.8	17.3	15.0	18.9	11.0
From 60 to 95 (years old)						
Female (n=2264)	30.0	9.8	29.3	23.8	35.4	
25(OH) D <20 ng/mL	15.1	3.8	16.1	12.2	18.4	13.6
25(OH) D <30 ng/mL	22.9	5.5	24.2	19.8	27.2	53.2
Male (n=1032)	30.6	9.9	29.8	23.8	35.8	
25(OH) D <20 ng/mL	15.9	3.4	16.7	14.9	18.6	12.7
25(OH) D <30 ng/mL	23.1	5.1	23.9	19.8	27.2	50.6

25(OH) D: 25 hydroxivitamin D; n: sample size by age and sex; SD: standard deviation; %: percentage.

BCPS/LM/BEMS for this age range (25(OH) D <30 ng/mL), we observed that 53.2% of women aged ⩾60 years and 50.6% of men aged ⩾60 years had inadequate levels of 25(OH) D (Table 2).

DISCUSSION

In this retrospective study that was accomplished by accessing the de-identified electronic database of the largest commercial laboratory in Latin America, the 25(OH) D serum levels of 24,074 presumably healthy individuals were evaluated to determine its distribution in different genders and age ranges, as well as the prevalence of hypovitaminosis D in residents of the urban metropolitan region of Rio de Janeiro, located at latitude 22°54′13″ South.

We observed that, in both sexes, the levels of 25(OH) D decreased from the first years of life to adolescence, then slightly increased, and then tended to stabilize during adulthood. Furthermore, we observed that during childhood, adolescence, adulthood, and elderly life, the levels of 25(OH) D appeared to be lower in women; we also saw that the 25(OH) D mean values were homogeneous in the different age ranges and subgroups of both sexes. Children, particularly male children, presented higher 25(OH) D mean values, while female teenagers presented lower mean values.

Regarding the children and adolescents' profiles, we verified that the results of the present study corroborated several important publications. In a populational study performed in the European continent involving 55,844 individuals, the authors observed that adolescents between 15 and 18 years of age had a higher risk of vitamin D deficiency (25(OH) D <12 ng/mL) than children aged between 1 and 14 years, adults, and even seniors (>61 years of age) (35). Additionally, in a recent study performed with 2,416 healthy children and teenagers, the authors documented that the nadir of 25(OH) D occurs between 14 and

16 years of age in male and female adolescents, respectively (36).

A reduction in serum levels of 25(OH) D in adolescents has been universally described, particularly in female adolescents, suggesting that this phase might be a risk for hypovitaminosis D presentation, which, when severe and/or for an extended period, may have negative consequences that can be relevant for both linear growth and peak bone mass (37–41). Therefore, the status of vitamin D may be an important factor to be considered in this age range (26,42).

The mechanisms responsible for an increase in the prevalence of hypovitaminosis D in the adolescent period have not yet been elucidated and may be related to biological parameters, low ingestion of foods rich in vitamin D (milk, oily fish, and specific mushrooms), reductions of sun exposure, and physical activity, all of which have been directly associated with lower levels of 25(OH) D (4,39,42). A Brazilian study conducted with 136 adolescents found that only 14.9% of this population ingested adequate quantities of vitamin D, 17.6% used topic sunscreen daily, and 27.9% did not practice regular outdoor physical activities (26). Reinforcing the importance of lifestyle, it was demonstrated that Italian teenagers who practiced outdoor activities for <3h per week presented a higher prevalence of hypovitaminosis D (16).

There is little information available concerning the levels of 25(OH) D in children. However, in a study performed in the Brazilian Southeast region, the authors observed, similarly to us, that the percentage of hypovitaminosis D is smaller in nurslings, suggesting that breastfeeding-associated vitamin D supplementation and regular sun exposure may elevate 25(OH) D levels in nurslings and young children (22).

In accordance with several publications, we observed that the mean values of 25(OH) D were lower in women at all life phases, with a significant difference in those <60 years old. This difference between genders is not well studied, and thus far, cannot be separately justified because of circulating concentrations of sexual steroids. The available studies that evaluate the correlation between serum levels of 25(OH) D, testosterone, free testosterone, estradiol, and sexual hormone binding globulin are controversial (43–47).

We also observed that female adolescents showed a decrease in 25(OH) D levels during the pubertal period and, as demonstrated by the Bonferroni test, a discrete increase after menopause, which was previously described by Katrinaki et al. (48). It is possible that the increase and decrease of tissue receptor expression to 1,25 hydroxivitamin D are linked, respectively, to increases and decreases of estrogen serum levels, and this hormonal process may contribute to vitamin D alterations (48,49). However, it is known that several other conditions may also influence 25(OH) D levels.

It is important to highlight that factors such as reduced insulin sensitivity, elevated body mass index (BMI), and increased bone turnover may be associated with the pubertal process in female adolescents and may contribute to decreases in 25(OH) D levels. In fact, Geserick et al. (36) demonstrated in their research that preceding the 25(OH) D nadir, there is a peak of bone formation and resorption markers mainly in the 3rd and 4th pubertal stages for girls and boys, respectively. Moreover, according to several reports in the literature (50,51), the authors reported an inverse correlation between serum levels of 25(OH) D and BMI, both in children and adolescents.

A review study published in 2009 indicated that factors such as reduction in time to sun exposure and epidermal production of vitamin D3, reduction of the expression and affinity to vitamin D receptors, lower intestinal vitamin D absorption, higher BMI, renal function deterioration, lower estrogen levels, and an increase in insulin-like growth factor 1 may all contribute to the decline of 25(OH) D levels in the elderly population (49).

Although the 25(OH) D mean value levels appear to be similar when we confront the totality of individuals over and under 60 years old, the detailed analysis of our distribution graphs suggests that values of 25 (OH) D in seniors decline with increasing age and that there is no difference in the averages between sexes in this age range. These findings, which could be confirmed by expanding our sample of older patients, are supported by the results of previous research conducted on a Mediterranean population, in which the average 25(OH) D concentrations in men and women aged >70 years were similar and significantly lower than other age range averages (48).

It is noteworthy that the mean 25(OH) D values were approximately 30 ng/mL in all subgroups evaluated in this study. Within this context, it is worth mentioning that, for the same values of 25(OH) D, parathyroid hormone concentrations were 1.5–2 times higher in senior individuals than in adolescents (52). Thus, similar serum levels of 25(OH) D may have different metabolic consequences in youth and seniors, and higher cutoff values are recognized for the elderly population.

Considering the cutoff values currently adopted in Brazil, we observed that only 6% of girls and 3.6% of boys aged <12 years showed inadequate vitamin D serum levels (25 (OH) D <20 ng/mL). This percentage increased to 13.4% and 12.6%in male and female adolescents, respectively, and was nearly 11% in adults of both genders up to 59 years of age. The percentage of seniors with 25(OH) D serum levels <20 ng/mL was very similar to that observed in adolescents; however, in 53.2% of women and 50.6% of men ⩾60 years of age, the serum concentrations of 25(OH) D were <30 ng/mL; therefore, they were considered inadequate for this age range.

The comparison of prevalence in the literature is not an easy task because of the large diversity of methods used for 25(OH) D dosage and the different cutoff values used in studies. Sample compositions (dimension, ethnicity, age range, comorbidities, and use of medications that interfere with absorption/action of vitamin D), geographical coordinates of the locations where the research was conducted, and seasonal differences can also impede accurate comparisons.

Data collected from studies with European populations from diverse ethnicities and residents at different latitudes showed that 8.3% and 17.7% of individuals presented levels of 25(OH) D <30 ng/mL in summer and winter, respectively. This percentage may reach 40.4% when considering Caucasians and varies according to age, with a range of 4–7% in children, 12–40% in adolescents, 9–24% in adults, and 1–8% in seniors (35).

In contrast, the results of an extensive study that involved 5,276 adults from the city of Hong Kong, located in the Northern (N) hemisphere at a similar latitude as ours (22° ° N), revealed that hypovitaminosis D (25(OH) D <30 ng/mL) was observed in 46.3% of individuals. The prevalence in patients around 20 years old was 62.5%, in those around 60 years old it was 44.5%, and in seniors aged

Table 3 - Brazilian studies regarding the prevalence of hypovitaminosis D.

	Place of Study	Study population	n	Assay 25(OH)	% Hypovitaminosis D
Premaor et al. (27)	Porto Alegre Latitude: 30°S	Males (54.3%) and women > 65 years of age, light skinned (58.8%) Most patients had been recently hospitalized	81	Radioimmunoassay	77.8% <20 ng/mL
Saraiva et al. (29)	São Paulo Latitude: 23°S	Institutionalized seniors (70.6% female; mean age 76.6 years) and no institutionalized seniors (69.1% female; mean age 79.1 years)	420	Radioimmunoassay	Institutionalized: 71.2% <20 ng/mL Outpatients: 43.8% <20 ng/mL
Scalco et al. (30)	Porto Alegre Latitude: 30°S	Seniors (> 65 years of age) residing in nursing homes	102	Chemiluminescence	85.7% <20 ng/mL
Silva et al. (31)	Belo Horizonte Latitude: 19°S	Women (91.6%) and men (14–91 years of age, mean age 58.87 years)	132	High performance liquid chromatography	0.8% <14 ng/mL 42.4% <32 ng/mL
Peters et al. (26)	Rural Town São Paulo Latitude: 23°S	Children and adolescents (6–20 years of age)	136	Radioimmunoassay	60.0% <30 ng/mL
Souto et al. (33)	Rio de Janeiro Latitude: 22°S	Patients with systemic lupus erythematosus (94.3% female; 67.9% caucasian)	159	High performance liquid chromatography	8.2% <20 ng/mL
Santos et al. (28)	Curitiba Latitude: 25°S Porto Alegre Latitude: 30°S	Apparently healthy girls (7–18 years of age)	234	Radioimmunoassay	36.3% <20 ng/mL
Cabral et al. (21)	Recife Latitude: 8°S	Male and female (69.4 ± 6.5 years of age; 66.7% had dark skin)	284	Electrochemiluminescent competitive immunoassay	66.7% <30 ng/mL
Ferreira et al. (18)	Maceió Latitude: 9°S	HIV-infected adults (≥20 years of age)	113	Chemiluminescence	23.9% <30 ng/mL
Lopes et al. (24)	Brasília Latitude: 15°S	Infertile women and controls (21–47 years of age)	369	Chemiluminescence	32.0% <20 ng/mL (infertile and control patients)
Araújo et al. (22)	João Pessoa Latitude: 7°S	Post-pubertal adolescents (15–19 years of age)	220	Chemiluminescence	8.2 <30 ng/mL 42.7% <20 ng/mL
Chrisostomo et al. (20)	Curitiba Latitude: 25°S	Pregnant women (18–40 years of age)	520	Microparticle chemiluminescence	43.7% <20 ng/mL
Peçanha et al. (25)	Viçosa Latitude: 20°S	Children, mostly biracial or black boys (5.8 ± 4.6 years of age)	124	Chemiluminescence	57.3% <20 ng/mL
Vivan et al. (34)	Porto Alegre Latitude: 30°S	Pre-bariatric patients (76.6% female; mean age 44.9 years)	291	Chemiluminescence	55.3% <20 ng/mL
Sousa et al. (32)	Natal Latitude: 5°S	Seniors (> 60 years of age) from nine nursing homes	153	Chemiluminescence	71.2% <30 ng/mL

n: sample size.

⩾70 years the prevalence was 44.5% (53). The elevated prevalence of hypovitaminosis D in the Asian population appears to be corroborated by a recent systematic review involving 21,236 post-menopausal women, in which 99.4% of the Chinese *versus* 29% of the American subjects presented 25(OH) D concentrations <30 ng/mL (54).

Information extracted from records of the *National Institutes of Health* showed that the prevalence of hypovitaminosis D in children varies from 7% in South America to 95% in Afghanistan, and that the prevalence of hypovitaminosis D appears to be higher in women living in the Middle East (19).

Of the 15 studies regarding the prevalence of hypovitaminosis D in the Brazilian population (Table 3), only Silva et al. (31) evaluated a broad age range sample of individuals aged 14–91 years who did not receive vitamin D supplementation. Using the cutoff values of 14 and 32 ng/mL to define deficiency and insufficiency, the authors found percentages of 0.8% and 42.4%, respectively.

Araújo et al. (22) evaluated 220 adolescents aged between 15 and 19 years (mainly black women) from the city of João Pessoa (latitude 7° S), reporting that 8.2% had insufficiency (25(OH) D <30 ng/mL) and 42.7% had vitamin D deficiency (25(OH) D<20 ng/mL), a much higher prevalence than that observed in our study.

Saraiva et al. (29) analyzed 25(OH) D concentrations in 420 elderly patients from the city of São Paulo, located at latitude 23°S, in which 69.7% were female, noting that 71.2% of the institutionalized seniors (n=177) and 43.8% of the ambulatorial seniors (n =243) presented with 25(OH) D levels <20 ng/mL. Similar to our observations, the 25(OH) D averages were considerably lower in women.

Taking into consideration the variability of the cutoff values used to define hypovitaminosis D, it is possible that the use of 25(OH) D averages might be a more adequate parameter for comparing different populations. In a large study conducted with a numerous sample size of individuals who were residents at Crete Island (latitude 35° N), the averages of 25(OH) D were 19.48 ± 9.51 ng/mL for men and 18.01 ± 9.01 ng/mL for women, comparable to the Polish population (18.0 ± 9.6 ng/mL), although much lower than the levels observed in our sample (48,55). The 25(OH) D average encountered in our adolescent population was also considerably higher than that reported in the Greek and Italian populations (16,48), which is equivalent to what was found in a study performed with Brazilian individuals in the rural zone of the state of São Paulo that demonstrated levels of 29.2 ± 0.8 ng/mL (26).

The comparatively higher mean value of 25(OH) D found in our study was expected because the city of Rio de Janeiro has favorable geographical coordinates, is coastal, presents abundant sunny days in all seasons, and offers several opportunities for outdoor activities. Furthermore, the blood samples used in the study were collected during the summer period, and the estimated 25(OH) D serum concentrations were measured in presumably healthy individuals.

It is important to emphasize, however, that the present study was conducted based on retrospective information from a laboratory database, which may contain misleading reports, including those regarding the use of drugs and supplements. Additionally, with the available data, it was not possible to analyze race, BMI, physical activity, and the use of sunscreen in the selected sample.

