UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA

NEW MARKERS, PREVALENCE AND BURDEN OF OSTEOPOROSIS AND FRAGILITY FRACTURES

ANA MARIA FERREIRA RODRIGUES

ORIENTADORES: PROF. DOUTORA HELENA CRISTINA DE MATOS CANHÃO

PROF. DOUTOR JOÃO EURICO CABRAL DA FONSECA

PROF. DOUTOR ARA NAZARIAN

TESE ESPECIALMENTE ELABORADA PARA A OBTENÇÃO DO GRAU DE DOUTOR EM MEDICINA

ESPECIALIDADE DE REUMATOLOGIA

2018

UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA

NEW MARKERS, PREVALENCE AND BURDEN OF OSTEOPOROSIS AND FRAGILITY FRACTURES

ANA MARIA FERREIRA RODRIGUES

ORIENTADORES: PROF. DOUTORA HELENA CRISTINA DE MATOS CANHÃO

PROF. DOUTOR JOÃO EURICO CABRAL DA FONSECA

PROF. DOUTOR ARA NAZARIAN

TESE ESPECIALMENTE ELABORADA PARA A OBTENÇÃO DO GRAU DE DOUTOR EM MEDICINA

ESPECIALIDADE DE REUMATOLOGIA

JÚRI - PRESIDENTE: DOUTOR JOSÉ AUGUSTO GAMITO MELO CRISTINO, PROFESSOR CATEDRÁTICO DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE LISBOA

VOGAIS: DOUTOR JOSÉ ANTÓNIO PEREIRA SILVA, PROFESSOR CATEDRÁTICO DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

DOUTOR JAIME DA CUNHA BRANCO, PROFESSOR CATEDRÁTICO DA FACULDADE DE CIÊNCIAS MÉDICAS DA UNIVERSIDADE NOVA DE LISBOA

DOUTOR HELENA CRISTINA DE MATOS CANHÃO, PROFESSORA CATEDRÁTICA DA FACULDADE DE CIÊNCIAS MÉDICAS DA UNIVERSIDADE NOVA DE LISBOA (ORIENTADORA)

DOUTOR JACINTO MANUEL DE MELO OLIVEIRA MONTEIRO, PROFESSOR CATEDRÁTICO DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE LISBOA

DOUTORA MARIA JOSÉ PARREIRA DOS SANTOS, PROFESSORA AUXILIAR CONVIDADA DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE LISBOA

2018

As opiniões expressas nesta publicação são da exclusiva responsabilidade do seu autor.

A impressão desta dissertação foi aprovada pelo Conselho Científico da Faculdade de Medicina da Universidade de Lisboa em reunião de 18 de setembro de 2018.

Ao abrigo do artigo nº 2 do Regulamento Geral para Ciclos de Estudos de 3º Ciclo na Faculdade de Medicina da Universidade de Lisboa, fazem parte desta tese as seguintes publicações e manuscritos submetidos para publicação:

I. **EpiReumaPt – the study of Rheumatic and Musculoskeletal diseases in Portugal: a detailed view of the methodology**.

Rodrigues AM, Gouveia N, da Costa LP, et al. 2015. Acta Reumatol Port. 40:110-124.

II. **Cohort profile: Epidemiology of Chronic Diseases Cohort (EpiDoC)**.

Dias SS*, Rodrigues AM*, Gregório MJ, et al. *both authors equally contributed. 2018. International Journal of Epidemiology. doi: 10.1093/ije/dyy185. [Epub ahead of print]

III. **Prevalence of rheumatic diseases and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt – a national health survey.**

Branco JC, Rodrigues AM, Gouveia N, et al. 2016. RMD Open. 2:e000166.

IV. **The burden of undertreatment fragility fractures among senior women.** Rodrigues AM, Eusébio M, Santos MJ, et al. 2018. Archives of Osteoporosis. 13:22.

V. **Smoking is a Predictor of worse trabecular mechanical performance in hip fragility fracture patients.**

Rodrigues AM, Caetano-Lopes J, Vale AC, et al. 2012. J Bone Miner Metab. 30(6): 692-699.

VI. **Low osteocalcin/collagen type I bone gene expression ratio is associated with hip fragility fractures.**

Rodrigues AM, Caetano-Lopes J, Vale AC, et al. 2012. Bone. 51:981-989.

VII. **Low Serum Levels of DKK2 are a potential serum marker of Incident Low Impact Fracture Risk in Older Women.**

Rodrigues AM, Eusébio M, Rodrigues AB et al. (*Submitted to JBMR Plus*).

VIII. **Multidisciplinary Portuguese recommendations on DXA request and indication to treat in the prevention of fragility fractures.**

Marques A, Rodrigues AM, Romeu JC, et al. 2016. Acta Reumatol Port. 41: 305-321.

IX. **Portuguese recommendations for the prevention, diagnosis and management of primary osteoporosis – 2018 update.**

Rodrigues AM, Canhão H, Marques A, et al. 2018. Acta Reumatol Port. 43:10-3

Figures Index

Tables Index

ALP - Alkaline phosphatase

BMD - Bone mineral density

BMI - Body mass index

BMPs - Bone morphogenetic proteins

BMUs -Basic multicellular units

BSP - Bone sialoprotein

COL1 - Type I collagen

CTSK - Cathepsin K

CTX - Carboxy-telopeptide of type I collagen

DKK - Dickkopf-related protein

DKK 1 - Dickkopf-related protein 1

DKK 2 - Dickkopf-related protein 2

DPD - Deoxypyridinoline

Dsh - Disheveled

DXA - Dual energy X-ray absorptiometry

EpiDoC - Epidemiology of Chronic Diseases

FGF-2 - Fibroblast growth factor

FZ - Receptor frizzled

GSK-3β - Glycogen synthase kinase

IGF-I - Insulin-like growth factor

LRPs - Low density lipoprotein receptor-related proteins

NTX – amino-telopeptide of type I collagen

NVNH - Non-vertebral non-hip

OCL - Osteocalcin

OCL/COL1a1 - Ratio of osteocalcin/ COL1a1 gene expression

OPG - Osteoprotegerin

OSX - Osterix

P1CP - Procollagen 1 carboxy-terminal peptide

P1NP - Procollagen 1 amino-terminal peptide

PTH - Parathyroid hormone

PYD - Pyridinoline

RANK - Receptor activator of nuclear factor-kB

RANKL - Receptor activator of nuclear factor-kB ligand

RANKL/OPG ratio - Ratio of receptor activator of nuclear factor-kB ligand and osteoprotegerin

RMDs - Rheumatic and Musculoskeletal Diseases

RT-PCR - Real-time polymerase chain reaction

RUNX2 - Runt-related transcription factor 2

SDs - Standard deviations

sFRP – Secreted frizzled-related protein

SOST - Sclerostin

TBS - Trabecular bone score

TNF - Tumour necrosis factor

TRAcP5b - Tartrate-resistant acid phosphatase 5b

WHO - World Health Organization

WIF-1 - Wnt inhibitory factor

Estudar a fisiopatologia e a epidemiologia da osteoporose foi um desafio e uma grande oportunidade que culmina nesta tese de Doutoramento. Com este projeto tive oportunidade de crescer enquanto investigadora clínica. As provas de Doutoramento a que agora me submeto são um passo importante neste meu percurso e muitos foram os que contribuíram para este projeto, sem os quais certamente este não teria avançado.

Aos meus orientadores, Prof. Doutora Helena Canhão e Prof. Doutor João Eurico Fonseca, agradeço aos dois em simultâneo porque tudo aquilo que sou profissionalmente devo-o a vós. Obrigada por me terem ensinado muito do que sei enquanto médica, docente e investigadora. Obrigada por me terem deixado percorrer o meu caminho mesmo quando não concordavam com as minhas escolhas. Obrigada por nunca terem desistido de mim! Admiro-vos profundamente e nutro por vós uma amizade que não tem fim. Espero estar sempre à altura dos vossos desafios e poder partilhar convosco todos os vossos sucessos.

Chefe, agradeço as inúmeras horas de discussão de ideias, a sua orientação e a sua exigência. A sua energia e capacidade de trabalho são difíceis de alcançar, mas eu não vou desistir! Agradeço também todas as oportunidades que me deu nos últimos anos, começámos um caminho novo e muito promissor!

Prof. Doutor João Eurico, quero expressar aqui a enorme gratidão pelo seu apoio, pela sua disponibilidade permanente e pela sua orientação ao longo destes anos, sabendo eu que tem inúmeras solicitações e escassez de tempo.

Agradeço a todos os meus colegas do Serviço de Reumatologia e Doenças Ósseas Metabólicas do Hospital de Santa Maria, foi lá que comecei este projeto. Muitos de vós ajudaram-me na recolha da informação clínica dos doentes, na discussão de ideias, na recolha de amostras de sangue, obrigada a TODOS pela generosidade e carinho. Obrigada Joaquim e Elsa, não posso deixar de vos destacar porque viveram comigo este projeto.

Agradeço também a todos os colegas do Serviço de Ortopedia do Hospital de Santa Maria pela colaboração e apoio, destacando o seu Diretor de Serviço, o Prof. Doutor Jacinto Monteiro que sempre apoiou e acarinhou este projeto.

Agradeço também aos elementos da Unidade de Investigação Clínica do Instituto de Medicina Molecular, que não só fizeram o trabalho de laboratório e estudo mecânico como foram parceiros em várias aventuras da descoberta da fisiopatologia do osso. Quero destacar o apoio e amizade de alguns porque sem eles este projeto não teria chegado ao fim. Agradeço à Joana Caetano Lopes por TUDO! Obrigada Ana Lopes, Inês Perpetuo, Inês Lopes, Bruno Vidal, Catarina Vale e Saba. Aos clínicos desta unidade que foram fundamentais neste meu percurso, Elsa Sousa, Joaquim Pereira, Maria José Santos e Ana Filipa Mourão. Obrigada pela troca de ideias, orientação e pela amizade.

Agradeço ao Prof. Doutor Jácome Armas, por todo o apoio na investigação e na atividade clínica dos últimos anos. Obrigada por ter sido o primeiro a acreditar em mim! Nunca me vou esquecer!

Prof. Doutor Jaime Branco, agradeço o facto de me ter acolhido na equipa do EpiReumaPt. Agradeço também a confiança que depositou em mim nas várias fases deste projeto. Admiro profundamente a sua capacidade de liderança, respeito e cuidado com os que o rodeiam.

À Equipa do EpiReumaPt agradeço toda a disponibilidade, carinho e amizade. Destaco o Prof. Doutor Jaime Branco, a Prof. Doutora Helena Canhão, a Nélia Gouveia, a Sofia Ramiro, o Pedro Machado e a Ana Filipa Mourão. Só uma equipa muito unida poderia ter ultrapassado todos os desafios que nos foram colocados. Estar no terreno foi difícil e exigente e eu não teria conseguido sem a Nélia Gouveia, a Susana Sousa, a Tânia Rego, a Sol, a Catarina, a Maria, a Inês, o Rafael, os irmãos Matias e o Sr. Simões. Agradeço ainda a todos os meus colegas reumatologistas com quem partilhei muitos destes dias no terreno. Muito obrigada, companheiros de viagem!

Mónica Eusébio, agradeço-te a amizade, disponibilidade, carinho e as infindáveis horas de trabalho conjunto na limpeza das bases de dados e na estatística. Crescemos juntas nesta área e aprendemos muito com este projeto!

Agradeço ao Prof. Doutor Pereira da Silva a oportunidade de ter colaborado consigo na elaboração das recomendações nacionais sobre osteoporose. Espero que este tenha sido o primeiro de muitos projetos em conjunto!

À equipa da Unidade EpiDoC, Prof. Doutora Helena Canhão, Prof. Doutor Jaime Branco, Rute de Sousa, Maria João Gregório e Sara Dias. O nosso lema é *"um por todos e todos por um"*! Penso que foi este lema que nos fez fazer tanto em tão pouco tempo! Agradeço também a todos os assistentes de investigação que colaboraram connosco no seguimento da nossa coorte! Obrigada Mónica, Ricardo, Pedro, Filipes, Tomás, Marta, Carina e Sofia, pela dedicação, profissionalismo e responsabilidade.

Agradeço profundamente a todos os doentes e participantes destes projetos, foi a vossa generosidade que permitiu compreendermos melhor estas doenças. Esperamos que este novo conhecimento contribua para melhorar a saúde de muitos.

Aos meus amigos, Susana Capela, Elsa Sousa, Maria João Gregório, Marina, Marisa, Patrícia e Thelma por todo o apoio, carinho e compreensão. Agradeço a vossa infindável amizade, apoio e paciência.

Por fim, agradeço à minha família, Paulo, Zé Pedro, Joana, mãe e pai, o apoio incondicional, o sacrifício, o respeito e a compreensão. Obrigada por terem partilhado comigo este meu sonho, mesmo sem o compreenderem.

É tão bom chegar ao fim destas linhas e perceber o privilégio que é ter tantas pessoas a quem AGRADECER! OBRIGADA A TODOS!

RESUMO/ABSTRACT

As fraturas de fragilidade são um problema de saúde publica com especial incidência na população idosa, sendo uma causa importante de incapacidade funcional permanente e morte precoce. Decorrem devido a um traumatismo de baixa energia e estão associadas a uma doença óssea metabólica, a osteoporose. Até há relativamente pouco tempo a identificação de pessoas em risco de fratura era feita através da medição da densidade óssea e de acordo com limiares de decisão de tratamento definidos pela Organização Mundial da Saúde. No entanto, esta medida demonstrou não identificar cerca de 40% dos indivíduos que sofrem uma fratura de fragilidade. Outros fatores de risco têm que ser considerados que não apenas a avaliação da quantidade de tecido mineralizado, nomeadamente, fatores ósseos (dinâmica celular, estrutura tecidular nano e microscópica, etc) e não ósseos (risco de quedas, impacto da queda, massa muscular, etc). Os fatores de risco clínico (FRC) (idade, sexo, índice massa corporal, doenças crónicas, álcool, tabaco) englobam vários fatores ósseos e não ósseos e são preditores independentes de fraturas. Foram criados algoritmos que utilizam FRC e que demonstraram ser tão bons ou melhores preditores do risco de fratura do que a avaliação da densidade mineral óssea, contudo ainda há um longo caminho a percorrer até encontrar a melhor ferramenta para identificar pessoas em risco.

Nesta tese de doutoramento tivemos por objetivos determinar a prevalência e impacto da osteoporose e das fraturas de fragilidade em Portugal e melhorar a estratificação de pessoas em risco de fratura através da identificação de novos biomarcadores. Para atingir este último objetivo, olhámos para o ambiente celular (em particular para os osteoblastos, células formadoras de osso) e para o tecido ósseo (nomeadamente para as propriedades mecânicas do osso) e procurámos identificar as suas disfunções em doentes com fraturas de fragilidade da anca. A nossa hipótese era a de que fragilidade mecânica do osso no idoso está associada a uma desregulação da mineralização óssea devido a uma perturbação na diferenciação terminal dos osteoblastos que por sua vez se associa a uma anormal expressão dos reguladores da via de sinalização WNT [*dickkopf-related protein* (DKK)1 e DKK2, *sclerostin* (SOST) e *secreted frizzled related protein-1* (sFRP-1)]. Colocámos ainda a hipótese de que os níveis séricos dos reguladores

da via de sinalização WNT poderiam ser marcadores de fraturas de fragilidade em idosos. Por fim, tínhamos por objetivo, melhorar a prática clínica na avaliação do risco de fratura e na identificação de indivíduos que devem ser tratados para a osteoporose através da realização de consensos clínicos nacionais.

Este doutoramento cruzou duas áreas distintas da investigação clínica, a epidemiologia e a investigação clínica aplicada, por isso, utilizámos duas amostras distintas de participantes/ doentes. Para analisar as associações entre os fatores de risco clínico, marcadores de diferenciação dos osteoblastos com as propriedades biomecânicas do osso e com fraturas, utilizámos uma amostra de conveniência, composta por osso e informação clínica dos doentes submetidos a artroplastia total da anca devido a fratura de fragilidade e a coxartrose durante 2008 a 2012 no Serviço de Ortopedia do Hospital de Santa Maria, Centro Hospitalar Lisboa Norte. A outra amostra é constituída por adultos residentes em Portugal avaliados num estudo epidemiológico sobre doenças reumáticas, o EpiReumaPt (2011-2013) que deu origem à coorte "Epidemiology of Chronic Diseases" (EpiDoC cohort) já com duas avaliações prospetivas (2011-2016). Na amostra do EpiReumaPt determinámos a prevalência e impacto da osteoporose em Portugal. Para além disso avaliámos especificamente as mulheres acima dos 65 anos de idade e determinámos a prevalência, fatores de risco e impacto das fraturas de fragilidade neste grupo vulnerável. Os dados da coorte EpiDoC foram utilizados para analisar a associação entre os níveis séricos dos reguladores da via de sinalização WNT (via reguladora da diferenciação terminal dos osteoblastos) com fraturas de fragilidade. Para a realização dos consensos clínicos, necessários na mudança de conceito de identificação de indivíduos em risco de fratura, procedemos a uma revisão da literatura e colaborámos na realização de recomendações nacionais de osteoporose.

De seguida, apresentamos os nossos principais resultados. O primeiro estudo desta tese de doutoramento, utilizou a amostra do EpiReumaPt e demonstrou que 10,2% dos adultos portugueses sofrem de osteoporose, sendo esta prevalência superior nas mulheres (17%) do que nos homens (2,6%) e aumenta significativamente com a idade. Cerca de 40% dos adultos portugueses com idade igual ou superior a 75 anos têm osteoporose. O diagnóstico de osteoporose mostrou estar associado a incapacidade

funcional (avaliada pelo *Health Assessment Questionnaire*) mas não se associou a sintomas de ansiedade e depressão. O segundo estudo, analisou as mulheres acima dos 65 anos do EpiReumaPt e verificou que a prevalência de fraturas de fragilidade neste grupo etário é de 20,7%. Contudo, apenas 13,9% das mulheres que referiram história de fratura de fragilidade reportaram ter algum dia efetuado terapêutica para osteoporose. Os locais de fratura mais frequentes foram: perna, punho, úmero, costelas e cotovelo. Os fatores de risco clínico que se mostraram estar associados à existência de fraturas de fragilidade foram o aumento de idade, obesidade e diminuição da densidade óssea do punho.

O terceiro trabalho desta dissertação de doutoramento, é de investigação clínica aplicada, utilizando a amostra de doentes submetidos a artroplastia total da anca (n=92). Neste trabalho testámos as diferentes propriedades mecânicas do osso trabecular da anca ex-vivo (rigidez, resistência e ductilidade) e a única propriedade mecânica que demonstrou ser significativamente diferente entre as pessoas com fratura de fragilidade (n=40) das com artrose (n=52) foi a baixa rigidez óssea (propriedade mecânica associada à mineralização). Os fatores de risco associados à baixa rigidez óssea nos doentes com fraturas foram o tabagismo e o sexo feminino. No quarto estudo, avaliámos 64 doentes submetidos a artroplastia total da anca, 25 por fratura de fragilidade e 39 por osteoartrose. Neste estudo, verificámos que no osso femoral, a expressão génica de osteocalcina (OC) e o rácio de OC/COL1A (marcador da expressão terminal de OB) estão diminuídos no grupo que sofreu uma fratura, quando comparado com o grupo de coxartrose. A reforçar este achado, a análise imunohistoquímica do osso de um subgrupo de doentes com fratura, demonstrou que os osteoblastos tinham menos marcação para OC do que no subgrupo de osteoartrose. Verificámos ainda que a baixa expressão génica de OC e o rácio OC/COL1A se associam a menor rigidez, resistência e ductilidade do osso trabecular dos doentes com fratura.

Após estes achados que reforçam a importância da perturbação da diferenciação terminal dos osteoblastos nos doentes com fraturas e sabendo que a via de regulação principal desta fase é a via WNT, considerámos relevante testar os níveis séricos dos reguladores da via WNT (DKK1, DKK2, SOST, WIF-1 e sFRP-1) enquanto marcadores de

risco de fratura em mulheres acima dos 65 anos, usando a coorte EpiDoC. Estas mulheres foram seguidas durante 2,3±1,0 anos e durante este período ocorreram 62 fraturas de baixo-impacto. Os baixos níveis séricos de DKK2 associaram-se a um aumento de risco de fraturas de baixo impacto independentemente da densidade óssea e dos fatores de risco clínico [HR (95% CI) 0,53 (0,32; 0,88)]. Por cada redução de 1 desvio padrão dos níveis de DKK2, o risco de fratura aumenta 1,5 vezes. Os outros reguladores da via WNT não demonstraram estar associados ao risco de fratura, no entanto o tempo de seguimento desta população é pequeno e o número de fraturas também. No futuro será necessário testar estes resultados noutras populações.

No final deste doutoramento, considerámos relevante incentivar a mudança de paradigma na identificação de indivíduos em risco para fratura de fragilidade na prática clínica em Portugal, até agora muito centrada na avaliação da densidade óssea, para a avaliação do risco de fratura individual calculada por um algoritmo (o FRAX) validado mundialmente e nacionalmente. Este algoritmo utiliza FRC com ou sem densidade óssea para a identificação de pessoas em risco e o limiar de tratamento foi estabelecido com base em estudos de custo-efetividade. Sendo esta a melhor ferramenta disponível em Portugal para a identificação de pessoas em risco, considerámos relevante implementar esta ferramenta na prática clínica através da inclusão da mesma nas recomendações clínicas da área da osteoporose.

Em conclusão, esta tese de doutoramento contribuiu para aumentar o conhecimento sobre a prevalência, fatores de risco, taxas de tratamento e impacto da osteoporose e das fraturas de fragilidade em Portugal, reforçando a sua importância em termos de saúde pública em particular na população idosa portuguesa.

Do ponto de vista mecanístico, contribuímos para o reconhecimento de que a fragilidade óssea do idoso se associa a uma perturbação da formação óssea devido a uma desregulação da diferenciação terminal do osteoblasto. Identificámos também que os níveis séricos de um dos reguladores da diferenciação terminal do osteoblasto (DKK2) se associam ao aumento do risco de fraturas de fragilidade em mulheres acima dos 65 anos, podendo ser mais uma ferramenta para melhor identificar pessoas em risco de fratura. Por fim, colaborámos na elaboração de consensos nacionais baseados na

melhor evidência científica atual de modo a modificar a prática clínica e a reduzir a incidência de novas fraturas de fragilidade em Portugal.

Palavras Chave: Osteoporose, fraturas de fragilidade, Epidemiologia, risco de fratura, Via de sinalização WNT

In this PhD thesis, we aimed to determine the prevalence and burden of osteoporosis and fragility fractures in Portugal to provide evidence in support of new health strategies to improve clinical care and reduce or prevent disability and mortality among elderly. We also aimed to better identify senior women at high risk for a fragility fracture through the use of novel noninvasive biomarkers. To achieve this goal, we evaluated cellular (osteoblast) and tissue (bone mechanical properties) mechanism dysfunction to identify potential serum markers of bone fragility. We hypothesized that bone fragility in the elderly is associated with dysregulation of mineralization because of osteoblast terminal differentiation and disturbances in Wnt regulators [dickkopf-related protein (DKK)1 and DKK2, sclerostin (SOST) and secreted frizzled related protein-1 (sFRP-1)]. Moreover, we hypothesized that serum levels of Wnt regulators are associated with bone fragility and fractures and can constitute new markers for osteoporosis treatment decision. Finally, to create awareness and reduce new fragility fractures in Portugal, we aimed to develop national clinical consensus recommendations for osteoporosis diagnosis and treatment**.**

The studies presented in this thesis crossed two main areas of clinical research, patient-oriented mechanistic research, and epidemiological research. We used two samples of participants/patients. One sample was composed of patients undergoing hip replacement surgery (for osteoarthritis or a fragility fracture) in the Orthopaedic Department of *Hospital de Santa Maria, Centro Hospitalar Lisboa Norte* from 2008 to 2012. It was used to analyse clinical determinants of bone fragility and fractures in the elderly, particularly the associations between osteoblast dysfunction, bone mechanical properties, and fragility fractures. The other sample was a population based on a nationwide sample evaluated in the EpiReumaPt (2011-2013) study. Using the EpiReumaPt sample, we determined the prevalence and individual burden of osteoporosis in Portugal. Moreover, we analysed the prevalence, burden, and risk factors of fragility fractures in a particularly vulnerable stratum, senior women. The EpiReumaPt population was then followed in two more waves of evaluation (2011-2016)

under the scope of the Epidemiology of Chronic Diseases (EpiDoC) cohort. Using data from the EpiDoC cohort, we analysed the association between serum markers of Wnt inhibitors (osteoblast regulators) and fragility fractures. Finally, a review of the literature was performed to develop national clinical recommendations regarding fracture risk assessment and osteoporosis treatment.

In the first study of this thesis, using the EpiReumaPt sample, we found that 10.2% of Portuguese adults have osteoporosis. The prevalence is higher in women (17.0%) than in men (2.6%) and increases with age. Almost half (40.0%) of Portuguese adults 75 years and older have osteoporosis, and an osteoporosis diagnosis was associated with substantial physical function impairment but not with anxiety or depression symptoms. The second study, also using EpiReumaPt data, showed that self-reported fragility fractures were highly prevalent among senior women (20.7%). This high prevalence was in stark contrast with the low rate of osteoporosis treatment (13.9%). Non-hip and non-vertebral fractures (i.e., lower leg, wrist, humerus, rib, clavicle, and elbow fractures) accounted for the majority of fragility fractures, and clinical risk factors independently associated with prevalent fragility fractures were increased age, obesity, and lower distal bone mineral density (BMD).

The challenge to better identify seniors (people aged ≥65 years old) at high risk for a fragility fracture led us to search for novel noninvasive biomarkers of bone fragility and fractures. To achieve this goal, we conducted patient-oriented mechanistic research, evaluating associations between cellular mechanism dysfunction and bone fragility among patients undergoing hip replacement surgery. In the third research work of this thesis, we demonstrated that when adjusted for differences in age, sex, and body mass index, the only macrostructural bone characteristic that remained significantly different between patients with hip fragility fractures and those with osteoarthritis was trabecular stiffness (which is linked to mineralization disturbances). Stiffness was lower in patients with fragility fractures. We also found that smoking habits and female sex were independently associated with lower stiffness in patients with a fragility fracture.

In the fourth study of this thesis, we evaluated markers of osteoblast differentiation, as these are the cells responsible for the production of mineralized tissue. We found that osteocalcin (OCL) relative bone expression and the OCL/type 1 collagen, alpha 1 chain (COL1A1) expression ratio in bone (a marker of osteoblast terminal differentiation) were significantly lower in patients with hip fractures than in those with osteoarthritis. Consistent with these results, in a subset of patients, fewer osteoblasts stained for OCL in patients with a fragility fracture than in those with osteoarthritis**.** We also demonstrated that in patients with hip fractures, a low bone OCL/COL1A1 expression ratio was associated with worse trabecular mechanical behaviour. This work reinforced the importance of osteoblast dysfunction in bone intrinsic properties and fractures among the elderly.

In our fifth study, we examined whether the Wnt inhibitors DKK1, DKK2, SOST, WIF-1, and sFRP-1 (regulators of osteoblast differentiation and bone formation) were associated with BMD or fragility fractures in a population-based cohort. Using EpiDoC cohort data, we found that low serum levels of DKK2 predicted low-impact fractures, independent of BMD, and clinical risk factors for fracture. For every 1 standard deviation decrease in DKK2, fracture risk increased by approximately 1.5-fold. Serum levels of DKK2 were not associated with vertebral or hip BMD. Our results suggest a possible interaction among BMD, FRAX score without BMD, and serum DKK2 levels in the assessment of fracture risk, which requires further investigation in a larger study with longer follow-up.

The final work of this PhD thesis involved performing a literature review and establishing national consensus recommendations regarding fracture risk assessment and osteoporosis clinical management and treatment to change clinical practice and reduce the incidence of fragility fractures in Portugal.

In conclusion, this thesis provided rigorous epidemiological data regarding the prevalence of osteoporosis and fragility fractures in Portugal. It contributed to refining fracture risk assessment through the identification of new serum markers (among regulators of osteoblast-mediated bone formation) of bone fragility and fractures.

Mechanistic research regarding bone biomechanics and osteoblast dysfunction showed that fragility fractures are associated with reduced bone stiffness, reflecting mineralization disturbances. Furthermore, reduced osteoblast terminal differentiation was associated with poor bone mechanics and fractures. Finally, national consensus recommendations were created to improve fracture risk assessment of individuals, as well clinical management and treatment of osteoporosis, with the goal of reducing fragility fractures in Portugal.

Key words: Osteoporosis, Fragility fractures, Epidemiology, Fracture risk, Wnt signalling

THESIS OUTLINE

This thesis describes the foundations and results of 7 years of work dedicated to improving fragility fracture prevention, which focused on aspects ranging from the assessment of individual patients to driving changes in national health policies. First, we aimed to provide rigorous epidemiological data regarding the prevalence of osteoporosis and fragility fractures. Then, we conducted rigorous research in a nationwide cohort to refine fracture risk assessment through the identification of new serum markers of bone fragility and fractures. The rationale for these candidate markers (which are regulators of osteoblast-mediated bone formation) originated from studying bone biomechanics and osteoblast dysfunction in senior patients with hip fractures. Finally, we created national consensus recommendations regarding fracture risk assessment, as well as osteoporosis clinical management and treatment, with the goal of changing clinical practice and reducing the incidence of fragility fractures in Portugal.

This thesis is divided into six chapters. In **Chapter I (Introduction),** we present the rationale for conducting this research in light of current knowledge. A general introduction to the problems of osteoporosis and fragility fractures is presented, including national and international epidemiological data and information regarding individual and societal burden and economic impact, focusing on national epidemiological unmet needs. We also discuss current knowledge regarding the cellular and biomechanical disturbances of osteoporosis and fragility fractures, emphasizing the identification of potential markers of bone fragility and fractures. Finally, we address the challenges of assessing an individual´s fracture risk and discuss strategies to improve fracture risk prediction.

In **Chapter II (Aims),** we describe the aims of this thesis. Both the general and specific aims are included.

In **Chapter III (Methodology),** we briefly describe the methodology used during the research. This thesis involves two groups of participants. One group is composed of patients undergoing hip replacement surgery (because of osteoarthritis or fragility

fractures) between 2008 and 2012 in the Orthopaedic Department of Centro Hospitalar Lisboa Norte. The other group is a population-based nationwide sample evaluated by survey in the EpiReumaPt study (2011-2013). The EpiReumaPt population was subsequently followed in two additional waves of evaluation (2011-2016) under the scope of the Epidemiology of Chronic Diseases (EpiDoC) cohort. We include two papers in the methodology section. The first is a comprehensive description of the EpiReumaPt methodology, which includes data collection, the attrition of participants, and data management. The second paper describes the EpiDoC cohort.

In **Chapter IV (Results),** we present the results of this thesis in four sections. **Section I** addresses the prevalence and burden of osteoporosis and fragility fractures in Portugal and treatment rates for osteoporosis in high-risk patients. It comprises two papers. The first (Part 1) estimates the prevalence and burden of osteoporosis among the adult Portuguese population. The second (Part 2) estimates the prevalence, burden, and undertreatment of osteoporosis among Portuguese senior women (aged ≥65 years old). **Section II** includes a set of studies identifying clinical risk factors and cellular disturbances associated with poor trabecular intrinsic mechanical behaviour and hip fractures. This section includes two papers. The first (Part 1) analyses clinical risk factors associated with bone fragility in patients with hip fractures. The second paper (Part 2) analyses the association between osteoblast terminal differentiation and poor bone quality, as well as fragility fractures. **Section III** addresses the hypothesis that serum levels of osteoblast terminal differentiation regulators could be surrogate markers of bone fragility and fractures. It includes one paper (Part 1) that evaluates serum markers of bone remodelling as risk factors of fragility fractures in a nationwide cohort of senior women. **Section IV** describes the development of national clinical consensus recommendations regarding individual fracture risk assessment as a strategy to reduce the occurrence of new fragility fractures**.** Part 1 involves multidisciplinary Portuguese recommendations regarding indications for dual energy X-ray absorptiometry (DXA) and indications for initiating treatment to prevent fragility fractures. Part 2 involves an update of the Portuguese recommendations for the prevention, diagnosis, and management of primary osteoporosis.

Chapter V (General Discussion and Conclusions) comprises an overall discussion of the main results of this thesis and possible implications for clinical practice and national health policies. The main conclusions and future perspectives are also included in this chapter.

In **Chapter VI (References),** we list the references cited in this work.

CHAPTER I

INTRODUCTION

Definition of Osteoporosis and Fragility Fractures

In 1993, the World Health Organization (WHO) defined osteoporosis as a metabolic skeletal disease characterized by low bone mass and microarchitecture deterioration, with consequent increased bone fragility and risk of fracture (1). Osteoporosis is one of the most common rheumatic and musculoskeletal diseases (RMDs) in the elderly (2). It is a multifactorial disease, and several conditions can cause loss of bone mass and compromised bone strength. In women after menopause, osteoporosis can occur because of a sudden high rate of bone loss resulting from a lack of oestrogen (3). Osteoporosis can also be secondary to a disease or treatment, such as hyperparathyroidism, hypogonadism, rheumatoid arthritis, malabsorption syndrome, or glucocorticoid therapy, that compromises the attainment of peak bone mass during skeletal growth or significantly accelerates the loss of bone mass (4).

Osteoporosis is clinically silent until a fracture occurs. Worldwide, approximately 9 million fragility fractures are caused by osteoporosis each year, of which more than half occur in the American continent and Europe (5, 6). Fragility fractures are defined as any fracture resulting from minimal or no trauma, such as those resulting from a fall from standing height or less. Fragility fractures are more frequent, and their occurrence increases exponentially in senior individuals (people aged ≥65 years old). In fact, before the age of 50 years, few fractures are reported (7-9). Although the most commonly studied fragility fractures are vertebral and hip fractures, several epidemiological studies have shown that the most common fragility fractures are non-hip and non-vertebral (e.g., wrist, humerus, pelvis, rib, tibia, clavicle) (7-9). Fragility fractures are associated with low bone mineral density (BMD) and a higher risk of recurrent fragility fractures (3, 10-14). This is true not only for vertebral and hip fractures but also for non-vertebral non-hip (NVNH) fractures. Recent studies have identified an association

between NVNH fractures and a low BMD, higher risk of subsequent fractures, and negative health outcomes (7, 8, 13, 14).

Epidemiology of Osteoporosis, Clinical Risk Factors, and Burden of Fragility Fractures

The increase in worldwide life expectancy has increased the prevalence of osteoporosis and the incidence of fragility fractures (15, 16). In Europe, the estimated prevalence of osteoporosis in 2010 was 5.5% of the general population. However, because osteoporosis prevalence increases with age and is more common in females, the prevalence of osteoporosis among people 50 years and older was 6.6% in men and 22.1% in women (6). The clinical significance of osteoporosis is a fracture resulting from bone fragility. In Europe, an osteoporotic fracture occurs every 30 seconds, and more than 3.5 million people suffer a fragility fracture each year (2, 17, 18). The individual lifetime risk of a hip, vertebral, or wrist fracture is 30% to 40%, which is similar to the risk of a cardiovascular event (19).

Several epidemiological studies have identified clinical risk factors for fragility fractures, such as age (> 65 years), female sex, low body mass index (BMI), prior fragility fracture, parental history of hip fracture, long-term use of oral glucocorticoids (> 5 mg prednisolone or equivalent for longer than 3 months), current smoking, high intake of alcohol (> 3 units/day), rheumatoid arthritis and other secondary causes of osteoporosis (e.g., diabetes mellitus, hypogonadism, anorexia nervosa, inflammatory bowel disease, calcium/vitamin D deficiency, hyperparathyroidism), prolonged immobilization and paralysis, medications (e.g., anticonvulsants, antiretroviral therapy) (20-23), and frequent falls (21, 24). These clinical risk factors are integrated in several fracture risk prediction tools that are now commonly used worldwide (25).

Fragility fractures are an important cause of morbidity, mortality, and healthcare costs (26-28). In Europe, 37 billion euros per year are spent in healthcare costs related to fragility fractures. After sustaining a fragility fracture, individuals experience pain and impaired physical function, and some require hospitalization. For the majority, recovery is slow, and rehabilitation is often incomplete, leading to permanent disability. In fact,

1% of disability-adjusted life years attributable to non-communicable diseases is due to fragility fractures (2, 17, 29).