Thus, although our results must be evaluated with proper caution, the large number of examinations included in our study, standardization of the assay for 25(OH) D dosages, and adopted criteria for both the methods and statistical analyses allow us to conclude that, in the studied population, and in accordance to the literature, the 25(OH) D mean values were higher in children and lower in adolescents and women. We also conclude that the majority of presumably healthy non-senior individuals, who resided in the metropolitan region of Rio de Janeiro, demonstrated satisfactory levels of 25(OH) D (⩾20 ng/mL) during the summer months. However, more than half of the elderly population had inadequate serum 25(OH) D concentrations (<30 ng/mL). Therefore, strategies for the prevention and monitoring of hypovitaminosis D should be considered for the senior population.

■ ACKNOWLEDGMENTS

Ethical approval was obtained on 21 November 2018, by the local Ethics Committee of University Hospital Pedro Ernesto of the State University of Rio de Janeiro (HUPE/UERJ) (registration N° 04191018.3.0000.5259). In addition, considering the cross-sectional and observational nature of this study, no registration as a clinical trial is required.

■ AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. Material preparation and writing of the manuscript were performed by Leão LMCSM and Rodrigues BC. The first draft of the manuscript was written by Leão LMCSM and Rodrigues BC, and all authors commented on previous versions of the manuscript. Souza TSP and Hirose CK collected the data, and Dias PTP was responsible for the statistical analysis. Gehrke B contributed to the manuscript writing and translated it from Portuguese to English. Freire MDC led the project at Diagnósticos da América SA and Leão LMCSM was in charge of the entire project. All of the authors have read and approved the final version of the manuscript.

■ REFERENCES

1. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clin Proc. 2011;86(1):50-60. https://doi.org/10.4065/mcp.2010.0567
2. Saraff V, Shaw N. Sunshine and vitamin D. Arch Dis Child. 2016;101(2):190-2. https://doi.org/10.1136/archdischild-2014-307214
3. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80(6 Suppl):1689S-96S. https://doi.org/10.1093/ajcn/80.6.1689S
4. Valtueña J, González-Gross M, Huybrechts I, Breidenassel C, Ferrari M, Mouratidou T, et al. Factors associated with vitamin D deficiency in European adolescents: the HELENA study. J Nutr Sci Vitaminol (Tokyo). 2013;59(3):161-71.
5. Casella CB, Seguro LP, Takayama L, Medeiros D, Bonfa E, Pereira RM. Juvenile onset systemic lupus erythematosus: a possible role for vitamin D in disease status and bone health. Lupus. 2012;21(12):1335-42. https://doi.org/10.1177/0961203312454929
6. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81. https://doi.org/10.1056/NEJMra070553
7. Al-Daghri NM, Alkharfy KM, Al-Othman A, El-Kholie E, Moharram O, Alokail MS, et al. Vitamin D supplementation as an adjuvant therapy for patients with T2DM: an 18-month prospective interventional study. Cardiovasc Diabetol. 2012;11:85. https://doi.org/10.1186/1475-2840-11-85
8. Min B. Effects of vitamin D on blood pressure and endothelial function. Korean J Physiol Pharmacol. 2013;17(5):385-92. https://doi.org/10.4196/kjpp.2013.17.5.385
9. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr. 2004;79(5):820-5. https://doi.org/10.1093/ajcn/79.5.820
10. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab. 2007;92(6):2017-29. https://doi.org/10.1210/jc.2007-0298
11. Mascitelli L, Goldstein MR, Pezzetta F. [Vitamin D deficiency and cardiovascular diseases]. Recenti Prog Med. 2010;101(5):202-11.
12. Herrmann M, Farrell CL, Pusceddu I, Fabregat-Cabello N, Cavalier E. Assessment of vitamin D status – a changing landscape. Clin Chem Lab Med. 2017;55(1):3-26. https://doi.org/10.1515/cclm-2016-0264

13. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30. https://doi.org/10.1210/jc.2011-0385
14. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab. 2016;101(2):394-415. https://doi.org/10.1210/jc.2015-2175
15. Płudowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe - recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol. 2013;64(4):319-27. https://doi.org/10.5603/EP.2013.0012
16. Vierucci F, Del Pistoia M, Fanos M, Erba P, Saggese G. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Ital J Pediatr. 2014;40:54. https://doi.org/10.1186/1824-7288-40-54
17. Maeda SS, Borba VZ, Camargo MB, Silva DM, Borges JL, Bandeira F, et al. Recommendations of the Brazilian Society of Endocrinology and Metabology (SBEM) for the diagnosis and treatment of hypovitaminosis D. Arq Bras Endocrinol Metab. 2014;58(5):411-33.
18. Ferreira CES, Maeda SS, Batista MC, Castro ML, Vasconcellos LS, Madeira M, et al. Posicionamento Oficial da Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial e da Sociedade Brasileira de Endocrinologia e Metabologia– Intervalos de Referência da Vitamina D – 25(OH)D. 2018. [Cited 9 October 2018] Available from: http://bibliotecasbpc.org.br/pags/view.archive.php?ID=2158&PATH=pdf
19. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014;144 Pt A:138-45. https://doi.org/10.1016/j.jsbmb.2013.11.003
20. Chrisostomo KR, Skare TL, Kulak J Jr, Urbanetz AA, Chrisostomo ER, Nisihara R. The prevalence and clinical associations of hypovitaminosis D in pregnant women from Brazil. Int J Gynaecol Obstet. 2018;143(1):66-70. https://doi.org/10.1002/ijgo.12575
21. Cabral MA, Borges CN, Maia JM, Aires CA, Bandeira F. Prevalence of vitamin D deficiency during the summer and its relationship with sun exposure and skin phototype in elderly men living in the tropics. Clin Interv Aging. 2013;8:1347—51.
22. Santos Araújo EPD, Queiroz DJM, Neves JPR, Lacerda LM, Gonçalves MDCR, Carvalho AT. Prevalence of hypovitaminosis D and associated factors in adolescent students of a capital of northeastern Brazil. Nutr Hosp. 2017;34(5):1416-23.
23. Ferreira SM, Lima MH, Omena AL, Canuto JM, Canuto VM, Morais TM, et al. Prevalence of hypovitaminosis D and its association with oral lesions in HIV-infected Brazilian adults. Rev Soc Bras Med Trop. 2016;49(1):90-4. https://doi.org/10.1590/0037-8682-0159-2015
24. Lopes VM, Lopes JR, Brasileiro JP, Oliveira I, Lacerda RP, Andrade MR, et al. Highly prevalence of vitamin D deficiency among Brazilian women of reproductive age. Arch Endocrinol Metab. 2017;61(1):21-7. https://doi.org/10.1590/2359-3997000000216
25. Peçanha MB, Freitas RB, Moreira TR, Silva LS, Oliveira LL, Cardoso SA. Prevalence of vitamin D deficiency and its relationship with factors associated with recurrent wheezing. J Bras Pneumol. 2019;45(1):e20170431. https://doi.org/10.1590/1806-3713/e20170431
26. Peters BS, dos Santos LC, Fisberg M, Wood RJ, Martini LA. Prevalence of vitamin D insufficiency in Brazilian adolescents. Ann Nutr Metab. 2009;54(1):15-21. https://doi.org/10.1159/000199454
27. Premaor MO, Alves GV, Crossetti LB, Furlanetto TW. Hyperparathyroidism secondary to hypovitaminosis D in hypoalbuminemic is less intense than in normoalbuminemic patients: a prevalence study in medical inpatients in southern Brazil. Endocrine. 2004;24(1):47-53. https://doi.org/10.1385/ENDO:24:1:047
28. Santos BR, Mascarenhas LP, Satler F, Boguszewski MC, Spritzer PM. Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. BMC Pediatr. 2012;12:62. https://doi.org/10.1186/1471-2431-12-62
29. Saraiva GL, Cendoroglo MS, Ramos LR, Araújo LM, Vieira JG, Maeda SS, et al. Prevalência da deficiência, insuficiência de vitamina D e hiperparatiroidismo secundário em idosos institucionalizados e moradores na comunidade da cidade de São Paulo, Brasil. Arq Bras Endocrinol Metab. 2007;51(3):437-42. https://doi.org/10.1590/S0004-27302007000300012
30. Scalco R, Premaor MO, Fröehlich PE, Furlanetto TW. High prevalence of hypovitaminosis D and secondary hyperparathyroidism in elders living in nonprofit homes in South Brazil. Endocrine. 2008;33(1):95-100. https://doi.org/10.1007/s12020-008-9061-2
31. Silva BC, Camargos BM, Fujii JB, Dias EP, Soares MM. Prevalência de deficiência e insuficiência de vitamina D e sua correlação com PTH, marcadores de remodelação óssea e densidade mineral óssea, em pacientes ambulatoriais. Arq Bras Endocrinol Metabol. 2008;52(3):482-8. https://doi.org/10.1590/S0004-27302008000300008
32. Sousa SES, Sales MC, Araújo JRT, Sena-Evangelista KCM, Lima KC, Pedrosa LFC. High Prevalence of Hypovitaminosis D in Institutionalized Elderly Individuals is Associated with Summer in a Region with High Ultraviolet Radiation Levels. Nutrients. 2019;11(7):1516. https://doi.org/10.3390/nu11071516
33. Souto M, Coelho A, Guo C, Mendonça L, Argolo S, Papi J, et al. Vitamin D insufficiency in Brazilian patients with SLE: prevalence, associated factors, and relationship with activity. Lupus. 2011;20(10):1019-26. https://doi.org/10.1177/0961203311401457
34. Vivan MA, Kops NL, Fülber ER, de Souza AC, Fleuri MASB, Friedman R. Prevalence of Vitamin D Depletion, and Associated Factors, among Patients Undergoing Bariatric Surgery in Southern Brazil. Obes Surg. 2019;29(10):3179-87. https://doi.org/10.1007/s11695-019-03963-9
35. Cashman KD, Dowling KG, Skrabáková Z, Kiely M, Lamberg-Allardt C, Durazo-Arvizu RA, et al. Standardizing serum 25-hydroxyvitamin D data from four Nordic population samples using the Vitamin D Standardization Program protocols: Shedding new light on vitamin D status in Nordic individuals. Scand J Clin Lab Invest. 2015;75(7):549-61. https://doi.org/10.3109/00365513.2015.1057898
36. Geserick M, Vogel M, Eckelt F, Schlingmann M, Hiemisch A, Baber R, et al. Children and adolescents with obesity have reduced serum bone turnover markers and 25-hydroxyvitamin D but increased parathyroid hormone concentrations – Results derived from new pediatric reference ranges. Bone. 2020;132:115124. https://doi.org/10.1016/j.bone.2019.115124
37. Ganji V, Zhang X, Tangpricha V. Serum 25-hydroxyvitamin D concentrations and prevalence estimates of hypovitaminosis D in the U.S. population based on assay-adjusted data. J Nutr. 2012;142(3):498-507. https://doi.org/10.3945/jn.111.151977
38. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: an examination in children and adults with and without supplement use. Am J Clin Nutr. 2011;94(1):128-35. https://doi.org/10.3945/ajcn.111.013268
39. González-Gross M, Valtueña J, Breidenassel C, Moreno LA, Ferrari M, Kersting M, et al. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. Br J Nutr. 2012;107(5):755-64. https://doi.org/10.1017/S0007114511003527
40. Kim SH, Oh MK, Namgung R, Park MJ. Prevalence of 25-hydroxyvitamin D deficiency in Korean adolescents: association with age, season and parental vitamin D status. Public Health Nutr. 2014;17(1):122-30. https://doi.org/10.1017/S1368980012004703
41. Siddiqui AM, Kamfar HZ. Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia. Saudi Med J. 2007;28(3):441-4.
42. Smith TJ, Tripkovic L, Lanham-New SA, Hart KH. Vitamin D in adolescence: evidence-based dietary requirements and implications for public health policy. Proc Nutr Soc. 2018;77(3):292-301. https://doi.org/10.1017/S0029665117004104
43. Zhao D, Ouyang P, de Boer IH, Lutsey PL, Farag YM, Guallar E, et al. Serum vitamin D and sex hormones levels in men and women: The Multi-Ethnic Study of Atherosclerosis (MESA). Maturitas. 2017;96:95-102. https://doi.org/10.1016/j.maturitas.2016.11.017
44. Rafiq R, van Schoor NM, Sohl E, Zillikens MC, Oosterwerff MM, Schaap L, et al. Associations of vitamin D status and vitamin D-related polymorphisms with sex hormones in older men. J Steroid Biochem Mol Biol. 2016;164:11-7. https://doi.org/10.1016/j.jsbmb.2015.11.013
45. Wang N, Han B, Li Q, Chen Y, Chen Y, Xia F, et al. Vitamin D is associated with testosterone and hypogonadism in Chinese men: Results from a cross-sectional SPECT-China study. Reprod Biol Endocrinol. 2015;13:74. https://doi.org/10.1186/s12958-015-0068-2
46. Chang EM, Kim YS, Won HJ, Yoon TK, Lee WS. Association between sex steroids, ovarian reserve, and vitamin D levels in healthy nonobese women. J Clin Endocrinol Metab. 2014;99(7):2526-32. https://doi.org/10.1210/jc.2013-3873
47. Pop LC, Shapses SA, Chang B, Sun W, Wang X. Vitamin D-Binding Protein In Healthy Pre- And Postmenopausal Women: Relationship With Estradiol Concentrations. Endocr Pract. 2015;21(8):936-42. https://doi.org/10.4158/EP15623.OR
48. Katrinaki M, Kampa M, Margioris A, Castanas E, Malliaraki N. Vitamin D levels in a large Mediterranean cohort: reconsidering normal cut-off values. Hormones (Athens). 2016;15(2):205-23.
49. Oudshoorn C, van der Cammen TJ, McMurdo ME, van Leeuwen JP, Colin EM. Ageing and vitamin D deficiency: effects on calcium homeostasis and considerations for vitamin D supplementation. Br J Nutr. 2009;101(11):1597-606. https://doi.org/10.1017/S0007114509338842
50. Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. J Clin Endocrinol Metab. 2005;90(7):4119-23. https://doi.org/10.1210/jc.2005-0216
51. Earthman CP, Beckman LM, Masodkar K, Sibley SD. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. Int J Obes (Lond). 2012;36(3):387-96.

52. Soares LM, Pedrosa W, Elói-Santos SM, Vasconcellos LS. 25-Hydroxyvitamin D threshold values should be age-specific. Clin Chem Lab Med. 2017;55(7):e140-e142. https://doi.org/10.1515/cclm-2016-0798
53. Leung RY, Cheung BM, Nguyen US, Kung AW, Tan KC, Cheung CL. Optimal vitamin D status and its relationship with bone and mineral metabolism in Hong Kong Chinese. Bone. 2017;97:293-8. https://doi.org/10.1016/j.bone.2017.01.030
54. Valladares T, Simões R, Bernardo W, Schmitt ACB, Cardoso MRA, Aldrighi JM. Prevalence of hypovitaminosis D in postmenopausal women: a systematic review. Rev Assoc Med Bras (1992). 2019;65(5):691-8. https://doi.org/10.1590/1806-9282.65.5.691
55. Płudowski P, Ducki C, Konstantynowicz J, Jaworski M. Vitamin D status in Poland. Pol Arch Med Wewn. 2016;126(7–8):530-9.

ANEXO F – Artigo publicado – Thyroid dysfunction in patients undergoing Nivolumab cancer treatment: Case reports and review of literature

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Thyroid dysfunction in patients undergoing Nivolumab cancer treatment: Case reports and review of literature

Bárbara Gehrke, Ricardo de Andrade Oliveira, Jorge Alexandre Fernandes Canedo, Paula Bruna Araujo, Maria Caroline Alves Coelho

ABSTRACT

Introduction: The recent development of anticancer treatments focused on disrupting the signaling pathways has arrived as a promising alternative for conditions previously untreatable. Nivolumab is a second-generation monoclonal antibody that works as a negative regulatory agonist of the programmed cell death protein 1 (PD-1) receptor expressed by B and T lymphocytes and natural killer cells, preventing the binding of PD-1 to its ligands programmed cell death ligand-1 (PD-L1) and programmed cell death ligand-2 (PD-L2), therefore protecting healthy tissues. As a result of an increased immune system activity, this may provide inflammatory side effects known as immune-related adverse events (IRAEs). Thyroid dysfunction (TD) is frequently observed in patients using Anti-PD-1.

Case Report: Our case report refers to two patients of 67 and 75 years old, feminine and masculine genders, respectively, that developed Nivolumab-induced TD with transient hyperthyroidism and posterior evolution to hypothyroidism. Both were treated with Levothyroxine after thyrotoxicosis phase.

Conclusion: Thyroid dysfunction has been a recurrent IRAE described in patients using Anti-PD-1 and it is usually a treatable condition. Our patients manifested a less common TD known as lymphocytic thyroiditis. Because there is an increased tendency of using immune checkpoint inhibitors, both cases highlight the importance of close monitoring to detect the development and progression of TD, avoiding preventable morbidity and allowing to maintain cancer therapy.

Keywords: Immune checkpoint inhibitors, Neoplasms, Nivolumab, Thyroiditis

How to cite this article

Gehrke B, Oliveira RA, Canedo JAF, Araujo PB, Coelho MCA. Thyroid dysfunction in patients undergoing Nivolumab cancer treatment: Case reports and review of literature. Int J Case Rep Images 2021;12:101223Z01BG2021.

Article ID: 101223Z01BG2021

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doi: 10.5348/101223Z01BG2021CR

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Received: 25 January 2021
Accepted: 30 April 2021
Published: 23 May 2021

INTRODUCTION

The recent development of anticancer treatments focused on disrupting the signaling pathways has arrived as a promising alternative for conditions previously untreatable [1]. Cancer cells have the capability to stimulate different immune checkpoint pathways that harbor immunosuppressive functions [2]. Therefore, the immune checkpoint inhibitors (ICIs) target regulatory pathways in T cells to enhance antitumor immune responses [3].

The antibody against programmed cell death 1 (Anti-PD-1) receptor called Nivolumab is a second-generation

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monoclonal antibody that works as a negative regulatory agonist of the PD-1 receptor expressed by B and T lymphocytes and natural killer (NK) cells, preventing the binding of PD-1 to its ligands programmed cell death ligand-1 (PD-L1) and programmed cell death ligand-2 (PD-L2). It also protects healthy tissues (Figure 1) [1]. The purpose is to block inhibitory pathways that act as an obstacle to adequate and efficient antitumor T cell responses [4]. Nivolumab was approved by the U.S. Food and Drug Administration (FDA) in 2014 for the treatment of various cancers including melanoma and non-small cell lung cancer (NSCLC) [4, 5].

As a result of an increased immune system activity, the immune checkpoint blockage may provide side effects which are usually called immune-related adverse events (IRAEs). The organs frequently affected by this Anti-PD-1 drug are: gastrointestinal tract, endocrine glands, skin, and liver [5]. Thyroid dysfunction (TD) is frequently observed in patients using Anti-PD-1 (Nivolumab and Pembrolizumab), and here we report two cases of patients who developed Nivolumab-induced TD [6].

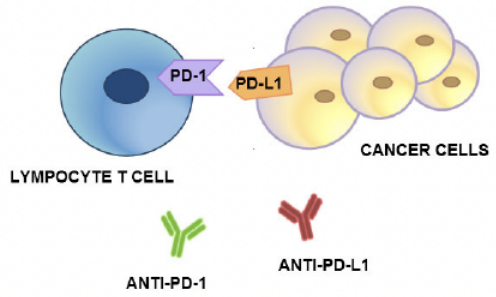

Figure 1: Mechanism of action of immune checkpoint inhibitors Anti-PD-1 and Anti-PD-L1. The surface of immune cells such as B and T lymphocytes and natural killer cells express the receptor programmed cell death 1 (PD-1). PD-1 binds to its ligand (programmed cell death ligand 1—PD-L1) and prevents T cells from exterminating cancer cells. Antitumor immune responses occur when antibodies Anti-PD-1 and/or Anti-PD-L1 connect to its respective receptor at the surface of the lymphocyte T cell, inhibiting the binding of PD-1 to PD-L1.

CASE SERIES

Case 1

A 67-year-old woman with diagnosis of melanoma at the dorsal region and metastasis to lungs who began Nivolumab treatment and developed moderate cutaneous reaction requiring therapy with prednisone 1 mg/kg/day. The patient had partial recovery of skin adverse event with glucocorticoid. A blood test was performed after initial administration of Nivolumab showing signs of hyperthyroidism: thyroid-stimulating hormone (TSH) of 0.013 mU/L [reference values (RV): 0.3–4.0] and free thyroxine (fT4) of 2.1 ng/dL (RV: 0.9–1.8). Because there were no clinical signs of thyroid hyperfunction, it was decided to observe and repeat the exam further along treatment. After eight months of Nivolumab, the patient developed overt hypothyroidism with TSH of 25.0 mU/L and fT4 of 0.3 ng/dL. Levothyroxine was initiated daily at the dose of 50 mcg, with satisfactory response.

Case 2

A 75-year-old man with diagnosis of NSCLC with metastasis to lungs and liver began treatment with Nivolumab and after two months showed alteration of thyroid function compatible with hyperthyroidism (TSH of 0.03 mU/L and fT4 of 2.2 ng/dL). It was decided to maintain clinical observation and repeat exams after three weeks. Results revealed hypothyroidism (TSH of 19.0 mU/L and fT4 of 0.5 ng/dL) with positivity of Anti-thyroperoxidase antibody (Anti-TPOAb). The TSH receptor antibody (Anti-TSIAb) was negative. Therapy with levothyroxine was started with initial dose of 25 mcg per day and progressively increased until 75 mcg per day, to achieve euthyroidism. The patient evolved to death due to the severity of his clinical condition.

DISCUSSION

The case reports showed two patients in use of Nivolumab that developed initially transient hyperthyroidism with posterior evolution to hypothyroidism. Thyroid dysfunction is described in patients using Nivolumab in a range of 0–18.5% [1]. The most common manifestations of Nivolumab TD are hypothyroidism, either overt or subclinical with an incidence of 8%, primary autoimmune hyperthyroidism or Graves' disease in up to 2.8%; and silent or painless thyroiditis, also known as lymphocytic thyroiditis, with incidence of 1.6% [7]. Thyroid dysfunction caused by ICI is more prevalent in females, being consistent with what is found in the general population [1].

Both hypothyroidism and painless thyroiditis have an autoimmune pathogenesis that involves humoral and cellular response mechanisms which need further research. Hypothyroidism, usually caused by Hashimoto's disease, consists of a chronic autoimmune inflammation of the thyroid gland [1]. The Graves' disease may be suggested due to the presence of Anti-TSIAb, which activates TSH receptors and increase thyroid hormone production [1]. Studies speculate that lymphocytic thyroiditis occurs due to the fact that Nivolumab induces the reduction of immune feedback in healthy thyroid tissue, leading to the development of an inflammatory process (thyroiditis) [1]. Frequently, this painless thyroiditis is expressed with an initial transient hyperthyroidism (destruction

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of follicular cells leading to the release of thyroid hormone) followed by hypothyroidism, and some patients return to euthyroidism or develop permanent hypothyroidism [1]. A study with 657 patients using ICI therapies (monotherapy or combinations) described hypothyroidism in 84% of the patients who had documented transient thyroiditis [8].

In order to differentiate primary autoimmune hyperthyroidism from lymphocytic thyroiditis, the gold standard exam is radioactive iodine uptake scintigraphy. A low uptake reveals thyroiditis, whereas an increased uptake of 25% or over indicates Graves' disease [1]. Unfortunately, our patients were not submitted to this exam during hyperthyroidism evaluation. Moreover, patients with TD due to ICI may express elevated levels of anti-TPOAbs in approximately 50% of the cases, which was observed in one of our cases [8].

Thyroid disorders are frequently asymptomatic and are monitored by routine laboratory tests during follow-up [9]. According to Gonzalez-Rodriguez et al. [1], 67% of patients who develop thyroiditis are asymptomatic during thyrotoxicosis phase and this is what we observed in our patients. Therefore, we may hypothesize that they developed an inflammatory process similar to lymphocytic thyroiditis as a result of Nivolumab therapy, considering that the first exam evidenced hyperthyroidism with rapid transition to hypothyroidism.

Van Kooten et al. [10] described two cases with thyroiditis onset 2–4 weeks after initiating treatment with Nivolumab, an evolution similar to that of our patients. The authors speculate that the underlying pathophysiological mechanism is a transient destructive thyroiditis, a conclusion based on the relatively rapid resolution and temporarily increased FDG uptake observed with F-fluorodeoxy glucose positron emission tomography (FDG-PET/CT). For this reason, thionamides are not recommended to treat the initial hyperthyroidism phase [10]. Orlov et al. [11] and de Filette et al. [12] also described painless thyroiditis with thyrotoxicosis and subsequent hypothyroidism in patients using Anti-PD-1 monoclonal antibody therapy.

The incidence of IRAE with PD-1 or PD-L1 inhibitors is different from that reported with cytotoxic T lymphocyte-associated antigen-4 monoclonal antibodies (Anti-CTLA-4-Ipilimumab) agents [13]. Hypophysitis has been more commonly described in patients using Ipilimumab reaching 5.6% [7]. Ipilimumab induces TD in a range of 0–7.4% of the patients treated. On the other hand, TD was more incidental in patients who used Anti-PD-1 and the most common manifestation was hypothyroidism with Pembrolizumab and Nivolumab reaching 8.5% and 8%, respectively [7]. For both medications, hypothyroidism is the most frequent thyroid dysfunction followed by hyperthyroidism [1]. PD1 is expressed by T and B lymphocytes and NK cells while CTLA4 is expressed only by T lymphocytes. For this reason, when these surface proteins are inhibited, the cells tend to proliferate. This elucidates the reason why Anti-PD1 therapies could lead to more thyroidopathies compared to Anti-CTLA4 [1].

A meta-analysis published in 2018, with 38 clinical trials including 7551 patients, revealed that the incidence of hypothyroidism and hyperthyroidism was higher in individuals using combination therapy. Also, those on PD-1 inhibitor therapy had an increased risk of developing hypothyroidism and the study evidenced that hyperthyroidism was significantly greater with anti-PD-1 compared to anti-PD-L1 [14].

Thyroid disorders are more likely to be observed in patients receiving Anti-PD-1 therapy who have positivity for antithyroid antibodies [5]. This occurs due to the additive effect of the T-cell-mediated immunity and the way that the Anti-PD-1 therapy modulates humoral immunity, intensifying preexisting thyroid autoimmunity [5]. In a study with Nivolumab, 26% of the patients who developed TD presented antithyroid antibodies at baseline, whereas 36% presented them along the treatment, which reinforces the immune-mediated determinant of this condition [1]. Interestingly, the histologic evaluation of Hashimoto's thyroiditis (autoimmune thyroiditis) shows evidence of infiltration by lymphocytic B cells and cytotoxic T cells. PD-1 is expressed by T and B lymphocytes and, for this reason, when these surface receptors are inhibited the cells tend to multiply, inducing immune response which exacerbates due to preexisting thyroid autoimmunity [1].

According to Kimbara et al. [15], in a study with 168 patients, the incidence of TD was significantly higher in those with thyroid autoimmunity compared to individuals without. Particularly in this analysis, the presence of antithyroglobulin antibodies (TgAb) before Nivolumab was associated with a large and significant hazard ratio of TD compared to that of TPOAb. In addition to that, 74% of 23 patients who developed TD presented increased levels of TgAb during Nivolumab treatment and 2 patients with TPOAb alone at baseline became positive for TgAb when developed TD. None of our patients had a previous history of TD and unfortunately, they had no previous dosage of antibodies against the thyroid.

There are strong recommendations in most papers that screening of thyroid function and its autoantibodies should be performed at baseline and every 4–6 weeks as Nivolumab is administered intravenously every 2 weeks, or if the patient presents any symptoms suggestive of TD [3, 16].

In a study with 657 patients undergoing therapy with ICI, in which 56 presented TD (14 were receiving Nivolumab), the median time to thyrotoxicosis was 6 weeks after starting the ICI therapy and the median time to hypothyroidism was 17 weeks [8]. In our case report the time to hypothyroidism after starting Nivolumab was 32 weeks in case 1 and 11 weeks in case 2.

If thyroiditis is speculated, it might be prudent to observe the transient hyperthyroidism and once established hypothyroidism initiate Levothyroxine. During thyrotoxicosis period therapy with beta blocker may be considered and although in some references

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steroids are not routinely recommended during this phase, in severe symptomatic hyperthyroidism we may consider suspending ICI and initiating corticosteroids [8–10]. Depending on the clinical condition of the patient, it might be necessary to evaluate cortisol levels aside from thyroid function, to exclude the possibility of adrenal insufficiency before initiating Levothyroxine [9].