Differences exist between countries regarding the prevalence of osteoporosis and the incidence of fragility fractures. Both osteoporosis and fragility fractures are more frequent in northern Europe than in southern Europe (30, 31). In Portugal, epidemiological data of fragility fractures are lacking, and only incidence rates of hip fractures are well characterized. It has been estimated that 10,000 hip fractures occur each year, with regional differences in incidence that must be taken into consideration by national healthcare system planners (32-34). Moreover, hip fractures in Portugal are responsible for 12% mortality excess and cost 216 million euros per year (34).

To prevent fractures and increase the quality of care of people sustaining a low-impact fracture, more precise data are required regarding the prevalence and burden of osteoporosis and fragility fractures in Portugal. In particular, it is important to increase the body of knowledge regarding NVNH fractures, which account for the majority of low-impact fractures (7). This thesis used the EpiReumaPt study, as well as prospective follow-up of this population (the EpiDoC study), to fill this knowledge gap.

Bone Biomechanics Disturbances in Osteoporosis and Fracture Risk

Bone is a highly dynamic, mineralized connective tissue. Its main functions are support and protection of soft tissues, attachment of tendons and ligaments for locomotion, storage of calcium and phosphate, and harbouring bone marrow. This living tissue is light, to allow movement; relatively flexible, to allow deformation and absorb energy during impact loads; and relatively stiff, to resist loads and ultimately prevent fractures (35). Overall bone strength depends on the homeostasis of bone mechanical properties, which are determined by its material composition and hierarchical structure (Figure 1). Bone is composed of an organic matrix, composed chiefly of type I collagen (COL1), as well as an inorganic phase, composed primarily of calcium hydroxyapatite crystals (36). The organic matrix provides flexibility, whereas the mineral content is responsible for

stiffness. Variations in tissue mineral density affect function and low BMD is associated with reduced bone strength (37). However, bone fragility can also be the result of failed material or structural adaptations, not only decreased bone mass.

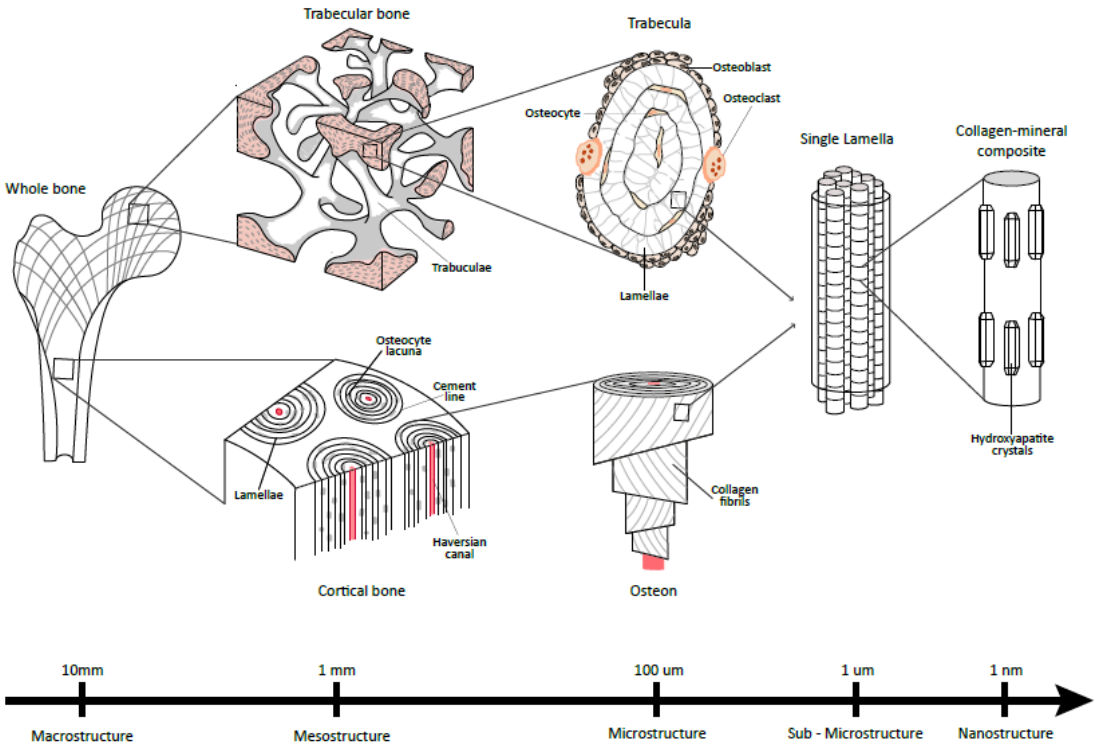

Figure 1. Hierarchical structure of bone.

To resist a fracture, bone composition in all dimensions is important. This includes the amount of bone (bone mass quantity), the spatial distribution of bone mass (e.g., trabecular vs cortical bone), and the intrinsic properties of bone material. At the nanoscale level, the triple helix of COL1 confers strength in tension, and collagen crosslinks keep the helices together. Too few crosslinks can lead to helix separation, whereas too many crosslinks diminish the ability to absorb energy (35, 38). At the microscopic level, less trabecular interconnectivity or more cortical porosity is related to bone fragility. Bone strength also depends on bone size and shape: long bones have higher resistance to a compressive load than vertebrae.

The role of the intrinsic properties of bone material in osteoporosis is emphasized by observations that in patients with osteoporosis treated with anti-osteoporotic agents,

fracture risk remains unchanged when there are no modifications of bone matrix volume or microarchitecture (39-43). Similarly, a 10% increase in areal BMD in women receiving anti-resorptive therapy was insufficient to explain the 40% reduction in fracture risk (44). Thus, the effect of both anti-resorptive and anabolic therapy in bone material properties (namely bone matrix mineralization) must be considered (40, 42, 43, 45).

Some recent studies showed that certain clinical risk factors, such as age, sex, race, and BMI, influence bone mechanical properties. The bones of elderly individuals have poorer mechanical properties than those of younger individuals (46). Men and women differ in bone size, and men consequently have a higher bone mineral content (47). There are also racial differences in bone strength that are only partially explained by the higher bone mineral content (48). However, little is known regarding other clinical risk factors for osteoporosis, such as alcohol consumption and smoking. Moreover, the effect size of each clinical risk factor on bone intrinsic mechanical properties is unknown. This thesis analysed the association between disturbances of bone intrinsic mechanical properties and clinical risk factors among the elderly.

Cellular Environment Disturbances in Osteoporosis and Fracture Risk

Bone is in constant adaptation through cellular mechanisms of bone modelling (construction) and remodelling (reconstruction). Bone modelling involves the construction of new bone without previous bone resorption; it produces changes in bone size and shape. By contrast, bone remodelling is the process of bone resorption and formation that occurs daily in the skeleton; it maintains bone strength throughout life (35). Bone is remodelled by the action of basic multicellular units (BMUs), which are composed of osteoblasts, the cells that form mineralized matrix, and osteoclasts, the cells that resorb mineralized matrix. As shown in Figure 2, osteoblast and osteoclast functions are coupled. Osteocytes (fully differentiated osteoblasts embedded in the mineralized matrix) are pivotal cells in both modelling and remodelling because they regulate differentiation and proliferation of both osteoclasts and osteoblasts (49). Bone

mass is maintained by a balance between the volume of bone resorbed and the volume of bone formed in each BMU, whereby bone resorption and new bone formation are temporally and spatially synchronized in a very finely regulated manner.

Bone modelling occurs mainly during growth and is necessary to achieve peak bone mass, which occurs at 18 to 25 years of age. Peak bone mass is mostly (60% to 80%) determined by genetic factors, but it is also influenced by sexual hormones and environmental factors, such as physical activity and nutrition (e.g., calcium intake) (50). Physiologically, bone remodelling is necessary for fracture healing and skeletal adaptation to mechanical forces, by removing damaged bone and forming new bone. It is also required for calcium homeostasis (Figure 2). During midlife, women have high rates of bone remodelling because of oestrogen deficiency, which leads to accelerated loss of bone mass and bone fragility. Later in life, both sexes have higher rates of bone remodelling and a decline in periosteal bone formation, compared with younger individuals, which results in structural deterioration (51).

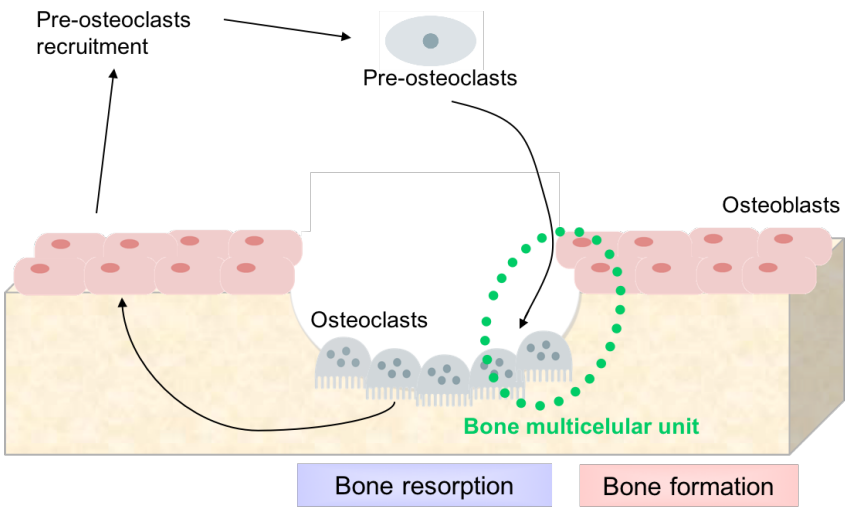

Figure 2. Bone remodelling.

Bone-resorbing multinucleated osteoclasts are derived from hematopoietic stem cells. Osteoclast progenitors differentiate into pre-osteoclasts in response to macrophage colony-stimulating factor. In addition, full differentiation and activation of osteoclasts depends on the receptor activator of nuclear factor-kB (RANK)–RANK ligand (RANKL)–

osteoprotegerin (OPG) axis in osteoblasts (Figure 3) (52). Oestrogen deficiency after menopause increases bone loss by activating RANKL-induced osteoclast differentiation (53). Similarly, the decline of both oestrogen and androgen in older men is associated with age-related osteoporosis by increasing osteoclast formation and function through this axis (54). In the elderly, osteoclastogenesis is also enhanced by low serum levels of vitamin D and calcium, which lead to increased production of parathyroid hormone (PTH) (55). In inflammatory diseases, such as rheumatoid arthritis, inflammatory cytokines also activate the RANK-RANKL-OPG axis, thereby enhancing osteoclastogenesis and bone resorption (56) (Figure 3).

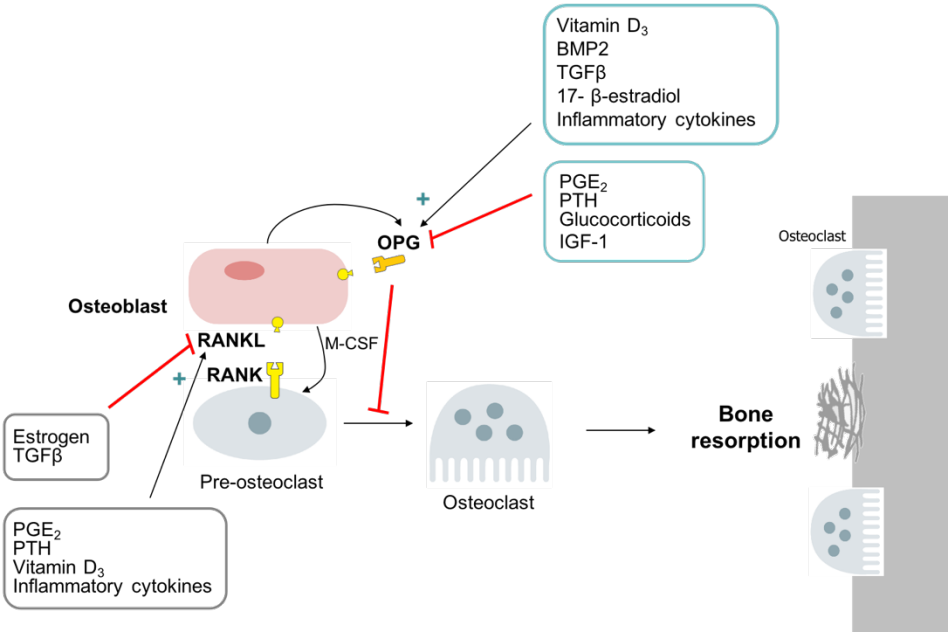

Figure 3. Factors influencing osteoclast differentiation and activation.

(RANK, receptor activator of nuclear factor kappa; RANKL, RANK ligand; OPG, osteoprotegerin; M-CSF, macrophage colony-stimulating factor; BMP2, bone morphogenetic protein 2; TGFβ, transforming growth factor beta; PGE_2, prostaglandin E2; PTH, parathyroid hormone; IGF1, insulin-like growth factor 1)

Osteoblasts are mononuclear cells specialized to secrete the collagenous bone matrix where hydroxyapatite crystals deposit. They are derived from mesenchymal stem cells and require runt-related transcription factor 2 (RUNX2), osterix (OSX), and β-catenin to differentiate into osteoblasts (Figure 4) (57, 58). Osteoblasts differentiate and mature

from their progenitors in response to several regulatory factors, including bone morphogenetic proteins (BMPs), insulin-like growth factor (IGF)-I, fibroblast growth factor (FGF)-2, PTH, vitamin D, tumour necrosis factor (TNF), Wnt signalling, and other extracellular signals (59-65).

The Wnt/β-catenin pathway is essential for osteoblast proliferation and differentiation (66, 67). Activation of this pathway also reduces osteoblast apoptosis, favouring bone formation, mineralization, and increased bone mass (68). Upon binding of a Wnt ligand to the receptor frizzled (FZ) and the co-receptors low density lipoprotein receptor-related proteins (LRPs) 5/6 (67, 69), glycogen synthase kinase (GSK)-3β is inhibited through mechanisms involving Axin, Frat-1, and disheveled (Dsh). β-catenin is then translocated to the nucleus, where it activates transcription of target genes (67). In the absence of a Wnt ligand, cytosolic β-catenin is degraded, and the expression of Wnt-responsive genes is suppressed (70). The Wnt signalling pathway is regulated by several antagonists, such as the secreted frizzled-related protein (sFRP) family and Wnt inhibitory factor (WIF)-1 (71, 72). Moreover, Dickkopf-related protein (DKK) 1 [29] and sclerostin (SOST) are both potent inhibitors of the Wnt pathway; they reduce LRPs 5/6 activity and consequently blunt osteoblast differentiation and bone formation (70). The Wnt/β-catenin pathway has been widely studied, and its inhibitors are now under investigation as new therapeutic targets for osteoporosis (73). In addition, LRP4 has recently been identified as a novel DKK1 and SOST receptor, facilitating the inhibition of Wnt signalling (74, 75). DKK2 is another molecule of interest since, depending on the cellular context, DKK2 inhibits Wnt signalling and stops osteoblast proliferation or acts to induce osteoblast maturation. In fact, DKK2-null mice are osteopenic and have defects in bone mineralization (76). Thus, the role of DKK2 is to act as a fine-tuning regulator of osteoblast maturation and bone mineralization (Figure 4).

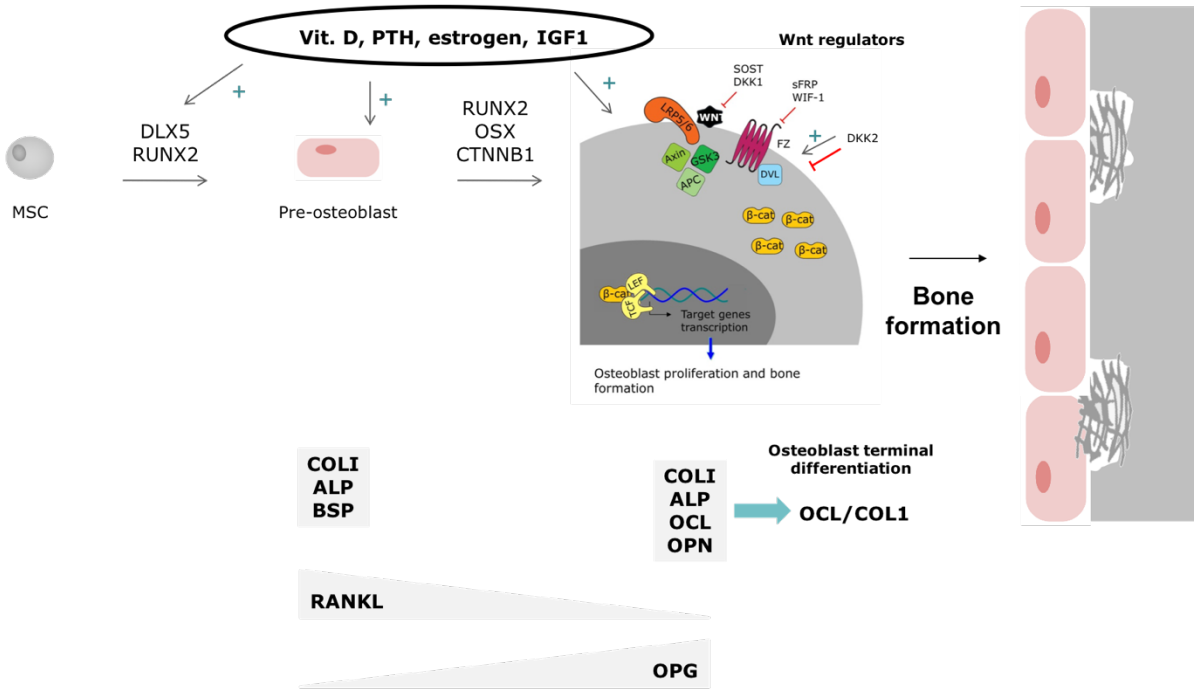

Figure 4. Regulators of osteoblasts differentiation, proliferation, and maturation.

(MSC, mesenchymal stem cells; DLX5, distal-less homeobox 5; RUNX2, runt-related transcription factor 2; OSX, osterix; CTNNB1, catenin beta 1; Vit. D, vitamin D3; PTH, parathyroid hormone; IGF1, insulin-like growth factor 1; WNT, wingless; SOST, sclerostin; DKK-1, Dickkopf-related protein 1; sFRP, secreted frizzled-related protein; WIF-1, Wnt inhibitory factor 1; DKK-2, Dickkopf-related protein 2; LRP5/6, low density lipoprotein receptor-related proteins 5/6; APC, activated protein C; GSK3, glycogen synthase kinase-3; FZ, frizzled protein; DVL, disheveled; LEF, lymphoid enhancer factor; TCF, T-cell factor; β-cat, beta catenin; ALP, alkaline phosphatase; BSP, bone sialoprotein; COL1, collagen type I; OPN, osteopontin; OCL, osteocalcin; RANKL, receptor activator of nuclear factor kappa ligand; OPG, osteoprotegerin)

Wnt signalling is associated with age-related loss of bone mass. In fact, serum levels of DKK1 and SOST increase with ageing and are associated with bone mass loss (77-79). Mirza and colleagues found that serum SOST levels were not only significantly higher in post-menopausal women, but they were also inversely associated with the free oestrogen index (80). In addition, treatment with either anti-SOST or anti-DKK1 antibodies in animal models of post-menopausal osteoporosis revealed an increase in bone formation, bone mass, and bone strength (81, 82).

One of the consequences of osteoblast dysfunction is impaired synthesis of collagen and osteocalcin (OCL) and, consequently, decreased mineralization ability. Differentiated osteoblasts synthesize COL1, which acts as a scaffold for deposition of minerals, as well as non-collagenous proteins (such OCL) that organize mineral orientation in the collagen

scaffold (83, 84). Osteoblast activity can be measured by gene expression of COL1a1 and OCL in bones (85). Osteoblasts express COL1a1 at the beginning of their differentiation and, thereafter, total collagen synthesis declines as maximal expression of OCL occurs when osteoblasts become terminally differentiated (76, 83-86). Therefore, the ratio of OCL/COL1a1 expression in bone is an indicator of the proportion of osteoblast terminal differentiation and mineralization impairment. Little is known regarding the importance of osteoblast terminal differentiation in mineralization disturbances and the deterioration of bone mechanical properties leading to fragility fractures. Questions regarding bone formation disturbances in post-menopausal osteoporosis have recently arisen with the finding that oestrogen deficiency is linked to mineralization disturbances secondary to osteoblast impairment (87-90). In the elderly, some studies have shown that osteoblast dysfunction due to age-dependent decreases in IGF-1 and FGF-2 was associated with osteoporosis (55). Further insight into the importance of impaired osteoblast function in fragility fractures among the elderly is needed. In this thesis, we have filled this knowledge gap by analysing the association between bone OCL/COL1a1 expression ratio and bone mechanical properties, as well as hip fractures.

Fragility Fractures Risk Assessment

A fragility fracture is not only caused by skeletal factors but also by extra-skeletal factors, such as a propensity to falls, soft tissue dysfunction, impact force, and impact surface. Clinical risk factors (e.g., age, sex, family history of fractures, chronic diseases, BMI) influence bone mechanical properties, propensity to falls, and muscle behaviour, but they do not explain entirely these other factors (Figure 5). Thus, assessment of fracture risk should involve a comprehensive approach accounting for the myriad of factors contributing to fragility fractures.

Several tools have been developed to identify people at risk of fragility fractures. The most widely used are measurement of BMD, determination of serum levels of bone turnover markers, and the use of algorithms for fracture risk prediction that include clinical risk factors for fractures and BMD (e.g., FRAX tool, QFracture, Garvan risk

calculator) (91, 92). The development of these tools, especially fracture risk prediction algorithms, has improved the identification of individuals at high risk of fracture; however, these tools still fail to identify a substantial portion of women and men who will have a fragility fracture (93-95).

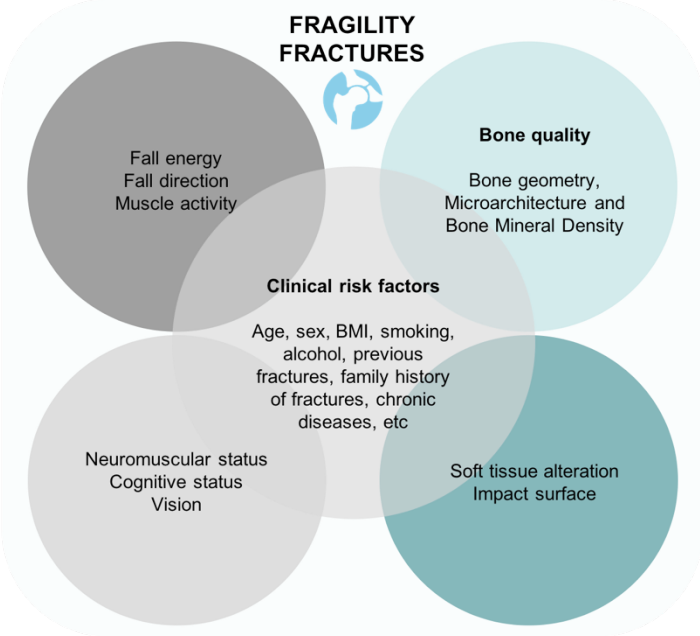

Figure 5. Skeletal and extra-skeletal risk factors for fragility fractures.

Dual x-ray absorptiometry

DXA is used to assess BMD at the most vulnerable sites for fractures: the lumbar spine and hips. The WHO operational definition of osteoporosis is based on a reduction of BMD to 2.5 or more standard deviations (SDs) below the young adult norm, the T-score (96) (Table 1). In both men and women, low BMD is associated with a higher risk of fragility fractures, independent of age or fracture site (93, 97). Fracture risk increases 2.6 fold for each SD decrease in hip BMD (97). Of note, site-specific BMD is a better predictor of fracture risk at that site; for example, hip fractures are better predicted by hip BMD than by vertebral BMD (93).

Table 1. World Health Organization diagnostic criteria for osteoporosis.

BMD T-score	Classification
T-score ≥ -1	Normal
- 2.5 < T-score < -1	Low bone mass
T-score ≤ -2.5	Osteoporosis
T-score ≤ -2.5 + fragility fracture	Severe osteoporosis

The association between BMD and fragility fractures is good but not perfect. In fact, the cut-off BMD value established by the WHO to clinically define osteoporosis fails to predict almost 50% of fragility fractures (93, 98).

Over the past decade, both the hardware and software of DXA have improved, enhancing the reliability of BMD measurements. Additionally, introduction of high-quality DXA 2-dimensional x-rays led to the development of the trabecular bone score (TBS) at the lumbar vertebra. TBS is a textural index that evaluates pixel grey-level variations in the lumbar spine DXA image, delivering an indirect index of trabecular microarchitecture (99). TBS is associated with bone mechanical intrinsic properties and bone strength (100, 101). Prospective studies revealed that TBS is also associated with fragility fractures, independent of BMD and clinical risk factors (102, 103). However, the predictive ability of TBS is modest. Like BMD, it can enhance fracture risk prediction if included in a comprehensive model of fracture risk determination (103, 104).

Bone turnover markers

Biochemical bone turnover markers reflect either the enzymatic activity of osteoblasts and osteoclasts or the breakdown products of bone tissue. Therefore, these markers can be valuable tools to investigate bone metabolism, monitor treatment efficacy, define treatment strategies, and assess fracture risk. The most widely used markers for bone formation are serum levels of alkaline phosphatase (ALP), bone-specific ALP, OCL,

and procollagen type I pro-peptides (P1CP and P1NP). Pyridinium crosslinks (PYD and DPD) and two type I collagen telopeptides (CTX and NTX) reflect bone resorption. Tartrate-resistant acid phosphatase 5b (TRAcP5b), cathepsin K (CTSK), bone sialoprotein (BSP), and the RANKL/OPG ratio (Figure 6) were recently identified as biomarkers for evaluating bone remodelling (105). Biochemical determination of bone turnover markers is an appealing way to identify people with a high risk of fracture, as samples of blood or urine are easily collected, and several inexpensive assays are now in the market.

Serum levels of bone turnover markers reflect bone remodelling (106) and are associated with BMD (95, 107-110). Some studies reported a modest association with fragility fractures (95, 107-110), although others found that the association with fragility fractures was not independent of BMD (107, 110-113). Moreover, the available bone turnover markers have wide biological variability and, in some cases, multiple methodologies are used to test the same analyte (95). Therefore, new biological markers of bone metabolism that demonstrate added value in individual fracture risk assessment are necessary.

Some studies suggested that serum levels of the Wnt regulators, in particular SOST and DKK1, could have a role in osteoporosis and in the identification of subjects at risk of developing osteoporosis. In fact, serum levels of DKK1 and SOST increase with age, and this is associated with loss of bone mass (77-79). Mirza and colleagues found that SOST serum levels were not only significantly higher in post-menopausal women, but they were also inversely associated with the free oestrogen index (80). In addition, treatment of post-menopausal osteoporosis (in both animal models and humans) with either anti-SOST or anti-DKK1 antibodies led to increased bone formation, bone mass, and bone strength (81, 82, 114). Anti-SOST treatment also demonstrated efficacy in reducing fracture risk (115). In this thesis, we aimed to uncover the clinical utility of serum measurements of Wnt regulators and determine their association with fracture risk in a population-based nationwide cohort, the EpiDoC cohort. More importantly, we sought to understand whether serum levels of Wnt regulators could be used to improve fracture risk prediction when added to BMD and clinical risk factors.

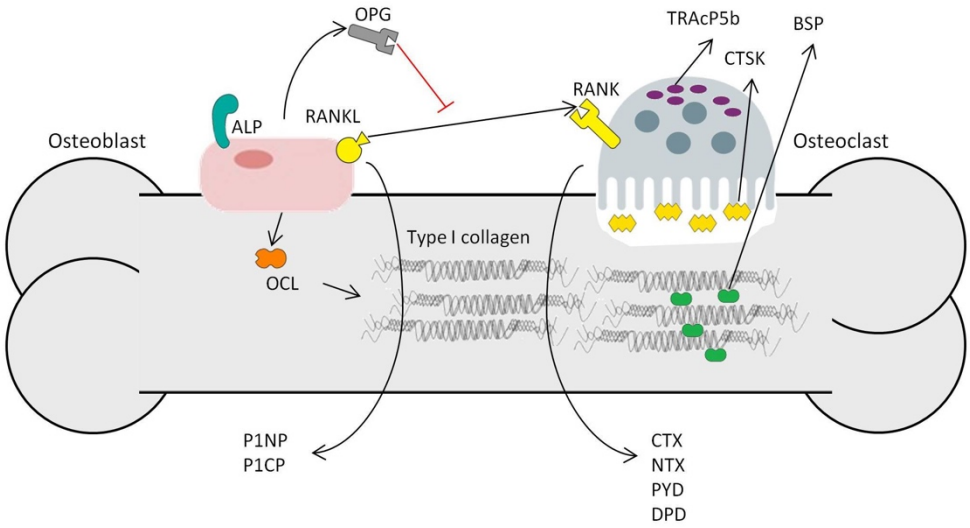

Figure 6. Bone turnover markers.

Bone turnover markers can be divided into bone formation and bone resorption markers. Formation markers derive from osteoblast metabolism, while resorption markers originate from osteoclast action. The RANKL/OPG ratio is a marker of osteoclastogenesis. The most reliable bone turnover markers are P1NP and CTX. (ALP, alkaline phosphatase; BSP, bone sialoprotein; CTSK, cathepsin K; CTX, carboxy-telopeptide of type I collagen; DPD, deoxypyridinoline; NTX, amino-telopeptide of type I collagen; OCL, osteocalcin; OPG, osteoprotegerin; P1CP, procollagen 1 carboxy-terminal peptide; P1NP, procollagen 1 amino-terminal peptide; PYD, pyridinoline; RANK, receptor activator of nuclear factor kappa B; RANKL, RANK ligand; TRAcP5b, tartrate-resistant acid phosphatase 5b.)

Fracture risk prediction algorithm: FRAX tool

Population-based cohorts from Europe, North America, Asia, and Australia identified clinical risk factors for fractures, which provided information about fracture risk independently of BMD (116-120) (Figure 7). Therefore, the importance of using clinical risk factors in addition to BMD information for predicting individual absolute fracture risk was clear to researchers (121). Several fracture prediction models have been created and validated; however, the FRAX model is the most widely validated and disseminated tool worldwide (92). The FRAX tool is an algorithm based on a multivariate model, which incorporates (in a weighted manner) the independent clinical risk factors for fracture (hip BMD, age, BMI, prior low-impact fracture, parental history of hip fracture, current smoking, alcohol intake, glucocorticoid use for more than 3 months,

rheumatoid arthritis, and other secondary causes of osteoporosis) in combination with the corresponding mortality rate for each country (Figure 7).

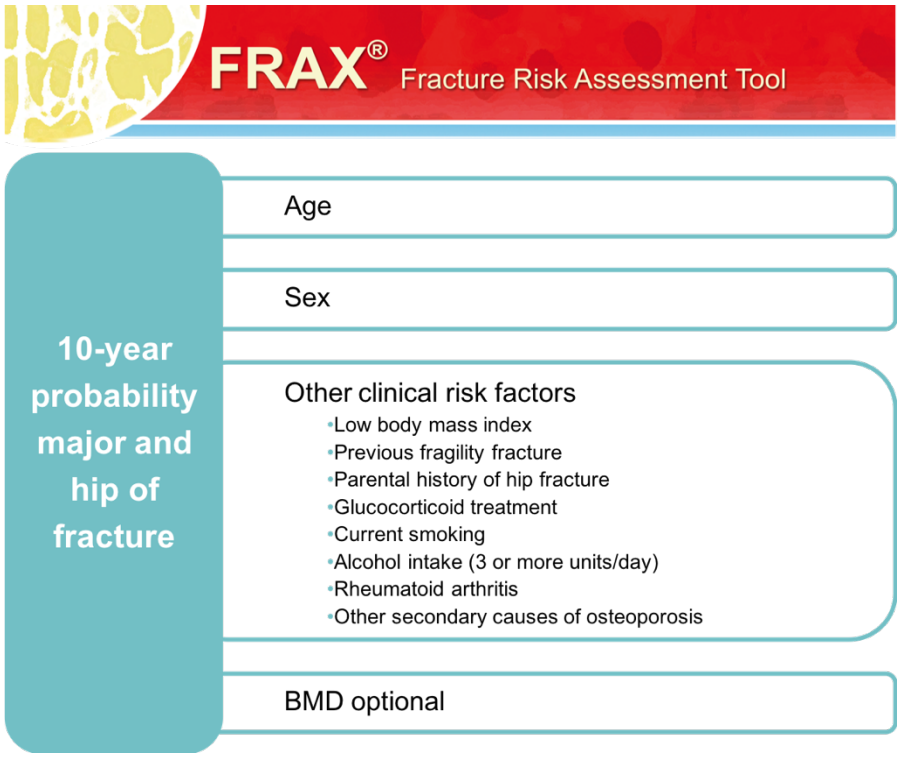

Figure 7. FRAX® fracture risk assessment tool.

FRAX was developed by researchers at Sheffield University to assess the 10-year probability of both major and hip fractures, with or without considering BMD. It was meant to be used for individuals more than 40 years of age with untreated osteoporosis. Overall, this tool demonstrated better performance than BMD in fragility fracture prediction (91). In fact, computing FRAX with only clinical risk factors has demonstrated similar performance to DXA alone in predicting non-hip fractures and better performance than DXA in predicting hip fractures (19, 122). If we consider just the senior population, FRAX calculated with only clinical risk factors is superior to BMD as a screening tool for identifying individuals with a high risk of fracture (91). A recent randomized controlled trial revealed that using the FRAX algorithm as a screening tool is feasible and effective in reducing the incidence of hip fractures. However, it has the same limitations as BMD screening in preventing other low-impact fractures (123). Recently, it became possible to adjust the FRAX output by incorporated vertebral spine

TBS measurements. This integrated model (FRAX+TBS) modestly increases the ability to predict fractures in individuals, compared with FRAX alone; it is most useful for younger adults (104).

The FRAX tool has been available online since 2008 and can be used in 53 countries worldwide, based on country-specific calibration according to the national epidemiology of fractures and mortality rates (92). To accurately treat patients, it was necessary to define country-specific treatment thresholds for the FRAX prediction model. Several countries conducted cost-effectiveness analyses and defined pharmacological treatment thresholds for FRAX (124, 125). In 2013, a research group led by Professor Pereira da Silva et al. calibrated a FRAX model for Portugal, allowing FRAX to be used in clinical practice in this country (33). A few years later, in 2016, Marques et al. performed a cost-effectiveness analysis for different pharmacological treatments to define FRAX treatment thresholds in Portugal (126). This work of validating and defining cost-effectiveness pharmacological treatment thresholds was of upmost importance to improve the selection of patients that would benefit from anti-osteoporotic therapy. To implement this new knowledge in clinical practice and improve the substantial constraints involved in the prevention, screening, and management of osteoporosis-related fractures, it was necessary to develop national consensus recommendations. The work under the scope of this thesis encompassed the collaborative efforts in establishing multidisciplinary recommendations regarding the indications for DXA and for initiating medical therapy aimed at preventing fractures, as well as the efforts involved in developing an update of the Portuguese Society of Rheumatology's recommendations regarding the prevention, diagnosis, and management of osteoporosis.

Treatment Strategies for Osteoporosis and Fragility Fractures

Osteoporosis is a multifactorial disease associated with low peak bone mass and/or rapid and persistent bone loss. In addition, overlapping effects of many concomitant chronic diseases and medications also contribute to excessive and/or imbalanced bone

remodelling, promoting further loss of bone mass and microarchitectural deterioration. Factors such as insufficient sun exposure, inadequate nutrient intake (of calcium, vitamin D, and protein), limited exercise, and high-risk behaviours (smoking and excessive alcohol consumption) also play a role in low peak bone mass and loss of bone (116, 120, 127-130). Therefore, the optimal approach for treating osteoporosis involves both pharmacological and non-pharmacological measures.

Adequate nutrition with a well-balanced diet, sufficient sun exposure, and regular weight-bearing exercise are important measures that promote bone health in the general population, and especially in patients with osteoporosis (Figure 8) (131).

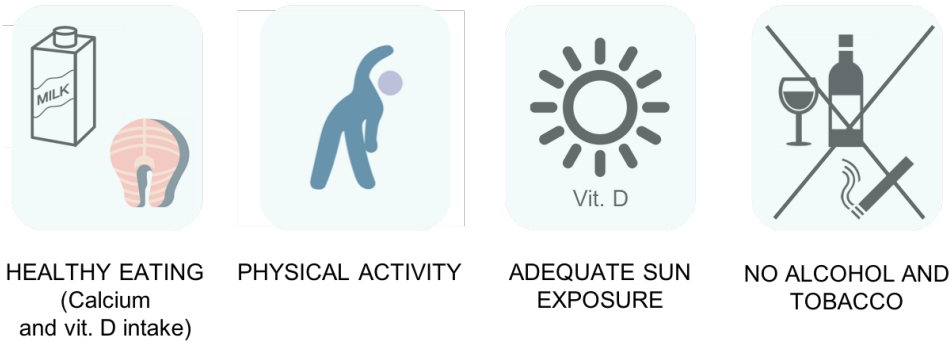

Figure 8. Non-pharmacological osteoporosis treatment.