It is uncommon for patients to discontinue Nivolumab treatment because of TD, as it can be controlled with thyroid drugs during the cancer treatment [3]. The discontinuation rate of Anti-PD-1/PD-L1 ranges from 3 to 8% due to IRAE, whereas for Ipilimumab it reaches up to 15% [9]. When administered Ipilimumab in combination with Nivolumab, the discontinuation rate increases to 36% [9].

Latest data suggested that the development of IRAEs might be associated with higher response rates to cancer treatment and a longer median duration of response, as seen in a study from 2019 where the response rate was 38.2% among the 34 patients in the thyroiditis group and 17.4% among the 69 patients from non-thyroiditis group ($p = 0.028$), while the median progression-free survival was 10.1 months in the thyroiditis group and 3.7 months in the non-thyroiditis group [17]. From that point of view, the presence of IRAE (specifically TD) might grant a better prognosis for the patient due to a more effective and potent immune-mediated response to therapy [9]. Yamauchi et al. [6] described a curious relationship between TD consequent to ICI and good prognosis in NSCLC patients, although sample sizes were small (approximately 50 patients in two different studies). The study explains that there is evidence that target antigens exist at the lung and the thyroid gland, so if the patient develops TD as an IRAE, immune responses to lung cancer are expected as antibodies recognize antigens in both sites [6]. Our second patient had NSCLC, but the disease aggravated and he didn't survive.

CONCLUSION

The use of ICI therapy has increased in the last decade and IRAEs have a high incidence with these medications. Endocrine adverse events, especially TD, are frequent in this scenario. Close monitoring is necessary to detect the development and progression of TD, avoiding preventable morbidity and allowing to maintain cancer therapy. Therefore, endocrinologists and oncologists must work together to make sure that the patient is being followed appropriately.

REFERENCES

1. Gonzalez-Rodríguez E, Rodríguez-Abreu D, Spanish Group for Cancer Immuno-Biotherapy (GETICA). Immune checkpoint inhibitors: Review and management of endocrine adverse events. Oncologist 2016;21(7):804–16.
2. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: Recent progress and potential biomarkers. Exp Mol Med 2018;50(12):1–11.
3. Tanaka R, Fujisawa Y, Maruyama H, et al. Nivolumab-induced thyroid dysfunction. Jpn J Clin Oncol 2016;46(6):575–9.
4. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348(6230):56–61.
5. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med 2018;378(2):158–68.
6. Yamauchi I, Yasoda A, Matsumoto S, et al. Incidence, features, and prognosis of immune-related adverse events involving the thyroid gland induced by nivolumab. PLoS One 2019;14(5):e0216954.
7. de Filette J, Andreescu CE, Cools F, Bravenboer B, Velkeniers B. A systematic review and meta-analysis of endocrine-related adverse events associated with immune checkpoint inhibitors. Horm Metab Res 2019;51(3):145–56.
8. Iyer PC, Cabanillas ME, Waguespack SG, et al. Immune-related thyroiditis with immune checkpoint inhibitors. Thyroid 2018;28(10):1243–51.
9. Ferrari SM, Fallahi P, Galetta F, Citi E, Benvenga S, Antonelli A. Thyroid disorders induced by checkpoint inhibitors. Rev Endocr Metab Disord 2018;19(4):325–33.
10. van Kooten MJ, van den Berg G, Glaudemans AWJM, et al. Transient thyrotoxicosis during nivolumab treatment. Neth J Med 2017;75(5):204–7.
11. Orlov S, Salari F, Kashat L, Walfish PG. Induction of painless thyroiditis in patients receiving programmed death 1 receptor immunotherapy for metastatic malignancies. J Clin Endocrinol Metab 2015;100(5):1738–41.
12. de Filette J, Jansen Y, Schreuer M, et al. Incidence of thyroid-related adverse events in melanoma patients treated with pembrolizumab. J Clin Endocrinol Metab 2016;101(11):4431–9.
13. Araujo PB, Coelho MCA, Arruda M, Gadelha MR, Neto LV. Ipilimumab-induced hypophysitis: Review of the literature. J Endocrinol Invest 2015;38(11):1159–66.
14. Barroso-Sousa R, Barry WT, Garrido-Castro AC, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens: A systematic review and meta-analysis. JAMA Oncol 2018;4(2):173–82.
15. Kimbara S, Fujiwara Y, Iwama S, et al. Association of antithyroglobulin antibodies with the development of thyroid dysfunction induced by nivolumab. Cancer Sci 2018;109(11):3583–90.
16. O'Malley G, Lee HJ, Parekh S, et al. Rapid evolution of thyroid dysfunction in patients treated with Nivolumab. Endocr Pract 2017;23(10):1223–31.
17. Lei M, Michael A, Patel S, Wang D. Evaluation of the impact of thyroiditis development in patients receiving immunotherapy with programmed cell death-1 inhibitors. J Oncol Pharm Pract 2019;25(6):1402–11.

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Author Contributions

Bárbara Gehrke – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Guarantor of Submission

The corresponding author is the guarantor of submission.

Source of Support

None.

Consent Statement

Written informed consent was obtained from the patient for publication of this article.

Conflict of Interest

Authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its Supporting Information files.

ANEXO G – Artigo publicado – Microarchitectural parameters and bone mineral density in patients with tumour-induced osteomalacia by HR-pQCT and DXA

Received: 11 January 2021 | Revised: 17 May 2021 | Accepted: 23 May 2021
DOI: 10.1111/cen.14533

ORIGINAL ARTICLE

WILEY

Microarchitectural parameters and bone mineral density in patients with tumour-induced osteomalacia by HR-pQCT and DXA

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Abstract

Objective: Tumour-induced osteomalacia (TIO) is a rare paraneoplastic condition characterized by decreased tubular phosphate reabsorption. The purpose of this study was to evaluate bone mineral density (BMD) and microarchitecture in six TIO patients compared with eighteen healthy controls.

Design: Volumetric BMD and microarchitecture were evaluated by high-resolution peripheral quantitative computerized tomography (HR-pQCT), and areal BMD was evaluated by dual-energy X-ray absorptiometry (DXA). Differences between groups were significant for p-value < .05.

Patients: All TIO subjects were healthy until development of diffuse bone pain and multiple skeletal fractures and deformities. At baseline, sPi and TmPi/GFR were low and patients were on vitamin D and phosphate replacement at the study.

Measurements and Results: Compared with controls, TIO patients had lower aBMD at lumbar spine and hip, and lower vBMD at trabecular, cortical and entire bone, at distal radius (R) and distal tibia (T): Dtrab (R = 118.3 × 177.1; T = 72.3 × 161.3 gHA/cm^3); Dcomp (R = 782.3 × 866.5; T = 789.1 × 900.9 gHA/cm^3); and Dtotal (R = 234.5 × 317; T = 167.1 × 295.8 gHA/cm^3). Bone microarchitecture was very heterogeneous among patients and significantly different from controls: lower Ct.Th (R = 0.59 × 0.80; T = 0.90 × 1.31 mm), BV/TV (R = 0.09 × 0.14; T = 0.06 × 0.13) and Tb.N (R = 1.46 × 2.10; T = 0.93 × 1.96 mm^{-1}); and also higher Tb.Sp (R = 0.70 × 0.41; T = 1.28 × 0.45 mm) and Tb.1/N.SD (R = 0.42 × 0.18; T = 0.87 × 0.20 mm).

Conclusion: In this original study of TIO patients, DXA and HR-pQCT evaluation identified lower areal and volumetric BMD and severely impaired microarchitecture at cortical and trabecular bones, which probably contribute to bone fragility and fractures.

KEYWORDS
bone density, bone microarchitecture, high-resolution peripheral quantitative computerized tomography (HR-pQCT), hypophosphatasia, osteomalacia

1 | INTRODUCTION

Oncogenic osteomalacia (OOM) or tumour-induced osteomalacia (TIO) is a rare paraneoplastic disorder associated with decreased tubular phosphate reabsorption, hypophosphataemia, low or low-to-normal levels of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and elevated alkaline phosphatase. Serum calcium, 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) levels are usually normal.[1] These changes result from the overproduction of fibroblast growth factor 23 (FGF 23) and other phosphatonins, which inhibit renal phosphate reabsorption by suppressing the type 2a and 2c Na–dependent phosphate cotransporters (NaPi-2a and NaPi-2c) present in the proximal tubules, in addition to inhibiting hydroxylation of 25(OH)D by negative action on 1α-hydroxylase enzyme.[2] The incidence and prevalence of the disease worldwide is still unknown, but there are approximately 500 cases reported in the literature.[1,3] The age at the diagnosis can vary, with an average of 40–45 years and with no predilection for gender.[3] The clinical picture is usually nonspecific, with musculoskeletal pain, progressive muscle weakness and weight loss. Patients may present a history of multiple fractures and bone deformities depending on the severity and duration of symptoms.[4]

Tumour-induced osteomalacia is usually due to tumours of mesenchymal origin that produce phosphatonins. These tumours may be located anywhere with a predilection for bone and soft tissues and in most cases occur in the appendicular extremities and skeleton.[3] Tumour localization is the major challenge of TIO as its excision is the only known cure. Conventional imaging tests are generally unable to locate the lesion due to its slow growth, reduced size and benign behaviour.[2] Although rare, there are reported cases of TIO caused by malignant tumours.[5] The best localization methods are functional imaging studies using somatostatin analogue–labelled radioisotopes such as OctreoScan, since most of these tumours have somatostatin receptors.[1,2] Functional studies can be complemented by anatomical studies to improve the accuracy of the lesion site, thus facilitating the surgical approach.[1,6]

Individuals with TIO have severe defects of bone mineralization caused by chronic hypophosphataemia, often associated with the negative influence of secondary hyperparathyroidism that may arise due to vitamin D deficiency or clinical treatment with oral phosphate supplementation.[1] X-rays may suggest reduced mineralization of the skeleton, deformities, fractures and pseudofractures. Dual-energy X-ray absorptiometry (DXA) may detect low bone density in patients with TIO, although central sites such as the lumbar spine and proximal femur areal bone mineral density (aBMD) may be falsely increased by fractures. HR-pQCT was employed to evaluate patients with genetic hypophosphataemic rickets [7,8] and vitamin D–dependent rickets[9] but has never been used to evaluate patients with TIO. The aim of this study was to evaluate patients with TIO in comparison with healthy control subjects using DXA and HR-pQCT.

2 | METHODS

2.1 | Subjects

The study protocol was approved by the Research Ethics Committee of the Hospital Universitário Clementino Fraga Filho (HUCFF—Federal University of Rio de Janeiro, Brazil). Written informed consent was obtained from all patients and controls before participating in the study, which was in accordance with the Second Declaration of Helsinki. The patients were selected at an outpatient clinic for osteometabolic diseases at HUCFF based on having a medical history compatible with acquired osteomalacia in adulthood (patients referred muscle weakness, diffuse bone pain, fractures), physical examination (skeletal deformities), laboratory tests confirming hyperphosphaturia as the sole renal tubular defect, and radiological findings compatible with the diagnosis of osteomalacia. The biochemical investigation was performed at diagnosis, at the moment of the study and after surgery, and the fracture history of each patient was acquired at the moment of the diagnosis and throughout follow-up. Adult patients previously identified as having genetic hypophosphataemic rickets were excluded from this protocol. Six male patients met the criteria for TIO, whereas eighteen healthy individuals were included as a control group. At the time of the study, all TIO patients were on vitamin D (calcitriol and/or cholecalciferol) and oral phosphate supplements. The dosage of vitamin D (cholecalciferol and/or calcitriol) administered was according to the necessity of each patient, in order to obtain clinical and laboratory improvement. Joulie's solution at the dosage of 2 g per day (fractioned in 4 to 5 times daily) was used to restitute the levels of phosphate. Control patients were matched for age and body mass index (BMI), between 40 and 70 years old, and were not using any medication or had any disease that interferes with bone density. The control group is part of a database of healthy individuals who performed the same examinations as the TIO group.

2.2 | Biochemistry

The mean values of serum calcium, phosphorus, creatinine, alkaline phosphatase (ALP), and 24-hour urine calcium, phosphorus and creatinine measured during the previous 6 months were obtained for analysis. Tubular phosphate reabsorption was calculated in all patients. Blood was drawn after an overnight fast between 8 and 10 a.m. on the same day DXA and HR-pQCT were performed. The samples were kept frozen at −40°C until measurement of PTH by chemiluminescence (Immulite 2000, Siemens)—normal range 1.16–7.105 pmol/L—and 25-hydroxyvitamin D by electrochemiluminescence (Elecsys 2010, Roche). The reference values used in Brazil to classify the serum levels of 25(OH) D as adequate were published as a position statement of the Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC). Values above 50 nmol/L are desirable

for the healthy population up to 64 years old, and values higher than 75 nmol/L are recommended for individuals aged 65 years and older and also for those classified as risk groups such as patients with recurrent falls, osteoporosis, fragility fractures, secondary hyperparathyroidism, sarcopenia, pregnant women, diabetes mellitus, cancer and chronic kidney disease and also patients in use of glucocorticoids, and anticonvulsant and antiretroviral therapies.[10]

2.3 | Areal bone density evaluation by DXA

All participants underwent DXA using Prodigy-GE equipment at the HUCFF (GE Lunar Prodigy Advance, GE Healthcare) for assessment of areal BMD at the lumbar spine (L1-L4), femoral neck (FN) and total hip (TH). The results were expressed as the absolute values (g/cm^2) and standard deviations (SDs) from the expected mean BMD for the reference age-matched population (Z-score). The patients with a BMD Z-score of −2.0 SD or lower were classified as lower-than-expected BMD for their age according to the International Society for Clinical Densitometry (ISCD). The BMD coefficient of variation in our institution is 1.8% at the lumbar spine and 2.3% at the hip. All DXA scans were performed by the same operator and evaluated by the same physician.