Several drugs with demonstrated efficacy, safety, and cost-effectiveness are available for the treatment of osteoporosis (Table 2) (126, 132-145). Fracture risk reduction associated with these pharmacological treatments varies between 20% and 83%, depending on the drug and fracture site. However, despite this plethora of osteoporosis treatments, in many European countries and the United States of America, fewer than 25% of patients with major osteoporosis-related fractures are treated for their underlying osteoporosis (146). In Portugal, no information is available regarding the diagnosis and treatment rates of osteoporosis in women who have sustained a fragility fracture. In this thesis, we aimed to improve knowledge and understanding of this highly relevant topic. Moreover, we developed clinical consensus recommendations regarding

osteoporosis treatment strategies, with the goal of contributing to improved osteoporosis treatment in Portugal.

Table 2. Evidence regarding efficacy and safety of pharmacological treatments for patients with osteoporosis (Adapted from the American College of Physicians Clinical Guideline of osteoporosis treatment (147)).

Treatment	Effect on fracture risk			Adverse effects
	Vertebral	Non-vertebral	Hip	
Alendronate	+	+	+	Mild gastrointestinal symptoms ¥
Ibandronate	+	?	?	Mild gastrointestinal symptoms; myalgia¥
Residronate	+	+	+	Mild gastrointestinal symptoms ¥
Zoledronic Acid	+	+	+	Mild gastrointestinal symptoms; hypocalcaemia; flu-like symptoms ¥
Denosumab	+	+	+	Mild gastrointestinal symptoms; infections; cutaneous rash¥
Teriparatide	+	+	?	Mild gastrointestinal symptoms; hypocalcaemia; headache
Raloxifene	+	-	-	Hot flashes; thromboembolic events

Non-vertebral refers to fractures not occurring in the spine or skull.

+ refers to efficacy demonstrated in randomized control trials. - refers to no efficacy demonstrated in randomized control trials. ? refers to unknown efficacy in original trials or efficacy shown only during post hoc analysis. ¥ indicates that atypical femoral fractures and jaw osteonecrosis are very rare adverse events with bisphosphonates and denosumab in patients with osteoporosis (148).

In conclusion, the adverse consequences of osteoporosis and fragility fractures are being increasingly recognized. However, several unmet research needs remain regarding fracture risk stratification of patients, disease mechanisms, and new treatment strategies. To overcome the complex problems of predicting and managing fragility fractures, we hypothesize that establishing national epidemiological knowledge regarding osteoporosis and fragility fractures will provide evidence to customize national health care solutions. We also hypothesize that fracture risk assessment can be refined by the use of serum biomarkers. Clinical consensus recommendations, using the best evidence in clinical practice, will be also an important tool to improve the identification and management of patients with a high risk of fragility fracture.

CHAPTER II

GENERAL AND SPECIFIC AIMS

In this thesis, we aimed to answer three questions, which reflect current unmet research needs:

1) What are the prevalence, burden, and risk factors of osteoporosis and fragility fractures in Portugal?
2) How can we better identify people at risk of fragility fractures?
3) Can we establish national clinical consensus recommendations to improve individual clinical assessment?

General Aims

With this PhD thesis, we aimed to address the prevalence and burden of osteoporosis and fragility fractures in Portugal to provide objective evidence to support new health strategies, improve clinical care, and reduce or prevent disability and mortality.

We also aimed to improve the identification of senior women (aged ≥65 years old) at high risk for a fragility fracture through the use of novel noninvasive biomarkers. To achieve this goal, we explored cellular (osteoblast) mechanism dysfunction to identify potential serum markers of bone fragility. We hypothesized that bone fragility in the elderly is associated with dysregulation of osteoblast terminal differentiation and disturbances in Wnt regulators (DKK1, DKK2, SOST, WIF-1, and sFRP-1). Moreover, we hypothesized that serum levels of Wnt regulators are associated with bone fragility and fractures and can constitute new markers for osteoporosis treatment decision-making.

Finally, we aimed to develop national clinical consensus recommendations for osteoporosis diagnosis and treatment to create awareness and reduce new fragility fractures in Portugal.

Specific Aims

AIM 1: To estimate the prevalence and burden of osteoporosis in Portugal.

AIM2: To estimate the prevalence, risk factors, and burden of fragility fractures, as well as the prescription of and compliance with anti-osteoporotic drug treatment, among older Portuguese women (a vulnerable high-risk stratum).

AIM 3: To identify clinical risk factors associated with poor mechanical properties among patients with fragility fractures.

AIM 4: To analyse whether osteoblast disturbances, measured by gene expression of OCL/COL1A1 in bone (a surrogate marker of osteoblast terminal differentiation), are associated with bone mechanical behaviour and fragility fractures.

AIM 5: To analyse whether serum levels of Wnt regulators (DKK1, DKK2, SOST, WIF-1, and sFRP-1), as controllers of osteoblast differentiation, are independently associated with axial bone mineral mass evaluated by DXA and incident fragility fractures, using a population-based nationwide cohort of senior women.

AIM 6: To develop national clinical consensus recommendations regarding osteoporosis diagnosis and treatment to reduce the incidence of fragility fractures in Portugal.

CHAPTER III

METHODOLOGY

EPIREUMAPT STUDY DESCRIPTION

EPIDOC COHORT DESCRIPTION

METHODOLOGY

For this thesis, we used two samples of participants/patients. One sample was composed of patients who underwent hip replacement surgery for osteoarthritis or fragility fracture from 2008 to 2012 at the Orthopaedic Department of *Centro Hospitalar Lisboa Norte*. The other sample was a population-based nationwide sample evaluated by survey in the EpiReumaPt study (2011-2013). The EpiReumaPt population was subsequently followed in two more waves of evaluation (2011-2016) under the scope of the Epidemiology of Chronic Diseases (EpiDoC) cohort. This chapter summarizes relevant aspects of the methodology used for this thesis. For each research question, a description is provided of the population of interest (i.e., subpopulations used), recruitment, study design, and case definition. This chapter also describes the work performed by the PhD student in each of these projects. For the EpiReumaPt survey and EpiDoC cohort, a comprehensive methodological approach was used and prepared for publication in separate manuscripts written by the PhD student as the first author. They are presented in distinct sections of this chapter.

1. Convenience sample of patients undergoing hip replacement surgery

In our research, we aimed to study bone biomechanics and cellular dysfunction in patients with hip fractures. This mechanistic approach enabled us to identify potential biomarkers of bone fragility and fractures. To achieve this aim, we studied a sample of consecutive patients who underwent total hip replacement surgery within 8 days after a hip fragility fracture at the Orthopaedic Department of *Hospital de Santa Maria* in Lisbon, between 2008 and 2012. We also included patients with osteoarthritis referred for total hip replacement surgery during the same period as a comparison group. A clinical protocol was used, which included determining the presence or absence of clinical risk factors for fracture. Fasting blood samples and spine and hip BMD measurements were obtained. Femoral epiphyses were collected, from which trabecular bone cylinders were obtained for use in compression mechanical tests. Gene

expression of bone matrix components was assessed by quantitative real-time polymerase chain reaction (RT-PCR) analysis.

Under the scope of this research, two manuscripts (Chapter IV, Section II, Part 1, and Part 2) were prepared and published in peer-reviewed journals. The manuscript presented in Chapter IV, Section II, Part 1 aimed to analyse clinical risk factors associated with poor mechanical behaviour in patients with hip fragility fractures. The manuscript presented in Chapter IV, Section II, Part 2 aimed to analyse the association between osteoblast terminal differentiation and fragility fractures, as well as poor trabecular mechanical behaviour.

Both studies used the same inclusion and exclusion criteria. The inclusion criteria were age 50 years or older and/or a post-menopausal woman, able to provide clinical information, and able to provide written informed consent. The exclusion criteria were receiving anti-osteoporotic therapy; being out of the range for calculating the 10-year risk of major/hip fracture using the FRAX algorithm (age and BMI); or having a personal history of other bone metabolic diseases (other than osteoporosis), bone metastasis, a primary tumour, or osteomyelitis. Although we used the same inclusion and exclusion criteria for both studies, the samples were not the same because in the first study (Chapter IV, Section II, Part 1), we used data collected between 2008 and 2009, and for the second study (Chapter IV, Section II, Part 2), we used data collected between 2009 and 2012.

The PhD student contributed to the design and conduct of the study, and applied the clinical protocol for all samples. The student also performed data management, data analysis (the PhD student takes responsibility for the integrity of the data analysis presented in these papers), and data interpretation. The student likewise drafted and revised the manuscripts and submitted the final version of each manuscript. Both papers were published in international peer-reviewed journals.

2. EpiReumaPt survey

For this thesis, we also aimed to determine the prevalence of osteoporosis in Portugal and define the burden of fragility fractures and the osteoporosis treatment rates among senior women (aged ≥65 years old). For this, we used data from the EpiReumaPt study (Chapter IV, Section I, Part 1, and Part 2).

The EpiReumaPt cohort is composed of a randomly selected, representative sample of the adult Portuguese population in mainland Portugal, the Azores Islands, and the Madeira Islands. Recruitment started in September 2011 and finished in December 2013. EpiReumaPt aimed to estimate the prevalence of RMDs in the adult Portuguese population. The selected diseases were hand, knee, and hip osteoarthritis; low back pain; rheumatoid arthritis; fibromyalgia; gout; spondyloarthritis; periarticular disease; systemic lupus erythematosus; polymyalgia rheumatica; and osteoporosis.

The study design involved a three-stage approach. The first step was a face-to-face survey performed by trained interviewers at the household of 10,661 subjects who were randomly selected by stratified multistage sampling. A highly sensitive screening questionnaire for RMDs was used. Secondly, participants who screened positive (64%) for at least one RMD, as well as 20% of those with a negative screening, were invited for an assessment by a rheumatologist and were asked to donate a blood sample to be stored at the Biobanco-IMM, Lisbon Academic Medical Centre. In total, 3,877 subjects participated in this second phase. The rheumatologist performed a medical history and physical examination and obtained appropriate laboratory and imaging tests. At the end of the visit, the rheumatologist established a diagnosis. Finally, a team of three experienced rheumatologists reviewed all of the clinical data and defined the diagnoses according to previously validated criteria.

The EpiReumaPt sample was composed of adults (≥ 18 years old) who were living in private households in Portugal (mainland, Madeira Islands, or Azores Islands). Osteoporosis was defined by the diagnosis of an expert (the rheumatologist), which was based on the presence of at least of one of the following: previous fragility fracture, previous diagnosis of osteoporosis, osteoporosis treatment, or fulfilment of WHO

criteria (when axial DXA data were available).

Our research question was under the scope of the EpiReumaPt study and results were published along with the prevalence and burden of other RMDs in a paper in which the PhD student was the second author and participated in the following: data collection (one-third of the EpiReumaPt clinical appointments), study conduct, data management, data analysis (the PhD student takes responsibility for the integrity of the data analysis presented in the paper), data interpretation, manuscript drafting and revisions, and approval of the final version of the manuscript (Chapter IV, Section I, Part 1).

In the manuscript presented in Chapter IV, Section I, Part 2, we also used EpiReumaPt data to study the prevalence, burden, risk factors, and osteoporosis treatment rates of fragility fractures among Portuguese senior women. The population of interest was defined as women 65 years and older who participated in the second phase of EpiReumaPt (Figure 9). Fragility fractures were defined as any self-reported, low-impact fracture (fractures resulting from a fall from a standing height or less or occurring in the absence of any trauma) in individuals older than 40 years. Fractures of the face, skull, foot, fingers, and toes were excluded.

In this work, the PhD student was responsible for formulation of the research question, data management, data analysis (the PhD student takes responsibility for the integrity of the data analysis presented in the paper), data interpretation, manuscript drafting and revisions, approval of the final version of the manuscript, and submission of the manuscript, which was published in an international peer-reviewed journal.

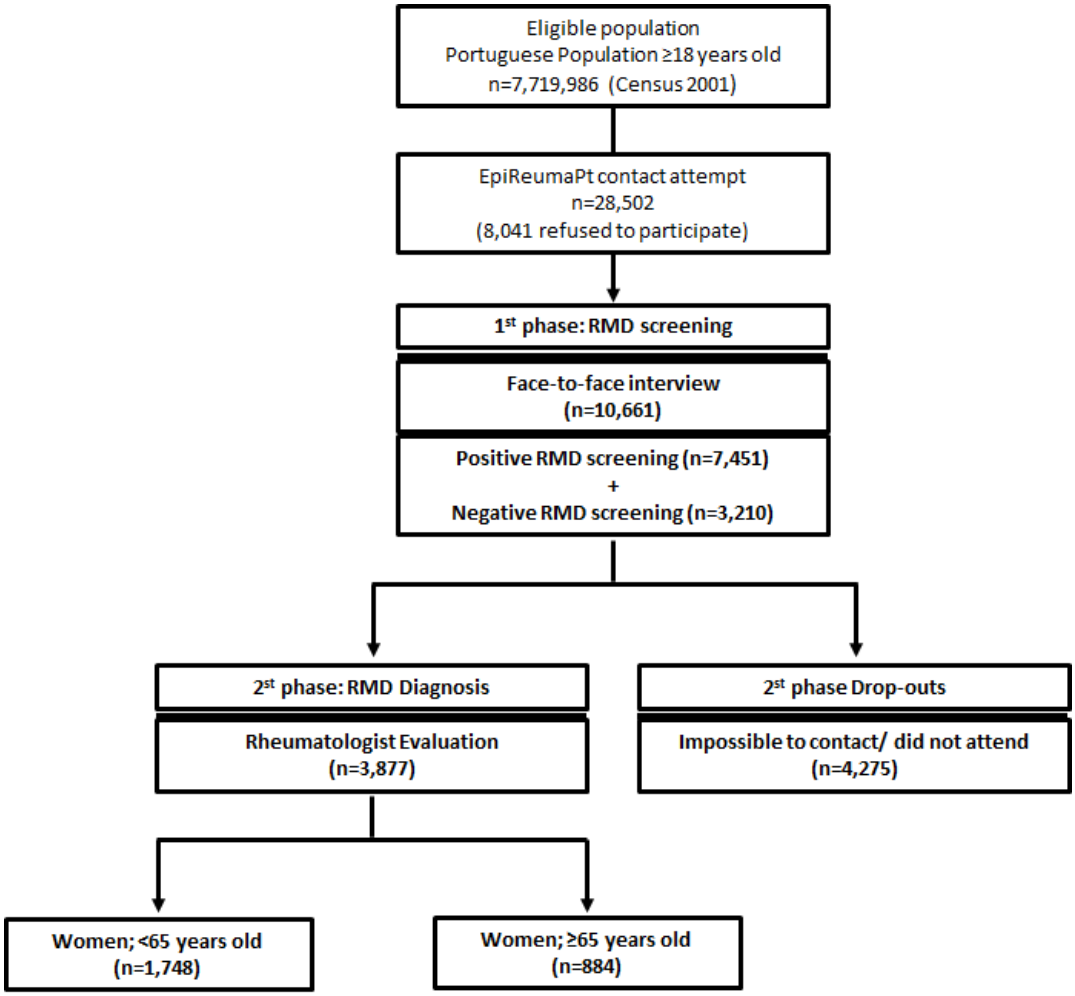

Figure 9. Study design flowchart of the research addressing the prevalence and burden of fragility fractures in senior Portuguese women.

3. EpiDoC cohort

Using data from the EpiDoC cohort, we analysed whether serum levels of Wnt regulators (masters of osteoblast differentiation)—including DKK1, DKK2, SOST, WIF-1, and sFRP—are independently associated with axial bone mineral mass evaluated by DXA or with incident fragility fractures in senior women.

The EpiDoC cohort was composed by EpiReumaPt participants who agreed to be followed (n=10,153) and who completed the three waves of the study. In each wave, a core questionnaire regarding socioeconomic status, RMDs, fractures, falls, other chronic diseases, quality of life, and healthcare resource consumption was employed to gather

longitudinal data. Each wave also had specific questions regarding other health and health-related issues, allowing the collection of cross-sectional and longitudinal data. The first wave, the EpiReumaPt study (2011-2013), was described above. The follow-up waves (second and third) involved telephone call interviews performed by research assistants. The second wave (EpiDoC 2, 2013–2015) collected data regarding lifestyles, lifestyle determinants, and innovative patient solutions for coping with disability, and the third wave (EpiDoC 3, 2015–2016) evaluated inequalities in access to food and healthcare services.

To identify new serum markers of bone fragility in senior women, we used a subpopulation of the EpiDoC cohort, defined as women 65 years and older who participated in the second phase of EpiReumaPt and agreed to be followed (Figure 10). The exclusion criteria were receiving osteoporosis treatment or having bone metastasis or any metabolic bone disease except osteoporosis. The manuscripts for this study are presented in Chapter IV, Section III, Part 1.

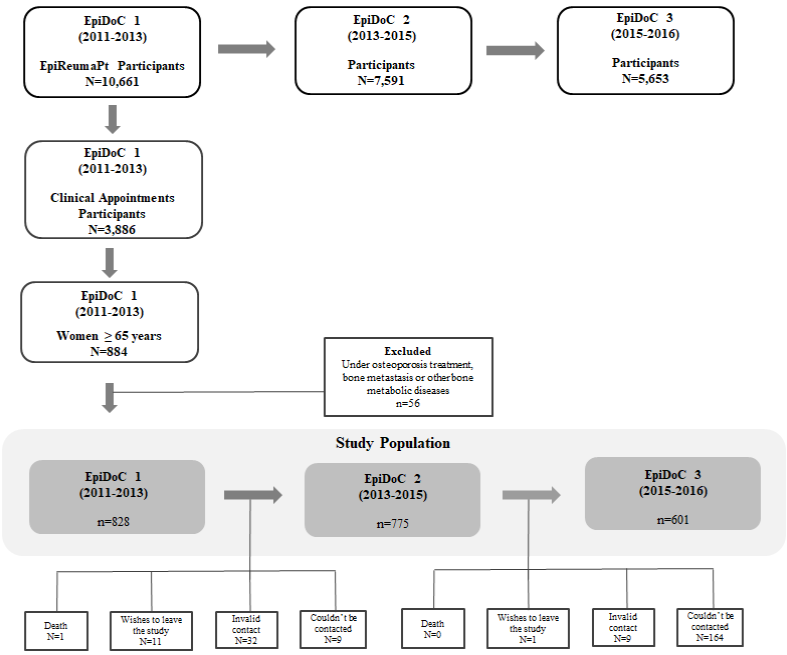

Figure 10. Flowchart of study population and participant retention during follow-up for research analysing Wnt regulators as biomarkers of bone mineral density and fragility fractures.

The PhD student was responsible for developing the follow-up protocol for the EpiDoC cohort and for designing and testing the questionnaires used in the phone call interviews for EpiDoC 2 and 3. The student also monitored data acquisition and developed strategies to reduce missing data. In addition, the student performed data management, data analysis, and data interpretation; drafted and revised the manuscript; and prepared and submitted the final version of the manuscript. The PhD student takes full responsibility for the integrity of the data analysis, data interpretation, and the manuscript presented in Chapter IV, Section III, Part 1.

EPIREUMAPT STUDY DESCRIPTION

RODRIGUES AM, GOUVEIA N, DA COSTA LP, ET AL. 2015. EPIREUMAPT – THE STUDY OF RHEUMATIC AND MUSCULOSKELETAL DISEASES IN PORTUGAL: A DETAILED VIEW OF THE METHODOLOGY. ACTA REUMATOL PORT. 40:110-124

ARTIGO ORIGINAL

EpiReumaPt – the study of Rheumatic and Musculoskeletal diseases in Portugal: a detailed view of the methodology

Ana M Rodrigues[1,2,3,4], Nélia Gouveia[1,2,5], Leonor Pereira da Costa[6], Mónica Eusébio[7], Sofia Ramiro[1,5,8] Pedro Machado[1,9], Ana Filipa Mourão[1,3,5,10], Inês Silva[1,10], Pedro Laires[1,4], Alexandre Sepriano[3,5,10], Filipe Araújo[10,11,12], Pedro Simões Coelho[13], Sónia Gonçalves[14,15], Ana Zhao[16], João Eurico Fonseca[3,4,16,17], JM Caldas de Almeida[5] Viviana Tavares[18,19], JAP da Silva[20] , Henrique Barros[21], Jorge Cerol[6], Jorge Mendes[13], Loreto Carmona[22], Helena Canhão[1,2,3,4,17], Jaime C Branco[1,2,5,10,23] on behalf of the EpiReumaPt study group*

ACTA REUMATOL PORT. 2015;40:110-124

ABSTRACT

Rheumatic and musculoskeletal diseases (RMD) are prevalent and a leading cause of disability and consumption of healthcare and social resources. EpiReumaPt is a national population-based survey developed by the Portuguese Society of Rheumatology that aimed to estimate the prevalence of RMDs and determine their impact on function, quality of life, mental health and use of healthcare resources.

This article describes in detail the design, methodology and planned analyses of EpiReumaPt.

Recruitment started in September 2011 and finished in December 2013. This study involved a three-stage approach. The first step was a face-to-face survey performed by trained interviewers at the household of

1. EpiReumaPt Study Group – Sociedade Portuguesa de Reumatologia, Lisboa, Portugal
2. EpiDoc Unit – Unidade de Epidemiologia em Doenças Crónicas (CEDOC, NMS/UNL), Lisboa, Portugal
3. Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Lisboa, Portugal
4. Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
5. Centro de Estudos de Doenças Crónicas (CEDOC) da NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
6. Centro de Estudos e Sondagens de Opinião da Universidade Católica Portuguesa (CESOP-CATÓLICA), Lisboa, Portugal
7. Sociedade Portuguesa de Reumatologia, Lisboa, Portugal
8. Leiden University Medical Center, Leiden, The Netherlands
9. MRC Centre for Neuromuscular Diseases, University College London, London, United Kingdom
10. Serviço de Reumatologia do Hospital Egas Moniz – Centro Hospitalar Lisboa Ocidental (CHLO- E.P.E.), Lisboa, Portugal
11. Institute of Microbiology, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
12. Unidade Curricular Especialidades Médico-Cirúrgicas I, NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
13. NOVA IMS, Universidade Nova de Lisboa, Lisboa, Portugal
14. Instituto Piaget, Lisboa, Portugal
15. Unidade de Epidemiologia do Instituto de Medicina Preventiva e Saúde Pública da Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
16. Biobanco-IMM, Instituto de Medicina Molecular, Lisboa, Portugal
17. Serviço de Reumatologia do Hospital de Santa Maria – Centro Hospitalar Lisboa Norte (CHLN-E.P.E), Lisboa, Portugal
18. Hospital Garcia de Orta, EPE, Almada, Portugal
19. APOROS – Associação Nacional contra a Osteoporose, Lisboa, Portugal
20. Clínica Universitária de Reumatologia, Faculdade de Medicina e Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
21. Departamento de Epidemiologia do Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal
22. Instituto de Salud Musculoesquelética, Madrid, Spain
23. Programa Nacional Contra as Doenças Reumáticas (2006-2014), Direcção-Geral da Saúde. Lisboa, Portugal

***EpiReumaPt study group**
SOCIEDADE PORTUGUESA REUMATOLOGIA:
Alexandra Bernardo, Alexandre Sepriano, Ana Filipa Mourão, Ana Maria Rodrigues, Ana Raposo, Ana Sofia Roxo, Anabela Barcelos, António Vilar, Armando Malcata, Augusto Faustino, Cândida Silva, Carlos Vaz, Carmo Afonso, Carolina Furtado, Catarina Ambrósio, Cátia Duarte, Célia Ribeiro, Cláudia Miguel, Cláudia Vaz, Cristina Catita, Cristina Ponte, Daniela Peixoto, Diana Gonçalves, Domingos Araújo, Elsa Vieira-Sousa, Eva Mariz, Fátima Godinho, Fernando Pimentel, Filipa Ramos, Filipa Teixeira, Filipe Araújo, Filipe Barcelos, Georgina Terroso, Graça Sequeira, Guilherme Figueiredo, Helena Canhão, Herberto Jesus, Inês Cunha, Inês Gonçalves, Inês Silva, José António P. da Silva, Jaime Cunha Branco, Joana Abelha, Joana Ferreira, João Dias, João Eurico Fonseca, João Ramos, João Rovisco, Joaquim Pereira, Jorge Silva, José Carlos Romeu, José Costa, José Melo Gomes, José Pimentão, Lúcia Costa, Luís Inês, Luís Maurício, Luís Miranda, Margarida Coutinho, Margarida Cruz, Margarida Oliveira, Maria João Gonçalves, Maria João Salvador, Maria José Santos, Mariana Santiago, Mário Rodrigues, Maura Couto, Miguel Bernardes, Miguel Sousa, Mónica Bogas, Patrícia Pinto, Paula Araújo, Paula Valente, Paulo Coelho, Paulo Monteiro, Pedro Madureira, Raquel Roque, Renata Aguiar, Ricardo O. Figueira, Rita Barros, Rita Fonseca, Romana Vieira, Rui André, Rui Leitão, Sandra Falcão, Sara Serra, Sílvia Fernandes, Sofia Pimenta, Sofia Ramiro, Susana Capela, Taciana Videira, Teresa Laura Pinto, Teresa Nóvoa, Viviana Tavares

10,661 subjects, who where randomly selected by a stratified multistage sampling. A highly sensitive screening questionnaire for RMDs was used. Secondly, participants who screened positive (64%) for at least one RMD, as well as 20% of individuals with a negative screening, were invited for assessment by a rheumatologist. In total, 3,877 subjects participated in this second phase, where they were also invited to donate a blood sample to be stored at the Biobanco-IMM. History and physical examination, followed by appropriate laboratory and imaging tests were performed. At the end of the visit, the rheumatologist established a diagnosis. Finally, a team of three experienced rheumatologists reviewed all the clinical data and defined the diagnoses according to previously validated criteria.

The EpiReumaPt dataset, containing data from several questionnaires, various clinical measurements and information from laboratory and imaging tests, comprises an invaluable asset for research. The large amount of information collected from each participant and the large number of participants, with a wide age range covering and being representative of the adult population from the entire country, makes EpiReumaPt the largest study of RMDs performed in Portugal.

Keywords: EpiReumaPt; Epidemiology; Rheumatic diseases; Methodology; Portugal; Study design.

INTRODUCTION

Rheumatic and Musculoskeletal diseases (RMDs) are among the most common diseases managed at the primary health care level. They are leading causes of disability in developed countries and consume a large amount of health and social resources [1-3].

As opposed to several other European countries, the prevalence of RMDs in Portugal is poor-defined due to the lack of well-designed and consistent epidemiologic studies[1-7]. A nationwide epidemiological study was the way to fulfill this unmet need, and it was also a specific objective of the National Program Against Rheumatic Diseases (PNCDR) (2004-2014)[8]. This program was part of the National Health Plan for 2004/2010 and a contribution of the Portuguese Government to the international "Bone and Joint Decade 2000/2010", an initiative of the United Nations, supported by the World Health Organization[9].

The Portuguese Society of Rheumatology (SPR) is a scientific society that has the mission to increase the knowledge and awareness of RMDs in Portugal. SPR combines its scientific expertise with excellent relationships with other stakeholders, including governmental and regulatory authorities and the pharmaceutical industry[10]. As a result, during the last few years, SPR has attained major achievements as a scientific society, for instance, with the development of national health registries, data collection and analyses of large databases[7,11]. SPR had previously recognized that an epidemiologic study of RMDs was an unmet need in Portugal, but it had been repeatedly postponed due to financial constraints. In 2011, the joint efforts of SPR, governmental entities, the pharmaceutical industry and the commitment of the investigators of the study allowed the development of the first large epidemiologic and population-based study of RMDs in Portugal (EpiReumaPt). The main aim of EpiReumaPt was to estimate the prevalence of RMDs, namely hand, knee and hip osteoarthritis (OA), low back pain (LBP), rheumatoid arthritis (RA), fibromyalgia (FM), gout, spondyloarthritis (SpA), periarticular diseases (PD), systemic lupus erythematosus (SLE), polymyalgia rheumatica (PMR), and osteoporosis (OP) in the adult Portuguese population. The secondary aims were to determine the impact of RMDs on function, quality of life, mental health, work status and use of health care resources, in line with the objectives of the PNCDR. The rigorous methodology and large scale of the study were unprecedented in Portugal represents an important contribution of rheumatology as a specialty moving towards excellence standards of epidemiological and clinical research in Portugal.

This paper describes in detail the methodology of EpiReumaPt, including its objectives and study design, how recruitment was conducted, and gives the first insight into study participation and data preparation for analyses. Specific practical issues and management strategies of EpiReumaPt are addressed in another article published in the same issue of this Journal[12].

GEOGRAPHICAL SETTING OF EPIREUMAPT

Portugal is a Southwestern European country that includes the mainland and the two archipelagos, Madeira and Azores. According to the 2011 census, Portugal has a resident population of 10,562,178 inhabitants, of which 8 million are adults (4,072,122 men and 4,585,118 women)[13]. As in other European countries, the age gap between young and older people increased in the last decade. In fact, according to Portuguese CENSUS the percentage of young adults (18-29 years-

FIGURE 1. Portuguese population density distribution according to NUTS II
NUTS II- Nomenclature of Territorial Units for Statistics (Norte, Centro, Alentejo, Algarve, Lisboa, Madeira and the Azores)

old) decreased from 16% in 2001 to 5.1% in 2011. Among the elderly population (>65 years-old) the opposite trend was observed, rising from 16% in 2001 to 19% in 2011[13].

Portugal is divided in 7 regions according to the Nomenclature of Territorial Units for Statistics II (NUTS II) - *Norte, Centro, Lisboa e Vale do Tejo, Alentejo, Algarve, Região Autónoma dos Açores* (the Azores) *and Região Autónoma da Madeira* (Madeira). At the NUTS II level, the *Norte* region has the largest population density (34.7 %) followed by *Lisboa e Vale do Tejo* (26.6%) and *Centro* (22.4%) (Figure 1). The others NUTS II regions (*Alentejo, Algarve, the* Azores and Madeira) encompass small towns and villages with a lower population density and higher desertification rates.

MATERIALS AND METHODS

STUDY POPULATION

The study population was composed by non-institutionalized adults (≥18 years-old) living in private households in Portugal (Mainland and the Islands - Madeira and the Azores).

Exclusion criteria were: residents in hospitals, nursing homes, military institutions or prisons, and individuals unable to speak Portuguese or unable to complete the questionnaire, despite being aided [7].

STUDY DESIGN

EpiReumaPt is a national, cross-sectional, population-based study conducted from September 2011 to December 2013 and involved a three-stage approach (Figure 2).

First phase (RMD disease screening): face to face interviews were performed by interviewers (non-physicians, trained for this purpose), at each participant's household. The interviews were conducted with a Computer Assisted Personal Interview (CAPI) system. A detailed and comprehensive questionnaire including a screening for RMDs symptoms was applied (available upon request). Participants were inquired about self-reported RMD and subsequently about specific rheumatic and musculoskeletal symptoms. Finally, an algorithm for the screening of specific RMD was applied. In addition, subjects were inquired about socio-demographics, socio-economics, life style, healthcare resources consumption, functional status, quality of life, mental health, work status, and other diseases.

An individual was considered to have a positive screening if the subject mentioned a previously known RMD, if any of the algorithms in the screening questionnaires was positive, or if the subject reported muscle, vertebral or peripheral joint pain in the previous 4 weeks. The overall performance of the screening algorithm was evaluated (the gold standard was considered the final diagnosis after revision, see phase 3) and the overall sensitivity of the screening questionnaire for RMD was 98%, with a specificity of 22%. The positive predictive value was 85% and the negative predictive value was 71%.

Second phase (RMD Diagnosis): In order to determine the RMD diagnosis, a clinical observation by a rheumatologist was offered to subjects who screened positive for at least one RMD and also to 20% of individuals with no rheumatic complaints, during the first phase of the study. In total, 95 rheumatologists were involved. They were blinded to the screening results and received instructions on how to conduct the history and physical examination, following a standardized protocol. They could also request for new laboratory and imaging tests during the appointment. Participants were asked to bring their previous imaging and labo-

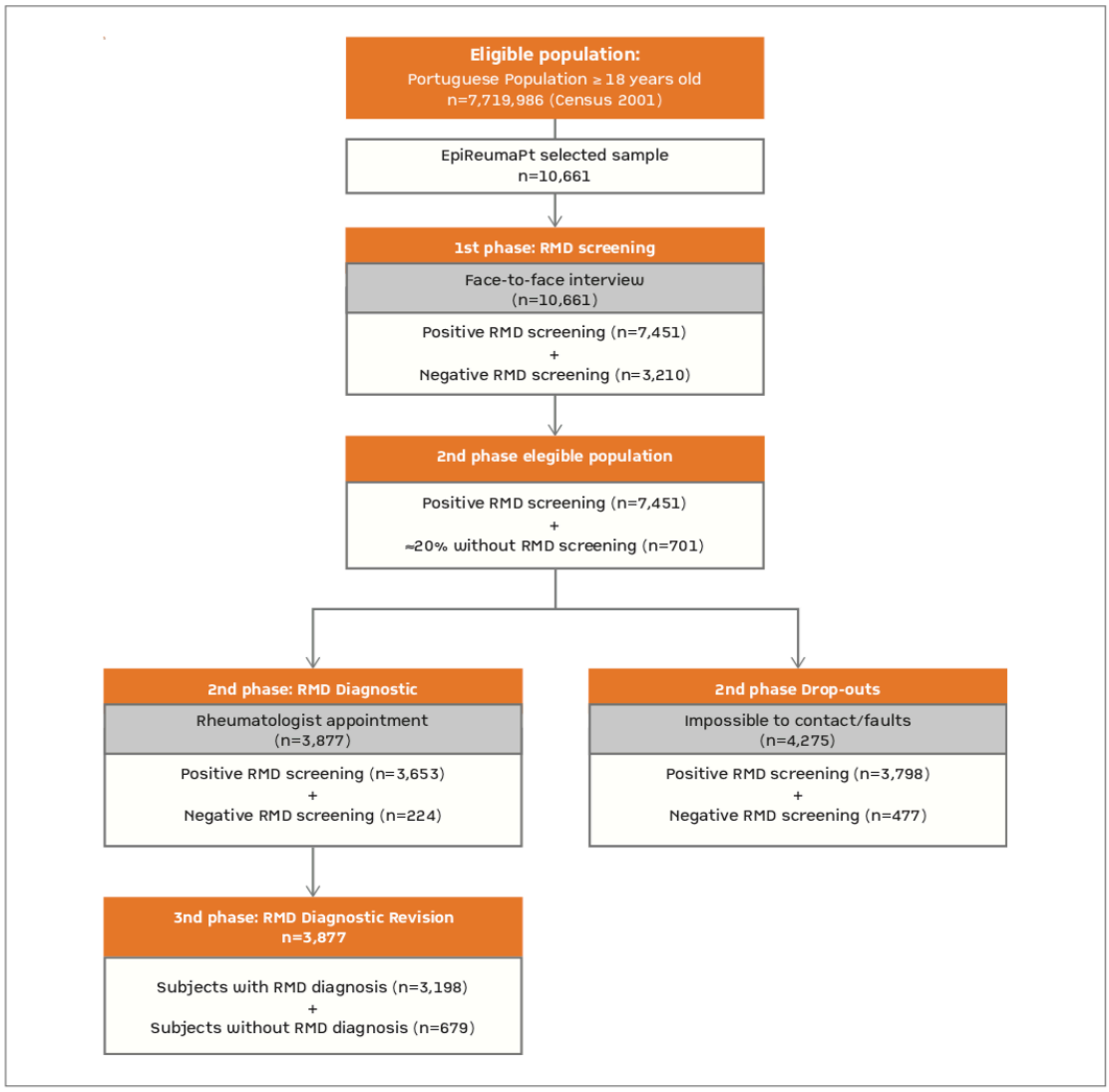

FIGURE 2. Flowchart of recruitment in the EpiReumaPt Study
RDM: Rheumatic and Musculoskeletal diseases

ratory results. Computed assisted software specifically designed for the study was used to support clinical appointment registries. First, the rheumatologist collected the clinical history in a standardized way and placed all the diagnostic hypotheses. The hypotheses where then selected in a dedicated EpiReumaPt s software and specific questions related to the possible diagnosis were asked. For each RMD that was studied in EpiReumaPt, the research team developed specific questions, including those related to validated classification criteria that should be completed, according to the diagnostic hypothesis previously selected. Finally, the physician had to go through a checklist, in order to verify if the patient fulfilled the pre-established diagnostic criteria (see case definition). If needed, laboratory testing and radiographic examinations were performed at the participant's Primary Care Center in order to confirm the diagnostic hypothesis.