2.4 | Volumetric BMD and microstructure evaluation by HR-pQCT

Bone volumetric density and microarchitecture were measured on the appropriately immobilized nondominant distal forearm and tibia using a 3D HR-pQCT system (Xtreme CT I, SCANCO Medical AG). This system employs a 2D detector combined with a 0.08-mm point-focus X-ray tube, which enables the acquisition of several CT sections with an 82-µm nominal resolution. A total of 110 sections were obtained at each site, generating a 9-mm 3D representation in the axial direction. The radiation dose was similar to that used in standard DXA procedures (less than 3 µSv per measurement). The attenuation data were transformed to equivalent hydroxyapatite (HA) densities. The variables included in the analysis were as follows: volumetric BMD (g HA/cm^3) in the trabecular (Dtrab), cortical (Dcomp) or total (Dtotal) region; cortical thickness (Ct. Th, mm); bone volume-to-total volume ratio (BV/TV, 1); trabecular thickness (Tb. Th, mm); trabecular number (Tb.N, mm^{-1}); trabecular separation (Tb. Sp, mm); and standard deviation of the Tb. Sp (tTb.1/N.SD, mm), which reflects the heterogeneity of the trabecular network. The HR-pQCT coefficient of variation in our institution is 1%–2% for density measurements and 3%–5% for microarchitectural parameters.

2.5 | Statistical analysis

The statistical analysis was performed using SPSS version 20.0 for MacOS (SPSS Inc). The Kolmogorov-Smirnov test was used to evaluate the distribution pattern of the numerical variables. The numerical variables had a normal distribution and were expressed as the mean ± standard deviation. Unpaired Student's *t*-test was performed to compare numerical variables between the two groups. A *p*-value <.05 was considered statistically significant.

3 | RESULTS

All TIO patients and control subjects were males. The descriptive characteristics of the TIO patients are shown in Table 1. At the time of the diagnosis, all patients reported being healthy until the onset of bone pain, multiple severe fractures, thoracic and/or pelvic deformities and height loss, leading to a major compromise in mobility and quality of life. The suspicion of oncogenic osteomalacia was based on the severity of symptom and laboratory results showing hypophosphataemia, low maximal tubular reabsorption of phosphate, high serum alkaline phosphatase, and normal serum levels of calcium and 25-hydroxyvitamin D. Renal tubular acidosis (and other chronic kidney diseases) and primary or secondary hyperparathyroidism were excluded. Most patients had fractures of the ribs, and four patients evidenced femoral fracture with osteosynthesis. One subject presented fracture at the tibia, and another subject had a fracture at the radius. The most affected patient presented multiple vertebral fractures (C6 to D11), had lost 32 centimetres in height, had severe thoracic deformities and was restricted to a wheelchair. All fractures occurred without major trauma and no other causes for bone fragility were identified. Four other patients walked with the aid of crutches. Four patients were submitted to surgery after evaluation with DXA and HR-pQCT and achieved cure. The locations of the tumours were as follows: sole of the foot, inguinal region, tongue and mediastinum. The histopathological analysis confirmed three mesenchymal tumours and one squamous carcinoma, with positive immunohistochemistry for vimentin in all of them. Other positive markers were CD34, CD68, CD99 and FLI 1. The remaining two patients, in whom tumour location was not possible, continued with follow-up and clinical treatment with supplementation of phosphate, calcium and vitamin D (see Table 1 for detailed information).

The age and body mass index were similar between the groups. The mean age of TIO patients was 58.67 ± 10.44 years and of control patients was 58.94 ± 3.13 years. TIO patients had lower values for areal BMD at all sites and lower TH Z-scores evaluated by DXA, although only three were classified as lower-than-expected BMD according to ISCD. No subject in the control group presented this alteration (Table 2).

No association was found between duration of treatment with phosphate plus vitamin D and serum alkaline phosphatase (S ALP). Patients with a longer duration of treatment did not necessarily present lower ALP levels suggesting a more compensated state of the disease.

Patient 1 presented the worse parameters on DXA, compatible with very low vBMD at trabecular and cortical compartments, and also very low cortical thickness, despite long treatment duration and normal serum values of S ALP. Patient 2 evidenced the worse bone

TABLE 1 Individual characteristics of tumour-induced osteomalacia (TIO) patients

TIO patients	1	2	3	4	5	6
Age at initial symptoms (years)	56	58	33	53	50	40
Age at study (years)	66	69	41	65	58	53
Fractures since initial symptoms	Multiple vertebrae, ribs, femur (osteosynthesis), radius	Multiple vertebrae collapse (severe kyphosis), femur (osteosynthesis)	Multiple vertebrae collapse (severe kyphosis), ribs, pseudofracture at left fibula; varus hip	Bilateral femoral neck (necrosis and osteosynthesis), ribs	Bilateral femoral fractures (prosthesis), pododactyl, face, ribs	Multiple vertebrae collapse (severe kyphosis), ribs, hip (pelvic deformity)
Mobility	Crutches	Crutches	Wheelchair	Crutches	Crutches	Wheelchair
Height before disease onset / at study (metres)	1.70 / 1.58	1.69 / 1.49	1.83 / 1.51	1.77 / 1.74	1.74 / 1.70	1.70 /1.60
Weight (kilograms)	52	41.8	58.8	62.3	72.5	78.6
Time on vit.D / P (months)	24 / 86	26 / 26	8 / 8	124 / 124	18 / 18	10 / 10
Biochemistry at diagnosis / at study						
S Pi (mmol/L)	0.58 / 0.71	0.64 / 0.64	0.45 / 0.54	0.61 / 0.77	0.58 / 0.48	0.61 / 0.74
S Ca (mmol/L)	2.07 / 2.39	2.14 / 2.19	2.14 / 2.19	2.24 / 2.39	2.09 / 2.37	2.27 / 2.56
S ALP (U/L)	606 / 74	510 / 390	476 / 420	181 / 143	262 / 138	261 / 95
TmPi/GFR (mmol/L)	1.6 / 1.8	1.4 / 1.5	1.2 / 1.4	1.1 / 1.4	1.3 / 0.9	1.0 / 1.4
25(OH) D (nmol/L)	115 / 167	78 / 151	137 / 162.5	125 / 175	92 / 100	85 / 94,5
PTH at diagnosis (pmol/L)	4.66	4.31	5.64	7.63	9.42	7.88
Tumour localization	Right calcaneal (sole)	Not found	Proximal medial region of right thigh (inguinal)	Posterior mediastinum	Tongue	Not found
Histopathology	Mesenchymal tumour	-	Mesenchymal tumour	Mesenchymal tumour	Squamous carcinoma	-
Clinical remission	Yes	persist on vit D+Pi	Yes	Yes	Yes	persist on vit D+Pi
S Pi post-op or during follow-up for those without surgery (mmol/L)	1.35	0.64	1.09	1.42	0.80	0.80
TmPi/GFR post-op (mmol/L)	1.27	-	1.13	1.31	0.95	-

Note: S Pi (serum phosphorus—normal range: 0.80–1.45 mmol/L); S Ca (serum calcium—normal range: 2.12–2.54 mmol/L); S ALP (serum alkaline phosphatase—normal range: 44 to 147 international units per litre (IU/L)); TmPi/GFR (maximal tubular reabsorption of phosphate—normal range per age: 25–35 years: 1.0 to 1.35; 45–55 years: 0.90–1.35; 65–75:0.80–1.35 mmol/L); 25(OH) D (25-hydroxyvitamin D—normal range: >75 nmol/L for patient with osteometabolic disease); PTH (parathyroid hormone—normal range: 1.16–7.105 pmol/L); height in metres; weight in kilograms; duration of treatment with vitamin D and phosphate (in months).

TABLE 2 Results of dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computerized tomography (HR-pQCT) of tumour-induced osteomalacia (TIO) patients

TIO patients	1	2	3	4	5	6
DXA						
LS BMD (g/cm^2)	1.000	1.201	0.866	1.229	1.102	0.934
LS Z-score	−3.6	+1.3	−2.5	+0.9	−0.6	−2.4
FN BMD (g/cm^2)	0.506	0.744	0.746	0.850		1.221
FN Z-score	−4.1	−0.5	−1.7	−0.3		+ 1.7
TH BMD (g/cm^2)	0.637	0.805	0.682	0.935		0.808
TH Z-score	−3.5	−0.6	−2.3	−0.3		−1.7
HR-pQCT						
Distal radius						
Dtotal (mg HA/cm^3)	142.1	192.4	285.8	268.7	240.3	277.7
Dcomp (mg HA/cm^3)	705	809.1	819.6	798.9	852.4	708.9
Ct. Th (mm)	0.39	0.58	0.67	0.64	0.75	0.53
Dtrab (mg HA/cm^3)	61.3	61.7	154.5	147	95.8	189.5
BV/TV (1)	0.051	0.051	0.129	0.122	0.08	0.158
Tb.N (mm^{-1})	1.01	0.78	1.31	1.85	1.73	2.08
Tb. Th (mm)	0.05	0.066	0.098	0.066	0.046	0.076
Tb. Sp (mm)	0.936	1.221	0.666	0.475	0.532	0.405
Tb.1/N.SD (mm)	0.647	0.873	0.34	0.254	0.221	0.197
Distal tibia						
Dtotal (mg HA/cm^3)	134.9	100.9	126.7	237.1	209.6	193.8
Dcomp (mg HA/cm^3)	773.2	806.9	766.9	743.2	757.7	887
Ct. Th (mm)	0.72	0.83	0.57	1.37	1.07	0.85
Dtrab (mg HA/cm^3)	66	−5.9	66.4	101.9	112.6	93.3
BV/TV (1)	0.055	−0.005	0.055	0.085	0.094	0.078
Tb.N (mm^{-1})	1.1	0.38	0.83	0.54	1.18	1.59
Tb. Th (mm)	0.05	−0.01	0.066	0.156	0.08	0.049
Tb. Sp (mm)	0.860	2.668	1.132	1.686	0.771	0.579
Tb.1/N.SD (mm)	0.526	1.521	0.615	1.732	0.583	0.257

Note: Data of patient #5 regarding femur DXA parameters are not presented due to the presence of bilateral prosthesis.
Abbreviations: BMD, bone mineral density; BV/TV, bone volume-to-total volume ratio; Ct. Th, cortical thickness; Dcomp, cortical vBMD; Dtotal, vBMD of entire bone; Dtrab, trabecular vBMD; FN, femoral neck; LS, lumbar spine; Tb.1/N.SD, inhomogeneity of trabecular network; Tb.N, trabecular number; Tb. Sp, trabecular separation; Tb. Th, trabecular thickness; TH, total hip; vBMD, volumetric bone mineral density.

parameters on HR-pQCT due to microarchitectural derangement, and DXA results did not reflect such a negative change compared with other TIO patients. Thus, areal BMD was probably inaccurate due to vertebral collapse and hip deformities, whereas volumetric density measured at distal radius and distal tibia more likely represented the severe skeletal disease. HR-pQCT analysis of patient 2 was repeated and confirmed negative values of trabecular bone indices at distal tibia, mainly vBMD. The interpretation, according to manufacturer, is that trabecular bone density (Dtrab) is possibly similar or lower than bone marrow density, which varies from −100 mgHA/cm^3 to +100 mgHA/cm^3. DXA and HR-pQCT data are shown in Table 2.

When compared to the control group, the TIO patients had lower values for Dtrab, cortical (Dcomp) and entire region (Dtotal) vBMD at the distal radius and distal tibia. They also showed lower values for cortical thickness (Ct. Th), trabecular number (Tb.N) and bone volume-to-total volume ratio (BV/TV), and higher trabecular separation (TbSp) and heterogeneity of the trabecular network (Tb.1/N.SD) at both regions. These data are shown in Table 3.

4 | DISCUSSION

Our study is the first to describe bone volumetric density and microarchitecture using HR-pQCT in male patients with oncogenic osteomalacia during adulthood. When compared to controls matched by sex, age and BMI, TIO patients had lower volumetric BMD at the trabecular and cortical bones, as well as derangements in microstructural parameters obtained by HR-pQCT at the distal radius and

TABLE 3 Comparisons between anthropometric characteristics and bone parameters obtained by DXA and HR-pQCT in male patients with tumour-induced osteomalacia (TIO) and controls

	TIO (*N* = 6)	Control (*N* = 18)	*p* value
Age at study (years)	58.67 ± 10.44	58.94 ± 3.13	.578
BMI (kg/m^2)	23.60 ± 4.44	27.30 ± 4.76	.172
DXA			
LS BMD (g/cm^2)	1.000 ± 0.217	1.188 ± 0.166	.047
LS Z-score	−1.15 ± 1.99	−0.16 ± 1.36	.197
FN BMD (g/cm^2)	0.813 ± 0.260	0.980 ± 0.110	.048
FN Z-score	-0.98 ± 2.12	0.03 ± 0.98	.186
TH BMD (g/cm^2)	0.773 ± 0.117	1.021 ± 0.118	.002
TH Z-score	−1.68 ± 1.30	−0.20 ± 0.99	.013
HR-pQCT			
Distal radius			
Dtotal (mg HA/cm^3)	234.50 ± 56.58	317.02 ± 60.81	.004
Dcomp (mg HA/cm^3)	782.31 ± 61.09	866.51 ± 54.38	.005
Ct. Th (μm)	0.59 ± 0.12	0.80 ± 0.21	.015
Dtrab (mg HA/cm^3)	118.30 ± 53.22	177.17 ± 32.18	.015
BV/TV (1)	0.09 ± 0.04	0.14 ± 0.02	.015
Tb.N (mm^{-1})	1.46 ± 0.50	2.10 ± 0.26	.004
Tb. Th (mm)	0.06 ± 0.01	0.07 ± 0.01	.417
Tb. Sp (mm)	0.70 ± 0.31	0.41 ± 0.06	.005
Tb.1/N.SD (mm)	0.42 ± 0.27	0.18 ± 0.06	.002
Distal tibia			
Dtotal (mg HA/cm^3)	167.16 ± 53.79	295.87 ± 49.65	.001
Dcomp (mg HA/cm^3)	789.15 ± 52.42	900.90 ± 40.29	.002
Ct. Th (μm)	0.90 ± 0.28	1.31 ± 0.23	.007
Dtrab (mg HA/cm^3)	72.38 ± 42.73	161.33 ± 27.33	<.0001
BV/TV (1)	0.06 ± 0.03	0.13 ± 0.02	<.0001
Tb.N (mm^{-1})	0.93 ± 0.44	1.96 ± 0.30	.001
Tb. Th (mm)	0.06 ± 0.05	0.06 ± 0.01	.251
Tb. Sp (mm)	1.28 ± 0.78	0.45 ± 0.07	.001
Tb.1/N.SD (mm)	0.87 ± 0.60	0.20 ± 0.04	.001

Abbreviations: BMD, bone mineral density; BMI, body mass index; BV/TV, bone volume-to-total volume ratio; Ct. Th, cortical thickness; Dcomp, cortical vBMD; Dtotal, vBMD of entire bone; Dtrab, trabecular vBMD; DXA, dual-energy X-ray absorptiometry; FN, femoral neck; HR-pQCT, high-resolution peripheral quantitative computerized tomography; LS, lumbar spine; Tb. N, trabecular number; Tb. Th, trabecular thickness; TH, total hip; tTb.1/N.SD, inhomogeneity of trabecular network; vBMD, volumetric bone mineral density.
The bold values were representing statistically significant values for p <.05.

distal tibia. The DXA analysis also showed a lower areal BMD at the lumbar spine, femoral neck and total hip.

Our data are in accordance with the low BMD and T-score in the two TIO patients described by Piemonte et al.[11] and Malabanan et al.[12] who also had low phosphorus and history of fractures at the time of diagnosis, with one of the patients using a wheelchair. Other studies that reported lower bone mineral density in individuals with other causes of hypophosphataemic osteomalacia included vitamin D deficiency, nutritional, malabsorption and renal tubular acidosis. One study evaluating a patient with autosomal dominant hypophosphataemic rickets found lower BMD at the lumbar spine.[13–15]

A low aBMD in patients with osteomalacia is mainly due to calcium, phosphate and/or vitamin D deficiency, since osteomalacia is primarily a process of replacement of the mineralized bone matrix by unmineralized bone matrix, named osteoid. The assessment of BMD by DXA in these individuals has its value in monitoring the effectiveness of pharmacological treatment[13] and of the postoperative follow-up after tumour excision in cases of TIO; however, the results from the DXA analysis can suffer from interference of pseudofractures, fractures and bone deformities. In this scenario, HR-pQCT can give more accurate information regarding volumetric bone density and microstructure using distal regions of the skeleton that are less

affected in these adults. On the other hand, it is not uncommon for TIO patients to present with symptomatic peripheral Looser zones, especially in the lower limbs.

High-resolution peripheral quantitative computerized tomography may predict the risk of fractures and evaluates a tridimensional BMD, which is different from what DXA provides, as it does not distinguish cortical from trabecular bone and analyses areal BMD. Through this study, we evaluated how the bone tissue of patients with TIO could behave concerning bone microarchitecture and therefore could influence treatment management and intensity of therapy. The presence of worse bone parameters in HR-pQCT could reflect an aggressive evolution of the disease and highlights the necessity of an intensified clinical management for these patients. HR-pQCT improved our knowledge in the bone derangements associated with bone fragility and fractures in patients not classified as osteoporosis using DXA. The same probably applies to TIO patients, in whom DXA not always detects such alterations.[16]

The lower volumetric BMD values in the trabecular, cortical and entire bone at the radius and tibia are in accordance with the lower areal BMD in all central sites in DXA detected in our TIO patients compared with controls; however, only the evaluation using HR-pQCT detected the deranged bone microstructure in trabecular and cortical bone compartments.

Using HR-pQCT to study 29 adults with genetic hypophosphataemic rickets, Shanbhogue et al also found a lower total vBMD at the radius and tibia and a low cortical vBMD at the radius.[8] Cheung et al also found a low cortical vBMD in 34 adults and children with X-linked hypophosphataemic rickets (XLH) upon pQCT analysis.[17] As pointed out by the authors, these results in the cortical bone are expected in patients with osteomalacia due to excess nonmineralized osteoid accumulated in the cortex, as demonstrated by previous histomorphometric studies.[18] In contrast to these findings, Colares Neto et al found no differences between adults with XLH and controls with respect to total and cortical vBMD at both sites.[7]

Cheung et al.[17] found a high Dtrab at the radius in all patients with XLH but mainly in children using calcitriol and phosphate salts, as the adults were not receiving treatment. One histomorphometric study of adult patients with XLH and moderate-to-severe osteomalacia did not show trabecular osteopenia either in symptomatic or in asymptomatic patients. The normal-to-increased calcified bone trabecular volume found in these subjects seemed to be due to low bone turnover with remodelling imbalance and preservation of the trabecular mass.[19] Colares Neto et al found the same results as Cheung et al at the distal radius analysis but not in the distal tibia where Dtrab was low in all patients.[7,17] The authors attributed these differences to distinct mechanical load at each site and the fact that deformities in the legs modify the strain points with irregular bone formation.

One study with bone biopsy of a patient with TIO using histomorphometry allied with analysis of bone mineralization density distribution (BMDD) showed reduced trabecular bone volume and slight increase in the osteoblastic surface, which was associated with a significant reduction in osteoclastic surface and resorptive activity, denoting a low bone turnover.[20] This finding is in line with our results of low Dtrab despite no significant alteration in Tb. Th, which suggests that TIO also leads to a low rate of bone turnover with a reduction in bone mass over time, despite the established clinical treatment. These differences between Dtrab values found in patients with hypophosphataemic rickets of genetic origin compared to those with acquired osteomalacia in adulthood due to a phosphaturic tumour may be related to greater disease severity in the TIO group. Many patients with XLH have mild disease, and some individuals are minimally affected even without phosphorus treatment and vitamin D supplementation.[21]

We found derangement of the trabecular bone considering the decreased BV/TV and Tb.N, and increased Tb. Sp and heterogeneity of the trabecular network, which highlights an important compromise of the trabecular architecture in addition to the reduction in the trabecular volume mentioned above. These architectural changes were also reported by Shanbogue et al and Colares Neto et al[7,8]

Our study is limited due to its cross-sectional design and small sample size, but the rarity of TIO must be considered. Another limitation is that although TIO seems to be the more probable aetiology for acquired severe hypophosphataemic-hyperphosphaturic osteomalacia in all six patients, only four patients had the hypophosphataemic tumours confirmed and removed. In the other two patients, tumour location was not achieved, although they were submitted to all imaging examinations. This type of tumour is usually small and characterized by slow growth, and probably for these reasons, the imaging examinations were not able to capture the lesions. However, the fact that all patients initiated osteomalacia in adulthood, the absence of family history of this disease and the exclusion of other tubulopathies favour the hypothesis of tumoral osteomalacia. Unfortunately, 1,25 $(OH)_2D$ and FGF-23 assays were not available in our centre. Also, the gonadal status was not evaluated since TIO patients and control subjects did not present symptoms or history of hypogonadism. To our knowledge, this is the first study to assess bone density and microstructure parameters in TIO patients based on HR-pQCT and DXA results compared with healthy controls. Our findings may help to better understand and treat this serious disease, and further prospective studies may evaluate the real impact of surgery and clinical treatment on the evolution of bone properties in these patients.

In conclusion, we demonstrated that patients with TIO have decreased bone density and impaired microarchitecture at both the cortical and trabecular compartments, as evaluated by HR-pQCT. These findings suggest that TIO severely affects bone health and may lead to fragility fractures and increased mortality.

ACKNOWLEDGEMENT

The authors have nothing to declare.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data; been involved in drafting of the manuscript or revised it critically for important intellectual content; approved the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of the Federal University of Rio de Janeiro and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Written informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Minisola S, Peacock M, Fukumoto S, et al. Tumour-induced osteomalacia. *Nat Rev Dis Primers*. 2017;3:17044.
2. Chong WH, Molinolo AA, Chen CC, Collins MT. Tumor-induced osteomalacia. *Endocr Relat Cancer*. 2011;18:R53-R77.
3. Florenzano P, Gafni RI, Collins MT. Tumor-induced osteomalacia. *Bone Rep*. 2017;7:90-97.
4. Feng J, Jiang Y, Wang OU, et al. The diagnostic dilemma of tumor induced osteomalacia: a retrospective analysis of 144 cases. *Endocr J*. 2017;64:675-683.
5. Uramoto N, Furukawa M, Yoshizaki T. Malignant phosphaturic mesenchymal tumor, mixed connective tissue variant of the tongue. *Auris Nasus Larynx*. 2009;36:104-105.
6. Dadoniene J, Miglinas M, Miltiniene D, et al. Tumour-induced osteomalacia: a literature review and a case report. *World J Surg Oncol*. 2016;14(4):1-10.
7. Colares Neto GP, Pereira RMR, Alvarenga JC, Takayama L, Funari MFA, Martin RM. Evaluation of bone mineral density and microarchitectural parameters by DXA and HR-pQCT in 37 children and adults with X-linked hypophosphatemic rickets. *Osteoporos Int*. 2017;28:1685-1692.
8. Shanbhogue VV, Hansen S, Folkestad L, Brixen K, Beck-Nielsen SS. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in adult patients with hypophosphatemic rickets. *J Bone Miner Res*. 2015;30:176-183.
9. Chiang CY, Zebaze R, Wang X, Ghasem-Zadeh A, Zajac JD, Seeman E. Cortical matrix mineral density measured noninvasively in pre- and postmenopausal women and a woman with vitamin D-dependent rickets. *J Bone Miner Res*. 2018;33:1312-1317.
10. Moreira CA, Ferreira CEDS, Madeira M, et al. Reference values of 25-hydroxyvitamin D revisited: a position statement from the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC). *Arch Endocrinol Metab*. 2020;64:462-478.
11. Piemonte S, Romagnoli E, Cipriani C, et al. Six-year follow-up of a characteristic osteolytic lesion in a patient with tumor-induced osteomalacia. *Eur J of Endocrinol*. 2014;170:K1-K4.
12. Malabanan A, Turner A, Rosenberg I, Holick M. Oncogenic osteomalacia: clinical presentation, densitometric findings, and response to therapy. *J Clin Densitom*. 1998;1:77-80.
13. Bhambri R, Naik V, Malhotra N, et al. Changes in bone mineral density following treatment of osteomalacia. *J Clin Densitom*. 2006;9:120-127.
14. Saghafi M, Azarian A, Hashemzadeh K, Sahebari M, Rezaieyazdi Z. Bone densitometry in patients with osteomalacia: is it valuable? *Clin Cases Miner Bone Metab*. 2013;10:180-182.
15. Negri AL, Bogado CE, Zanchetta JR. Bone densitometry in a patient with hypophosphatemic osteomalacia. *J Bone Miner Metab*. 2004;22:514-517.
16. Boutroy S, Brouxein M, Munoz F, Delmas P. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab*. 2005;90:6508-6515.
17. Cheung M, Roschger P, Klaushofer K, et al. Cortical and trabecular bone density in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*. 2013;98:E954-E961.
18. Marie PJ, Glorieux FH. Stimulation of cortical bone mineralization and remodeling by phosphate and 1,25-dihydroxyvitamin D in vitamin D-resistant rickets. *Metab Bone Dis Relat Res*. 1981;3:159-164.
19. Marie PJ, Glorieux FH. Bone histomorphometry in asymptomatic adults with hereditary hypophosphatemic vitamin D-resistant osteomalacia. *Metab Bone Dis Relat Res*. 1982;4:249-253.
20. Nawrot-Wawrzyniak K, Varga F, Nader A, et al. Effects of tumor-induced osteomalacia on the bone mineralization process. *Calcif Tissue Int*. 2009;84:313-323.
21. Carpenter TO, Imel EA, Holm IA, et al. A clinician's guide to X-linked hypophosphatemia. *J Bone Miner Res*. 2011;26:1381-1388.

How to cite this article: Mendes DAB, Coelho MCA, Gehrke B, et al. Microarchitectural parameters and bone mineral density in patients with tumour-induced osteomalacia by HR-pQCT and DXA. *Clin Endocrinol*. 2021;00:1-8. https://doi.org/10.1111/cen.14533

ANEXO H – Artigo aceito para publicação – Long-term consequences of osteoporosis therapy with bisphosphonates

review

Long-term consequences of osteoporosis therapy with bisphosphonates

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Received on Aug/9/2022
Accepted on Nov/10/2022

DOI: 10.20945/2359-3997000000629

ABSTRACT

Bisphosphonates (BPs) are medications widely used in clinical practice to treat osteoporosis and reduce fragility fractures. Its beneficial effects on bone tissue have been consolidated in the literature for the last decades. They have a high affinity for bone hydroxyapatite crystals, and most bisphosphonates remain on the bone surface for a long period of time. Benefits of long-term use of BPs: Large and important trials (Fracture Intervention Trial Long-term Extension and Health Outcomes and Reduced Incidence with Zoledronic acid Once Yearly-Pivotal Fracture Trial) with extended use of alendronate (up to 10 years) and zoledronate (up to 6 years) evidenced significant gain of bone mineral density (BMD) and vertebral fracture risk reduction. Risks of long-term use of BPs: The extended use of antiresorptive therapy has drawn attention to two extremely rare, although severe, adverse events. That is, atypical femoral fracture and medication-related osteonecrosis of the jaw are more common in patients with high cumulative doses and longer duration of therapy. BPs have demonstrated safety and effectiveness throughout the years and evidenced increased BMD and reduced fracture risks, resulting in reduced morbimortality, and improved quality of life. These benefits overweight the risks of rare adverse events.

Keywords
Bisphosphonate; osteoporosis; fracture; osteonecrosis of the jaw; atypical femoral fracture

INTRODUCTION

Bisphosphonates (BPs) are medications with consolidated evidence of anti-osteoporotic effect, and they have already been used for more than two decades in the clinical field (1). The benefits of fracture prevention and bone mineral density (BMD) increase are maintained for years during and after discontinuation of treatment with BP (1-5). Other positive aspects of these agents are lower healthcare costs, reduced morbidity, and significantly increased survival rates (6). Three-year use of zoledronic acid (ZOL) reduces mortality in individuals with low trauma hip fractures by 28% (6).

BPs are derivatives of inorganic pyrophosphate (PPi), which are chemically stable compounds. Studies accomplished in the 1960s evidenced that these compounds are able to hinder calcification by connecting to hydroxyapatite crystals. Therefore, the hypothesis that regulating PPi levels could control bone mineralization emerged (7). BPs differ from PPi in that a hydroxyl group is connected to the central carbon, which increases the ability to bind to calcium (7). The antiresorptive potency of BPs is determined by the presence of a nitrogen or amino group. These components increase the potency from 10 to 10.000 when comparing the first generation (clodronate and tiludronate) from the second and third generations (alendronate (ALN), risedronate (RIS), ibandronate (IBN), pamidronate, and ZOL) (7).

The Food and Drug Administration approved ALN in 1996, and since then, throughout several years, studies have been conducted to evaluate the efficacy and safety of BPs (1-5). ALN, RIS, and ZOL could reduce the risk of vertebral, nonvertebral, and hip fractures (1). IBN has been demonstrated to reduce the risk of vertebral fractures, and there is a paucity of data to prove the beneficial effect on hip and nonvertebral fractures (8,9).