The clinical assessments were performed at the Primary Care Center of the participant's neighborhood. A mobile van, fully equipped, was used to perform imaging and laboratory tests: X-ray of the affected joint(s), peripheral dual energy X-ray and blood tests. A multidisciplinary team with a rheumatologist, an X-Ray technician, a nurse, a staff coordinator and a driver supported the clinical visits.

Third phase (RMD Diagnostic Validation): Using the results from the laboratorial and imaging tests previously requested, a team of three experienced rheumatologists reviewed all the clinical data from each participant in order to validate the diagnostic decision made in the second phase. Moreover, when a patient was referred to a rheumatology center due to a suspected inflammatory disease in the second phase, follow-up information from that center was also used. A specific protocol was developed to support these tasks. When data were insufficient to fulfill international classification criteria, a meeting with 5 rheumatologists took place in order to reach an agreement on the final diagnosis based on expert opinion. When doubts persisted regarding the final diagnosis, the opinion of the rheumatologist that performed the clinical assessment (second phase) prevailed. Diagnostic agreement between the 3 reviewers was 98.3% with a Cohen s K coefficient of 0.87 (95%CI from 0.83 to 0.91).

Sampling and recruitment

The sample size was calculated by taking into account the prevalence of RA, as described in the study protocol [7]. The participants were selected through a process of multistage random sampling. The sample was stratified according to the Portuguese statistic regions NUTS II in the 2001 Census and the size of the population (less than 2,000; 2,000-9,999; 10,000-19,999; 20,000-99,999; and ≥100,000 inhabitants). The number of participants of each stratum was proportional to the actual distribution of the population. In Madeira and the Azores we increased the sample size (oversampling) to allow separate analyses in these regions.

Candidate households were selected through a random route process: sampling points were randomly selected on the maps of each locality, where the interviewer began a systematic step count (defined for each locality according to its size), granting each household and each individual an equal probability of being chosen. Dwellings with commercial or industrial purposes, private or public institutions and visibly unoccupied buildings were considered ineligible. In the household, the individual over 18 years old with permanent residence and with the most recently completed birthday was selected. The population recruitment was led by *Centro de Estudos e Sondagens de Opinião da Universidade Católica Portuguesa* (CESOP-UCP). Each interviewers team worked daily on the field (week and weekend) in groups of 4 or 5 elements, and covering a different route. When no subject was found in a first visit of the selected household, he/she could not be replaced, unless that household had been visited in three different times, including evenings and weekends.

Quality control of interviews was performed through a random evaluation of the interviews and recheck of the participants' eligibility criteria. Specifically, each interviewer had 25% of his interviews submitted to a quality control telephone contact, in order to assess the reliability of the answers. The selection of households and the selection of respondents were also submitted to a quality control.

MEASUREMENTS AND ASSESSMENTS

Case definition

RMD diagnoses were performed according to the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for RA[14]; the ACR criteria for knee OA[15], hip OA[16], hand OA[17], FM[18] , SLE[19] and gout[20]; the Assessment of SpondyloArthritis international Society (ASAS) criteria for axial and peripheral SpA[21-23]; and the Bird criteria for PMR[24]. PD was defined as a regional pain syndrome affecting muscles, tendons, bursas or periarticular soft tissues, with or without evidence of joint or bone involvement. The following PDs were specifically searched: tenosynovitis, adhesive capsulitis of the shoulder, enthesopathies, bursitis, palmar or plantar fasciitis, and carpal or tarsal tunnel syndrome, present at the time of the interview. The PD diagnosis was established based on expert opinion after reviewing clinical history, physical exam, ultrasound and electromyography (when available). OP was defined by decision of the rheumatologist based on the presence of at least one of the following: previous fragility fracture, self-reported OP diagnosis, current OP treatment or fulfillment of the WHO criteria[25] when lumbar and/or femoral neck dual energy X-ray absorptiometry (DEXA) was available. Low back pain (LBP) was defined solely by self-reported symptoms.

SECONDARY VARIABLES DESCRIPTION

In the 1st phase of EpiReumaPt, subjects were asked about their socio-demographic data (age, gender, ethnicity, education, marital status), socio-economic profile (measures of wealth [used to generate income quintiles], household income, work status) and life style habits (alcohol and coffee intake, current smoking and physical exercise). Work disability was evaluated by absenteeism, presenteeism, early retirement and unemployment due to work disability. Healthcare resource consumption data was collected considering the number and type of outpatient clinic visits, hospitalizations, homecare assistance and other needs for healthcare services in the previous 12 months.

Health-related quality of life was evaluated using the European Quality of Life questionnaire with five dimensions and three levels (EQ-5D-3L)[26,27] and also the Short Form (36) Health Survey (SF-36)[28]. Physical function was assessed by the Health Assessment Questionnaire (HAQ)[29], anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HADS)[30]. We used Portuguese validated versions of all these assessment scales. Anthropometric data (self-reported weight and height) and self-reported chronic diseases (high cholesterol level, high blood pressure, allergy, gastrointestinal disease, mental disease, cardiac disease, diabetes, thyroid and parathyroid disease, urolithiasis, pulmonary disease, hyperuricemia, neoplastic disease, neurologic disease, hypogonadism) were also searched. Finally, information regarding pharmacological and non-pharmacological therapies was collected.

In the 2nd phase of EpiReumaPt, data concerning the medical history and physical examination were collected. Questions about previous diagnosis of RMD, medication and the need for medical visits due to RMD symptoms in the previous year were also performed. Validated instruments (eg. disease activity score 28 (DAS28) for RA and knee injury and osteoarthritis outcome score (KOOS) for knee OA) were applied by the rheumatologist according to the patient diagnosis.

BLOOD SAMPLING

A blood sample was drawn whenever subjects attended the second phase of the EpiReumaPt study and signed the informed consent for the procedure. Patients with known hepatitis C, HIV infection or debilitating conditions were excluded. A 15-25 ml whole blood sample was obtained; serum was separated by centrifuging (800g, 10 minutes) the sample in the mobile van and kept kept in the fridge at 4ºC. Blood samples from 3,664 participants were sent in a cooler on the same day or within two days[12] to Biobanco-IMM. Serum and whole blood samples where aliquoted in 250µL and 2mL respectively and stored at -80ºC. DNA extraction was performed by Qiacube (Qiagen, Venlo, Netherlands) from 200µL of the whole blood. The DNA was stored at -80ºC in 100µL aliquots. The content of the EpiReumaPt biobank is described in Table III. Serum and whole blood samples were also sent to the Central Diagnostic Laboratory Germano de Sousa (Lisbon, Portugal), if deemed necessary by the rheumatologist to perform laboratory tests.

LABORATORY PROCEDURES

The different laboratorial paramethers were measures according to the respective manufacturer's instructions: rheumatoid factor was measured by chemiluminescence; uric acid was quantified by a modification of uricase method first published by Bulger and Johns, modified by Kalckar; C-reactive protein was determined by immunoturbidimetric method; urea was measured by kinetic enzymatic method urease / glutamate dehydrogenase; total creatine kinase (CK) was measured by creatinine phosphate method; and complement fractions C3 and C4 were detected by turbidimetry, on an Dimension Vista 1500 Intelligent Lab System (Siemens, Erlangen, Germany), applying reagents from Siemens (Siemens, Erlangen, Germany). Thyroid stimulating hormone (TSH) and Free thyroxine (FT4) were detected by chemiluminescence, on an Advia Centaur XP (Siemens, Erlangen, Germany), applying reagents from Siemens (Siemens, Erlangen, Germany). Antibodies against Cyclic Citrullinated Peptides (anti-CCP) and antibodies against double stranded DNA (anti-dsDNA) were measured by automated fluoroimmunoassay, on an Immunocap250 (Thermo Scientific, Uppsala, Sweden), applying reagents from ELiA-Phadia (Thermo Scientific, Uppsala, Sweden). Human Leukocyte Antigen-B27 (HLA-B27) was measured flow cytometry, on a FacsCalibur (Becton Dickinson, New Jersey, USA), applying reagents from BD Bioscience (Becton Dickinson, New Jersey, USA). Antinuclear antibodies (ANA) were measured by indirect fluoroimmunoassay, applying reagents from Euroimmun (Euroimmun, Luebeck, Germany). Full blood count and erythrocyte sedimentation rate (ESR) were measured in whole blood samples. Hemoglobin was quantified by Surfactant Sodium Lauryl Sulfate Colorimetric, Mean Corpuscular Volume (MCV) was

measured by flow cytometry with hydrodynamic focusing, and leukocytes, lymphocytes and neutrophils were measured by flow cytometry with side light scatter, forward scatter and fluorescence intensity. ESR was measured by microphotometry capillary flow.

Peripheral DXA procedures

All participants who attended the second phase of the study had a wrist DXA at the mobile unit on a PIXI™ LUNAR device (a peripheral Instantaneous X-ray Imager). This procedure provided precise assessment of bone mineral density (BMD) with excellent image resolution (0.2 mm pixels). PIXI is a peripheral densitometer that allows the operator to examine both the *calcaneus* and the forearm. PIXI employs the dual-energy x-ray absorptiometry technique. A total of 3,342 participants had a forearm bone mineral density evaluation.

X- Ray procedures

Participants who attended the second phase had performed wrist and calcaneus X-ray and bone mineral assessment on a high resolution digital X-Ray machine (D3A, France) in the mobile unit, in order to assess bone microanalysis (BMA). Moreover, X-rays of the affected joint or joints were also performed on BMA high-resolution digital X-ray machine (D3A, France) as requested by the rheumatologist. The content of the EpiReumaPt imaging reservoir is described in Table III.

STATISTICAL ANALYSIS

EpiReumaPt was designed to obtain a representative sample of the Portuguese population. This population will be subject of many other future analyses. Exactly in order to guarantee its representativity, the design effect will need to be taken into account. This can be achieved by using weighted proportions that have, for this matter, been computed.

For the main sample, the initial extrapolation weights were calculated as the inverse of the inclusion probabilities, taking into account the sampling design, i.e., a stratified two-stage cluster sampling design. The stratification was based on the seven NUTS II regions and on five classes of the number of inhabitants per locality (<2,000; 2,000-9,999; 10,000-19,999; 20,000-99,999; >99,999). In each stratum, the first sampling stage consisted in the selection of localities with a probability proportional to its size (number inhabitants aged 18 years old or more), except for localities where the number of inhabitants was larger than 20,000, where all the localities were selected. In the second stage, households were selected using a pseudo-random selection procedure equivalent to the equal probability selection. These weights were submitted to a calibration process by crossing region (seven classes), size of locality (five classes), gender (two classes) and seven age categories (18-25, 26-35, 36-45, 46-55, 56-65, 66-75 and ≥76 years old). This procedure was used to reproduce the known population totals for the crossing margins of these four variables.

A sub-sample was drawn selecting all individuals with positive screening for RMDs and 20% of those with negative screening. For this sub-sample, inclusion probabilities were calculated considering the result of the screening and adjustment for non-response. This last adjustment was used because not all individuals selected for the second phase actually attended the assessment by the rheumatologist. The basic extrapolation weights obtained from these procedures were again submitted to a calibration process by crossing two classes of region (one collecting all the mainland regions and a different one gathering the two autonomic regions), gender (2 classes), four age categories (resulting from the aggregation of the original classes in 18-35, 36-55, 56-75 and ≥76 years old) and result of the RMD screening (positive/negative) in order to reproduce the known national totals for the crossing margins of these four variables. The decision on the variables used for this second stage calibration was based on a generalized linear model (positive diagnostic for several rheumatic diseases was used as dependent variable) that identified the most important criteria related to the prevalence of RMDs. These weighted proportions will be used in several future analyses, including the estimation of the prevalence of the RMDs (study's primary objective), which will be a matter of a separate manuscript.

ETHICAL ISSUES AND PERSONAL PROTECTION

The EpiReumaPt study was performed according to the principles established by the Declaration of Helsinki. The study was reviewed and approved by the National Committee for Data Protection (*Comissão Nacional de Proteção de Dados*) and by the NOVA Medical School Ethics Committee. Ethical Committees of Regional Health Authorities (ARS) also reviewed and approved the study. According to the Portuguese law, all

subjects provided informed consented to participate in the EpiReumaPt study. Individuals also consented to give a blood sample for storage in Biobanco-IMM and to be re-contacted if needed. Data protection was assured by a data encryption process, which kept the confidentiality and anonymity of each study subject. Decryption was only possible with a secure password only known by the Principal Investigator. This study was conducted according to the good practices in research.

REPORTING OF DIAGNOSIS AND TEST RESULTS

During the assessment by the rheumatologist in phase 2, all patients with a new diagnosis of a chronic inflammatory rheumatic disease were referred to a rheumatology center for follow-up. Other non-inflammatory newly diagnosed RMDs were referred to the primary care physician. Each participant who performed laboratory tests received a letter reporting the test results. If a clinically significant abnormality was depicted in the laboratorial results or X-rays, the participant was also advised to see his/her doctor for further investigation.

RESULTS

The EpiReumaPt population is comparable to the Portuguese population, as confirmed with data from the Portuguese National Institute of Statistics (Census 2011)[13, 31] (Table I).

PARTICIPATION ANALYSIS

The EpiReumaPt study recruited 10,661 subjects and 64% had a positive screening for at least one RMD. Moreover, out of the 8,152 eligible subjects, 3,877 entered the second phase and were evaluated by a rheumatologist. Individuals who attended the observation by the rheumatologist did not differ from those who did not except for the screening diagnosis, age group, gender and residence region according to the NUTS II (Table II). These variables were considered in the weighted model used to calculate the prevalence of RMD. Furthermore, a sensitivity analysis was performed and no differences in health status (including quality of life and functional status) were found between participants and dropouts of the second phase according to age groups, NUTS II and comorbidities (data not shown).

DISCUSSION

EpiReumaPt is the first large-scale epidemiological population-based study that evaluated RMDs in Portugal. EpiReumaPt has a unique study design: the first phase with a face to face questionnaire that aimed at screening for the presence of RMD symptoms and specific RMDs; the second phase, comprising a clinical observation performed by rheumatologists in primary care units near the participants' residence in order to have the RMD diagnosis firmly established by a specialist; and the third phase, consisting of a rigorous case review that aimed to homogenize the diagnostic criteria and validate the definitive RMD diagnosis. With this study design we were able to diagnosis new RMDs, to correct the misinformation of some self-reported diagnosis and to refine RMDs with a standardized case definition.

EpiReumaPt has also unique features when compared to other studies performed in Portugal and abroad[1,2,4,32-36]: It is a population-based study, with a representative sample of the Portuguese population and it covers an extensive range of topics that go beyond rheumatology. Unlike the recruitment performed by mail as in the Spanish (Episer)[37] and the Greek studies[5, 38] that also evaluated the prevalence of RMD, recruitment in EpiReumaPt was done by a random route technique with a face to face interview, which reduced selection bias. The EpiReumaPt screening algorithm was specifically developed for this study and designed to be highly sensitive in order to capture the maximum number of RMDs cases. Finally, our case definition included the most recent classification criteria for several RMDs such as the classification criteria of the ACR/EULAR for RA[14] and the ASAS criteria for SpA[21,23]. A comparison with Census 2011 allowed the development of different weights to be applied in the samples from 1st and 2nd phases, which will improve the accuracy of further analyses and estimates.

The concerted action from research groups, health and governmental authorities, pharmaceutical companies, the SPR and the population has resulted in a very large database and has triggered extensive research activities and collaborations. EpiReumaPt has initiated collaboration with various research groups in Portugal and other European countries and in the USA. Procedures for data access are established, and a dedicated team of researchers is currently working on EpiReumaPt data covering studies within a wide range of medical topics. Moreover, the EpiReumaPt image

TABLE I. SOCIO-DEMOGRAPHIC AND HEALTH RELATED CHARACTERISTICS OF THE ADULT PORTUGUESE POPULATION: EPIREUMAPT (1ST AND 2ND PHASE POPULATIONS) AND CENSUS 2011 POPULATIONS (PORTUGUESE POPULATION)

Demographic characteristics	1st phase study population n=10,661	2nd phase study population n=3,877	CENSUS 2011
Gender (female)	6,551 (52.6%)	2,630 (52.5%)	4,585,118 (53.0%)
Age group			
18-29	1,182 (22.1%)	190 (21.0%)	1,470,782 (17.0%)
30-39	1,511 (18.8%)	403 (19.3%)	1,598,250 (18.5%)
40-49	1,906 (17.3%)	680 (18.2%)	1,543,392 (17.8%)
50-59	1,801 (14.8%)	818 (14.7%)	1,400,011 (16.2%)
60-69	1,915 (12.9%)	914 (13.4%)	1,186,442 (13.7%)
70-74	849 (5.8%)	376 (5.3%)	496,438 (5.7%)
≥75	1,497 (8.4%)	496 (8.0%)	961,925 (11.1%)
Ethnicity/Race			
Caucasian	10,342 (96.0%)	3,786 (93.3%)	
Black	221 (3.4%)	64 (6.1%)	
Asian	8 (0.1%)	2 (0.0%)	No comparable data
Gipsy	20 (0.3%)	3 (0.1%)	
Other	38 (0.3%)	13 (0.5%)	
Education level			
>12 years	1,764 (20.4%)	508 (21.1%)	1,741,567 (20.1%)
10-12 years	1,920 (23.8%)	575 (23.2%)	1,560,958 (18.0%)
5-9 years	2,175 (22.6%)	775 (22.4%)	2,134,401 (24.6%)
0-4 years	4,726 (33.2%)	1,997 (33.4%)	3,239,724 (37.4%)
NUTS II			
Norte	3,122 (34.9%)	1,050 (37.2%)	3,007,823 (34.7%)
Centro	1,997 (22.8%)	856 (19.8%)	1,938,815 (22.4%)
Lisboa	2,484 (26.7%)	708 (29.6%)	2,300,053 (26.6%)
Alentejo	669 (7.3%)	273 (5.8%)	633,691 (7.3%)
Algarve	352 (3.8%)	144 (3.1%)	370,704 (4.3%)
Azores	1,029 (2.2%)	420 (2.3%)	192,357 (2.2%)
Madeira	1,008 (2.3%)	426 (2.2%)	213,797 (2.5%)
Marital status			
Single	1,935 (29.4%)	456 (32.2%)	
Married	6,111 (50.2%)	2,460 (49.9%)	
Divorced	810 (7.4%)	310 (7.3%)	No comparable data
Widower	1,414 (8.2%)	550 (7.6%)	
Consensual union	382 (4.8%)	99 (3.1%)	
BMI			
Underweight	167 (2.2%)	46 (1.1%)	
Normal	4,063 (45.5%)	1,234 (46.4%)	No comparable data
Overweight	3,799 (35.1%)	1,485 (34.3%)	
Obese	2,080 (17.1%)	924 (18.1%)	
Socio-economics			
Household income*			
<500€	1,994 (19.9%)	795 (21.8%)	
501€to 750€	1,707 (21.7%)	710 (20.4%)	
751€to 1000€	1,268 (18.8%)	511 (18.9%)	No comparable data
1001€to 1500€	1,141 (17.2%)	403 (15.9%)	
1501€to 2000€	657 (9.9%)	246 (10.3%)	

Continues on the next page

TABLE I. SOCIO-DEMOGRAPHIC AND HEALTH RELATED CHARACTERISTICS OF THE ADULT PORTUGUESE POPULATION: EPIREUMAPT (1ST AND 2ND PHASE POPULATIONS) AND CENSUS 2011 POPULATIONS (PORTUGUESE POPULATION) – (CONTINUE)

Demographic characteristics	1st phase study population n=10,661	2nd phase study population n=3,877	CENSUS 2011
2001€to 2500€	379 (5.9%)	118 (4.7%)	
2501€to 3000€	222 (3.0%)	73 (4.7%)	
3001€to 4000€	146 (1.8%)	43 (16%)	
>4000€	99 (1.9%)	26 (1.7%)	
Employment status			
Employed full-time	3,993 (42.8%)	1,221 (42.6%)	
Employed part-time	345 (4.6%)	117 (3.5%)	
Domestic worker	660 (3.9%)	286 (3.3%)	
Unemployed	1,087 (12.0%)	390 (13.7%)	No comparable data
Student	428 (8.4%)	58 (4.8%)	
Temporally work disabled	160 (1.2%)	80 (12.5%)	
Retired	3,758 (24.9%)	1,636 (26.4%)	
Others	229 (2.2%)	89 (4.5%)	
Quality of life EQ5D Score	0.83 ± 0.23	0.81 ± 0.24	No comparable data
HAQ (0-3)	0.26 ± 0.54	0.27 ± 0.53	
Life Style Habits			
Current coffee intake			
None	3,374 (29.1%)	1,263 (30.2%)	
1 to 3	6,364 (59.1%)	2,331 (59.5%)	No comparable data
More than 3	908 (11.9%)	277 (10.4%)	
Current alcohol intake			
Daily	2,050 (20.2%)	773 (20.8%)	
Occasionally	3,967 (42.6%)	1,305 (46.0%)	No comparable data
Never	4,625 (37.1%)	1,794 (33.2%)	
Current smoking habits			
Daily	1,854 (23.2%)	526 (20.8%)	
Occasionally	246 (2.7%)	67 (2.2%)	No comparable data
Never	8,554 (74.1%)	3,282 (77.0%)	
Physical exercise	3,499 (37.0%)	1,182 (37.3%)	No comparable data
Number of comorbidities (self-reported)	1.55 ± 1.80	1.71 ± 1.83	No comparable data
High cholesterol level	3,360 (24.4%)	1,556 (25.4%)	
High blood pressure	3,369 (23.1%)	1,528 (23.2%)	
Allergy	2,287 (21.3%)	985 (23.6%)	
Gastrointestinal disease	1,837 (14.9%)	907 (17.4%)	
Mental disease	1,619 (12.9%)	764 (11.1%)	
Cardiac disease	1,366 (10.5%)	641 (11.7%)	
Diabetes	1,217 (8.3%)	539 (8.8%)	No comparable data
Thyroid and parathyroid disease	941 (7.0%)	484 (10.5%)	
Renal colic	885 (7.0%)	426 (8.8%)	
Pulmonary disease	637 (5.4%)	295 (6.0%)	
Hyperuricemia	690 (5.2%)	332 (4.7%)	
Neoplasic disease	439 (3.4%)	208 (3.6%)	
Neurologic disease	418 (3.3%)	183 (3.7%)	
Hypogonadism	90 (0.7%)	40 (0.6%)	

*household income in the last month

Sample size is not constant due to missing data in: **1st Phase EpiReumaPt study:** Ethnicity (n=10,629), Education level (n=10,585), Marital status (n=10,652), BMI (n=10,109), Household income (n=7,613), EQ5D Score (n=10,596), Current coffee intake (n=10,646), Current alcohol intake (n=10,646), Current smoking habits (n=10,645), Physical exercise (n=10,654), Number of Comorbidities (n=9,601), High cholesterol level (n=10,514), High blood pressure (n=10,582), Allergy (n=10,570), Gastrointestinal disease (n=10,572), Mental disease (n=10,593),

Continues on the next page

Continuation
Cardiac Disease (n=10,563), Diabetes (n=10,587), Thyroid and parathyroid disease (n=10,557), Renal colic (n=10,543), Pulmonary disease (n=10,594), Hyperuricemia (n=10,458), Neoplasic disease (n=10,602), Neurologic disease (n=10,581), Hypogonadism (n=10,445)
2nd phase EpiReumaPt study: Ethnicity (n=3,868), Education level (n=3,855), Marital status (n=3,875), BMI (n=3,689), Household income (n=2,925), EQ5D Score (n=3,846), Current coffee intake (n=3,871), Current alcohol intake (n=3,871), Current smoking habits (n=3,871), Physical exercise (n=3,874), Number of Comorbidities (n=3,398), High cholesterol level (n=3,825), High blood pressure (n=3,851), Allergy (n=3,845), Gastrointestinal disease (n=3,835), Mental disease (n=3,855), Cardiac Disease (n=3,833), Diabetes (n=3,840), Thyroid and parathyroid disease (n=3,834), Renal colic (n=3,835), Pulmonary disease (n=3,855), Hyperuricemia (n=3,799), Neoplasic disease (n=3,854), Neurologic disease (n=3,847), Hypogonadism (n=3,785)
The data presented in the CENSUS 2011 columns was obtained from the National Institute of Statistics.
NUTS II- Nomenclature of Territorial Units for Statistics (Norte, Centro, Alentejo, Algarve, Lisboa, Madeira and the Azores); **BMI**- Body Mass Index; **EQ5D**- European Quality of Life questionnaire five dimensions three levels; **HAQ**- Health Assessment Questionnaire
The estimated values for the characteristics were obtained considering study design.

TABLE II. COMPARISON BETWEEN EPIREUMAPT SUBJECTS INCLUDED IN PHASE 2 WITH THOSE NOT PARTICIPATING DESPITE BEING ELIGIBLE

	Second phase participants n=3,877	Second phase drop-outs n=4,275
Individuals without Rheumatic Disease (701 individuals selected to medical consultation)	**224** (31.9%)	**477** (68.0%)
Gender		
Female	**2,628** (67.8%)	**2,784** (65.1%)
Age	**57.10** (±15.48)	**55.24** (±18.95)
NUTSII		
Norte	**1,050** (27.1%)	**1,313** (30.7%)
Centro	**856** (22.1%)	**765** (17.9%)
Lisboa	**708** (18.3%)	**1,146** (26.8%)
Alentejo	**273** (7.0%)	**247** (5.8%)
Algarve	**144** (3.7%)	**132** (3.1%)
Azores	**420** (10.8%)	**335** (7.8%)
Madeira	**426** (11.0%)	**337** (7.9%)
Years of education	**6.81 (±3.94)**	**6.98 (±4.17)**
Household income		
<500€	**795** (27.2%)	**862** (28.9%)
501€to 750€	**710** (24.3%)	**674** (22.6%)
751€to 1000€	**511** (17.5%)	**463** (15.5%)
1001€to 1500€	**403** (13.8%)	**435** (14.6%)
1501€to 2000€	**246** (8.4%)	**232** (7.8%)
2001€to 2500€	**118** (4.0%)	**142** (4.8%)
2501€to 3000€	**73** (2.5%)	**81** (2.7%)
3001€to 4000€	**43** (1.5%)	**55** (1.8%)
>4000€	**26** (0.9%)	**41** (1.4%)
Employment status		
Full-time employee	**1,221** (31.8%)	**1,493** (35.2%)
Unemployed	**390** (10.2%)	**391** (9.2%)
Retired	**1,636** (42.6%)	**1,679** (39.6%)
Student	**58** (1.5%)	**149** (3.5%)
EQ5D	**0.72 (±0.27)**	**0.75 (±0.27)**
HAQ	**0.50 (±0.64)**	**0.43 (±0.65)**

Continues on the next page

TABLE II. COMPARISON BETWEEN EPIREUMAPT SUBJECTS INCLUDED IN PHASE 2 WITH THOSE NOT PARTICIPATING DESPITE BEING ELIGIBLE (CONTINUE)

	Second phase participants n=3,877	Second phase drop-outs n=4,275
Positive RMD screening diagnosis		
Low back pain	648 (53.3%)	567 (46.7%)
Inflammatory low back pain	1,263 (55.4%)	1,015 (44.6%)
Spondyloarthritis	2,119 (52.5%)	1,919 (47.5%)
Rheumatoid arthritis	2,002 (54.2%)	1,694 (45.8%)
Osteoarthritis	2,660 (51.9%)	2,465 (48.1%)
Fibromyalgia	822 (56.9%)	623 (43.1%)
SLE	694 (54.2%)	587 (45.8%)
Gout	624 (53.6%)	539 (46.3%)
PMR	300 (59.3%)	206 (40.7%)
Osteoporosis	983 (52.4%)	894 (47.6%)
Periarticular disease	2,405 (53.1%)	2,127 (46.9%)
Self-reported previous RMD diagnosis	1,604 (43.1%)	1,310 (31.7%)
Rheumatoid arthritis	221 (59.1%)	153 (40.9%)
Spondyloarthritis	93 (60.4%)	61 (39.6%)
Psoriatic arthritis	14 (60.9%)	9 (39.1%)
Osteoarthritis	635 (54.3%)	535 (45.7%)
Osteoporosis	393 (54.5%)	328 (45.5%)
Gout	57 (65.5%)	30 (34.5%)
Polymyalgia rheumatica	11 (45.8%)	13 (54.2%)
SLE	11 (47.8%)	12 (52.2%)
Fibromyalgia	66 (68.0%)	31 (32.0%)
Periarticular diseases	224 (62.2%)	136 (37.8%)
Comorbidities		
High cholesterol level	1,556 (40.7%)	1,410 (33.6%)
High blood pressure	1,528 (39.7%)	1,446 (34.2%)
Allergy	985 (25.6%)	910 (21.5%)
Gastrointestinal disease	907 (23.6%)	782 (18.5%)
Mental disease	764 (19.8%)	713 (16.8%)
Cardiac disease	641 (16.7%)	615 (14.5%)
Diabetes	539 (14.0%)	528 (12.4%)
Thyroid and parathyroid disease	484 (12.6%)	386 (9.1%)
Urolithiasis	426 (11.1%)	382 (9.1%)
Pulmonary disease	295 (7.6%)	259 (6.1%)
Hyperuricemia	332 (8.7%)	323 (7.7%)
Neoplasic disease	208 (5.4%)	192 (4.5%)
Neurologic disease	183 (4.8%)	203 (4.8%)
Hypogonadism	40 (1.1%)	43 (1.0%)
Rheumatic diseases	1,604 (43.1%)	1,310 (31.7%)
Number of Comorbidities	2.61 ± 2.10	2.09 ± 1.98

Sample size is not constant due to missing data in
Second phase Participants: Years of Education (n=3, 867), Household income (n=2,925), Employment status (n=3, 839), EQ5D (n=3,846), Self-reported previous RMD diagnosis (n=3,171), Self-reported previous RMD diagnosis per disease (n=1,604), High cholesterol level (n=3,825), High blood pressure (n=3,851), Allergy (n=3,845), Gastrointestinal disease (n=3,835), Mental disease (n=3,855), Cardiac Disease (n=3,833), Diabetes (n=3,840), Thyroid and parathyroid disease (n=3,834), Renal colic (n=3,835), Pulmonary disease (n=3,855), Hyperuricemia (n=3,799),

Continues on the next page

Continuation
Neoplasic disease (n=3,854), Neurologic disease (n=3,847), Hypogonadism (n=3,785), Rheumatic diseases (n=3,717), Number of Comorbidities (n=3,398).
Second phase drop-outs: Years of education (n=4,253), Household income (n=2,985), Employment status (n=4,237), EQ5D (n=4,250), Self-reported previous RMD diagnosis (n=4,131), Self-reported previous RMD diagnosis per disease (n=1,307), High cholesterol level (n=4,202), High blood pressure (n=4,233), Allergy (n=4,233), Gastrointestinal disease (n=4,235), Mental disease (n=4,235), Cardiac Disease (n=4,228), Diabetes (n=4,250), Thyroid and parathyroid disease (n=4,227), Renal colic (n=4,211), Pulmonary disease (n=4,238), Hyperuricemia (n=4,172), Neoplasic disease (n=4,249), Neurologic disease (n=4,233), Hypogonadism (n=4,177), Rheumatic diseases (n=4,131), Number of Comorbidities (n=3,793).
Positive Screening Low Back Pain (n=1,215), Inflammatory Low Back Pain (n=2,278), Spondyloarthritis (n=4,038), Rheumatoid Arthritis (n=3,696), Osteoarthritis (n=5,125), Fibromyalgia (n=1,445), SLE (n=1,281), Gout (n=1,163), PMR (n=506), Osteoporosis (n=1,877), Periarticular Pathology (n=4,532).
Regarding the acronyms **NUTS II** stands for the Nomenclature of Territorial Units for Statistics (Norte, Centro, Alentejo, Algarve, Lisboa, Madeira and the Azores), **EQ5D** refers to European Quality of Life questionnaire five dimensions three levels, **HAQ** stands for Health Assessment Questionnaire, and **SLE** - systemic lupus erythematosus.

TABLE III. THE EPIREUMAPT BIOBANK AND IMAGING RESERVOIR

EpiReumaPt biobank	n	Volume per aliquot
Serum	21,219	250 µL
Whole blood	7,476	2 mL
DNA	3,608	100 µL

EpiReumaPt imaging reservoir X-ray area	n
Wrists (BMA)	2,422
Calcaneus (BMA)	2,228
Hands	438
Hips	122
Knees	479
Lumbar spine	1,265
Thoracic spine	691
Cervical spine	206

BMA: bone mineral assessment

and biobank reservoirs constitute a valuable tool to perform a comprehensive approach to the pathophysiology and outcome research of several diseases.

A fundamental premise for population-based studies is high confidence and legitimacy felt by the study population. The strategy to achieve and withhold this confidence in the Portuguese population has been successful, and resulted in high participation rates and enthusiastic public and political support for EpiReumaPt[12]. The confidence and supportive attitude from the population was the trigger to develop an ongoing cohort study with EpiReumaPt subjects[39]. The follow-up of this population goes beyond RMDs. Several other diseases and health related topics are being explored in this cohort.

In conclusion, the strict and robust methodology of EpiReumaPt allowed for a large amount of information to be collected from each participant, and the inclusion of a large number of participants with a wide age range covering an entire country adult population, making EpiReumaPt the largest study on RMDs performed in Portugal. Moreover, the follow-up of this population is ongoing and now goes beyond RMDs. EpiReumaPt will answer several health-related questions and will generate important evidence useful to support health policies in Portugal.

ACKNOWLEDGEMENTS
The EpiReumaPt Study Group would like to aknowledge the invaluable help of: Francisco George, MD; *Faculdade de Medicina da Universidade de Coimbra; Faculdade de Medicina da Universidade de Lisboa; Faculdade de Medicina da Universidade Porto; Biobanco- IMM;* Patient Associations with RMD; Regional Government of Azores; Regional Government of Madeira; Regional Health Administrations of *Norte, Centro, Alentejo, Algarve,* and *Lisboa e Vale do Tejo*; *Centro Hospitalar do Médio Tejo; Hospital de S. João; Câmara Municipal de Lisboa*; *Associação Nacional de Freguesias.*

UNRESTRICTED GRANTS
EpiReumaPt was endorsed by the Alto *Patrocínio da Presidência da República* and was supported by a Grant from Directorate-General of Health. The project was also sponsored by: Fundação Calouste Gulbenkian, Fundação Champalimaud, Fundação AstraZeneca, Abbvie, Merck Sharp & Dohme, Pfizer, Roche, Servier, Bial, D3A Medical Systems, Happybrands, *Centro de Medicina Laboratorial Germano de Sousa, Clínica Médica da Praia da Vitória, CAL-Clínica, Galp Energia, Açoreana Seguros* and individual support of Rheumatologists.