BPs have been widely used in clinical practice to treat osteoporosis for several years. However, doubts concerning the duration of treatment and drug holidays remain (1). This article will review the beneficial effects and risks of long-term treatment with BPs.

MECHANISM OF ACTION OF BPS

BPs are known to have an affinity for bone hydroxyapatite crystals and, therefore, bind to this component at the surface of the bone matrix. Moreover, BPs are incorporated into areas where bone remodeling is active (1,7,10,11). This group of drugs can prevent bone resorption and increase BMD and strength (10). The reduction of fracture in postmenopausal women, when compared to placebo, occurs after 1-2 years of treatment with BPs (1).

Those containing nitrogen in their composition act by binding and inhibiting the activity of farnesyl pyrophosphate synthase. This enzyme regulates the mevalonic acid pathway and plays an important role in producing cholesterol, other sterols, and lipids. The inhibition of farnesyl pyrophosphate synthase modifies the post-translational protein, ultimately leading to the inhibition of osteoclast recruitment and activity at the bone surface, as well as osteoclast apoptosis (7). BPs, therefore, inhibit the recruitment of osteoclasts and induce the apoptosis of these cells, resulting in the suppression of osteoclast-mediated bone resorption (1,10). They attach to the bone mineral surface owing to their high affinity to hydroxyapatite (Table 1), and the remaining dosage fraction is excreted by the kidneys (7,10,12-14). Patients with impaired renal function have decreased excretion of the medication; therefore, its use is not recommended in patients with a glomerular filtration rate of <30 mL/min (10). Moreover, they are hydrophilic and, therefore, have poor absorption in the gastrointestinal tract when administered orally (less than 1% absorption per oral dose) (7).

BPs remain in the bone structure for a long period of time (10). Pieces of evidence show that, when the bone that contains BP resorbs, a part of this medication is released and recirculates locally and systemically, thereby attaching once again to other surfaces and inhibiting bone resorption (15). This retention of BPs in the bone tissue explains the slow bone loss after therapy discontinuation as the drug may be retained for up to 10 years in the bone matrix (11,15).

BENEFICIAL PIECES OF EVIDENCE FOR LONG-TERM USE OF BPS

Bone and cols. published in 2004 a multicenter study concerning the use of ALN in postmenopausal women for 10 years. Compared with baseline measurements, the ALN treatment resulted in a significant increase of BMD at the lumbar spine (13.7%), femoral neck (5.4%), and total hip (6.7%). The beneficial effect on BMD and bone remodeling markers was maintained, and the safety of the long-term therapy was confirmed. Nevertheless, a progressive loss of effect of the drug suspension was observed (5).

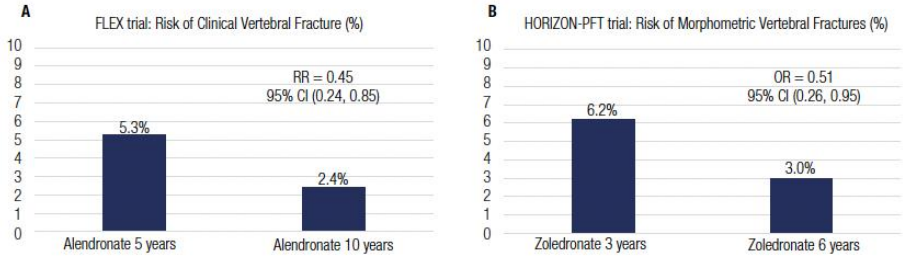
The Fracture Intervention Trial Long-term Extension (FLEX) is another multicentric study that evaluated the effects of extended treatment with oral BPs (10 years) compared with the 5-year use in 1099 postmenopausal women (15). Women participating in the FLEX trial had to have a T-score of –1.6 standard deviation (SD) or less but not necessarily be in the osteoporosis range. Women who discontinued ALN after 5 years of therapy and switched to placebo presented a statistically significant ($P < 0.001$) increase in serum levels of bone turnover markers when compared with those that followed receiving ALN therapy (raise of 28.1% for alkaline phosphatase specific for bone (BSAP), 55.6% for C-telopeptide of type 1 collagen (β-CTX), and 59.5% for N-propeptide of type 1 collagen (P1NP)), although these serum levels remained lower than pretreatment levels. Moreover, individuals treated with ALN for 5 years followed by placebo had a statistically significant ($P < 0.001$) decrease in BMD at the lumbar spine and total hip compared with the group that maintained ALN for 10 years. The BMD at the spine and total hip decreased by –3.7% and –2.4%, respectively, in 10

Table 1. Pharmacological characteristics of bisphosphonates considering affinity to hydroxyapatite and relative anti-resorptive potency. The table illustrates affinity to hydroxyapatite and relative anti-resorptive potency from lowest to highest, in order, from left to right

Characteristics	
Affinity	Risedronate < Ibandronate < Alendronate< Zoledronic acid
Relative anti-resorptive potency	Alendronate < Ibandronate < Risedronate < Zoledronic acid

years. Furthermore, as an exploratory outcome, the study documented the incidence of fractures for up to 10 years. The risk of clinical vertebral fractures in those treated with ALN for 5 years and switched to placebo was over two times higher than those using 5-10 mg daily of ALN for up to 10 years (5.3% versus 2.4%, respectively) (Figure 1A). Therefore, the relative risk reduction was 55%, and the absolute risk reduction was 2.9%. Concerning all clinical and nonvertebral fractures, no significant differences were documented. There was no increase in risk of nonvertebral fractures, and the risk of clinical vertebral fractures in the group treated with ALN for 10 years decreased compared to the group that switched to placebo at year 5. This result indicates that extended therapy (10 years) does not implicate adverse effects on bone strength. The high-risk groups comprise those with larger benefits in preventing vertebral clinical fractures: women with preexisting fractures or those with very low BMD (T-score of –2.5 SD or less) at the baseline. The number needed for extended 10-year therapy is 25 in high-risk groups, compared with 50-300 in lower-risk groups (with no previous vertebral fracture or osteoporosis diagnosis) (1). Data from a post hoc subgroup analysis showed that, for women without vertebral fracture but with an osteoporotic range (T-score of –2.5 SD or less) at the femoral neck at the FLEX baseline, extended therapy for up to 10 years with ALN decreased the risk of nonvertebral fractures (3). The results of the FLEX study call attention to the fact that a 5-year therapy with BP has a long-term effect on bone tissue. The reason is that, based on observational studies, the cumulative effect on the total hip BMD was a 1%-3% gain compared with the 5%–10% loss in untreated women of the same age (16).

The Health Outcomes and Reduced Incidence with Zoledronic acid Once Yearly-Pivotal Fracture Trial (HORIZON-PFT) studied the long-term effects of ZOL 5 mg. A total of 1,233 osteoporotic postmenopausal women using ZOL for 3 years were randomized into two separate groups: placebo (n = 617) and 3 more years of ZOL (n = 616). The main endpoint analyzed was femoral neck BMD, and the other secondary endpoints were fractures, other BMDs, bone turnover markers, and safety issues. Bone turnover markers elevated in a 3-year ZOL group followed by 3 years of a placebo, whereas in the 6-year ZOL group, they remained steady although both presented smaller values than the baseline. Regarding bone turnover markers, P1NP increased in both groups with a 14% difference between them (P = 0.0001), and values remained under pretreatment. The 3-year ZOL group followed by placebo resulted in a statistically significant (P < 0.0009) inferior BMD at the femoral neck compared with the 6-year ZOL group, with a difference of 1.04%, although it still remained above baseline values. At the lumbar spine, the difference in BMD was 2.03% higher in the 6-year ZOL group compared with the 3-year ZOL group (P = 0.002). The risk of morphometric vertebral fractures in women treated with ZOL for 3 years and switched to placebo was approximately two times higher when compared with those using 5 mg yearly of ZOL for up to 6 years (6.2% versus 3.0%, respectively, odds ratio of 0.51 with P = 0.035) (Figure 1B). Regarding the incidence of all clinical fractures, nonvertebral fractures, and



RR: relative risk; CI: confidence interval; OR: odds ratio.

Figure 1. Vertebral fracture risk reduction in long-term use of bisphosphonates evidenced at FLEX and HORIZON-PFT trials. **A.** illustrates the smaller risk of clinical vertebral fractures at the group that used Alendronate for up to 10 years when compared to the group that used Alendronate for 5 years and switched to placebo for 5 years. **B.** illustrates the smaller risk of morphometric vertebral fractures at the group that used Zoledronate for up to 6 years when compared to 3 years of treatment with Zoledronate plus 3 years of placebo.

clinical vertebral fractures, no significant differences were documented, although confidence intervals were vast, and thus, we cannot rule out a possible benefit. Based on the results of this trial, we may conclude that postmenopausal women diagnosed with osteoporosis and high risk of fractures (particularly vertebral fractures) may benefit from continuous treatment with 5 mg yearly of ZOL for up to 6 years (2). A second extension of the HORIZON-PFT trial observed the results of 9-year extension therapy with ZOL. Women were randomized to a 3-year placebo after a 6-year therapy with ZOL or maintenance of treatment for up to 9 years with ZOL. No significant differences were observed in BMD values, bone turnover markers, and the number of fractures. Therefore, the results suggest that the extended treatment with ZOL for up to 9 years has no additional benefit, although the sample size evaluated was much smaller than the previous extension study (190 women) owing to follow-up loss (17).

Furthermore, a smaller study with extended use of RIS published in 2004 observed the effects of a 7-year treatment in postmenopausal women with osteoporosis. The endpoints were BMD analysis, bone turnover marker levels, and assessment of vertebral, and nonvertebral fractures. The groups included 5 years of placebo followed by 2 years of RIS (n = 81) and 7 years of RIS group (n = 83); all 136 women completed the study. The study evidenced an increase in BMD measurements after 5 years of treatment with RIS when compared with the baseline and remained stable or increased in patients treated with 7 years of RIS. BMD at the lumbar spine raised 8.8% in the 5-year RIS group and 11.5% in the 7-year RIS group when compared with the study onset. Considering bone turnover markers, in the 7-year RIS group, N-telopeptide measured in urine decreased by 54% at 3 months and 63% at 7 years, when compared with the baseline. The incidence of vertebral fractures was similar in groups using RIS for 5 years, suspended therapy, and RIS for up to 7 years, although a significant reduction in vertebral fractures was observed in the 5-year placebo group that began treatment with RIS for 2 years. The incidence of nonvertebral fractures in a 5-year placebo followed by 2-year RIS was 7.4%, whereas the 7-year RIS group presented a lower incidence of 6.0% but was not significant. Further analysis demonstrated that once patients started receiving treatment with RIS after the 5-year placebo therapy, fracture incidence was diminished. The reason is that there was a statistically significant reduction of new fractures when compared with 4-5 years of placebo (2 *vs.* 12 patients, with P = 0.008). The study concluded that extended use of RIS maintains anti-fracture efficacy and is well tolerated for a long period of time (18).

Therefore, the task force of the American Society for Bone and Mineral Research (ASBMR) reaffirms the results and conclusions of the studies described above. The ASBMR also suggests that postmenopausal women receiving oral BP for ≥ 5 years or intravenous BP for ≥ 3 years and with hip, spine, or multiple other osteoporotic fractures before or during therapy or hip BMD T-score of ≤ –2.5 SD or high risks of fracture (obtained using fracture risk calculators) will benefit from maintaining BP or switching to another anti-fracture therapy and should be reassessed every 2-3 years (19).

RISKS ASSOCIATED WITH THE USE OF BPS

Adverse events non-related to extended use of BP are gastrointestinal symptoms with oral BPs, flu-like symptoms when administered intravenously, hypocalcemia, musculoskeletal pain, and atrial fibrillation (Table 2) (7,11). Individuals with known gastroesophageal reflux disease or with esophageal stricture are most affected by oral BP administration owing to esophageal irritation and erosion (7). Concerning flu-like symptoms, approximately 10%-30% of individuals that received the first infusion of nitrogen-containing BP present an acute phase reaction marked by influenza-like symptoms (headache, arthralgia, myalgia in association with transient pyrexia) (7). Moreover, hypocalcemia may occur after intravenous BP infusion, most frequently in patients with high rates of bone resorption mediated by osteoclasts, renal dysfunction, hypovitaminosis D, or undiagnosed cases of hypoparathyroidism (7). Severe musculoskeletal pain is a rare adverse event that may occur at any phase of intravenous or oral BP treatment (7).

Table 2. Bisphosphonates adverse events and duration of therapy

Short-term adverse events	Long-term adverse events
Gastrointestinal symptoms (oral administration)	Medication-related osteonecrosis of the jaw
Hypocalcemia	Atypical femoral fracture
Musculoskeletal pain	
Atrial fibrillation	
Flu-like symptoms (intravenous administration)	

Approximately 10 years after BP approved the osteoporosis treatment, post-marketing reports based on the long-term treatment of millions of patients revealed the occurrence of two initially unexpected adverse events: atypical femoral fracture (AFF) and medication-related osteonecrosis of the jaw (MRONJ). Both conditions are extremely rare and, therefore, must not hinder the long-term use of these antiresorptive agents when indicated (6).

Concerns regarding the long-term use of BP draw attention to the possibility that normal physiological bone turnover and repair may be reduced. This case may lead to the accumulation of microdamage or microcracks and reduced bone strength leading to AFF (1,15). Table 3 presents the major and minor criteria of AFF (6,20). The locations where AFF occur are considered atypical as they are regarded as the most resistant region of the femur, thereby being improbable to fracture with low trauma in the absence of fragility (1). Subtrochanteric and diaphyseal fractures account for 7%-10% of all femoral fractures, and among them, 75% are related to major trauma (20). Other risk factors for the occurrence of AFF are low 25-hydroxyvitamin D concentrations (less than 20 ng/mL), diabetes, use of a glucocorticoid, low total hip BMD, history of falls, older age, and femoral shape (20,21).

Commonly, AFF begins with a periosteal callus shown as a blurred formation, posteriorly becoming solidified. The periosteal calluses reflect an attempt to cure bone tissue before evolving into a fracture, and they occur in AFF near the developing fracture on the lateral cortex of the bone (20). Patients with this complication may present contralateral fracture in 28%, healing delay in 26%, and thigh pain in 30%-70% of cases (22). Therefore, whenever the patient with extended use of BP complains of groin or thigh pain, an urgent bilateral femoral imaging evaluation is required to detect partial fracture, stress reaction, or total hip fracture (20). Imaging may be used to diagnose AFF, and conventional radiography seems convenient, although advanced imaging technologies (scintigraphy, computerized tomography scan, and magnetic resonance imaging) present higher specificity and sensitivity for detection in the initial phases (20).