CORRESPONDENCE TO
Ana M. Rodrigues
Unidade de Investigação em Reumatologia,
Instituto de Medicina Molecular, Lisboa, Portugal
E-mail: anamfrodrigues@gmail.com

REFERENCES

1. Badley EM, Rasooly I, Webster GK. Relative importance of musculoskeletal disorders as a cause of chronic health problems, disability, and health care utilization: findings from the 1990 Ontario Health Survey. J Rheumatol 1994; 21: 505-514.
2. Yelin E, Callahan LF. The economic cost and social and psychological impact of musculoskeletal conditions. National Arthritis Data Work Groups. Arthritis Rheum 1995; 38: 1351-1362.
3. Makela M, Heliovaara M, Sievers K, Knekt P, Maatela J, Aromaa A. Musculoskeletal disorders as determinants of disability in Finns aged 30 years or more. J Clin Epidemiol 1993; 46: 549-559.
4. Carmona L, Ballina J, Gabriel R, Laffon A, Group ES. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. Ann Rheum Dis 2001; 60: 1040104-1040105.
5. Andrianakos A, Trontzas P, Christoyannis F, et al. Prevalence of rheumatic diseases in Greece: a cross-sectional population based epidemiological study. The ESORDIG Study. J Rheumatol 2003; 30: 1589-1601.
6. Hill CL, Parsons J, Taylor A, Leach G. Health related quality of life in a population sample with arthritis. J Rheumatol 1999; 26: 2029-2035.
7. Ramiro S, Canhao H, Branco JC. EpiReumaPt Protocol - Portuguese epidemiologic study of the rheumatic diseases. Acta Reumatol Port, 2010; 35: 384-390.
8. D.G.d. Saúde. Programa Nacional Contras as Doenças Reumáticas. s.l. 2004.
9. The Bone and Joint Decade. G.A.f.M. Health. http://bjdonline.org/. May 2015
10. Sociedade Portuguesa de Reumatologia. http://www.spreumatologia.pt. May 2015
11. Canhao H, Rodrigues AM, Mourao AF, et al. Comparative effectiveness and predictors of response to tumour necrosis factor inhibitor therapies in rheumatoid arthritis. Rheumatology (Oxford), 2012; 51: 2020-2026.
12. Gouveia N, Rodrigues AM, Ramiro S, et al. EpiReumaPt: how to perform a national population based study – a practical guide. Acta Reumatol Port. 2015;40:128-136
13. Relatório: Resultados definitivos dos CEnsus 2011, Portugal. I.N.d. Estatística.
14. Aletaha A, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010; 69: 1580-1588.
15. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29: 1039-1049.
16. Altman R, Alarcon G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. Arthritis Rheum 1991; 34: 505-514.
17. Altman R, Alarcon G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. Arthritis Rheum 1990; 33: 1601-1610.
18. Wolfe F, Clauw DJ, Fitzcharles MA, et al. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care Res (Hoboken) 2010; 62: 600-610.
19. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40: 1725.
20. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977; 20: 895-900.
21. Rudwaleit M, Landewe R, van der Heijde D, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. Ann Rheum Dis 2009; 68: 770-776.
22. Rudwaleit M, van der Heijde D, Landewe R, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 2009; 68: 777-783.
23. Rudwaleit M, van der Heijde D, Landewe R, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis 2011; 70: 25-31.
24. Bird HA, Esselinckx W, Dixon AS, Mowat AG, Wood PH. An evaluation of criteria for polymyalgia rheumatica. Ann Rheum Dis 1979; 38: 434-439.
25. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994; 9: 1137-1141.
26. Ferreira LN, Ferreira PL, Pereira LN, Oppe M. EQ-5D Portuguese population norms. Qual Life Res 2014; 23: 425-430.
27. Ferreira LN, Ferreira PL, Pereira LN, Oppe M. The valuation of the EQ-5D in Portugal. Qual Life Res, 2014; 23: 413-23.
28. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992; 30: 473-483.
29. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980; 23: 137-145.
30. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M. Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. Psychol Health Med 2007; 12: 225-235; quiz 235-237.
31. Hyphantis T, Kotsis K, Tsifetaki N, et al. The relationship between depressive symptoms, illness perceptions and quality of life in ankylosing spondylitis in comparison to rheumatoid arthritis. Clin Rheumatol 2013; 32: 635-644.
32. Riise T, Moen BE, Nortvedt MW. Occupation, lifestyle factors and health-related quality of life: the Hordaland Health Study. J Occup Environ Med 2003; 45: 324-332.
33. Truelsen T, Nielsen N, Boysen G, Gronbaek N. Self-reported stress and risk of stroke: the Copenhagen City Heart Study. Stroke 2003; 34: 856-862.
34. Matos AA, Silva JC, Queiroz MV, Pádua F. Inquérito epidemiológico das doenças reumáticas numa amostra da população portuguesa (Resultados Preliminares). Acta Reumatol Port 1991; 16: 98.
35. Figeirinhas J. Estudo Epidemiológico dos reumatismos. Acta Reumatol Port 1976; IV: 23.56.
36. Figueirinhas J. Alguns dados definitivos obtidos através do recente inquérito epidemiológico de reumatismos. Acta Reumatol Port 1976; IV: 373-380.
37. Carmona L, Gonzalez-Alvaro I, Balsa A, Angel Belmonte M, Tena

X, Sanmarti R. Rheumatoid arthritis in Spain: occurrence of extra-articular manifestations and estimates of disease severity. Ann Rheum Dis 2003; 62: 897-900.
38. Anagnostopoulos I, Zinzaras E, Alexiou I, et al. The prevalence of rheumatic diseases in central Greece: a population survey. BMC Musculoskelet Disord 2010; 11: 98.
39. Laires PA, Canhao H, Araujo D, et al. CoReumaPt protocol: the Portuguese cohort of rheumatic diseases. Acta Reumatol Port 2012; 37: 18-24.

EPIDOC COHORT DESCRIPTION

DIAS SS*, RODRIGUES AM*, GREGÓRIO MJ, ET AL. 2018 COHORT PROFILE: EPIDEMIOLOGY OF CHRONIC DISEASES COHORT (EPIDOC). INTERNATIONAL JOURNAL OF EPIDEMIOLOGY.

International Journal of Epidemiology, 2018, 1–12
doi: 10.1093/ije/dyy185
Cohort Profile

Cohort Profile

Cohort Profile: The Epidemiology of Chronic Diseases Cohort (EpiDoC)

Sara Simões Dias,[1,2,3]*† Ana Maria Rodrigues,[1,3]† Maria João Gregório,[1,3] Rute Dinis de Sousa,[1,3] Jaime Cunha Branco[1,3,4] and Helena Canhão[1,3]

[1]EpiDoC Unit, CEDOC, NOVA Medical School, Universidade Nova de Lisboa (NMS-UNL), Lisboa, Portugal, [2]Center for Innovative Care and Health Technology (ciTechCare), Polytechnic Institute of Leiria, Leiria, Portugal, [3]Episaúde – Associação Científica, Évora, Portugal and [4]Serviço de Reumatologia, CHLO, EPE | Hospital Egas Moniz, Lisboa, Portugal

*Corresponding author. CEDOC I, Epidoc Unit, Faculdade ciências médicas, Campus Sant'Ana, Pólo de Investigação, NMS, UNL, Edifício Amarelo, Rua do Instituto Bacteriológico, n° 5, Lab 2.9, 1150–082 Lisboa, Portugal. E-mail: sara.dias@nms.unl.pt

†Both authors contributed equally.

Editorial decision 5 August 2018; Accepted 20 August 2018

Why was the cohort set up?

Non-communicable chronic diseases are the leading cause of death and the main contributor to disease burden worldwide, accounting for 86% of all deaths in Portugal.[1] Several modifiable behavioural risk factors, such as unhealthy dietary habits, physical inactivity, tobacco use and harmful use of alcohol, are the main risk factors for these diseases. Thus, the existence of epidemiological data on chronic diseases and their determinants (i.e. socioeconomic and demographic factors), associated factors and consequences are important public health tools for designing and developing strategies to tackle the burden of non-communicable diseases.

In 2011, a prospective cohort study called Epidemiology of Chronic Diseases (EpiDoC) aimed to create a large population database for medical and health-related research in Portugal. To our knowledge, the EpiDoC study constitutes one of the first Portuguese prospective large cohort studies, including a representative sample of the Portuguese population, with the primary aim of examining the health determinants and outcomes of chronic non-communicable diseases and their impact on health care resource consumption. The EpiDoC study was designed by researchers from NOVA Medical School in Lisbon with close collaboration between social and biomedical scientists, ensuring a thorough multidisciplinary approach.

The first wave of this cohort study, named EpiDoC 1 (EpiReumaPt), occurred between September 2011 and December 2013. Its primary aim was to assess rheumatic and musculoskeletal disease (RMD) prevalence and its burden in Portugal. This wave had two phases: the first consisted of a face-to-face interview, and the second included a detailed clinical evaluation of RMD performed by rheumatologists. This baseline assessment also enabled the creation of a population-based biobank (i.e. DNA, serum and total blood samples) for identifying genetic predictors and serum risk factors for chronic diseases. Musculoskeletal imaging data were also collected, in particular peripheral dual energy X-ray (DXA) in all second phase participants and X-ray of the affected joint(s).

Similar to other cohort studies,[1,2] the scope of the EpiDoC study has expanded over time. So far, two subsequent waves have been completed: EpiDoC 2 (March 2013–July 2015) and EpiDoC 3 (September 2015–July 2016). In both waves, data were collected through a phone interview. EpiDoC 2 (CoReumaPt) focused on lifestyle behaviours and their determinants, with a secondary goal of identifying innovative patient solutions for coping with disability.

EpiDoC 3 (Saúde.Come) assessed inequalities in access to healthy food and health services, with a focus on food insecurity and its determinants and health consequences.

The EpiDoC study was performed according to the principles established by the Declaration of Helsinki and revised in 2013 in Fortaleza. Ethical approval was obtained from the National Committee for Data Protection (Comissão Nacional de Proteção de Dados) and NOVA Medical School Ethics Committee. Ethical committees of regional health authorities also approved the study.

Who is in the cohort?

Setting

EpiDoC is a prospective closed cohort study including a nationally representative sample of adults (≥18 years old) who were non-institutionalized and living in private households in Portugal Mainland and Islands (Azores and Madeira).[3] Portugal is a south-western European country with a resident population of 10 562 178, of whom 8 million are adults (4 072 122 men and 4 585 118 women).[4] During the past two decades, life expectancy in Portugal has been increasing. Data from the World Health Organization indicate that life expectancy in Portugal was 83.9 years for women and 78.2 years for men in 2015. In addition, as in other European countries, the Portuguese population has been undergoing demographic changes. The Portuguese population pyramid shows an increasing number of individuals at the top and a decreasing number at the bottom, indicating a new structure of the Portuguese population with fewer young people and more elderly. In 2015, the old-age dependency ratio was 31.1 per 100 persons of working age, which is the ratio between the number of persons aged ≥65 years (i.e. when individuals are generally economically inactive) and those aged 15–64 years.[5]

Portugal is divided into seven regions according to the Nomenclature of Territorial Units for Statistics II (NUTS II): Norte, Centro, Lisboa e Vale do Tejo, Alentejo, Algarve, Região Autónoma dos Açores (the Azores) and Região Autónoma da Madeira (Madeira). At the NUTS II level, the Norte region has the largest population density (34.7%), followed by Lisboa e Vale do Tejo (26.6%) and Centro (22.4%) (Figure 1). The other NUTS II regions (Alentejo, Algarve, the Azores, and Madeira) encompass small towns and villages with lower population densities and higher desertification rates.

Participant recruitment

Considering the primary aim of EpiDoC 1, the sample size was calculated based on the estimated prevalence of rheumatic diseases with a 95% confidence interval (CI), and

Figure 1. Portuguese population density distribution according to the 7 NUTS II.

standardized for age and sex according to the total adult population of the studied areas. Assuming that the expected prevalence of rheumatic diseases was between 0.5% and 1%, and expecting a drop-out rate of 50%, it was estimated that a total of 9000 individuals should be recruited. To obtain regional representativeness, the sample size was stratified according to dimensions and characteristics of the seven Portuguese regions. Population recruitment was conducted by Centro de Estudos e Sondagens de Opinião da Universidade Católica Portuguesa (CESOP-UCP), and multistage random sampling was used for participant selection.

In EpiDoC 1, candidates for participation were visited at their homes by a team of trained interviewers. Locations were selected as the primary unit of sampling according to the Census 2001. Selected households and their addresses were identified using a random selection of points in the map of each location, where the interviewer began a systematic step count (defined for each locality based on its size). Each selected household was visited, with no previous contact, up to three times (including evenings and weekends) if no candidate participant was present during the first visit. In each household, an individual ≥18 years old with permanent residence and the most recently completed birthday was selected to be a participant in the EpiDoC study. Before participant interviews, the EpiDoC team gave information about study details and aims at local churches, primary care centres and municipalities. Local priests, health providers and municipality employers helped us to spread the information and motivate participation.

EpiDoC 1 (2011–13)

EpiDoC 1 enrolled 10 661 participants and was primarily designed to estimate the prevalence of RMDs. To provide a comprehensive understanding of the burden of RMDs, this wave had the secondary aim of evaluating quality of life, physical function, mental health, work status and health care resource consumption, with the purpose of identifying differences in these and other outcomes between individuals with and without RMDs.[3]

EpiDoC 1 data collection consisted of two phases. Phase 1 involved face-to-face interviews conducted by a team of trained interviewers (non-physicians) through door-to-door visits. Phase 2 involved clinical observations with physical examination performed by rheumatologists, for participants identified as potentially having an RMD (using a screening questionnaire applied at Phase 1) and 20% of asymptomatic individuals. All procedures occurred between September 2011 and December 2013.

Of the 10 661 participants selected in Phase 1, 7451 had a positive RMD screening and 3210 had a negative RMD screening. A total of 8152 participants were contacted in Phase 2: 7451 with a positive RMD and 701 (~20%) without an RMD as previously defined in the study protocol. Of these, 4275 did not attend a clinical observation by a rheumatologist. Therefore, at the end of Phase 2, there were 3877 clinical observations with physical examination performed by rheumatologists; 3198 participants received validation of an RMD diagnosis and 679 did not have an RMD diagnosis.

In Phase 1, a structured questionnaire using a computer-assisted personal interview (CAPI) system was used to collect data. Questions on rheumatic symptoms were asked, and an algorithm for screening each RMD was applied. An individual was considered to have a positive screening: if he/she mentioned a previously known RMD; if any of the specific disease algorithms in the screening questionnaires were positive; or if the participant reported muscle, vertebral or peripheral joint pain in the previous 4 weeks.[3] Phase 2 was performed by rheumatologists at the local primary care centre for all participants who were identified as having a positive RMD screening. All clinical laboratory and imaging data were verified by a team of three experienced rheumatologists, and diagnoses were confirmed according to validated criteria.[3]

All participants enrolled in EpiDoC 1 (10 661 participants) were invited to participate in a follow-up study, of whom 10 153 (95.2%) signed consent forms and agreed to participate. For follow-up waves (EpiDoC 2 and 3), data were collected using a structured questionnaire administered by phone call interviews using a CAPI system. A core questionnaire was used in each EpiDoC wave, with additional questions added according to the focus of each wave. In EpiDoC 2 and 3, when a participant was not available, additional attempts were made at different times up to a maximum of six attempts. The last contact attempt had to follow the previous contact by least 1 month; only then was the contact attempt abandoned.

EpiDoC 2 (2013–15)

EpiDoC 2 was the first follow-up wave, with data collected between March 2013 and July 2015. EpiDoC 2 included 7591 participants (out of 10 153 eligible participants) representative of the adult Portuguese population, resulting in a response rate of 71.2% from EpiDoC 1. Considering that the main risk factors for non-communicable diseases are unhealthy lifestyle behaviours, EpiDoC 2 employed the core structured questionnaire but included more detailed questions on lifestyle behaviours, such as physical activity, dietary habits, tobacco and alcohol use and sleeping habits. Questions regarding innovative patient solutions for coping with disability were also included.

EpiDoC 3 (2015–16)

EpiDoC 3 occurred between September 2015 and July 2016 and included 5653 participants, resulting in a response rate of 55.7% from EpiDoC 1. This wave continued to employ the core structured questionnaire but included questions on food insecurity, its determinants and its health consequences. This particular interest in food insecurity was based on a growing awareness of social inequalities in health and modifiable risk factors for chronic diseases, such as dietary patterns, as well as the economic crisis faced by Portugal in previous years.

Cohort characteristics

The participation rate declined from EpiDoC 1 to EpiDoC 3, similar to most other population-based studies.[2,6] Table 1 presents the characteristics of participants in the cohort. There were no significant differences in any categories of variables between the three waves.

How often have they been followed up?

The EpiDoC study employed cross-sectional and longitudinal study designs (Figure 2). As it used a closed cohort, no new participants were added in any wave. Table 2 presents the attrition rates between EpiDoC 1 and 2, EpiDoC 1 and 3, and EpiDoC 2 and 3.

Table 1. Characteristics of the participants in the cohort

	EpiDoC 1	EpiDoC 2	EpiDoC 3	Census 2011
Sex	$n = 10\,661$	$n = 7591$	$n = 5653$	$n = 8\,657\,240$
Female	6551 (52.6%)	4784 (52.2%)	3607 (52.5%)	4 585 118 (53.0%)
Age group	$n = 10\,661$	$n = 7591$	$n = 5648$	
18–29	1182 (22.1%)	621 (18.4%)	355 (15.4%)	1 470 782 (17.0%)
30–39	1511 (18.8%)	975 (18.7%)	605 (19.1%)	1 598 250 (18.5%)
40–49	1906 (17.3%)	1437 (18.2%)	1049 (18.3%)	1 543 392 (17.8%)
50–59	1801 (14.8%)	1437 (16.2%)	1143 (15.9%)	1 400 011 (16.2%)
60–69	1915 (12.9%)	1440 (13.2%)	1112 (13.7%)	1 186 442 (13.7%)
70–74	849 (5.8%)	645 (6.2%)	491 (6.7%)	496 438 (5.7%)
≥75	1497 (8.4%)	1036 (9.1%)	893 (11.0%)	961 925 (11.1%)
Ethnicity/race	$n = 10\,629$	$n = 7574$	$n = 5638$	
Caucasian	10 342 (96.0%)	7423 (97.1%)	5536 (97.2%)	No comparable data
Black	221 (3.4%)	119 (2.5%)	81 (2.3%)	
Asian	8 (0.1%)	3 (0.0%)	2 (0.1%)	
Romany	20 (0.3%)	7 (0.1%)	5 (0.1%)	
Other	38 (0.3%)	22 (0.3%)	14 (0.3%)	
Years of education				
(mean ± SD)	7.41 ± 4.1	8.66 ± 3.90	8.80 ± 3.94	
Education level	$n = 10\,585$	$n = 7546$	$n = 5615$	
0–4 years	4726 (33.2%)	3272 (31.7%)	2392 (30.9%)	3 239 724 (37.4%)
5–9 years	2175 (22.6%)	1547 (21.3%)	1122 (19.6%)	2 134 401 (24.6%)
10–12 years	1920 (23.8%)	1391 (24.8%)	1049 (25.6%)	1 560 958 (18.0%)
>12 years	1764 (20.4%)	1336 (22.2%)	1052 (24.0%)	1 741 567 (20.1%)
NUTS II	$n = 10\,661$	$n = 7591$	$n = 5648$	
Norte	3122 (34.9%)	2240 (35.8%)	1659 (36.5%)	3 007 823 (34.7%)
Centro	1997 (22.8%)	1504 (23.3%)	1087 (23.2%)	1 938 815 (22.4%)
Lisboa	2484 (26.7%)	1588 (25.4%)	1131 (24.8%)	2 300 053 (26.6%)
Alentejo	669 (7.3%)	422 (7.2%)	320 (7.2%)	633 691 (7.3%)
Algarve	352 (3.8%)	245 (3.8%)	183 (3.7%)	370 704 (4.3%)
Azores	1029 (2.2%)	793 (2.1%)	657 (2.5%)	192 357 (2.2%)
Madeira	1008 (2.3%)	799 (2.4%)	611 (2.4%)	213 797 (2.5%)
Marital status	$n = 10\,652$	$n = 7586$	$n = 5644$	
Single	1935 (29.4%)	1285 (28.4%)	922 (28.5%)	No comparable data
Married	6111 (50.2%)	4591 (53.2%)	3457 (53.4%)	
Divorced	810 (7.4%)	556 (6.8%)	391 (6.1%)	
Widow(er)	1414 (8.2%)	910 (7.3%)	697 (7.6%)	
Consensual union	382 (4.8%)	244 (4.2%)	177 (4.4%)	
BMI	$n = 10\,109$	$n = 6922$	$n = 5174$	
Underweight	167 (2.2%)	111 (2.0%)	88 (2.1%)	No comparable data
Normal	4063 (45.5%)	2670 (45.5%)	2009 (44.5%)	
Overweight	3799 (35.1%)	2788 (37.1%)	2098 (37.7%)	
Obese	2080 (17.1%)	1353 (15.4%)	979 (15.7%)	
Monthly household income	$n = 7613$	$n = 5558$	$n = 4167$	
<500€	1994 (19.9%)	1331 (18.0%)	945 (16.66%)	No comparable data
501€ to 750€	1707 (21.7%)	1257 (20.8%)	949 (20.91%)	
751€ to 1000€	1268 (18.8%)	943 (19.0%)	717 (19.89%)	
1001€ to 1500€	1141 (17.2%)	852 (17.5%)	638 (16.97%)	
1501€ to 2000€	657 (9.9%)	511 (10.9%)	386 (11.08%)	
2001€ to 2500€	379 (5.9%)	295 (5.7%)	246 (6.37%)	
2501€ to 3000€	222 (3.0%)	188 (3.8%)	148 (3.98%)	
3001€ to 4000€	146 (1.8%)	108 (2.1%)	83 (1.94%)	
>4000€	99 (1.9%)	73 (2.2%)	55 (2.20%)	

SD, standard deviation.

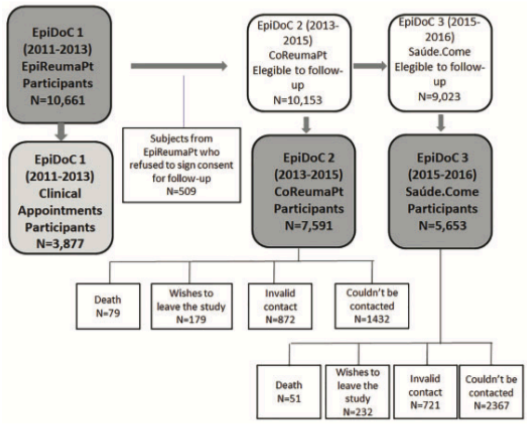

Figure 2. Flowchart of EpiDoC study.

Loss to follow-up

The participation rate in EpiDoC 3 was of 53.03%. The attrition was most pronounced in younger adults (18–29 years old). Of the 10 661 participants in EpiDoC 1,509 (4.8%) refused to sign the consent form for follow-up. Of the resulting 10 153 eligible participants for EpiDoC 2, 79 (0.8%) had died, 179 (1.8%) wished to leave the study and 917 (9.0%) had an invalid contact. Thus, a total of 1639 participants were lost to follow-up; these subjects had a mean age of 55 years, and 962 (58.7%) were women. Between EpiDoC 2 and 3, 51 (0.6%) participants had died, 232 (2.6%) wished to leave the study and 721 (8.0%) had an invalid contact. Thus, a total of 1004 participants were lost to follow-up; these individuals had a mean age of 56 years, and 620 (61.8%) were women. Figure 1 shows the flowchart of the EpiDoC study.

What has been measured?

Data collection included measures for five domains that were central to the longitudinal study: sociodemographic characteristics, lifestyle characteristics, health and clinical characteristics, health care resource consumption, a population-based biobank (total blood, serum and DNA) and imaging data (peripheral DXA and X-ray of the affected joint) (Table 3). For reasons of longitudinal comparison, most measurement tools were used consistently across waves. However, some measurement tools were updated or improved, new measurement tools were added and old measurement tools were removed as needed.

Of the measurements obtained across all three waves, lifestyle variables included smoking habits, alcohol intake and physical exercise. Health variables included anthropometric measures, self-reported chronic diseases, rheumatic diseases, a health assessment questionnaire (HAQ)[7] and the European Quality of Life Survey with five dimensions and three levels (EQ-5D-3L).[8,9] Employment variables included employment status, retirement due to disease, retirement due to RMD, work absenteeism due to disease, work disability due to RMD, unemployment due to disease, unemployment due to RMD, number of working hours/week and changed employment status due to RMD. Health care resource variables included hospitalization events (in previous 12 months since last contact), their reason and their duration. Concerning falls and bone fractures, variables included any falls or bone fractures and the number and location of bone fractures. Sociodemographic data, including sex, age, ethnicity, years of education and education level, and marital status, were collected only in EpiDoC 1, based on the assumption that these characteristics would not change over time. Other information obtained only in EpiDoC 1 were household income, household composition, coffee intake and health information from the 36-item Short Form Survey (SF-36).[10]

Information obtained only in EpiDoC 1 and 2 were the Hospital Anxiety and Depression Scale (HADS)[11] and home care assistance (in previous 12 months or since last contact), its provider and its payer.

Information obtained only in EpiDoC 2 and 3 included: sleep habits; frequency of watching TV, using computer/videogames/tablets and using the internet; number of meals per day; frequency of soup, vegetable, fruit, meat, fish, milk/dairy and water consumption; numbers of medical appointments (in previous 12 months or since last contact), private versus public medical appointments, private medical appointments with or without insurance, public medical appointments in a hospital/health care centre and private or public medical appointments by specialty.

Information obtained only in EpiDoC 3 were frequency of olive oil, wine, beans, fat and sugar consumption; attitudes toward food; a food insecurity scale; and characteristics of food acquisition and preparation.

Population-based biobank and imaging data were collected in EpiDoC 1 during medical appointments at the local primary care centre. Blood samples were collected from 3608 participants (DNA, serum and whole blood). Taking into consideration the imaging reservoir, there were a total of 3342 participants who had a forearm bone mineral density evaluation through peripheral DXA. Also, bone mineral assessment (BMA) using a high-resolution digital X-ray machine (D3A, France) was collected from 2422 wrists and 2228 calcaneus bones. Simple X-rays were performed to examine 438 hands, 122 hips, 479 knees, 1265 lumbar spines, 691 thoracic spines and 206 cervical spines, according to participants' musculoskeletal complaints. All data collected, including biobank and imaging data, are detailed in Table 3.

Table 2. Characteristics of the participants in the cohort (attrition rate)

	EpiDoC 1 vs EpiDoC2 (attrition rate)	EpiDoC 2 vs EpiDoC3 (attrition rate)	EpiDoC 1 vs EpiDoC3 (attrition rate)
Total	28.80%	25.53%	46.97%
Sex			
Female	26.97%	24.60%	44.94%
Age group			
18–29	47.46%	42.83%	69.97%
30–39	35.47%	37.95%	59.96%
40–49	24.61%	27.00%	44.96%
50–59	20.21%	20.46%	36.54%
60–69	24.80%	22.78%	41.93%
70–74	24.03%	23.88%	42.17%
≥75	30.79%	13.80%	40.35%
Ethnicity/race			
Caucasian	28.22%	25.42%	46.47%
Black	46.15%	31.93%	63.35%
Asian	62.50%	33.33%	75.00%
Romany	65.00%	28.57%	75.00%
Other	42.11%	36.36%	63.16%
Education level			
0–4 years	30.77%	26.89%	49.39%
5–9 years	28.87%	27.47%	48.41%
10–12 years	27.55%	24.59%	45.36%
>12 years	24.26%	21.26%	40.36%
NUTS II			
Norte	28.25%	25.94%	46.86%
Centro	24.69%	27.73%	45.57%
Lisboa	36.07%	28.78%	54.47%
Alentejo	36.92%	24.17%	52.17%
Algarve	30.40%	25.31%	48.01%
Azores	22.93%	17.15%	36.15%
Madeira	20.73%	23.53%	39.38%
Marital status			
Single	33.59%	28.25%	52.35%
Married	24.87%	24.70%	43.43%
Divorced	31.36%	29.68%	51.73%
Widow(er)	35.64%	23.41%	50.71%
Consensual union	36.13%	27.46%	53.66%
BMI			
Underweight	33.53%	20.72%	47.31%
Normal	34.29%	24.76%	50.55%
Overweight	26.61%	24.75%	44.77%
Obese	34.95%	27.64%	52.93%
Monthly household income			
<500€	33.25%	29.00%	52.61%
501€ to 750€	26.36%	24.50%	44.41%
751€ to 1000€	25.63%	23.97%	43.45%
1001€ to 1500€	25.33%	25.12%	44.08%
1501€ to 2000€	22.22%	24.46%	41.25%
2001€ to 2500€	22.16%	16.61%	35.09%
2501€ to 3000€	15.32%	21.28%	33.33%
3001€ to 4000€	26.03%	23.15%	43.15%
>4000€	26.26%	24.66%	44.44%

Table 3. Data collected over EpiDoC study

	EpiDoC 1 EpiReumaPt (CESOP) 10 661	EpiDoC 1 EpiReumaPt (medical appointments) 3877	EpiDoC 2 CoReumaPt 7591	EpiDoC 3 Saúde.Come 5653
Sociodemographic and economic data				
Sex	X			
Age	X			
Ethnicity	X			
Nationality	X			
Years of education and educational level	X			
Marital status	X			
Employment status	X		X	X
Household income	X			X
Household composition	X			X
Number of people <18 y in household	X			X
Number of people >65 y in household				X
Region (NUT II)	X			
Location and district	X			
Home & neighbourhood characteristics	X			
Single-parent families				X
Income perception				X
Anthropometric data				
Self-reported height (in cm)	X	X	X	X
Self-reported weight (in kg)	X	X	X	X
Body mass index (kg/m^2)	X	X	X	X
Self-reported chronic diseases				
High blood pressure, diabetes, high cholesterol level, pulmonary disease, cardiac disease, gastrointestinal disease, neurological disease, allergies, mental disease, neoplastic disease, thyroid and parathyroid disease, hyperuricaemia and urinary disease	X	X	X	X
Rheumatic diseases				
Rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, osteoarthritis, osteoporosis, gout, polymyalgia rheumatica, systemic lupus erythematosus, fibromyalgia, periarticular diseases, low back pain, inflammatory low back pain, chondrocalcinosis and other RMD	X	X	X	X
Who diagnosed RMD	X		X	X
Rheumatic complaints	X	X	X	X
Medical history		X		
Physical examination		X		
Anxiety, depression, physical function and quality of life				
Hospital Anxiety and Depression Scale (HADS)	X		X	
Health Assessment Questionnaire (HAQ)	X		X	X
Short Form Health Survey (SF-36)	X			
European Quality of Life questionnaire (EQ-5D-3L)	X		X	X
Falls and bone fractures				
Suffered any fall, where the fall happened (home, street, work), number of falls (home, street, work), suffered any bone fracture, number of bone fractures and location of bone fracture	X		X	X
Health and employment				
Retired due to disease, retired due to RMD, work absenteeism due to disease, work disabled due to	X		X	X

(Continued)

Table 3. Continued

	EpiDoC 1 EpiReumaPt (CESOP) 10 661	EpiDoC 1 EpiReumaPt (medical appointments) 3877	EpiDoC 2 CoReumaPt 7591	EpiDoC 3 Saúde.Come 5653
RMD, unemployed due to disease, unemployed due to RMD, number working h/week and changed employment status (past year) due to RMD				
Health and economic				
Chronic disease management difficulties, medication non-adherence due to economic constraints, and reduction in visits to medical appointments due to economic constraints				X
Hospitalizations, home care assistance and medical appointments				
Was hospitalized (past 12 months/since last contact), reason and duration of hospitalization, home care assistance (past 12 months/since last contact, currently), who provides and who pays for home care assistance, medical appointments (past 12 months/since last contact), number private/public medical appointments, private medical appointments with/without insurance, public medical appointments in hospital/health care centre, number private/public medical appointments by specialty, health care system (ADSE, subsystems, private insurance), medications and other treatments, medicine(s) currently taking, other treatments (physical and rehabilitation medicine, behavioural therapy etc.) and alternative treatments (acupuncture, homeopathy etc.)	X		X	X
Lifestyle data				
Smoking habits (current/past smoker, number of cigarettes, smoking duration)	X		X	X
Alcohol intake (frequency, number of units)	X		X	X
Coffee intake	X			
Physical exercise (frequency, type, age when started)	X		X	X
Sleep habits (h/day)			X	X
Frequency of watching TV			X	X
Frequency of using computer/videogames/tablets			X	X
Frequency of using internet			X	X
Dietary intake and behaviours				
Frequency of soup, vegetables, fruit, meat, fish, milk/dairy, water consumption			X	X
Adherence to Mediterranean diet				X
Food insecurity				X
Patient innovation to cope with disability			X	
Biobank and imaging data				
Serum, whole blood, DNA, peripheral BMD (wrist), X-ray of the affected joint (hand, hip, knee), calcaneus and wrist BMA		X		

What has it found? Key findings and publications

Over 24 peer-reviewed journal publications based on EpiDoC data have been published to date, covering a wide range of scientific domains. A full list of publications can be found on our website[http://cedoc.unl.pt/epidoc-unit/]. Sample overviews of study data are shown in Tables 1, 2 and 4. Here, we summarize key findings.

In EpiDoC 1, we characterized socioeconomic features of the Portuguese adult population. From a social and health point of view, an alarming finding was that one-fifth of the adult Portuguese population had a monthly

Table 4. Prevalence and 95% of confidence interval of reported chronic diseases and lifestyle habits

	EpiDoC 1		EpiDoC 2		EpiDoC 3	
	$n = 10\,661$		$n = 7591$		$n = 5653$	
Reported diseases						
Chronic diseases	$n = 10\,661$	95% CI	$n = 7591$	95% CI	$n = 5653$	95% CI
High blood pressure	3369 (23.1%)	21.9–24.9	2538 (24.1%)	22.7–25.5	1872 (24.8%)	23.1–26.7
Diabetes	1217 (8.3%)	7.6–9.1	877 (8.6%)	7.8–9.5	690 (9.2%)	8.1–10.4
High cholesterol level	3360 (24.4%)	23.2–.25.7	2595 (25.9%)	24.5–27.4	1831 (25.3%)	23.6–27.2
Lung disease	637 (5.4%)	4.6–6.3	496 (5.7%)	4.8–6.7	213 (2.8%)	2.4–3.3
Cardiac disease	1366 (10.5%)	9.4–11.6	1034 (11.9%)	10.5–13.4	704 (9.8%)	8.7–11.1
Gastrointestinal disease	1837 (14.9%)	13.8–16.1	1411 (16.1%)	14.7–17.6	544 (8.8%)	7.6–10.3
Neurological disease	418 (3.3%)	2.8–3.9	311 (3.4%)	2.8–4.1	212 (2.9%)	2.4–3.4
Allergies	2287 (21.2%)	19.9–22.7	1720 (22.8%)	21.2–24.5	548 (10.3%)	8.6–12.3
Mental disease	1619 (12.9%)	11.7–14.1	1274 (14.1%)	12.4–16.0	1008 (13.4%)	12.3–14.5
Cancer	439 (3.4%)	2.8–4.2	364 (4.0%)	3.3–4.9	318 (4.6%)	3.8–5.5
Hyperuricaemia	690 (5.2%)	4.7–5.8	514 (5.4%)	4.8–5.9	130 (1.9%)	1.5–2.4
Renal colic	885 (7.0%)	6.4–7.8	716 (8.4%)	7.3–9.6	250 (4.3%)	3.4–5.4
Rheumatic disease	2994 (21.2%)	20.0–22.5	2552 (25.5%)	24.0–27.1	2096 (29.5%)	27.5–31.5
Lifestyle habits						
Alcohol						
Never	4625 (37.2%)	35.6–38.8	3150 (37.1%)	35.2–39.2	1945 (30.6%)	28.2–33.2
Occasionally	3967 (42.6%)	40.9–44.3	2437 (39.6%)	37.7–41.7	2020 (39.6%)	37.4–42.0
Daily	2050 (20.2%)	18.9–21.6	1693 (23.2%)	21.7–24.8	1565 (29.8%)	27.7–31.9
Smoking habits						
Never/occasionally	8800 (76.8%)	75.1–78.4	4447 (54.4%)	52.3–56.4	3584 (58.8%)	56.3–61.3
Past smoking[a]	Not applicable		1522 (21.1%)	19.6–22.6	1149 (21.1%)	19.4–23.0
Present smoker	1854 (23.2%)	21.6–24.9	1289 (24.5%)	22.4–26.7	802 (20.0%)	17.6–22.7
Physical activity						
Regular	3499 (37.0%)	35.3–38.6	3442 (50.1%)	48.1–52.1	2147 (40.8%)	38.5–43.2
Not regular	7155 (63.0%)	61.3–64.6	3976 (49.8%)	47.9–51.9	3498 (59.2%)	56.8–61.5

[a]Past smoker was not included at baseline.

family income of <500€.[3] Indeed, data from EpiDoC 2 showed that poverty and a low education level are associated with an unhealthy lifestyle and higher prevalence of chronic diseases.[12]

Social inequality in health is a major concern within public health, with food insecurity being one of its main drivers. Food insecurity is defined as a difficulty in achieving a healthy diet due to economic constraints, and is a well-known determinant of health. EpiDoC 3 showed a high prevalence of food insecurity and its associations and unhealthy dietary behaviours. Food insecurity was associated with several non-communicable diseases, lower quality of life and higher health care resource consumption.[13] Publications using EpiDoC data have raised questions and informed policy makers about the need to reduce food insecurity, not only to improve individual health status but also to reduce public health costs.

Considering health and health-related characteristics, high blood pressure, high cholesterol level, allergies and RMDs were frequently self-reported among the Portuguese adult population. The prevalence of RMDs in Portugal is similar to that reported in other countries,[14–19] namely Portugal's close neighbour Spain.[20] Another interesting finding was the high proportion of individuals presenting typical features of one or more RMDs, who did not have a previous diagnosis (1532 out of 3877 participants).[21] This could be explained by the scarce number of rheumatologists in Portugal (1: 100 000 inhabitants)[22] and the lack of awareness among the population about these diseases, as RMD symptoms are frequently accepted as part of the normal ageing process. These results helped support a new national network for hospital reference of rheumatology, developed by the National Directorate General of Health in collaboration with the EpiDoC research team.

The RMD with the highest prevalence in Portugal was low back pain (26.4%; 95% CI, 23.3-29.5%), which was significantly more frequent in women than in men (29.6% vs 22.8%; $P = 0.040$). Low back pain increased with age, and its prevalence was highest in the 46–55-year age group (27.7%; 95% CI 23.1-32.4%).[21]

Regarding the impact of RMDs on health-related quality of life, physical function and mental health among the Portuguese population, EpiDoC data showed that patients with RMDs have more health care resource consumption, were more often hospitalized and had more homecare support needs in the previous 12 months, compared with participants with no RMDs.[12,21,23] In EpiDoC 1, a meaningful number ($n = 488$, 30.9%) of people claimed to have retired prematurely due to RMDs.[24] This translates to many years of working life already lost and many others still potentially lost. Indirect costs due to self-reported RMDs are also substantial, equivalent to at least 0.5% of the gross domestic product.[21] These results emphasize the burden of RMDs and the need to develop RMD awareness, which is a strong argument encouraging policy makers to increase the amount of resources allocated to the treatment of rheumatic patients.

EpiDoC 1 also showed a high prevalence of other chronic diseases among Portuguese adults such as dyslipidaemia (24.4–25.9%), hypertension (23.1–24.8%) and diabetes (7.6–9.1%). The elderly are a particularly vulnerable population for chronic diseases, among whom the coexistence of two or more chronic diseases is particularly high (78.3%), leading to low quality of life and disability.[25] The most common chronic diseases in the elderly were hypertension (57.3%), rheumatic disease (51.9%), hypercholesterolaemia (49.4%) and diabetes (22.7%). Among older adults, 66.6% were physically inactive and 22.3% were obese, particularly among Azoreans (33.0%). Similar results were found for Portuguese adults, of whom more than half did not exercise (63.0%) and more than 15% were obese.[25]

EpiDoC 2 estimated a prevalence of anxiety and depression among Portuguese elderly of 9.6% and 11.8%, respectively. Seniors with anxiety or depression were more likely to self-report higher levels of physical disability and lower quality of life.[26]

Biological and clinical data have been used in national studies of older adult lifestyles,[23,27] the impact of falls and fractures, vitamin D level, sun exposure, dairy consumption and oral health, as well as international collaborative projects on mitochondrial DNA and BMA and bone texture in osteoarthritis.[28]

In conclusion, EpiDoC publications have improved our understanding of socioeconomic and health inequalities among Portuguese adults, particularly the elderly. These studies demonstrate that unhealthy lifestyles are more prevalent among the most socioeconomically vulnerable groups and are associated with a higher prevalence of chronic non-communicable diseases and higher health care resource consumption. The EpiDoC study has also shed light on the burden of rheumatic diseases in Portugal. It shows a need to rethink the rheumatology support network and to provide better care to rheumatic patients. EpiDoC ongoing work is aimed at revealing the determinants and burden of multimorbidity and other chronic non-communicable diseases, namely mental and cardiovascular diseases. Particular attention will be directed at better understanding unmet elderly health needs.

What are the main strengths and weakness?

The main strengths of the EpiDoC study are its general population base and sample size, availability of repeated measures and extensive biobank blood collection. Another strength is its interdisciplinary research cooperation, with a team comprising physicians, psychologists, epidemiologists, nutritionists, statisticians, laboratory technicians and others. The different purposes of the three waves are also a strength, as they have expanded the scope of the EpiDoC study to become a more complete cohort study.

The EpiDoC study also has some weaknesses, such as its attrition rate, which is similar to that of other studies[29,30] and was not significantly different between the three waves. In EpiDoC 2 and EpiDoC 3, data were collected by phone interviews; however, we attempted to reduce attrition bias by using reminders for scheduled visits and sending periodic newsletters and reminders to all participants. Another limitation is that diseases were self-reported, although a detailed and comprehensive questionnaire included a screening for RMD symptoms. All measurement tools (HADS, EQ-5D-3L, SF-36 and HAQ) were validated and the screening of RMDs was validated by an algorithm supplemented by expert rheumatologist opinion. Each wave survey was composed of a structured comprehensive questionnaire which was tested for feasibility, participant comprehension and language.[3,12]

Can I get hold of the data? Where can I find more information?

The EpiDoC Unit promotes research networking—both national and international—and develops collaborative projects. Data from our cohort studies and projects are freely available for researchers who submit a research proposal to the scientific committee. More details about questionnaire content and clinical measurements can be found on our website [http://cedoc.unl.pt/epidoc-unit/]. A research proposal editable form can be downloaded and sent to [rute.sousa@nms.unl.pt]. An EpiDoC steering committee will evaluate all proposals for future studies and collaborations, to access data and use of biological samples.

Profile in a nutshell

- EpiDoC is a prospective population-based closed cohort study that collects health information. The study primarily aimed to address rheumatic diseases, but its scope has broadened to other chronic diseases, namely cardiovascular, gastroenterological, pulmonary, anxiety and depression and neurological diseases.
- Three health surveys of the general adult population (aged ≥18 years) in Portugal were completed: EpiDoC 1 (September 2011–December 2013), EpiDoC 2 (March 2013–July 2015) and EpiDoC 3 (September 2015–July 2016).
- EpiDoC surveys have spanned a total of 5 years, with an attrition rate of approximately 25%. EpiDoC 1, 2 and 3 had 10 661, 7591 and 5663 participants, respectively.
- The EpiDoC sample is representative of the Portuguese population. In EpiDoC 1, 6551 (52.6%) participants were women, and most were Caucasian (n = 10 342, 96.0%) and married (n = 6111, 50.2%). In EpiDoC 2, 4784 (52.2%) participants were women, and the mean age of all participants was 48.0 ± 18.0 years. In EpiDoC 3, 3607 (52.5%) participants were women, and the mean age of all participants was 49.64 ± 18.11 years.
- EpiDoC data are available to researchers who submit research proposals to the scientific committee. More details can be found on our website [http://cedoc.unl.pt/epidoc-unit/].

Funding

The EpiDoC study has had several sources of financial support. EpiDoC 1 and EpiDoC 2 were supported by grants from the Portuguese Directorate-General of Health and sponsorships from Fundação Calouste Gulbenkian, Fundação Champalimaud, Fundação AstraZeneca, Abbott, Merck Sharp & Dohme, Pfizer, Roche, Servier, Bial, D3A Medical Systems, Happybrands, Centro de Medicina Laboratorial Germano de Sousa, CAL-Clínica, Galp Energia, Açoreana Seguros and individual rheumatologists. EpiDoC 3 was supported by the Public Health Initiatives Programme (PT06) financed by EEA Grants Financial Mechanism 2009–14.

Conflict of interest: None declared.

References

1. Green MA, Li J, Relton C *et al*. Cohort Profile: The Yorkshire Health Study. *Int J Epidemiol* 2016;**45**:707–12.
2. Krokstad S, Langhammer A, Hveem K *et al*. Cohort Profile: The HUNT study, Norway. *Int J Epidemiol* 2013;**42**:968–77.
3. Rodrigues AM, Gouveia N, da Costa LP *et al*. EpiReumaPt—the study of rheumatic and musculoskeletal diseases in Portugal: a detailed view of the methodology. *Acta Reumatol Port* 2015;**40**:110–24.
4. Statistics Portugal INE. *Censos—Resultados Definitivos*. Lisbon: INE, 2012.
5. Eurostat. *Projected Old-Age Dependency Ratio*. 2016. http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tps00200&plugin=1 (26 April 2018, date last accessed).
6. Galea S, Tracy M. Participation rates in epidemiologic studies. *Ann Epidemiol* 2007;**17**:643–53.
7. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;**23**:137–45.
8. Ferreira LN, Ferreira PL, Pereira LN, Oppe M. EQ-5D Portuguese population norms. *Qual Life Res* 2014;**23**:425–30.
9. Ferreira LN, Ferreira PL, Pereira LN, Oppe M. The valuation of the EQ-5D in Portugal. *Qual Life Res* 2014;**23**:413–23.
10. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Med Care* 1992;**2**:98–83.
11. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M. Validation study of a Portuguese version of the hospital anxiety and depression scale. *Psychol Health Med* 2007;**12**:225–37.
12. Gregório MJ, Rodrigues AM, Eusebio M *et al*. Dietary patterns characterized by high meat consumption are associated with other unhealthy life styles and depression symptoms. *Front Nutr* 2017;**4**.
13. Gregório MJ, Rodrigues AM, Graça P *et al*. Food insecurity is associated with low adherence to the mediterranean diet and adverse health conditions in Portuguese adults. *Front Public Health* 2018;**6**.
14. Hoy D, March L, Brooks P *et al*. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;**73**:968–74.
15. Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis Res Ther* 2009;**11**:229.
16. Helmick CG, Felson DT, Lawrence RC *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part I. *Arthritis Rheum* 2008;**58**:15–25.
17. Anagnostopoulos I, Zinzaras E, Alexiou I *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;**11**:98.
18. Peláez-Ballestas I, Sanin LH, Moreno-Montoya J *et al*. Epidemiology of the rheumatic diseases in Mexico. A study of 5 regions based on the COPCORD methodology. *J Rheumatol* 2011;**86**:3–8.
19. Lawrence RC, Felson DT, Helmick CG *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. *Arthritis Rheum* 2008;**58**:26–35.
20. Carmona L, Ballina J, Gabriel R, Laffon A. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. *Ann Rheum Dis* 2001;**60**:1040–45.
21. Branco JC, Rodrigues AM, Gouveia N *et al*. Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt – a national health survey. *RMD Open* 2016;**2**:e000166.

22. Central Administration of the Health System (ACSS). *Actuais e Futuras Necessidades Previsionais de Médicos (SNS)*. Lisboa: ACSS, 2011.
23. Fernandes S, Rodrigues AM, Nunes C *et al*. Food insecurity in older adults: results from the EpiDoC3 study. *Front Med* 2018; 5:203.
24. Laires P. *The Impact of Rheumatic Diseases on Early Retirement*. Lisboa: Universidade de Lisboa, 2017.
25. Rodrigues AM, Gregório MJ, Sousa RD *et al*. Challenges of ageing in Portugal—data from the EpiDoC cohort. *Acta Med Port* 2018;**31**:80–93.
26. Sousa RD, Rodrigues AM, Gregório MJ *et al*. Anxiety and depression in the Portuguese older adults: prevalence and associated factors. *Front Med* 2017;**4**.
27. Duarte N, Rodrigues AM, Branco JC, Canhão H, Hughes SL, Paúl C. Health and lifestyles factors associated with osteoarthritis among older adults in Portugal. *Front Med* 2017;**4**.
28. Hladůvka J, Phuong BTM, Ljuhar R *et al*. Femoral ROIs and entropy for texture-based detection of osteoarthritis from high-resolution knee radiographs. *Comput Vis Pattern Recognit* 2017; arXiv:1703.09296.
29. Huisman M, Poppelaars J, van der Horst M *et al*. Cohort Profile: The Longitudinal Aging Study Amsterdam. *Int J Epidemiol* 2011;**40**:868–76.
30. Hasselhorn HM, Peter R, Rauch A *et al*. Cohort Profile: The LIDA Cohort Study—a German cohort study on work, age, health and work participation. *Int J Epidemiol* 2014; **43**: 1736–49.

CHAPTER IV

RESULTS

SECTION I – PREVALENCE, BURDEN AND UNDERTREATMENT OF OSTEOPOROSIS AND FRAGILITY FRACTURES IN PORTUGAL

SECTION II – CLINICAL RISK FACTORS AND CELLULAR DISTURBANCES ASSOCIATED WITH POOR TRABECULAR MECHANICAL BEHAVIOUR AND WITH HIP FRACTURES

SECTION III – SURROGATE MARKERS OF BONE MINERAL DENSITY AND FRACTURES IN A POPULATION BASED LONGITUDINAL COHORT

SECTION IV – STRATEGIES TO REDUCE NEW FRAGILITY FRACTURES IN PORTUGUESE POPULATION

SECTION I

PREVALENCE, BURDEN AND UNDERTREATMENT OF OSTEOPOROSIS AND FRAGILITY FRACTURES IN PORTUGAL

PART 1 – BRANCO JC, RODRIGUES AM, GOUVEIA N, ET AL. 2016. PREVALENCE OF RHEUMATIC DISEASES AND MUSCULOSKELETAL DISEASES AND THEIR IMPACT ON HEALTH-RELATED QUALITY OF LIFE, PHYSICAL FUNCTION AND MENTAL HEALTH IN PORTUGAL: RESULTS FROM EPIREUMAPT – A NATIONAL HEALTH SURVEY. RMD OPEN. 2:E000166.

PART 2 – RODRIGUES AM, EUSÉBIO M, SANTOS MJ, ET AL. 2018. THE BURDEN OF UNDERTREATMENT FRAGILITY FRACTURES AMONG SENIOR WOMEN. ARCHIVES OF OSTEOPOROSIS. 13:22.

Epidemiology

RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL ARTICLE

Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt– a national health survey

Jaime C Branco,[1,2,3,4,5] Ana M Rodrigues,[1,4,6,7] Nélia Gouveia,[1,3,4] Mónica Eusébio,[8] Sofia Ramiro,[1,3,9] Pedro M Machado,[1,10,11] Leonor Pereira da Costa,[12] Ana Filipa Mourão,[1,2,3,6] Inês Silva,[1,2] Pedro Laires,[1,7] Alexandre Sepriano,[2,3,6] Filipe Araújo,[2,13,14] Sónia Gonçalves,[15,16] Pedro S Coelho,[17] Viviana Tavares,[18,19,20] Jorge Cerol,[8] Jorge M Mendes,[17] Loreto Carmona,[21] Helena Canhão,[1,4,6,7,22] on behalf of the EpiReumaPt study group

To cite: Branco JC, Rodrigues AM, Gouveia N, *et al*. Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt– a national health survey. *RMD Open* 2016;**2**:e000166. doi:10.1136/rmdopen-2015-000166

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2015-000166).

Received 10 August 2015
Revised 3 December 2015
Accepted 18 December 2015

For numbered affiliations see end of article.

Correspondence to
Dr Ana Rodrigues;
anamfrodrigues@gmail.com

ABSTRACT

Objectives: To estimate the national prevalence of rheumatic and musculoskeletal diseases (RMDs) in the adult Portuguese population and to determine their impact on health-related quality of life (HRQoL), physical function, anxiety and depression.

Methods: EpiReumaPt is a national health survey with a three-stage approach. First, 10 661 adult participants were randomly selected. Trained interviewers undertook structured face-to-face questionnaires that included screening for RMDs and assessments of health-related quality of life, physical function, anxiety and depression. Second, positive screenings for ≥1 RMD plus 20% negative screenings were invited to be evaluated by a rheumatologist. Finally, three rheumatologists revised all the information and confirmed the diagnoses according to validated criteria. Estimates were computed as weighted proportions, taking the sampling design into account.

Results: The disease-specific prevalence rates (and 95% CIs) of RMDs in the adult Portuguese population were: low back pain, 26.4% (23.3% to 29.5%); periarticular disease, 15.8% (13.5% to 18.0%); knee osteoarthritis (OA), 12.4% (11.0% to 13.8%); osteoporosis, 10.2% (9.0% to 11.3%); hand OA, 8.7% (7.5% to 9.9%); hip OA, 2.9% (2.3% to 3.6%); fibromyalgia, 1.7% (1.1% to 2.1%); spondyloarthritis, 1.6% (1.2% to 2.1%); gout, 1.3% (1.0% to 1.6%); rheumatoid arthritis, 0.7% (0.5% to 0.9%); systemic lupus erythaematosus, 0.1% (0.1% to 0.2%) and polymyalgia rheumatica, 0.1% (0.0% to 0.2%). After multivariable adjustment, participants with RMDs had significantly lower EQ5D scores (β=−0.09; p<0.001) and higher HAQ scores (β=0.13; p<0.001) than participants without RMDs. RMDs were also significantly associated with the presence of anxiety symptoms (OR=3.5; p=0.006).

Conclusions: RMDs are highly prevalent in Portugal and are associated not only with significant physical function and mental health impairment but also with poor HRQoL, leading to more health resource

Key messages

What is already known about this subject?

- Rheumatic and musculoskeletal diseases (RMD) are among the most common chronic non-communicable diseases.

What does this study add?

- EpiReumaPt is the first population-based study on rheumatic diseases in Portugal and we demonstrated that low back pain and osteoarthritis are the two most prevalent RMD.
- We have used the new ACR/EULAR classification criteria for RA and the ASAS criteria for SpA and found a prevalence of 0.7% for RA and 1.6% for SpA with similar proportion of males and females with the disease.
- RMDs patients have poorer quality of life, higher health consumption and significant mental health impairment as compared to non-RMDs subjects.

How might this impact on clinical practice?

- EpiReumaPt study emphasizes the burden of RMDs in Portugal and the need to increase RMD awareness.

RMD Open

consumption. The EpiReumaPt study emphasises the burden of RMDs in Portugal and the need to increase RMD awareness, being a strong argument to encourage policymakers to increase the amount of resources allocated to the treatment of rheumatic patients.

INTRODUCTION

Rheumatic and musculoskeletal diseases (RMDs) are among the most common chronic non-communicable diseases. They are the leading cause of disability in developed countries, and consume a large amount of health and social resources.[1–3] So far, comparative factors on the impact on health-related quality of life (HRQoL), physical function and mental health status between RMD and non-RMD participants, have been unknown.[4 5]

The prevalence of RMDs has been determined in several countries,[6–13] however, epidemiological data in Portugal are scarce.[14–16] EpiReumaPt is a national health-survey conducted to estimate the prevalence of hand, knee and hip osteoarthritis (OA), low back pain (LBP), rheumatoid arthritis (RA), fibromyalgia (FM), gout, spondyloarthritis (SpA), periarticular disease (PD), systemic lupus erythaematosus (SLE), polymyalgia rheumatica (PMR) and osteoporosis (OP), in the adult Portuguese population. Another aim was to assess the burden of RMDs by determining their impact on HRQoL, physical function and mental health. Both aims address the needs and objectives identified in a recent governmental initiative—the National Program Against Rheumatic diseases.[17]

METHODS

The study protocol has been previously published,[18] as has a separate manuscript extensively describing the methodological details of the project.[19] An outline of the methodology is presented below.

Setting

Portugal is a southwestern European country, including the mainland and the Autonomous Regions of Azores and Madeira. According to a census performed in 2011, Portugal has a resident population of 10 562 178 inhabitants,[20] of whom 8 657 240 are adults.[18 21]

Study population

EpiReumaPt is a national, cross-sectional and population-based study. The study population was composed of adults (≥18 years old) who were non-institutionalised and living in private households in the Mainland and the Islands (Azores and Madeira). Exclusion criteria were: residents in hospitals, nursing homes and military institutions or prisons, and individuals unable to speak Portuguese or unable to complete the questionnaires.[21]

Sampling

Participants were selected through a process of multistage random sampling. The sample was stratified according to the Portuguese Nomenclature of Territorial Units for Statistics (NUTS II; seven territorial units: *Norte, Centro, Alentejo, Algarve, Lisboa e Vale do Tejo*, Madeira and Azores) and the size of the population (<2000; 2000–9999; 10 000–19 999; 20 000–99 999; and ≥100 000 inhabitants).

Recruitment

Recruitment took place between September 2011 and December 2013. EpiReumaPt involved a three-stage approach. First, candidate households were selected using a random route process. The adults with permanent residence in the selected household with the most recently completed birthday were recruited (one adult per household). Trained interviewers undertook structured face-to-face questionnaires in participants' households, collecting a vast number of variables (sociodemographic, socioeconomic, HRQoL (EQ-5D-3L), physical function (HAQ), anxiety and depression symptoms, lifestyle habits, chronic non-communicable diseases, healthcare resources utilisation) and performing a screening for RMDs. Questions were asked about several rheumatic symptoms and an algorithm for the screening of each RMD was applied. An individual was considered to have a positive screening if the subject mentioned a previously known RMD, if any one of the specific disease algorithms (covering disease characteristic and respective signs and symptoms) in the screening questionnaires was positive, or if the subject reported muscle, vertebral or peripheral joint pain in the previous 4 weeks. The overall performance of the screening algorithm was evaluated (the gold standard was considered to be the final diagnosis after revision, see phase 3) and the overall sensitivity of the screening questionnaire for RMDs was 98%, with a specificity of 22%. The positive predictive value was 85% and the negative predictive value was 71%.[21]

Second, all participants who screened positive for at least one RMD plus 20% of individuals with no rheumatic symptoms (negative screening) were invited for a structured evaluation by a rheumatologist at the local primary care centre. Finally, a team of three experienced rheumatologists revised all the clinical, laboratorial and imaging data, and confirmed the diagnoses according to validated criteria (figure 1).[21]

Measurements

In the first phase of EpiReumaPt, participants were asked about their sociodemographic data (age, gender, ethnicity, education, marital status), socioeconomic profile (measures of wealth, household income, current professional status) and lifestyle habits (alcohol, tobacco and coffee intake, physical exercise). Information on work status was also collected. Healthcare resource consumption data were collected through the number and type of outpatient

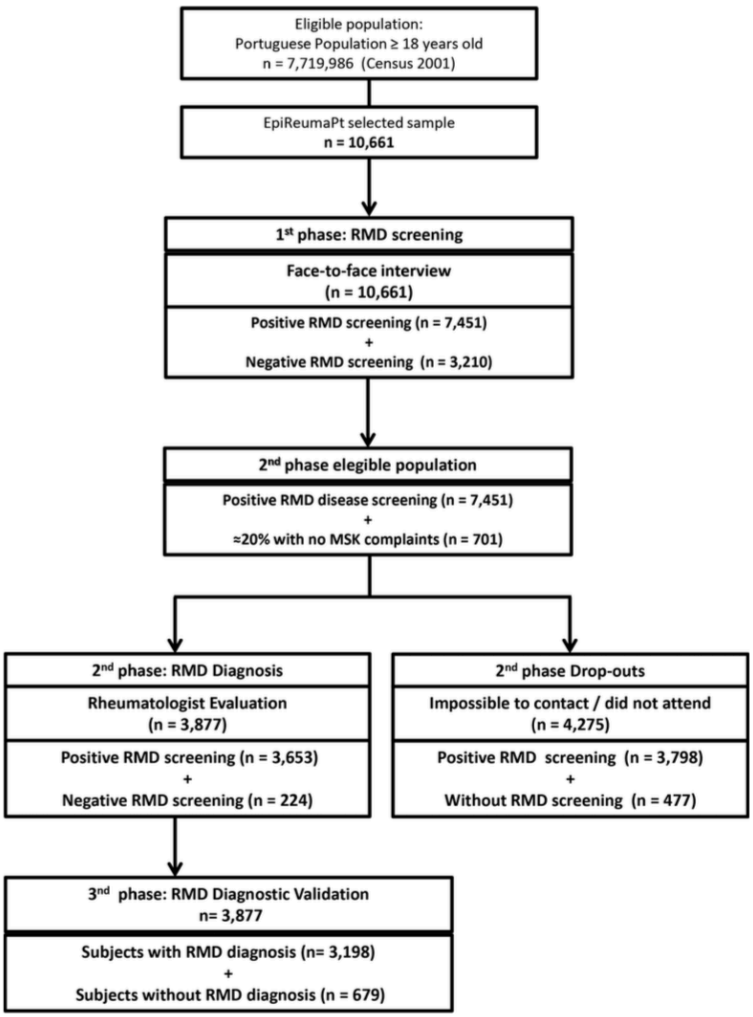

Figure 1 Flowchart of recruitment in the EpiReumaPt Study. RMD, rheumatic and musculoskeletal disease; MSK, musculoskeletal disease.

clinic visits, hospitalisations, homecare assistance and other needs for healthcare services in the previous 12 months.

To evaluate generic HRQoL, we used the Portuguese validated version of the European Quality of Life questionnaire, five dimensions, three levels (EQ-5D-3L).[22 23] Physical function was assessed by the Health Assessment Questionnaire (HAQ).[24] Anxiety and depression symptoms, as aspects of mental health, were assessed by the Portuguese validated version of the Hospital Anxiety and Depression Scale (HADS).[25] HADS is divided into an Anxiety subscale (HADS-A) and a Depression subscale (HADS-D), both containing seven intermingled items. We also assessed anthropometric data (self-reported weight and height) and self-reported chronic diseases (high cholesterol, high blood pressure, allergies, gastrointestinal disease, mental disease, cardiac disease, diabetes, thyroid and parathyroid disease, urolithiasis, pulmonary disease, hyperuricaemia, cancer, neurological disease, hypogonadism). Information regarding pharmacological and non-pharmacological therapies was also collected.

In the second phase of EpiReumaPt, thorough history-taking and physical examination were performed. Previous diagnoses of RMDs and current medications were also assessed.[21]

Case definition

The presence of a RMD was considered if a subject, after the clinical appointment of the second phase, had a positive expert opinion combined with the fulfilment of validated classification criteria to establish a diagnosis of knee OA, hip OA, hand OA, FM, SLE, gout, RA, SpA or PMR.[21] We used the American College of Rheumatology (ACR) classification criteria for knee OA,[26] hip OA,[27] hand OA,[28] FM,[29] SLE[30] and gout;[31]

the ACR/European League Against Rheumatism (ACR/EULAR) criteria for RA;[32] the Assessment of SpondyloArthritis international Society (ASAS) criteria for axial and peripheral SpA;[33–35] and the Bird criteria for PMR.[36]

PD was defined as a regional pain syndrome affecting muscles, tendons, bursas or periarticular soft tissues, with or without evidence of joint or bone involvement. The following PDs were specifically investigated: tenosynovitis, adhesive capsulitis of the shoulder, enthesopathies, bursitis, palmar or plantar fasciitis and carpal or tarsal tunnel syndrome present at the time of the assessment. The diagnosis was established based on expert opinion in the second phase of the study.

OP was defined by the clinical decision of the rheumatologist who observed the subject in the second phase of the study based on the presence of at least one of the following: previous fragility fracture, previous OP diagnosis, current OP treatment or fulfilment of the WHO criteria[37] when axial dual energy X-ray absorptiometry was available.

LBP was defined solely by self-report and clinical history.

Statistical analysis

Prevalence estimates for RMDs were computed as weighted proportions, in order to take into account the sampling design.[21]

Participants with and without RMDs were compared. Univariable analyses were first performed considering the study design. Multivariate regression models were used to assess the differences between individuals with and without RMDs, regarding: HRQoL and physical function (EQ5D and HAQ), mental health (presence of symptoms of anxiety (HADS-A ≥11 vs <11), presence of symptoms of depression (HADS-D ≥11 vs <11)[25]) and health resources consumption (number of medical visits (general practitioner, rheumatologist, orthopaedic surgeon and any other specialists), and home care in the previous 12 months (yes/no), hospitalisations in the previous 12 months (yes/no), early retirement due to disease (yes/no), absence from work due to disease in the previous 12 months (yes/no) and number of days of absence). Significantly different variables in the univariable analysis were included in the multivariable model. In order to adjust the differences between groups, the following potential confounders were included in the model: age, gender, NUTS II, education level, employment status, household income, alcohol intake, current smoking, physical exercise, body mass index (BMI), physical exercise and number of comorbidities.

To assess the independent relationship of each RMD with disability (HAQ), HRQoL (EQ5D), presence of symptoms of anxiety and presence of symptoms of depression, four multivariable regression models were performed. For the first two outcomes—continuous variables—linear regression was used; and for the last two—dichotomous outcomes—logistic regression was performed. Multivariable models were constructed using a backward selection method. The following independent variables were tested: age, gender, NUTS II, years of education, work status, BMI, alcohol intake, current smoking, regular physical activity and number of comorbidities. All RMDs were included in the models and were forced to stay there. For the models with HAQ and EQ5D, the presence of symptoms of anxiety or depression was also considered. Possible interactions between each RMD and gender and age were tested for the four outcomes.

Significance level was set at 0.05. All analyses were weighted and performed using STATA IC V.12 (StataCorp, 2011. Stata Statistical Software: Release 12. College Station, Texas, USA: StataCorp LP).

Ethical issues

EpiReumaPt was performed according to the principles established by the Declaration of Helsinki. The study was reviewed and approved by the National Committee for Data Protection (*Comissão Nacional de Proteção de Dados*) and by the NOVA Medical School Ethics Committee. All participants provided informed consent to participate in all phases of the study.[18] Further details of ethical issues of EpiReumaPt have been described elsewhere.[19]

RESULTS

Prevalence of RMDs in the Portuguese adult Population

The EpiReumaPt population did not differ from the Portuguese population (table 1).[20 38] In the EpiReumaPt study, 21.2% (95% CI 19.9% to 22.5%) of the Portuguese population self-reported a RMD. During the second phase of the study, we observed 3877 participants and detected 1532 new RMD diagnoses; 2670 individuals were found to have more than one RMD. Moreover, of the 3877 participants evaluated in the second phase, only 85 (9.6%) previously reporting a RMD had no identifiable target disease.

The prevalence of each RMD, overall and stratified by gender, and the estimated number of patients in the Portuguese population are shown in table 2. The RMD with the highest prevalence in Portugal was LBP (26.4%; 95% CI 23.3% to 29.5%), significantly more frequent in women than in men (29.6% vs 22.8%; p=0.040) (table 2). LBP increased with age and its prevalence was highest in the 46–55-year age group (27.7%; 95% CI 23.1% to 32.4%) (figure 2). PD was also a frequent RMD with an overall prevalence of 15.8% (95%CI 13.5% to 18.0%) and women were also significantly more affected than men (19.1% vs 12.0%; p=0.005). This RMD had the highest prevalence in the working-age population (46–55 years) (21.5%; 95% CI 17.4 to 25.5%) (figure 2). OA was also common among Portuguese individuals; particularly knee OA, with a prevalence of 12.4% (95% CI 11.0% to 13.8%). Of note, the combined prevalence of hip and/or knee and/or hand OA in Portugal is 19.1%

Table 1 Sociodemographic and health related characteristics of the adult Portuguese population: EpiReumaPt population (first and second phase) and Census 2011 population (Portuguese population)

Demographic characteristics	First phase study population n=10 661	Second phase study population n=3877	CENSUS 2011
Gender (female)	6551 (52.6%)	2630 (52.5%)	4 585 118 (53.0%)
Age group (years)			
18–29	1182 (22.1%)	190 (21.0%)	1 470 782 (17.0%)
30–39	1511 (18.8%)	403 (19.3%)	1 598 250 (18.5%)
40–49	1906 (17.3%)	680 (18.2%)	1 543 392 (17.8%)
50–59	1801 (14.8%)	818 (14.7%)	1 400 011(16.2%)
60–69	1915 (12.9%)	914 (13.4%)	1 186 442 (13.7%)
70–74	849 (5.8%)	376 (5.3%)	496 438 (5.7%)
≥75	1497 (8.4%)	496 (8.0%)	961 925 (11.1%)
Ethnicity/race			
Caucasian	10 342 (96.0%)	3786 (93.3%)	No comparable data
Black	221 (3.4%)	64 (6.1%)	
Asian	8 (0.1%)	2 (0.0%)	
Gipsy	20 (0.3%)	3 (0.1%)	
Other	38 (0.3%)	13 (0.5%)	
Education level (years)			
>12	1764 (20.4%)	508 (21.1%)	1 741 567 (20.1%)
10–12	1920 (23.8%)	575 (23.2%)	1 560 958 (18.0%)
5–9	2175 (22.6%)	775 (22.4%)	2 134 401 (24.6%)
0–4	4726 (33.2%)	1997 (33.4%)	3 239 724 (37.4%)
NUTS II			
Norte	3122 (34.9%)	1050 (37.2%)	3 007 823 (34.7%)
Centro	1997 (22.8%)	856 (19.8%)	1 938 815 (22.4%)
Lisboa	2484 (26.7%)	708 (29.6%)	2 300 053 (26.6%)
Alentejo	669 (7.3%)	273 (5.8%)	633 691 (7.3%)
Algarve	352 (3.8%)	144 (3.1%)	370 704 (4.3%)
Azores	1029 (2.2%)	420 (2.3%)	192 357 (2.2%)
Madeira	1008 (2.3%)	426 (2.2%)	213 797 (2.5%)

NUTS II, Nomenclature of Territorial Units for Statistics (Norte, Centro, Alentejo, Algarve, Lisboa, Madeira and the Azores).

(95% CI 17.1 to 21.1%). Noteworthy, gout had an overall prevalence of 1.3% (95% CI 1.0% to 1.6%) (table 2). The age stratum with the highest gout prevalence corresponded to the elderly (>85 years old) with a 3.2% prevalence (95% CI 2.0% to 4.4%) (figure 2). As expected, men had the highest gout prevalence (2.6% vs 0.1% in women, p<0.001). Moreover, 22.2% (95% CI 8.2 to 36.2) of gout patients had poliarticular disease and

Table 2 Prevalence of rheumatic and musculoskeletal diseases (RMDs) in Portugal, overall and stratified by gender

	Total prevalence (95% CI) n=3877	Women (95% CI) n=2630	Men (95% CI) n=1247
Low back pain (n=1393)	26.4% (23.3% to 29.5%)	29.6% (25.8% to 33.5%)	22.8% (17.9% to 27.8%)
Periarticular disease (n=929)	15.8% (13.5% to 18.0%)	19.1% (16.2% to 22.0%)	12.0% (8.4% to 15.6%)
Knee osteoarthritis (n=981)	12.4% (11.0% to 13.8%)	15.8% (13.7% to 18.0%)	8.6% (6.9% to 10.3%)
Osteoporosis (n=858)	10.2% (9.00% to 11.3%)	17.0% (14.7% to 19.2%)	2.6% (1.9% to 3.4%)
Hand osteoarthritis (n=625)	8.7% (7.5% to 9.9%)	13.8% (11.6% to 15.9%)	3.2% (2.2% to 4.1%)
Hip osteoarthritis (n=199)	2.9% (2.3% to 3.6%)	3.0% (2.3% to 3.7%)	2.9% (1.7% to 4.1%)
Fibromyalgia n=149)	1.7% (1.3% to 2.1%)	3.1% (2.4% to 3.9%)	0.0% (−0.0% to 0.2%)
Spondyloarthritis (n=92)	1.6% (1.2% to 2.1%)	2.0% (1.3% to 2.7%)	1.2% (0.7% to 1.8%)
Gout (n=92)	1.3% (1.0% to 1.6%)	0.1% (−0.0% to 0.2%)	2.6% (1.9% to 3.3%)
Rheumatoid arthritis (n=61)	0.7% (0.5% to 0.9%)	1.2% (0.8% to 1.5%)	0.3% (0.1% to 0.4%)
SLE (n=13)	0.1% (0.1% to 0.2%)	0.2% (0.1% to 0.4%)	0.0% (−0.0% to 0.1%)
Polymyalgia rheumatica (n=8)	0.1% (0.0% to 0.2%)	0.13% (0.0% to 0.2%)	0.1% (−0.0% to 0.2%)

The sample was calculated considering a minimum prevalence of 0.5%.[18] For rare diseases the estimated number of Portuguese participants with the disease could be overestimated.
RMD, rheumatic and musculoskeletal disease; SLE, systemic lupus erythaematosus.

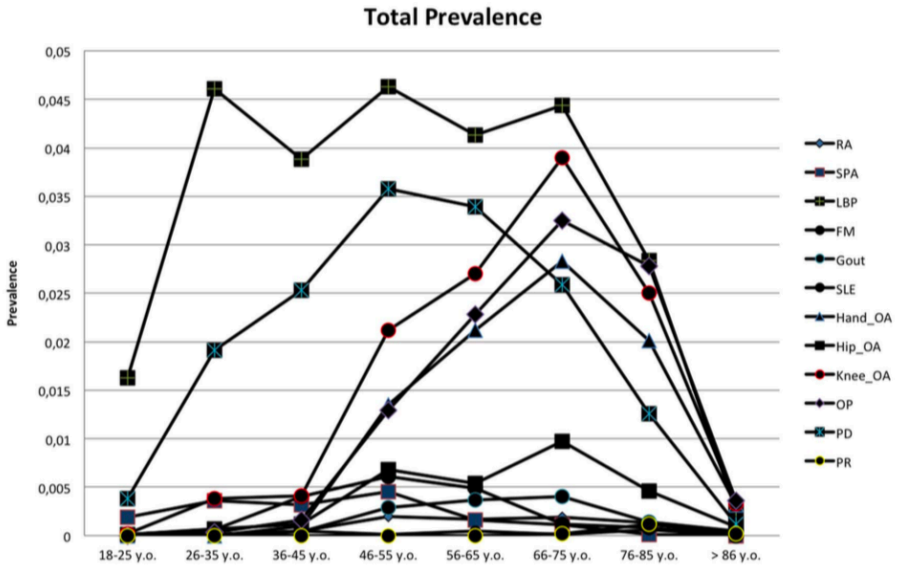

Figure 2 Prevalence of RMDs, stratified by age group. RMD, rheumatic and musculoskeletal disease.