The absolute risk of AFF in patients undergoing BP treatment ranges from 3.2 to 50 cases per 100.000 person-years with short-term use (<5 years) and approximately 113 per 100.000 person-years with long-term use (>5 years) (23). A recently published study evidenced that, in women treated with BP for over 3 years, the 5-year, and 10-year cumulative incidences of AFF were above fourfold and 10-fold, respectively, compared with women treated with BP for less than 3 years (24). Schilcher and cols. carried out a Swedish study and observed that for each additional year of BP use, the adjusted odds of AFF increase by 2.5-fold (25). However, although the relative risk of AFF is higher in patients on BP treatment, the absolute risk is uniformly very low among studies (20).

The risk of AFF should be analyzed alongside the benefits of preventing osteoporotic fractures. The use of amino-BPs in high-risk patients (with clinical vertebral fractures) is estimated to avoid 3300 clinical fractures per 100.000 person-years of treatment, whereas its use in moderate-risk patients (femoral neck BMD T-score < –2.0) prevents 1,700 clinical fractures per 100.000 person-years (20,26). Moreover, for each 100 typical femoral neck or intertrochanteric fractures prevented by BP use, one subtrochanteric atypical fracture occurs (27). Therefore, we may conclude that the risk-benefit analysis favors BP therapy for women at high risk for fracture.

MRONJ is an uncommon (prevalence varies from 0% to 0.04%) but threatening condition described in patients undergoing antiresorptive treatment with BP (11). This condition occurs in approximately 1.04-69 cases per 100.000 person-years. Patients with diagnosed cancer associated with malignant hypercalcemia or bone metastasis present a significantly higher incidence of MRONJ, ranging from 1% to 10% (28). This complication is defined as the "presence of exposed jaw bone, or bone that can be probed,

Table 3. Minor and Major criteria of atypical femoral fractures (AFF). Four of the five major criteria must be present for the diagnosis of AFF

Minor criteria:	Major criteria:
• Use of medications or comorbidities that increase risk of AFF	• Spontaneous or minimal trauma fracture
• Symptoms of groin or thigh pain	• Absence of comminution
• Bilateral fractures	• Located at femoral shaft or subtrochanteric region
• Increase in cortical thickness of diaphysis	• Short oblique or transverse orientation
• Lateral cortex periosteal reaction	• Incomplete fractures involving lateral cortex, while complete fractures extend through both cortices
• Delayed healing	

through an intraoral or extraoral fistula, for at least 8 weeks in patient with history of antiresorptive and/or antiangiogenic therapy, and in the absence of previous radiation therapy to head and neck". The diagnosis of MRONJ is clinical, although imaging may be accomplished to assist in doubtful cases (11).

Similar to AFF, the duration of antiresorptive treatment, including adherence to therapy, has been identified as a risk factor for MRONJ (11,29-31). Other risk factors are a history of cancer, chronic use of glucocorticoids (or antiangiogenic, antithrombotic, immunosuppressants, proton pump inhibitors, or antiresorptive agents), smoking, hypertension, rheumatoid arthritis, or *diabetes mellitus*. In addition, local risk factors may contribute to the development of this adverse event, such as invasive oral procedures, trauma, poor oral hygiene, inflammatory oral diseases, and dental infection (11).

A recently published position paper reunites the recommendations of three important societies: the Brazilian Society of Endocrinology and Metabolism (SBEM), the Brazilian Society of Stomatology and Oral Pathology (SOBEP), and the Brazilian Association for Bone Evaluation and Osteometabolism (ABRASSO). The paper recommends that patients considered to initiate therapy with an antiresorptive agent must be referred to dentists for prior orientation and preventive procedures (11). Moreover, no evidence supports the interruption of BP treatment prior to dental procedures to inhibit MRONJ (11,32), including the use of β-CTX serum levels to predict the risk of MRONJ before oral procedures (11). Regular preventive dental evaluations, orientations for oral hygiene, and prophylactic use of antibiotics before procedures, such as extraction, should be considered. Moreover, plain amoxicillin or its association with clavulanate is highly recommended (11). For those who are allergic to penicillin, clindamycin may be used. The literature described several prophylactic protocols regarding the period of use of antibiotics. Data suggest initiating therapy 48-72 hours prior to the dental procedure and maintaining treatment for 1-3 weeks after the procedure (11).

Patients that need to maintain anti-osteoporotic treatment owing to the high risk of fracture may use anabolic treatment to enhance regeneration of MRONJ (improvement of bone regeneration ratio and bone turnover markers), according to data described in the literature (33,34). In addition, more favorable outcomes were described when recombinant human bone morphogenetic protein 2 (rhBMP-2) was used in association with teriparatide, suggesting that rhBMP-2 boosts bone regeneration in MRONJ cases (33). Similarly, patients suffering from AFF may consider using teriparatide as an adjunct therapy, particularly those with no evidence of healing after 4-6 weeks following the surgical procedure (20,35). The literature described few cases of healing improvement owing to the anabolic effect of the drug (20). After 2 years of treatment with an anabolic agent, antiresorptive therapy may be resumed and must be individualized, considering the risk of fracture, resolution of adverse event (MRONJ or AFF), and type of treatment (surgical or not). Anti-osteoporotic agents that may be used are raloxifene, denosumab, BPs, and hormone replacement therapy (36).

In conclusion, through this review of the benefits and risks of the extended use of BP, we may conclude that the effects on BMD and the risk of fracture observed in long-term treatment far outweigh the risks of rare and severe adverse events. These positive effects are not restricted to bone changes once there is a reduction of morbimortality and an increase in patients' quality of life who receive adequate treatment for osteoporosis. Extended therapy with BP should be considered in patients with a high risk of fragility fractures (2,6,15,22). Thus, the decision to continue or interrupt therapy with BPs is a challenge and should follow the recommendations described above.

Sponsorship: the authors did not receive any financial support.

Acknowledgments: the authors have nothing to declare.

Disclosure: no potential conflict of interest relevant to this article was reported.

REFERENCES

1. Schmidt GA, Horner KE, McDanel DL, Ross MB, Moores KG. Risks and benefits of long-term bisphosphonate therapy. Am J Health Syst Pharm. 2010;67(12):994-1001.
2. Black DM, Reid IR, Boonen S, Bucci-Rechtweg C, Cauley JA, Cosman F, et al. The effect of 3 versus 6 years of zoledronic acid treatment of osteoporosis: a randomized extension to the HORIZON-Pivotal Fracture Trial (PFT). J Bone Miner Res. 2012;27(2):243-54.
3. Schwartz AV, Bauer DC, Cummings SR, Cauley JA, Ensrud KE, Palermo L, et al. Efficacy of continued alendronate for fractures in women with and without prevalent vertebral fracture: the FLEX trial. J Bone Miner Res. 2010;25(5):976-82.
4. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, et al. Randomised trial of effect of alendronate on risk

of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet. 1996;348(9041):1535-41.
5. Bone HG, Hosking D, Devogelaer JP, Tucci JR, Emkey RD, Tonino RP, et al. Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med. 2004;350(12):1189-99.
6. McClung M, Harris ST, Miller PD, Bauer DC, Davison KS, Dian L, et al. Bisphosphonate therapy for osteoporosis: benefits, risks, and drug holiday. Am J Med. 2013;126(1):13-20.
7. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc. 2008;83(9):1032-45.
8. Delmas PD, Recker RR, Chesnut CH 3rd, Skag A, Stakkestad JA, Emkey R, et al. Daily and intermittent oral ibandronate normalize bone turnover and provide significant reduction in vertebral fracture risk: results from the BONE study. Osteoporos Int. 2004;15(10):792-8.
9. Chesnut CH 3rd, Skag A, Christiansen C, Recker R, Stakkestad JA, Hoiseth A, et al. Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. J Bone Miner Res. 2004;19(8):1241-9.
10. Licata AA. Discovery, clinical development, and therapeutic uses of bisphosphonates. Ann Pharmacother. 2005;39(4):668-77.
11. Madeira M, Rocha AC, Moreira CA, Aguiar AMM, Maeda SS, Cardoso AS, et al. Prevention and treatment of oral adverse effects of antiresorptive medications for osteoporosis – A position paper of the Brazilian Society of Endocrinology and Metabolism (SBEM), Brazilian Society of Stomatology and Oral Pathology (Sobep), and Brazilian Association for Bone Evaluation and Osteometabolism (Abrasso). Arch Endocrinol Metab. 2021;64(6):664-72.
12. Pazianas M, van der Geest S, Miller P. Bisphosphonates and bone quality. Bonekey Rep. 2014;3:529-10.
13. Coffman AA, Basta-Pljakic J, Guerra RM, Ebetino FH, Lundy MW, Majeska RJ, et al. A Bisphosphonate With a Low Hydroxyapatite Binding Affinity Prevents Bone Loss in Mice After Ovariectomy and Reverses Rapidly With Treatment Cessation. JBMR Plus. 2021;5(4):e10476.
14. Eriksen EF, Diez-Perez A, Boonen S. Update on long-term treatment with bisphosphonates for postmenopausal osteoporosis: a systematic review. Bone. 2014;58:126-35.
15. Black DM, Schwartz AV, Ensrud KE, Cauley JA, Levis S, Quandt SA, et al. Effects of continuing or stopping alendronate after 5 years of treatment: the Fracture Intervention Trial Long-term Extension (FLEX): a randomized trial. JAMA. 2006;296(24):2927-38.
16. Ensrud KE, Palermo L, Black DM, Cauley J, Jergas M, Orwoll ES, et al. Hip and calcaneal bone loss increase with advancing age: longitudinal results from the study of osteoporotic fractures. J Bone Miner Res. 1995;10(11):1778-87.
17. Black DM, Reid IR, Cauley JA, Cosman F, Leung PC, Lakatos P, et al. The effect of 6 versus 9 years of zoledronic acid treatment in osteoporosis: a randomized second extension to the HORIZON-Pivotal Fracture Trial (PFT). J Bone Miner Res. 2015;30(5):934-44.
18. Mellstrom DD, Sorensen OH, Goemaere S, Roux C, Johnson TD, Chines AA. Seven years of treatment with risedronate in women with postmenopausal osteoporosis. Calcif Tissue Int. 2004;75(6):462-8.
19. Adler RA, El-Hajj Fuleihan G, Bauer DC, Camacho PM, Clarke BL, Clines GA, et al. Managing Osteoporosis in Patients on Long-Term Bisphosphonate Treatment: Report of a Task Force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2016;31(10):1910.
20. Shane E, Burr D, Ebeling PR, Abrahamsen B, Adler RA, Brown TD, et al. Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2010;25(11):2267-94.
21. Odvina CV, Zerwekh JE, Rao DS, Maalouf N, Gottschalk FA, Pak CY. Severely suppressed bone turnover: a potential complication of alendronate therapy. J Clin Endocrinol Metab. 2005;90(3):1294-301.
22. Radominski SC, Bernardo W, Paula AP, Albergaria BH, Moreira C, Fernandes CE, et al. Brazilian guidelines for the diagnosis and treatment of postmenopausal osteoporosis. Rev Bras Reumatol Engl Ed. 2017;57 Suppl 2:452-66.
23. Starr J, Tay YKD, Shane E. Current Understanding of Epidemiology, Pathophysiology, and Management of Atypical Femur Fractures. Curr Osteoporos Rep. 2018;16(4):519-29.
24. Lo JC, Neugebauer RS, Ettinger B, Chandra M, Hui RL, Ott SM, et al. Risk of complete atypical femur fracture with Oral bisphosphonate exposure beyond three years. BMC Musculoskelet Disord. 2020;21(1):801-10.
25. Schilcher J, Koeppen V, Aspenberg P, Michaelsson K. Risk of atypical femoral fracture during and after bisphosphonate use. Acta Orthop. 2015;86(1):100-7.
26. Brown JP, Morin S, Leslie W, Papaioannou A, Cheung AM, Davison KS, et al. Bisphosphonates for treatment of osteoporosis: expected benefits, potential harms, and drug holidays. Can Fam Physician. 2014;60(4):324-33.
27. Wang Z, Bhattacharyya T. Trends in incidence of subtrochanteric fragility fractures and bisphosphonate use among the US elderly, 1996-2007. J Bone Miner Res. 2011;26(3):553-60.
28. Fassio A, Bertoldo F, Idolazzi L, Viapiana O, Rossini M, Gatti D. Drug-induced osteonecrosis of the jaw: the state of the art. Reumatismo. 2017;69(1):9-15.
29. Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J Bone Miner Res. 2015;30(1):3-23.
30. Eiken PA, Prieto-Alhambra D, Eastell R, Abrahamsen B. Surgically treated osteonecrosis and osteomyelitis of the jaw and oral cavity in patients highly adherent to alendronate treatment: a nationwide user-only cohort study including over 60,000 alendronate users. Osteoporos Int. 2017;28(10):2921-8.
31. Zavras AI, Shanmugham JR. Bisphosphonates, Osteoporosis, and Osteonecrosis of the Jaw: A Critical Review of a Large Nested Case-Control Study. J Evid Based Dent Pract. 2016;16(2):136-8.
32. Hayashida S, Yanamoto S, Fujita S, Hasegawa T, Komori T, Kojima Y, et al. Drug holiday clinical relevance verification for antiresorptive agents in medication-related osteonecrosis cases of the jaw. J Bone Miner Metab. 2020;38(1):126-34.
33. Jung J, Yoo HY, Kim GT, Lee JW, Lee YA, Kim DY, et al. Short-Term Teriparatide and Recombinant Human Bone Morphogenetic Protein-2 for Regenerative Approach to Medication-Related Osteonecrosis of the Jaw: A Preliminary Study. J Bone Miner Res. 2017;32(12):2445-52.
34. Kakehashi H, Ando T, Minamizato T, Nakatani Y, Kawasaki T, Ikeda H, et al. Administration of teriparatide improves the symptoms of advanced bisphosphonate-related osteonecrosis of the jaw: preliminary findings. Int J Oral Maxillofac Surg. 2015;44(12):1558-64.
35. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022;80(5):920-43.
36. van de Laarschot DM, McKenna MJ, Abrahamsen B, Langdahl B, Cohen-Solal M, Guanabens N, et al. Medical Management of Patients After Atypical Femur Fractures: a Systematic Review and Recommendations From the European Calcified Tissue Society. J Clin Endocrinol Metab. 2020;105(5).