11.0% had chronic tophaceous gout. The mean number of gout attacks in the 12 months preceding the clinical evaluation was 2.0±1.7.

Regarding inflammatory rheumatic diseases, SpA had the highest prevalence in the adult population (1.6%; 95% CI 1.2% to 2.0%), with 51.8% of cases being axial SpA. We found no significant gender predominance in SpA (p=0.094). Among SpA subtypes according to the classical nomenclature, undifferentiated SpA accounted for 44.3% of cases, ankylosing spondylitis (AS) 29.6%, psoriatic arthritis 18.7% and SpA associated with inflammatory bowel disease 12.0%. These results correspond to a national prevalence rate of 0.7% (95% CI 0.4% to 1.0%) for undifferentiated SpA, 0.5% (95% CI 0.3% to 0.7%) for AS, 0.3% (0.1% to 0.5%) for psoriatic arthritis and 0.2% (0.0% to 0.4%) for SpA associated with inflammatory bowel disease. Finally, the prevalence of RA was 0.7% (95% CI 0.5% to 0.9%).

Participants with RMDs had significantly lower HRQoL, physical function and mental health and consumed more healthcare resources

Regarding HRQoL, we found that participants with RMD had significantly lower EQ5D scores (β=−0.09; p<0.001) when compared to participants without RMD, adjusted for demographic factors, socioeconomic factors, lifestyle and comorbidities. Furthermore, patients with RMD had significantly higher disability (HAQ score) (β=0.13; p<0.001).

We also found that, in participants with RMD, there was a significantly higher prevalence of anxiety symptoms (OR=3.5; p=0.006) but no significant differences were found regarding depressive symptoms (OR=1.9; p=0.173) (table 3).

Considering healthcare resource consumption (table 3), patients with RMD had been more often hospitalised and had more homecare support needs in the previous 12 months when compared to participants without any RMD (OR=2.5, p=0.027 and OR=13.2, p=0.001, respectively). Finally, we found no differences between the two groups regarding sick leave or early retirement due to disease (table 3).

Disease-specific associations with worse HRQoL and higher disability

Several RMDs were significantly and independently associated with worse QoL in the Portuguese population. By decreasing order of effect, PMR (β=−0.33; p=0.027), RA (β=−0.13; p=0.001), FM (β=−0.10; p<0.001), LBP (β=−0.07; p<0.001), knee OA (β=−0.06; p<0.001) and PD (β=−0.04; p=0.029) were associated with worse QoL. Moreover, participants retired or on sick leave (β=−0.04; p=0.016) and those with a higher number of comorbidities (β=−0.03; p<0.001) were also associated with worse QoL. The presence of anxiety and depressive symptoms (HADS≥11) were also associated with worse QoL (β=−0.14; p<0.001 and β=−0.14; p<0.001, respectively). On the other hand, alcohol consumption was significantly associated with better QoL (β=0.045; p<0.001) (table 4).

Regarding the HAQ score, and by decreasing order of effect, PMR (β=1.03; p<0.001), RA (β=0.38; p<0.001), FM (β=0.27; p=0.001), knee OA (β=0.11; p=0.002), LBP (β=0.09; p<0.001), OP (β=0.08; p=0.033) and PD

Table 3 Comparison of sociodemographic, socioeconomic, health status and health resources consumption between participants with and without RMD: adjusted analysis

HRQoL and physical function	RMD n=3195	Non-RMD n=682	β estimates	95% CI	Adjusted p Value
EQ5D (0–1)	0.7±0.3	0.9±0.1	−0.09	(−0.13 to −0.05)	<0.001*
HAQ (0–3)	0.4±0.7	0.1±0.2	0.13	(0.08 to 0.17)	<0.001*
Mental health	**RMD**	**Non-RMD**	**OR**	**95% CI**	**Adjusted p value**
Anxiety (yes vs no)	600 (16.7%)	63 (5.3%)	3.5	(1.4 to 8.0)	0.006*
Depression (yes vs no)	349 (8.3%)	29 (1.3%)	1.9	(0.8 to 4.6)	0.173
Healthcare resources consumption	**RMD**	**Non-RMD**	**OR**	**95% CI**	**Adjusted p value**
Physician visits in the past 12 months					0.010*
General practitioners	2661 (78.8%)	502 (71.5%)	0.5	(0.3 to 0.8)	<0.001*
Rheumatology visits	206 (4.6%)	11 (1.0%)	30.5	(7.4 to 126.2)	0.010*
Orthopaedic visits	475 (14.9%)	46 (6.5%)	3.2	(1.3 to 7.8)	0.825
Other visits	1758 (57.1%)	347 (53.5%)	0.9	(0.6 to 1.5)	
Healthcare resources consumption	**RMD**	**Non-RMD**	**β estimates**	**95% CI**	**Adjusted p value**
Number of physician appointments in the past 12 months					
General practitioners	2.5±5.9	4.0±19.0	−4.01	(−11.37 to 3.34)	0.285
Rheumatology appointments	0.1±0.8	0.0±0.1	0.08	(0.05 to 0.11)	<0.001*
Orthopaedic appointments	0.4±1.4	0.1±0.4	0.27	(0.10 to 0.43)	0.002*
Other appointments	1.9±8.0	1.5±1.5	0.01	(−0.47 to 0.50)	0.961
Healthcare resources consumption	**RMD**	**Non-RMD**	**OR**	**95% CI**	**Adjusted p value**
Home care in the past 12 months	100 (2.7%)	5 (0.1%)	13.2	(2.7 to 63.6)	0.001*
Hospitalisations in the past 12 months	324 (11.4%)	53 (5.5%)	2.5	(1.1 to 5.8)	0.027*
Early retirement due to disease	488 (30.9%)	33 (22.0%)	2.3	(0.9 to 6.0)	0.101
Absent from work due to disease in the past 12 months	323 (29.9%)	76 (24.8%)	1.7	(0.8 to 3.5)	0.163
Healthcare resources consumption	**RMD**	**Non-RMD**	**β estimates**	**95% CI**	**Adjusted p value**
Number of days absent from work due to disease in the past 12 months	31.5±83.9	22.5±14.1	14.11	(−4.72 to 32.94)	0.141

Sample size is not constant due to missing data in RMD: EQ5D (n=3168), Early retirement due to disease (n=1419), absent from work due to disease in the past 12 months (n=1010), number of days absent from work due to disease in the past 12 months (n=318).
Non-RMD: EQ5D (n=678), Early retirement due to disease (n=142), absent from work due to disease in the past 12 months (n=359), number of days absent from work due to disease in the past 12 months (n=75).
p Values were adjusted for age, gender, Nomenclature of Territorial Units for Statistics (North, Centre, Alentejo, Algarve, Lisbon, Madeira and the Azores), years of education, work status, household income, alcohol intake, physical exercise, Body Mass Index and number of comorbidities. For continuous variables, a multivariable regression was used to assess the differences between the groups (individuals with Rheumatic Diseases and those without Rheumatic Diseases). The estimated values were obtained considering study design.
*Adjusted p values <0.05.
EQ5D, European Quality of Life questionnaire five dimensions three levels; HAQ, Health Assessment Questionnaire; HRQoL, health-related quality of life; RMD, rheumatic and musculoskeletal disease.

Table 4 Factors associated with health-related quality of life (EQ5D) and physical function (HAQ) considering each RMD as a variable of interest: multivariable models

	EQ5D		HAQ	
Demographic characteristics	**β coefficient (95% CI)**	**p Value**	**β coefficient (9 5%CI)**	**p Value**
Gender (female)	−0.03 (−0.06 to 0.00)	0.058	0.11 (0.07 to 0.15)	<0.001*
Age (years)	0.00 (−0.0 to 0.01)	0.902	0.00 (−0.00 to 0.00)	0.857
BMI				
Underweight vs normal	0.09 (−0.01 to 0.16)	0.021*	−0.02 (−0.16 to 0.12)	0.802
Overweight vs normal	0.03 (−0.00 to 0.52)	0.067	−0.00 (−0.04 to 0.04)	0.975
Obese vs normal	0.01 (−0.02 to 0.04)	0.526	−0.08 (0.02 to 0.14)	0.005*
Years of education	−0.01 (−0.0 to 0.00)	0.788	−0.01 (−0.02 to −0.00)	0.002*
Employment status				
Employed vs retired or sick leave	−0.04 (−0.09 to −0.00)	0.046*	0.14 (0.06 to 0.21)	<0.001*
Employed vs unemployment	−0.00 (−0.04 to 0.05)	0.946	0.04 (−0.02 to 0.10)	0.170
NUTS II				
Norte vs Lisboa	0.0 (−0.03 to 0.04)	0.832	0.03 (−0.01 to 0.08)	0.168
Centro vs Lisboa	0.0 (−0.03 to 0.04)	0.777	0.04 (−0.02 to 0.10)	0.167
Alentejo vs Lisboa	0.02 (−0.2 to 0.05)	0.414	0.11 (0.05 to 0.18)	0.001*
Algarve vs Lisboa	0.04 (−0.00 to 0.09)	0.078	0.01 (−0.06 to 0.07)	0.836
Azores vs Lisboa	0.11 (−0.03 to 0.05)	0.572	−0.00 (−0.05 to 0.05)	0.938
Madeira vs Lisboa	0.01 (−0.03 to 0.04)	0.763	0.11 (0.02 to 0–19)	0.011*
Number of comorbidities (0–15)	−0.03 (−0.04 to −0.03)	<0.001*	0.06 (0.05 to 0.08)	<0.001*
Life-style habits				
Alcohol intake (yes/no)	0.05 (0.02 to 0.07)	0.001*	−0.06 (−0.10 to −0.01)	0.023*
Regular physical exercise (yes/no)	0.02 (−0.01 to 0.05)	0.152	−0.03 (−0.07 to 0.01)	0.139
Mental disorders				
Anxiety (yes/no)	−0.14 (−0.20 to −0.08)	<0.001*	0.15 (0.07 to 0.22)	<0.001*
Depression (yes/no)	−0.14 (−0.19 to −0.09)	<0.001*	0.32 (0.20 to 0.44)	<0.001*
RMD diagnosis				
Low back pain (yes/no)	−0.07 (−0.10 to −0.04)	<0.001*	0.09 (0.04 to 0.13)	<0.001*
Periarticular disease (yes/no)	−0.04 (−0.08 to −0.01)	0.016*	0.06 (0.01 to 0.11)	0.019*
Knee osteoarthritis (yes/no)	−0.06 (−0.09 to −0.03)	<0.001*	0.11 (0.04 to 0.18)	0.002*
Osteoporosis (yes/no)	−0.01 (−0.04 to 0.02)	0.676	0.08 (0.01 to 0.15)	0.033*
Hand osteoarthritis (yes/no)	−0.00 (−0.04 to 0.03)	0.831	−0.00 (−0.08 to 0.07)	0.903
Hip osteoarthritis (yes/no)	−0.05 (−0.10 to 0.01)	0.083	−0.30 (−0.70 to 0.10)	0.145
Fibromyalgia (yes/no)	−0.10 (−0.16 to −0.05)	<0.001*	0.27 (0.10 to 0.43)	<0.001*
Spondyloarthritis (yes/no)	−0.05 (−0.11 to 0.01)	0.120	0.08 (−0.35 to 0.19)	0.180
Gout (yes/no)	0.05 (−0.01 to 0.11)	0.085	−0.06 (−0.19 to 0.07)	0.387
Rheumatoid arthritis (yes/no)	−0.13 (−0.21 to −0.06)	0.001*	0.38 (0.20 to 0.56)	<0.001*
SLE (yes/no)	0.03 (−0.072 to 0.13)	0.585	0.23 (−0.07 to 0.53)	0.137
Polymyalgia rheumatica (yes/no)	−0.33 (−0.63 to −0.04)	0.027*	1.03 (0.46 to 1.60)	<0.001*
Hip osteoarthritis×age	–	–	0.01 (0.00 to 0.01)	0.016*

Two multivariable regression models were used: one to identify possible factors that have an impact on the HRQoL, and another to identify possible factors that have an impact on the functional capacity. The estimates were obtained considering study design.
*Adjusted p value<0.05.
BMI, body mass index; EQ5D, European Quality of Life questionnaire five dimensions three levels; HAQ, Health Assessment Questionnaire; NUTS II, Nomenclature of Territorial Units for Statistics (North, Centre, Alentejo, Algarve, Lisbon, Madeira and the Azores); RMD, rheumatic and musculoskeletal disease; SLE, systemic lupus erythaematosus.

(β=0.06; p=0.019) were significantly associated with disability.

Certain characteristics, such as female gender (β=0.11; p<0.001), low educational level (β=−0.01; p=0.002) and sick leave or retirement (β=0.14; p<0.001), were significantly associated with higher HAQ scores. The number of comorbidities (β=0.06; p<0.001) and symptoms of anxiety (β=0.15; p<0.001) or depression (β=0.32; p<0.001) were also significantly associated with disability. Daily or occasional alcohol intake was significantly associated with lower HAQ scores (β=−0.06; p=0.023) (table 4).

Disease-specific associations with depression and anxiety symptoms

Several RMDs were significantly and independently associated with the presence of anxiety (HADS-A ≥11) and depressive symptoms (HADS-D ≥11) (table 5). By order of effect, FM (OR=3.4; p<0.001), SpA (OR=3.0; p=0.008) and LBP (OR=1.9; p=0.005) were significantly and independently associated with the presence of anxiety symptoms (table 5). On the other hand, PMR (OR=14.3; p=0.012), FM (OR=4.0; p=0.001) and LBP (OR=1.6; p=0.014) and knee OA (OR=1.5; p=0.047), were

Table 5 Factors associated with anxiety and depression symptoms (HADS) considering each RMD as a variable of interest: multivariable models

	Anxiety		Depression	
Demographic characteristics	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
Gender (female)	3.1 (1.7 to 5.9)	0.001*	2.8 (1.6 to 4.9)	<0.001*
Age	0.98 (0.956 to 0.997)	0.024*	1.03 (1.0 to 1.1)	0.004*
BMI				
Underweight vs normal	0.4 (0.1 to 1.5)	0.183	0.1 (0.1 to 0.5)	0.010*
Overweight vs normal	0.8 (0.5 to 1.2)	0.240	0.6 (0.4 to 1.0)	0.059
Obese vs normal	0.5 (0.3 to 0.9)	0.026*	0.8 (0.5 to 1.3)	0.309
Years of education	0.9 (0.86 to 0.99)	0.027*	0.9 (0.8 to 0.998)	0.044*
Employment status				
Employed vs retired or leave	0.9 (0.5 to 1.5)	0.602	0.8 (0.5 to 1.5)	0.580
Employed vs unemployment	2.9 (1.4 to 5.9)	0.003*	1.9 (0.9 to 3.9)	0.080
NUTS II				
Norte vs Lisboa	1.8 (1.0 to 3.3)	0.035*	0.9 (0.5 to 1.6)	0.820
Centro vs Lisboa	1.1 (0.6 to 1.9)	0.739	0.9 (0.5 to 1.7)	0.746
Alentejo vs Lisboa	1.1 (0.6 to 2.1)	0.791	1.0 (0.4 to 2.2)	0.972
Algarve vs Lisboa	1.0 (0.5 to 2.2)	0.972	2.0 (0.5 to 8.0)	0.340
Azores vs Lisboa	1.2 (0.7 to 2.2)	0.502	1.0 (0.6 to 1.8)	0.987
Madeira vs Lisboa	1.0 (0.4 to 2.1)	0.922	0.6 (0.3 to 1.1)	0.101
Number of comorbidities (0–15)	1.5 (1.4 to 1.7)	<0.001*	1.3 (>1.2 to 1.5)	<0.001*
Life style habits				
Present alcohol intake (yes/no)	0.6 (0.3 to 0.9)	0.020*	0.8 (0.4 to 1.5)	0.505
Regular physical exercise (yes/no)	0.7 (0.4 to 1.2)	0.182	0.4 (0.2 to 0.6)	0.001*
RMD diagnosis				
Low back pain (yes/no)	1.9 (1.2 to 2.9)	0.005*	1.6 (1.1 to 2.4)	0.014*
Periarticular disease (yes/no)	1.1 (0.8 to 1.6)	0.599	0.7 (0.4 to 1.1)	0.082
Knee osteoarthritis (yes/no)	0.95 (0.6 to 1.4)	0.813	1.5 (1.0 to 2.4)	0.047*
Osteoporosis (yes/no)	1.2 (0.8 to 1.8)	0.344	1.1 (0.7 to 1.8)	0.745
Hand osteoarthritis (yes/no)	0.94 (0.5 to 1.6)	0.831	1.0 (0.7 to 1.6)	0.903
Hip osteoarthritis (yes/no)	0.9 (0.5 to 1.6)	0.628	0.8 (0.4 to 1.7)	0.600
Fibromyalgia (yes/no)	3.4 (1.8 to 6.1)	<0.001*	4.0 (1.8 to 8.9)	0.001*
Spondyloarthritis (yes/no)	3.0 (1.3 to 6.7)	0.008*	1.7 (0.5 to 5.2)	0.365
Gout (yes/no)	1.7 (0.6 to 4.8)	0.335	0.6 (0.1 to 4.8)	0.621
Rheumatoid arthritis (yes/no)	2.0 (0.7 to 5.8)	0.197	1.9 (0.8 to 4.7)	0.155
SLE (yes/no)	1.6 (0.2 to 11.0)	0.608	0.1 (0.0 to 0.8)	0.031*
Polymyalgia rheumatica (yes/no)	3.2 (0.3 to 40.1)	0.364	14.3 (>1.8 to 114.3)	0.012*

Two logistic regression models were used: one to identify possible factors that have an impact on the presence of anxiety symptoms, and another to identify possible factors that have an impact on presence of depression symptoms. The estimated values were obtained considering study design.
*Adjusted p value<0.05.
BMI, body mass index; NUTS II, Nomenclature of Territorial Units for Statistics (North, Centre, Alentejo, Algarve, Lisbon, Madeira and the Azores); RMD, rheumatic and musculoskeletal disease; SLE, systemic lupus erythaematosus.

significantly and independently associated with the presence of depressive symptoms. SLE was significantly associated with the absence of depressive symptoms (OR=0.1; p=0.031) (table 5).

DISCUSSION

EpiReumaPt has been the first large-scale epidemiological population-based study to evaluate RMDs in Portugal. In this study, we determined the prevalence of 12 target diseases (LBP, FM, OP, PD, hand, knee and hip OA, RA, SpA, SLE, gout and PMR). Moreover, we aimed to determine the impact of RMDs on physical and mental health.

We found that RMDs are highly prevalent in Portugal and that their prevalence is similar to that reported in other countries,[8–11 39–43] namely our close neighbour Spain.[7] However, in the EpiReumaPt study, LBP was the most prevalent RMD as opposed to other epidemiological studies[9 10 12] where OA was the most prevalent disease. This finding may be due to the different methodology used in the EpiReumaPt study in which OA was considered separately according to body region (hand, knee and hip). In fact, if we consider the combined prevalence of hip and/or knee and/or hand OA, it reaches 19.1%, which is indeed similar to that reported in other epidemiological studies. Moreover, the prevalence of gout (1.3%) was higher in the EpiReumaPt

study than that estimated for Europe in the Global Burden of Disease study,[44] but similar to the prevalence in the UK.[45] This finding may relate to the increasing prevalence of metabolic syndrome in Portugal, as a result of recent dietary changes including the decline of the Mediterranean food pattern.[46]

In the EpiReumaPt study, we used the new ACR/EULAR classification criteria for RA[32] and the ASAS criteria for SpA,[33 35] and found a prevalence of 0.7% for RA and 1.6% for SpA, with a similar proportion of males and females having the disease. Global prevalence values for SpA calculated before the introduction of the ASAS criteria were reported to be ≈1%,[47] but ranged substantially from 0.001 in Japan[48] to 2.5% in Northern Arctic Natives.[49] In fact, the new ASAS classification criteria for axial SpA cover a larger disease spectrum, from no structural damage to advanced disease. Importantly, these criteria include not only radiographic but also MRI-detected abnormalities of the sacroiliac joints.[33] To our knowledge, only one study has used the ASAS classification criteria to estimate the overall prevalence of SpA.[50] Constantino *et al* used a large population-based cohort—the GAZEL cohort—to estimate SpA prevalence in the French population (0.43%). Unlike the study by Constantino *et al*, in EpiReumaPt, the use of the new criteria confirmed a higher prevalence of SpA in Portugal than that previously reported.[14]

Another interesting finding in our study was the high proportion of individuals presenting with typical features of one or more RMD, who did not have a previous diagnosis (1532 participants). This could be explained by the scarce number of rheumatologists in Portugal (1:100 000 inhabitants)[51] and by the lack of awareness of the population to these diseases, being frequently accepted as part of the normal ageing process.

Regarding the impact of RMDs on HRQoL, physical function and mental health of the Portuguese population, we confirmed that patients with RMDs have significantly worse HRQoL and more disability when compared to participants without RMDs. We found that PMR, RA and FM were the conditions with the worst impact on function and HRQoL. When we compared those participants with and without RMDs regarding mental distress symptoms, we found a significantly higher proportion of patients with RMD with anxiety symptoms but not with depressive symptoms. This could be due to the unexpectedly low proportion of anxiety (16.7%) and depression (8.3%) symptoms among Portuguese patients with RMDs. In fact, in our study, we have shown that only LBP and FM were independently associated with anxiety as well as depressive symptoms. SpA was only associated with anxiety symptoms and PMR with depressive symptoms. In contrast, several other studies have shown higher prevalence of anxiety and depressive symptoms associated with several RMDs.[38 52 53] One explanation could be that many of these studies were performed in a hospital environment and were not population-based studies.

The EpiReumaPt study has some limitations, for example, we used the last birthday within-unit respondent selection method for recruitment. This method has been used by many survey research organisations since the early 1980s. The advantages of this method is that it takes little time to administer, is non-intrusive and, in theory, provides a true random selection of one adult within a multiple adult household. A drawback with the birthday method is that it generates a sample with too many respondents having their birthdays close to the survey date. In EpiReumaPt, we decided to use this method because few variables that we have used are related with birthday.[54 55] Moreover, we had a high dropout rate from the first phase to the second phase. In order to assure that we did not over/underestimate the disease prevalence due to eventual sample bias, we performed a detailed participation analysis considering several subject domains (demographic, socioeconomic, lifestyle, healthcare resource consumption, RMD screening result and self-report of other chronic diseases), which is described elsewhere.[21] Another possible study weakness is related to the definition of PD. We opted for clinical diagnosis after careful history-taking and physical evaluation. Previously structured approaches such as the upper limb MS regional syndrome schedule validated by Palmer *et al*[56] have been used and these could have benefits particularly for epidemiological studies in which physical examination is performed by different healthcare professionals. Moreover, densitometric measurements were not included in the OP definition, which could have led to an underestimation of the prevalence. This study also has several strengths—it is the first population-based study on RMDs in Portugal, and RMDs were accessed and validated by a rheumatologist, and captured various clinical measurements that allowed addressing of the burden of these diseases.

In conclusion, in EpiReumaPt, we have demonstrated that RMDs are highly prevalent in Portugal, as in other southern European countries. Moreover, RMDs are associated not only with significant physical function and mental health impairment but also with poor HRQoL, leading to more health resource consumption. EpiReumaPt also provided valuable data to researchers, healthcare providers and patient organisations. Results of EpiReumaPt emphasise the burden of RMDs in Portugal and the need to increase RMD awareness, being a strong argument to encourage policymakers to increase the amount of resources allocated to the treatment of rheumatic patients.

Author affiliations
[1]EpiReumaPt Study Group—Sociedade Portuguesa de Reumatologia, Lisboa, Portugal
[2]Serviço de Reumatologia do Hospital Egas Moniz—Centro Hospitalar Lisboa Ocidental (CHLO-EPE), Lisboa, Portugal
[3]Centro de Estudos de Doenças Crónicas (CEDOC) da NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
[4]EpiDoc Unit—Unidade de Epidemiologia em Doenças Crónicas (CEDOC, NMS/UNL), Lisboa, Portugal
[5]Programa Nacional Contra as Doenças Reumáticas (2006–2014), Direcção Geral da Saúde, Lisboa, Portugal

[6]Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Lisboa, Portugal
[7]Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[8]Sociedade Portuguesa de Reumatologia, Lisboa, Portugal
[9]Leiden University Medical Center, Leiden, The Netherlands
[10]Clínica Universitária de Reumatologia, Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal
[11]Centre for Rheumatology Research & MRC Centre for Neuromuscular Diseases, University College London, London, UK
[12]Centro de Estudos e Sondagens de Opinião da Universidade Católica Portuguesa (CESOP-CATÓLICA), Lisboa, Portugal
[13]Instituto de Microbiologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[14]Unidade Curricular Especialidades Médico-Cirúrgicas I, NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
[15]Instituto Piaget, Lisboa, Portugal
[16]Unidade de Epidemiologia do Instituto de Medicina Preventiva e Saúde Pública da Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[17]NOVA IMS, Universidade Nova de Lisboa, Lisboa, Portugal
[18]Hospital Garcia de Orta, Almada, Portugal
[19]Hospital Garcia de Orta, EPE, Almada, Portugal
[20]APOROS—Associação Nacional contra a Osteoporose, Lisboa, Portugal
[21]Instituto de Salud Musculoesquelética, Madrid, Spain
[22]Serviço de Reumatologia do Hospital de Santa Maria, Centro Hospitalar Lisboa Norte (CHLN-EPE), Lisboa, Portugal

Acknowledgements The EpiReumaPt Study Group would like to acknowledge the invaluable help of Henrique de Barros, MD PhD; João Eurico da Fonseca, MD PhD; José António Pereira da Silva, MD PhD; Francisco George, MD; Rui André, MD; Luís Maurício, MD; Faculdade de Medicina da Universidade de Coimbra, Faculdade de Medicina da Universidade de Lisboa, Faculdade de Medicina da Universidade do Porto, Liga Portuguesa Contra as Doenças Reumáticas, Associações de Doentes com Doença Reumática, Administrações Regionais de Saúde (Norte, Centro, Lisboa e Vale do Tejo, Alentejo e Algarve), Governo Regional da Madeira, Governo Regional dos Açores, Associação Nacional de Freguesias, Associação Nacional dos Municípios Portugueses, Câmara Municipal de Lisboa.

Collaborators EpiReumaPt study group. Sociedade Portuguesa Reumatologia: Alexandra Bernardo, Alexandre Sepriano, Ana Filipa Mourão, Ana Maria Rodrigues, Ana Raposo, Ana Sofia Roxo, Anabela Barcelos, António Vilar, Armando Malcata, Augusto Faustino, Cândida Silva, Carlos Vaz, Carmo Afonso, Carolina Furtado, Catarina Ambrósio, Cátia Duarte, Célia Ribeiro, Cláudia Miguel, Cláudia Vaz, Cristina Catita, Cristina Ponte, Daniela Peixoto, Diana Gonçalves, Domingos Araújo, Elsa Sousa, Eva Mariz, Fátima Godinho, Fernando Pimentel, Filipa Ramos, Filipa Teixeira, Filipe Araújo, Filipe Barcelos, Georgina Terroso, Graça Sequeira, Guilherme Figueiredo, Helena Canhão, Herberto Jesus, Inês Cunha, Inês Gonçalves, Inês Silva, JA Pereira da Silva, Jaime Branco, Joana Abelha, Joana Ferreira, João Dias, João Eurico Fonseca, João Ramos, João Rovisco, Joaquim Pereira, Jorge Silva, José Carlos Romeu, José Costa, José Melo Gomes, José Pimentão, Lúcia Costa, Luís Inês, Luís Maurício, Luís Miranda, Margarida Coutinho, Margarida Cruz, Margarida Oliveira, Maria João Gonçalves, Maria João Salvador, Maria José Santos, Mariana Santiago, Mário Rodrigues, Maura Couto, Miguel Bernardes, Miguel Sousa, Mónica Bogas, Patrícia Pinto, Paula Araújo, Paula Valente, Paulo Coelho, Paulo Monteiro, Pedro Madureira, Raquel Roque, Renata Aguiar, Ricardo O Figueira, Rita Barros, Rita Fonseca, Romana Vieira, Rui André, Rui Leitão, Sandra Falcão, Sara Serra, Sílvia Fernandes, Sofia Pimenta, Sofia Ramiro, Susana Capela, Taciana Videira, Teresa Laura Pinto, Teresa Nóvoa, Viviana Tavares.

Contributors JCB, SR, PMM, PL, LPdC JC, LC and HC developed the study design. JCB, HC, AMR, NG, AFM, IS, AS, FA and VT contributed substantially to data acquisition. JCB, AMR, ME, SR, PMM, SG, PSC, JMM and HC were responsible for data analysis and interpretation. JCB, AMR, SR, PMM and HC wrote the draft of this paper. All the co-authors have reviewed and made significant contributions to the paper and to its final approval.

Funding EpiReumaPt was supported by a grant from Direcção-Geral da Saúde and was sponsored by: Fundação Calouste Gulbenkian, Fundação Champalimaud, Fundação AstraZeneca, Abbott, Merck Sharp & Dohme, Pfizer, Roche, Servier, Bial, D3A Medical Systems, Happybrands, Centro de Medicina Laboratorial Germano de Sousa, CAL-Clínica, Galp Energia, Açoreana Seguros and individual Rheumatologists.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was reviewed and approved by the National Committee for Data Protection (Comissão Nacional de Proteção de Dados) and by the NOVA Medical School Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Badley EM, Rasooly I, Webster GK. Relative importance of musculoskeletal disorders as a cause of chronic health problems, disability, and health care utilization: findings from the 1990 Ontario Health Survey. *J Rheumatol* 1994;21:505–14.
2. Yelin E, Callahan LF. The economic cost and social and psychological impact of musculoskeletal conditions. National Arthritis Data Work Groups. *Arthritis Rheum* 1995;38:1351–62.
3. Makela M, Heliovaara M, Sievers K, *et al*. Musculoskeletal disorders as determinants of disability in Finns aged 30 years or more. *J Clin Epidemiol* 1993;46:549–59.
4. Wolfe F, Michaud K, Li T, *et al*. EQ-5D and SF-36 quality of life measures in systemic lupus erythematosus: comparisons with rheumatoid arthritis, noninflammatory rheumatic disorders, and fibromyalgia. *J Rheumatol* 2010;37:296–304.
5. Birtane M, Uzunca K, Tastekin N, *et al*. The evaluation of quality of life in fibromyalgia syndrome: a comparison with rheumatoid arthritis by using SF-36 Health Survey. *Clin Rheumatol* 2007;26:679–84.
6. Chopra A. COPCORD—an unrecognized fountainhead of community rheumatology in developing countries. *J Rheumatol* 2004;31:2320–2.
7. Carmona L, Ballina J, Gabriel R, *et al*. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. *Ann Rheum Dis* 2001;60:1040–5.
8. Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis Res Ther* 2009;11:229.
9. Pelaez-Ballestas I, Sanin LH, Moreno-Montoya J, *et al*. Epidemiology of the rheumatic diseases in Mexico. A study of 5 regions based on the COPCORD methodology. *J Rheumatol Suppl* 2011;86:3–8.
10. Lawrence RC, Felson DT, Helmick CG, *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum* 2008;58:26–35.
11. Helmick CG, Felson DT, Lawrence RC, *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum* 2008;58:15–25.
12. Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.
13. Saraux A, Guedes C, Allain J, *et al*. Prevalence of rheumatoid arthritis and spondyloarthropathy in Brittany, France. Societe de Rhumatologie de l'Ouest. *J Rheumatol* 1999;26:2622–7.
14. Matos AA, Branco JC, Silva JC, *et al*. Inquérito epidemiológico das doenças reumáticas numa amostra da população portuguesa (Resultados Preliminares). *Acta Reumatol Port* 1991;16:98.
15. Figeirinhas J. Estudo Epidemiológico dos reumatismos. *Acta Reumatol Port* 1976;IV:23.56.
16. Figeirinhas J. Alguns dados definitivos obtidos através do recente inquérito epidemiológico de reumatismos. *Acta Reumatol Port* 1976; IV:373–80.
17. Programa Nacional Contras as Doenças Reumáticas. s.l. Direção Geral de Saúde, 2004. www.dgs.pt/upload/membro.id/ficheiros/i006345.pdf

18. Ramiro S, Canhao H, Branco JC. EpiReumaPt Protocol—Portuguese epidemiologic study of the rheumatic diseases. *Acta Reumatol Port* 2010;35:384–90.
19. Gouveia N, Rodrigues AM, Ramiro S, *et al.* EpiReumaPt: how to perform a national population based study—a practical guide. *Acta Reumatol Port* 2015;40:128–36.
20. Estatística INd. Relatório: Resultados definitivos dos CEnsus 2011, Portugal. Secondary Relatório: Resultados definitivos dos CEnsus 2011, Portugal, 2012.
21. Rodrigues AM, Gouveia N, da Costa LP, *et al.* EpiReumaPt- the study of rheumatic and musculoskeletal diseases in Portugal: a detailed view of the methodology. *Acta Reumatol Port* 2015;40:110–24.
22. Ferreira LN, Ferreira PL, Pereira LN, *et al.* EQ-5D Portuguese population norms. *Qual Life Res* 2014;23:425–30.
23. Ferreira LN, Ferreira PL, Pereira LN, *et al.* The valuation of the EQ-5D in Portugal. *Qual Life Res* 2014;23:413–23.
24. Fries JF, Spitz P, Kraines RG, *et al.* Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;23:137–45.
25. Pais-Ribeiro J, Silva I, Ferreira T, *et al.* Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. *Psychol Health Med* 2007;12:225–35; quiz 35–7.
26. Altman R, Asch E, Bloch D, *et al.* Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;29:1039–49.
27. Altman R, Alarcon G, Appelrouth D, *et al.* The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. *Arthritis Rheum* 1991;34:505–14.
28. Altman R, Alarcon G, Appelrouth D, *et al.* The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. *Arthritis Rheum* 1990;33:1601–10.
29. Wolfe F, Clauw DJ, Fitzcharles MA, *et al.* The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res (Hoboken)* 2010;62:600–10.
30. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
31. Wallace SL, Robinson H, Masi AT, *et al.* Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum* 1977;20:895–900.
32. Aletaha D, Neogi T, Silman AJ, *et al.* 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
33. Rudwaleit M, Landewe R, van der Heijde D, *et al.* The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann Rheum Dis* 2009;68:770–6.
34. Rudwaleit M, van der Heijde D, Landewe R, *et al.* The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
35. Rudwaleit M, van der Heijde D, Landewe R, *et al.* The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. *Ann Rheum Dis* 2011;70:25–31.
36. Bird HA, Esselinckx W, Dixon AS, *et al.* An evaluation of criteria for polymyalgia rheumatica. *Ann Rheum Dis* 1979;38:434–9.
37. Kanis JA, Melton LJ III, Christiansen C, *et al.* The diagnosis of osteoporosis. *J Bone Miner Res* 1994;9:1137–41.
38. Hyphantis T, Kotsis K, Tsifetaki N, *et al.* The relationship between depressive symptoms, illness perceptions and quality of life in ankylosing spondylitis in comparison to rheumatoid arthritis. *Clin Rheumatol* 2013;32:635–44.
39. Hoy D, March L, Brooks P, *et al.* The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:968–74.
40. Cross M, Smith E, Hoy D, *et al.* The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. *Ann Rheum Dis* 2014;73:1316–22.
41. Cross M, Smith E, Hoy D, *et al.* The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *Ann Rheum Dis* 2014;73:1323–30.
42. Smith E, Hoy DG, Cross M, *et al.* The global burden of other musculoskeletal disorders: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:1462–9.
43. Sanchez-Riera L, Carnahan E, Vos T, *et al.* The global burden attributable to low bone mineral density. *Ann Rheum Dis* 2014;73:1635–45.
44. Smith E, Hoy D, Cross M, *et al.* The global burden of gout: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:1470–6.
45. Annemans L, Spaepen E, Gaskin M, *et al.* Gout in the UK and Germany: prevalence, comorbidities and management in general practice 2000–2005. *Ann Rheum Dis* 2008;67:960–6.
46. Rodrigues SS, Caraher M, Trichopoulou A, *et al.* Portuguese households' diet quality (adherence to Mediterranean food pattern and compliance with WHO population dietary goals): trends, regional disparities and socioeconomic determinants. *Eur J Clin Nutr* 2008;62:1263–72.
47. Reveille JD, Witter JP, Weisman MH. Prevalence of axial spondylarthritis in the United States: estimates from a cross-sectional survey. *Arthritis Care Res (Hoboken)* 2012;64:905–10.
48. Hukuda S, Minami M, Saito T, *et al.* Spondyloarthropathies in Japan: nationwide questionnaire survey performed by the Japan Ankylosing Spondylitis Society. *J Rheumatol* 2001;28:554–9.
49. Boyer GS, Templin DW, Cornoni-Huntley JC, *et al.* Prevalence of spondyloarthropathies in Alaskan Eskimos. *J Rheumatol* 1994;21:2292–7.
50. Costantino F, Talpin A, Said-Nahal R, *et al.* Prevalence of spondyloarthritis in reference to HLA-B27 in the French population: results of the GAZEL cohort. *Ann Rheum Dis* 2015;74:689–93.
51. Administração Central do Sistema de Saúde I. Actuais e Futuras Necessidades Previsionais de Médicos (SNS). 2011. https://saudeimpostos.files.wordpress.com/2011/.../actuais-e-futuras-necessidades
52. Poleshuck EL, Bair MJ, Kroenke K, *et al.* Psychosocial stress and anxiety in musculoskeletal pain patients with and without depression. *Gen Hosp Psychiatry* 2009;31:116–22.
53. Krishnan KR, France RD, Pelton S, *et al.* Chronic pain and depression. II. Symptoms of anxiety in chronic low back pain patients and their relationship to subtypes of depression. *Pain* 1985;22:289–94.
54. Oldendick RW, Bishop GF, Sorenson SB, *et al.* A comparison of the Kish and last-birthday methods of respondent selection in telephone surveys. *J Official Stat* 1988;4:307–18.
55. Gaziano C. Comparative analysis of within-household respondent selection techniques public opinion quarterly. 2005;69:124–57.
56. Palmer K, Walker-Bone K, Linaker C, *et al.* The Southampton examination schedule for the diagnosis of musculoskeletal disorders of the upper limb. *Ann Rheum Dis* 2000;59:5–11.

Archives of Osteoporosis (2018) 13:22
https://doi.org/10.1007/s11657-018-0430-z

ORIGINAL ARTICLE

The burden and undertreatment of fragility fractures among senior women

Ana M. Rodrigues[1,2] · Mónica Eusébio[3] · Maria José Santos[2,4] · Nélia Gouveia[5] · Viviana Tavares[4,6] · Pedro S. Coelho[7] · Jorge M. Mendes[7] · Jaime C. Branco[5,8] · Helena Canhão[1,9]

Received: 25 July 2017 / Accepted: 31 December 2017

Abstract

Summary Using a large population database, we showed that fragility fractures were highly prevalent in senior women and were associated with significant physical disability. However, treatment rates were low because osteoporosis treatment was not prescribed or not agreed to by the majority of women with prevalent fragility fractures.

Purpose The purpose of the study is to estimate prevalence of fragility fractures (FF), risk factors, and treatment rates in senior women and to assess impact of FF on physical function and quality of life.

Methods Women aged 65 years and older from the EpiReumaPt study (2011–2013) were evaluated. Rheumatologists collected data regarding FF, clinical risk factors for fractures, and osteoporosis (OP) treatment. Health-related quality of life (EQ5D) and physical function (HAQ) were analyzed. Peripheral dual-energy X-ray absorptiometry was performed. FF was defined as any self-reported low-impact fracture that occurred after 40 years of age. Prevalence estimates of FF were calculated.

Results Among 3877 subjects evaluated in EpiReumaPt, 884 were senior women. The estimated prevalence of FF was 20.7%. Lower leg was the most frequent fracture site reported (37.8%) followed by wrist (18.6%). Only 7.1% of the senior women reporting a prevalent FF were under treatment for OP, and 13.9% never had treatment. OP treatment was not prescribed in 47.7% of FF women, and 23.4% refused treatment. Age (OR = 2.46, 95% CI 1.11–5.47), obesity (OR = 2.05, 95% CI 1.14–3.70), and low wrist BMD (OR = 2.29; 95% CI 1.20, 4.35; $p = 0.012$) were positively associated with prevalent FF. A significantly higher proportion of women in the lowest quintile of wrist bone mineral density reported FF (OR = 2.29, 95% CI 1.20–4.35). FF were associated with greater physical disability ($\beta = 0.33$, 95% CI 0.13–0.51) independent of other comorbidities.

Conclusion FF was frequently reported among senior women as an important cause of physical disability. However, the prevalence of OP treatment was low, which constitutes a public health problem in this vulnerable group.

Keywords Fragility fractures · Osteoporosis treatment · Epidemiology · Women

✉ Ana M. Rodrigues
anamfrodrigues@gmail.com

[1] Campus Sant' Ana, Polo de Investigação, Nova Medical School, Edifício Amarelo, Rua do Instituto Bacteriológico no. 5, Universidade Nova de Lisboa, 1150-082 Lisbon, Portugal

[2] Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

[3] Sociedade Portuguesa de Reumatologia, Lisbon, Portugal

[4] Hospital Garcia de Orta, Almada, Portugal

[5] Chronic Diseases Research Centre (CEDOC), NOVA Medical School, Universidade Nova de Lisboa (NMS-UNL), Lisbon, Portugal

[6] APOROS—Associação Nacional Contra a Osteoporose, Lisbon, Portugal

[7] NOVA Information Managment School (IMS), Universidade Nova de Lisboa, Lisbon, Portugal

[8] Centro Hospitalar Lisboa Ocidental-EPE, Serviço de Reumatologia do Hospital Egas Moniz—Lisboa, Lisbon, Portugal

[9] NOVA National School of Public Health, Universidade Nova de Lisboa, Lisbon, Portugal

Published online: 07 March 2018

Introduction

Osteoporosis is a metabolic skeletal disease characterized by low bone mass and microarchitecture deterioration [1]. Clinically, osteoporosis manifests as the occurrence of fragility fractures, which represents a public health problem and results in increased mortality and morbidity. Fragility fractures are also a major and growing economic burden on healthcare systems worldwide [2].

Fragility fractures are defined by any low trauma fracture (those resulting from a fall from standing height or less) and are associated with low bone mineral density (BMD) and higher subsequent fracture risk [3, 4]. The most common fragility fractures occur in the wrist, spine, hip, humerus, pelvis, and ribs [5–7]. In Europe, more than 3.5 million fragility fractures are observed each year, accounting for 37 billion euros in healthcare costs. One percent of the disability-adjusted life year (DALY) attributable to non-communicable diseases is due to fragility fractures [8, 9].

With the increase in worldwide life expectancy, the number of individuals who will have a fragility fracture is expected to increase [10, 11]. In fact, the individual lifetime risk for sustaining a fragility fracture from the age of 50 years is estimated to be one in two for women, and one in five for men. Indeed, postmenopausal women are at particularly high risk for fragility fractures due to the sudden estrogen drop in menopause, which leads to bone loss and microarchitectural deterioration [12, 13].

To identify individuals at high risk for fragility fractures, clinical risk factors such as BMD, age, body mass index, and prior fractures must be considered. Accordingly, algorithms to predict individual fracture risk should include several risk factors. A number of algorithms for fragility fracture prediction have been validated, with FRAX algorithm being the most widely used [14]. The identification of the most frequent modifiable fracture risk factors in a certain population is important for public health policymakers. It is still unknown exactly what the risk factors are in some European countries, including Portugal. Moreover, considering the effectiveness of available therapeutic options in decreasing fracture risk [15], it is of paramount importance to understand if osteoporosis treatments are appropriately provided to high-risk patients, such as those who have sustained a previous fragility fracture.

EpiReumaPt is a population-based study performed in Portugal in 2011–2013 to assess rheumatic diseases including osteoporosis. From this survey, the estimated prevalence of osteoporosis among the Portuguese adult population was determined to be 10.2% [16]. As part of this study, we looked specifically at the high-risk population of senior women (65 years and older) and estimated the prevalence of fragility fractures, risk factors for fragility fractures, and treatment rates. We also assessed the impact of prevalent fragility fractures on physical function and quality of life.

Material and methods

Data source

This study was developed under the scope of EpiReumaPt, a national cross-sectional study conducted in Portugal from September 2011 to December 2013. The main objective of EpiReumaPt was to estimate the prevalence of 12 rheumatic and musculoskeletal diseases (RMDs), including osteoporosis [17]. In EpiReumaPt, a representative sample of the adult Portuguese population (10,661 participants) was assessed to capture and characterize all cases of RMDs [18]. The study included non-institutionalized adults (≥ 18 years old) living in private households in the Portuguese Mainland and Islands (Madeira and Azores). The study sample was stratified by administrative territorial units [(NUTS II) (Norte, Centro, Lisboa and Vale do Tejo, Alentejo, Algarve, Açores Islands (Azores) and Madeira Islands (Madeira)], and the size of the population within each locality (< 2000; 2000–9999; 10,000–19,999; 20,000–99,999; and ≥ 100,000 inhabitants, respectively). Of the 28,502 households we attempted to contact, 8041 refused to participate in the study, and 10,661 completed interviews. The EpiReumaPt population was similar to the Portuguese population (CENSUS 2011) in age strata, gender, and NUTII distribution [16].

We followed the EpiReumaPt methodology as previously described, which consisted of a three-phase approach [18]. In the first phase, a survey was administered through a face-to-face interview of households (10,661 participants) random selected by route methodology to screen for RMDs. This study assessed health-related quality of life and physical function. In the second phase, all subjects who screened positive for at least one RMD during the first phase, as well as 20% of randomly selected individuals without rheumatic complaints, were examined by rheumatologists. The selected phase 1 participants were invited to bring current medication, imaging, and medical records for the clinical appointment. The rheumatologists assessed second-phase participants ($n = 3877$) in a structured evaluation that included standardized physical examination, and laboratory and imaging tests (when needed) at a mobile unit to establish RMD diagnosis and evaluate disease-related information. The rheumatologists were blind for all health-related information and screening result collected in EpiReumaPt first phase. The second phase occurred a maximum of 1 month after the face-to-face interview conducted in the first phase. Finally, in the third phase, a team of three experienced rheumatologists reviewed all clinical, laboratory,

and imaging data and confirmed the diagnoses according to validated criteria for the different RMDs [15].

Study population

The population of interest for the present study was defined as all women 65 years and older who participated in the second phase of EpiReumaPt (Fig. 1).

Case definition

Fragility fracture was defined as any self-reported low-impact fracture (fractures that resulted from a fall from a standing height or less, or that occurred in the absence of trauma) in individuals older than 40 years [19, 20]. Fractures of the face, skull, foot, fingers, and toes were excluded. The accuracy of self-reported fragility fracture was previously shown to be acceptable [21–23]. We analyzed the overlap of self-reported previous fragility fractures captured in the first phase of EpiReumaPt and previous fractures as diagnosed by the rheumatologist in the second phase. Using this data, we computed Cohen's kappa (overall agreement was 82.68% with a kappa coefficient of 0.51). The overall sensitivity of the self-reported previous fragility fracture was 61.2% with a specificity of 89.2%. The positive predictive value was 63.2%, and the negative predictive value was 88.4%.

Measurement, assessment, and instruments

Sociodemographic and economic data (age, gender, ethnicity, education, marital status, household income, and composition), anthropometric data (self-reported weight and height), and self-reported chronic diseases (high cholesterol, high blood pressure, gastrointestinal disease, cardiac disease, diabetes, thyroid and parathyroid disease, pulmonary disease, hyperuricemia, cancer, neurologic disease, and hypogonadism) were collected during the first phase of EpiReumaPt. Anxiety and depression symptoms were assessed by the Portuguese-validated version of the Hospital Anxiety and Depression Scale (HADS). HADS is divided into an Anxiety subscale (HADS-A) and a Depression subscale (HADS-D), both containing seven related considerations (in both subscales, a score ≥ 11 translates into the presence of symptoms of anxiety or depression) [24]. Clinical risk factors (CRFs) for fractures, other than the risk factor of age, also were collected: body mass index (BMI) [categorized as underweight (BMI < 18.5 kg/m^2), normal weight (BMI between 18.5 and 24.9 kg/m^2), overweight (BMI between 25 and 29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2)], parental history of hip fracture, long-term use of oral glucocorticoids (≥ 3 months), rheumatoid arthritis, current smoking, alcohol intake (≥ 3 units/day), and the presence of other secondary causes of osteoporosis. The 10-year probability of major fractures and hip fractures was calculated using the FRAX tool available online [25], without using axial dual-energy X-ray absorptiometry (DXA) information. The

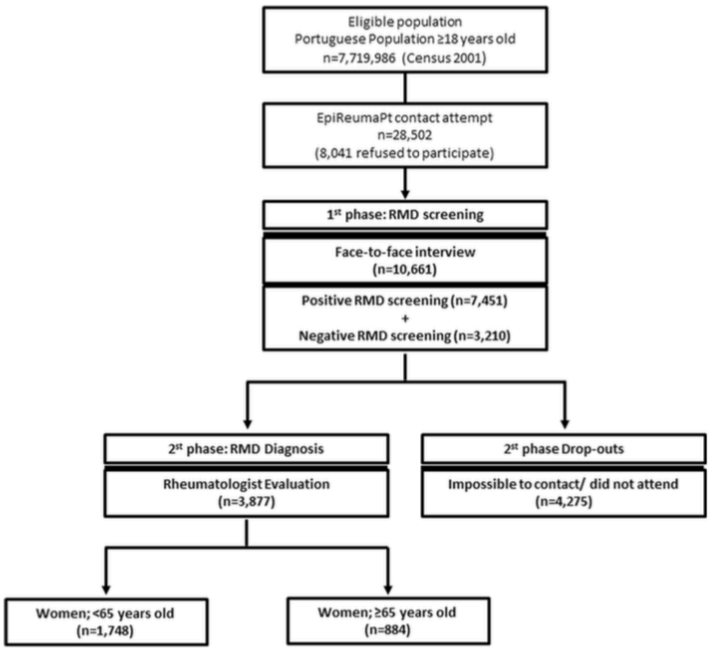

Fig. 1 Flow chart of study design. *RMD* rheumatic and musculoskeletal diseases

appropriateness of the osteoporosis treatment decision was judged according to the 2016 Multidisciplinary Portuguese Recommendations on DXA Request and Indication to Treat in the Prevention of Fragility Fractures (10-year risk probability of major fracture ≥ 11% or 10-year risk probability of hip fracture ≥ 3%) [26].

To evaluate generic health-related quality of life (HRQoL), a Portuguese-validated version of the European Quality of Life questionnaire, with five dimensions and three levels (EQ-5D-3L) [27, 28], was applied. Physical function was assessed by the Health Assessment Questionnaire (HAQ) [29]. Information regarding pharmacological therapies was also collected.

Fragility fractures and current medications were assessed by rheumatologists in the second phase of EpiReumaPt [18]. Fragility fracture diagnosis made by rheumatologists was based on a structured interview, physical examination, and medical and imaging record when it was available. Osteoporosis diagnosis was based on the presence of at least one of the following: previous self-reported fragility fractures, previous osteoporosis diagnosis, current osteoporosis treatment, or fulfillment of the World Health Organization criteria, when DXA was available. The presence of inflammatory rheumatic diseases was assessed in this stage (rheumatoid arthritis, spondyloarthritis, systemic lupus erythematosus, and polymyalgia rheumatica).

To reduce recall bias, pharmacological treatment for osteoporosis was reassessed via a phone call questionnaire specifically designed for this purpose. The questionnaire was used for all women 65 years and older who underwent the second phase of the EpiReumaPt study, which was performed no more than 3 months after the physical examination performed in the second phase. Trained interviewers asked questions regarding present and past pharmacological treatment (bisphosphonates, strontium ranelate, selective estrogen receptor modulators, denozumab, and teriparatide), treatment duration, and adverse events.

Peripheral DXA procedures

All participants who participated in the second phase of the study had a wrist DXA on a Lunar PIXI™ device (a peripheral instantaneous X-ray imager; GE Medical Systems, Florence, SC, USA) at the mobile unit. This procedure provided assessment of distal BMD at 0.2-mm pixels of image resolution.

Biochemical assessment

Blood samples were collected during the second phase of the EpiReumaPt study and were sent to a central lab [24]. Levels of bone remodeling markers, 25-hydroxyvitamin D3 (vitamin D), and creatinine were determined using fresh serum samples in all women 65 years and older. Laboratory parameters were measured according to the manufacturers' instructions. Serum levels of creatinine were measured by rate-blanked creatinine method on a Dimension Vista System (Siemens, Lisbon, Portugal) with Siemens reagents, and an estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [30].

Serum levels of intact parathyroid hormone (iPTH), osteocalcin, cross-linked C-telopeptide of type I collagen (CTX-I), and serum amino-terminal propeptides of type I procollagen (P1NP) were measured on fully automated Immulite 2000® electrochemiluminescent immunoassay analyzers (Siemens). Serum levels of vitamin D were measured using the LIAISON competitive immunoassay (DiaSorin, Sallugia, Italy).

Statistical analysis

Prevalence estimates for fragility fractures, osteoporosis, and fracture sites were computed as weighted proportions taking sampling design into account as described elsewhere[18]. In fact, the second-phase sub-sample inclusion probabilities were calculated considering NUTII region, size of locality, gender age stratum, and the different proportion of participants with positive screening for RMD (80%) and negative screening for RMD (20%) according to sampling design (stratified two-stage cluster sampling). Second-phase weight was also calibrated to account differences between second-phase participants and non-responders. Second-phase participants did not differ the second-phase non-responders except for the presence of positive screening for RMD, age group, gender, and residence region according to the NUTS II [18].

Subjects were divided in two groups, with and without prevalent fragility fractures. The characterization of sociodemographic, non-communicable chronic diseases, and risk factors for fractures, quality of life, and physical function were performed for the study population, and for the two groups as described. All categorical variables are presented as counts and proportions, while continuous variables are presented as means and standard deviations. Comparisons between groups were also weighted according to study design. Weighted treatment rates were computed in accordance with the existence of a prevalent fragility fracture, fracture site, and individual 10-year fracture risk (using the FRAX algorithm).

To evaluate risk factors associated with prevalent fragility fractures, univariable logistic regression analysis was first performed to assess differences between the groups with and without prevalent fragility fracture. Then, the association was assessed using multivariable analysis with variables selected in the previous step and according to the study design. For the majority of the risk factors, the adjustment was made

for age, NUTSII (Nomenclature of Territorial Units for Statistics), peripheral BMD (wrist), and categorical BMI. This was not the case for categorical age (≥65 to 69 as reference, categories from 70 to 79, and > 80 years old), dichotomous BMI (obesity vs other categories), and peripheral BMD (wrist). The adjustment for vitamin D also included the season of the year.

To assure a better clinical interpretation, some variables were subjected to categorical transformation. For chronic renal insufficiency, a new dichotomous variable was created with the cutoff set at moderate to severe loss of kidney function (30 ml/min/1.73 m^2) (yes/no). Vitamin D was categorized as vitamin D insufficiency (< 30 ng/ml), vitamin D deficiency (10 ng/ml) (yes/no), and normal levels of vitamin D (> 30 ng/ml). For peripheral BMD, the variable was categorized according to quintiles (the lower category vs. the four higher quintiles grouped as one category). Lastly, for all serum markers of bone fragility (CTX, P1NP, and osteocalcin) and PTH, the variables were categorized as terciles (the lower tercile vs. the two higher terciles).

To assess the independent relationship between prevalent fragility fracture, HRQoL (EQ5D), and physical function (HAQ), linear multivariable regression models were constructed (continuous outcomes), adjusted for age, NUTSII, years of education, married status [dichotomized by married/consensual union and single/widow(er)/divorced], cardiac disease, and categorical BMI.

The cutoff value for significance was at $p < 0.05$. All analyses were weighted and performed using Stata IC version 12 (StataCorp. 2011 Stata Statistical Software: Release 12, College Station, TX, USA).

Ethical issues

EpiReumaPt study was approved by the Ethics Committee of NOVA Medical School and by the Portuguese Data Protection Authority (*Comissão Nacional de Proteção de Dados*). Written informed consent, in accordance with the principles established by the Declaration of Helsinki, was obtained from all participants. Further details of ethical issues of EpiReumaPt were previously described [31].

Results

Among the 3877 subjects evaluated clinically in EpiReumaPt, 884 were women older than 65 years of age (Fig. 1). In this age stratum, the estimated prevalence of fragility fractures was 20.7% and the prevalence of osteoporosis was 49.5%. The average time since the last fragility fracture was 10.2 ± 12 years, and only 5.15% of women who reported a fragility fracture reported its occurrence in the previous year. Prevalent fragility fractures increased significantly with age. In fact, 33.6% of women 80 years and older had at least one fragility fracture, and 58.2% were diagnosed with osteoporosis by a rheumatologist (Fig. 2a). Non-vertebral, non-hip fractures (lower leg, wrist, elbow, humerus, clavicle, or rib) were the most prevalent fracture sites (Table 1). When considering only women with a prevalent fragility fracture, the lower leg was the most frequently reported fracture site (37.8%), followed by the wrist (18.6%), elbow (17.5%), and humerus (16.8%). Hip fractures were reported in 6.6% of women with fragility fractures, and clinical vertebral fractures were reported in 5.3% (Fig. 2b). Of note, the combined frequency of lower leg, and/or wrist, and/or elbow, and/or humerus, and/or clavicle, and/or rib fractures among women with fragility fractures was 85.4%.

Regarding the prevalent fragility fractures, 56.3% of women reported one prevalent fragility fracture, 27.3% reported two prevalent fragility fractures, and 16.4% reported three or more prevalent fragility fractures. We verified that only 7.1% of the women with prevalent fragility fractures were currently being treated for osteoporosis, and only 13.9% had previously been under osteoporosis treatment for a mean duration of 130.23 ± 171.76 months. When considering women who had a prevalent fragility fracture and had never had osteoporosis treatment, the treatment was not prescribed in 54.7% and treatment was prescribed but not used in 23.4%. Treatment rates were low regardless of the fracture site reported (Fig. 2b). Finally, the individual risk of a new fragility fracture was calculated for women with a prior prevalent fragility fracture using the FRAX algorithm. We verified that 54.4% had a 10-year risk of major osteoporosis ≥ 11% and 64.4% had a 10-year risk of hip fracture ≥ 3%, which are the cutoff standards for osteoporosis treatment decision according to the 2016 Multidisciplinary Portuguese Recommendations on DXA Request and Indication to Treat in the Prevention of Fragility Fractures [26] (Fig. 2c). Of interest, treatment rates among women without prevalent fragility fractures were lower, and 10-year risk of a fragility fracture was higher (16.0%) than in women with a previous fragility fracture.

Table 2 summarizes the sociodemographic, economic, and health characteristics of participants according to the existence of a prevalent fragility fracture. The majority of Portuguese senior women have low literacy, have low household income per month, and have a high prevalence of chronic non-communicable diseases, namely high blood pressure, diabetes, and high cholesterol level. Women who had a prevalent fragility fracture were more frequently older and widows. Low prevalence of inflammatory rheumatic diseases was found among senior women. Regarding lifestyles, the majority of Portuguese senior women (with and without a prevalent fragility fractures) do not smoke, do not have alcohol intake above 3 units per day but are physical inactive (81.3%).

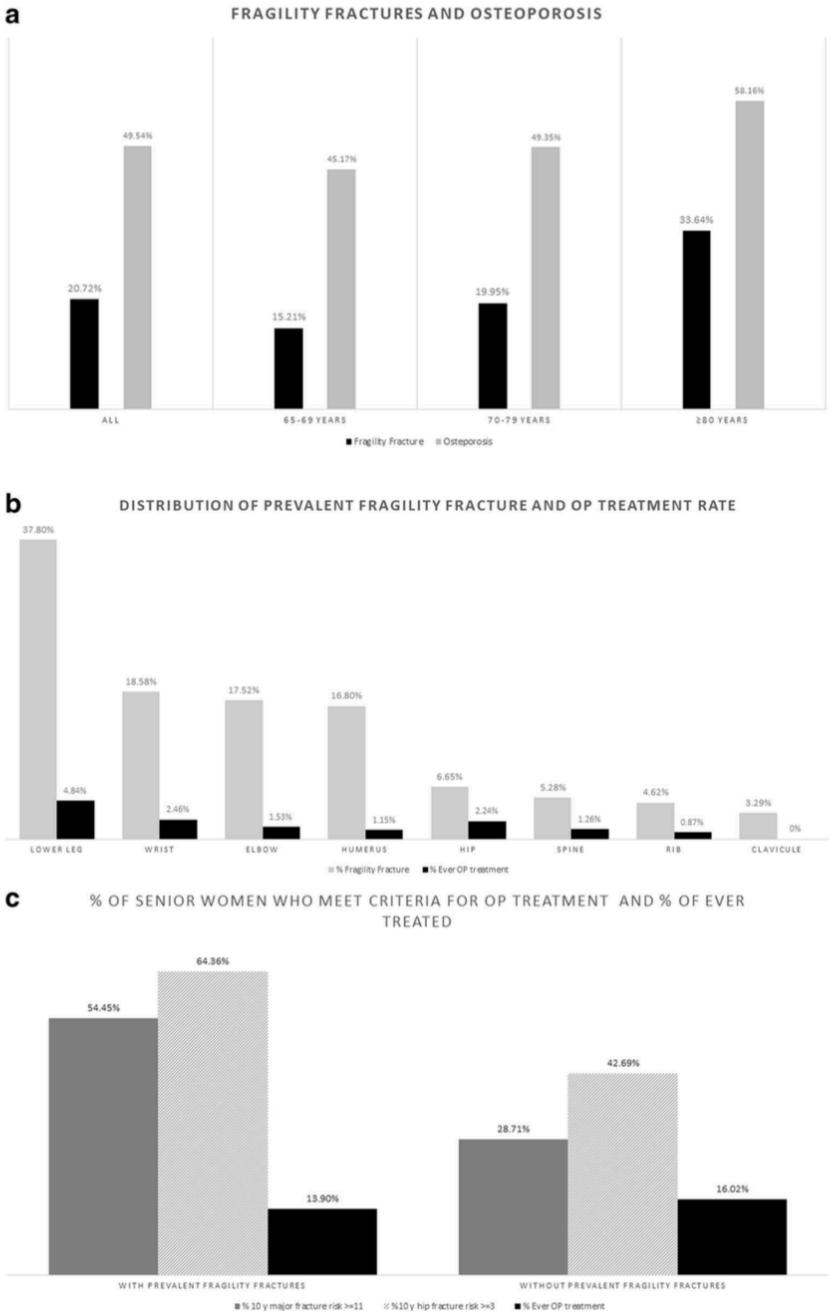

Fig. 2 Fragility fractures, fracture site, and treatment rates in women + 65 years old. **a** Fragility fractures and osteoporosis by age group. **b** Distribution of prevalent fragility fractures and OP treatment rate. **c** Proportion of senior women who met criteria for OP treatment and proportion of ever-treated subjects

Risk factors of fragility fractures among senior women

The clinical risk factors for fractures that were significantly and independently associated with prevalent fragility fracture were age (OR = 2.46, 95% CI 1.11, 5.47; $p = 0.027$) and obesity (OR = 2.05, 95% CI 1.14, 3.70; $p = 0.017$) (Table 3). No other clinical risk factors were found significantly different between women with and without prevalent fragility fractures. Regarding distal BMD, a significantly higher proportion of women in the lowest quintile of wrist BMD reported a fragility fracture (OR = 2.29; 95% CI 1.20, 4.35; $p = 0.012$) (Table 3).

No independent association was verified between prevalent fragility fractures and serum levels of vitamin D (Table 3). The prevalence of vitamin D insufficiency (< 30 ng/ml) was found in 32.5% of the patient cohort. Of women who reported a prevalent fragility fracture, 34.3% had vitamin D insufficiency. A similar result was found among women who did not report prevalent fragility fractures (35.1%). No independent association was found with serum markers of bone turnover (CTX, P1NP, and osteocalcin) and prevalent fragility fracture either (Table 3).

Association of fragility fractures, quality of life, physical disability

To address the burden of fragility fractures, we studied the association between fragility fractures and physical function and quality of life. Women with a prevalent fragility fractures reported greater physical disability than those without prevalent fragility fractures (HAQ score 1.04 ± 1.19 vs 0.74 ± 0.99) (Table 2). In fact, prevalent fragility fractures among elderly women was associated with greater physical disability in general (β = 0.33, 95% CI 0.13, 0.51; $p \leq 0.001$) after adjustment for age, NUTSII, years of education, marital status, cardiac disease, and BMI (Table 4). Further, we performed a sensitivity analysis and tested for interaction between the independent association between prevalent fragility fracture and HAQ score, and we found that time since the last fragility fracture is indeed an effect modifier and the association between fragility fractures and HAQ score is higher among the ones with lower time since last fracture (data not showed).

Regarding HRQoL, although women with prevalent fragility fractures reported lower quality of life compared to those with no prevalent fragility fractures (Table 2), this result was not statistically significant after adjustment for confounders (Table 4).

Discussion

In this large population-based study through EpiReumaPt, reported fragility fractures (20.7%) and diagnoses of osteoporosis (49.5%) were both highly prevalent among senior women. However, the high prevalence of these conditions was in stark contrast with the low rates of OP treatment (13.9%). Non-hip, non-vertebral (NHNV), lower leg, wrist, humerus, rib, clavicle, and elbow fractures accounted for the majority of fragility fractures. Moreover, the clinical risk factors independently significantly associated with prevalent fragility fractures were increased age, obesity, and lower distal BMD.

We have characterized all prevalent fragility fractures and included clinical vertebral, hip, and NHNV fractures, because of recent evidence showing that all fragility fractures, including NHNV, are associated with an increased risk of subsequent fracture, and higher morbidity and mortality. The most prevalent sites of self-reported fragility fracture among women older than 65 years were NHNV fractures. These results are in line with other studies reporting that NHNV fractures

Table 1 Estimates of the prevalence of fragility fracture site by age group

Fragility fracture site		Age group		
	All, *n* (%)	65–69 y.o., *n* (%)	70–79 y.o., *n* (%)	> 80 y.o., *n* (%)
Lower leg	55 (6.06%)	19 (7.55%)	24 (4.57%)	12 (7.90%)
Wrist	39 (3.85%)	9 (2.09%)	21 (4.33%)	9 (5.87%)
Elbow	38 (3.42%)	10 (2.70%)	18 (3.28%)	10 (5.18%)
Humerus	38 (3.28%)	6 (1.55%)	22 (3.64%)	10 (5.39%)
Hip	11 (1.38%)	3 (0.80%)	3 (1.23%)	5 (2.92%)
Spine	11 (1.09%)	3 (1.10%)	5 (0.99%)	3 (1.36%)
Rib	9 (0.90%)	2 (0.71%)	6 (1.12%)	1 (0.60%)
Clavicle	7 (0.64%)	0 (0%)	4 (0.66%)	3 (1.79%)

y.o. years old, % percentage

Table 2 Crude analysis of sociodemographic, economic characteristics, risk factors for fractures, and health status of the Portuguese women 65 years and older with and without prevalent fragility fracture

	Women ≥ 65 y.o. ($n = 884$) n (%)	Women with prevalent fragility fractures ($n = 189$) n (%)	Women without prevalent fragility fractures ($n = 654$) n (%)	p value
Sociodemographic				
Age				
65–69 y.o.	295 (31.52%)	53 (24.34%)	239 (36.46%)	0.017*
70–79 y.o	443 (51.40%)	94 (47.91%)	319 (50.23%)	
> 80 y.o	146 (17.08%)	42 (27.75%)	96 (14.31%)	
NUTS II				
Norte	235 (33.82%)	54 (31.61%)	162 (32.50%)	0.642
Centro	201 (22.81%)	37 (21.85%)	151 (22.87%)	
Lisboa	150 (25.60%)	36 (29.30%)	108 (25.56%)	
Alentejo	71 (8.45%)	17 (9.31%)	54 (8.88%)	
Algarve	46 (5.36%)	8 (3.81%)	37 (6.02%)	
Azores	88 (1.46%)	12 (1.01%)	74 (1.65%)	
Madeira	93 (2.50%)	25 (3.12%)	68 (2.53%)	
Ethnicity/race				
Caucasian	851 (99.12%)	165 (99.43%)	646 (99.13%)	0.294
Black	3 (0.33%)	0 (0%)	3 (0.43%)	
Other	2 (0.23%)	0 (0%)	2 (0.31%)	
Did not know/ did not answer	3 (0.32%)	1 (0.57%)	1 (0.13%)	
Education level (years)				
> 12	46 (7.35%)	9 (4.42%)	33 (7.49%)	0.176
10–12	32 (5.89%)	5 (2.62%)	25 (6.80%)	
5–9	69 (8.20%)	18 (13.19%)	47 (7.00%)	
0–4	719 (78.57%)	149 (79.77%)	541 (78.71%)	
Marital status				
Single	46 (5.99%)	7 (3.66%)	36 (6.54%)	0.011*
Married	425 (47.94%)	73 (39.95%)	332 (49.57%)	
Divorced	46 (7.38%)	6 (2.76%)	37 (8.58%)	
Widow(er)	341 (38.66%)	80 (53.63%)	246 (35.27%)	
Consensual union	1 (0.03%)	0 (0%)	1 (0.0.4%)	
Household income per month				
< 500€	285 (38.67%)	66 (36.03%)	211 (39.12%)	0.264
501€ to 750€	186 (25.72%)	39 (30.68%)	142 (24.33%)	
751€ to 1000€	73 (9.74%)	17 (12.15%)	53 (8.82%)	
1001€ to 1500€	62 (12.83%)	16 (15.72%)	44 (12.33%)	
1501€ to 2000€	35 (7.44%)	5 (3.71%)	30 (8.69%)	
2001€ to 2500€	11 (3.85%)	1 (0.69%)	10 (4.81%)	
2501€ to 3000€	7 (0.91%)	2 (1.03%)	4 (0.82%)	
3001€ to 4000€	4 (0.55%)	0 (0%)	4 (0.71%)	
> 4000€	2 (0.29%)	0 (0%)	2 (0.37%)	
Non-communicable chronic diseases (self-reported)				
High blood pressure	554 (59.38%)	127 (64.12%)	407 (58.77%)	0.425
Diabetes	199 (20.99%)	43 (27.21%)	150 (20.24%)	0.162
High cholesterol level	512 (56.15%)	104 (51.52%)	380 (56.63%)	0.412
Pulmonary disease	97 (10.57%)	25 (14.03%)	66 (9.59%)	0.156
Cardiac disease	282 (32.79%)	68 (43.03%)	194 (29.90%)	0.032*
Gastrointestinal disease	305 (35.86%)	72 (42.03%)	219 (34.85%)	0.242
Neurologic disease	68 (7.20%)	14 (7.94%)	50 (7.06%)	0.713

Table 2 (continued)

	Women ≥ 65 y.o. ($n = 884$) n (%)	Women with prevalent fragility fractures ($n = 189$) n (%)	Women without prevalent fragility fractures ($n = 654$) n (%)	p value
Neoplastic disease	74 (8.06%)	16 (8.66%)	53 (7.71%)	0.722
Thyroid and parathyroid disease	166 (19.86%)	42 (25.30%)	116 (17.55%)	0.094
Hypogonadism	11 (1.58%)	4 (1.98%)	6 (0.89%)	0.269
Mental disease				
Anxiety symptoms (HADS score ≥ 11)	182 (17.90%)	43 (21.74%)	127 (16.47%)	0.158
Depression symptoms (HADS score ≥ 11)	174 (19.31%)	42 (25.71%)	121 (16.80%)	0.089
Inflammatory rheumatic diseases				
Rheumatoid arthritis	22 (2.00%)	6 (1.87%)	15 (2.04%)	0.886
Spondyloarthritis	9 (0.95%)	1 (0.62%)	8 (1.11%)	0.590
Systemic lupus erythematosus	1 (0.12%)	1 (0.60%)	0 (0%)	NA
Polymyalgia rheumatica	4 (0.55%)	0 (0%)	4 (0.74%)	NA
Secondary osteoporosis				
Yes	29 (2.79%)	6 (2.98%)	22 (2.77%)	0.899
No	854 (97.21%)	183 (97.02%)	631 (97.23%)	
Glucocorticoid intake				
Yes	35 (3.79%)	6 (3.05%)	26 (3.81%)	0.658
No	848 (96.21%)	183 (96.95%)	627 (96.19%)	
Parent hip fracture				
Yes	55 (7.10%)	13 (6.87%)	36 (6.79%)	0.976
No	828 (92.90%)	176 (93.13%)	617 (93.21%)	
Anthropometric data				
Body mass index (kg/m^2)				
Underweight	7 (0.81%)	3 (1.61%)	4 (0.67%)	0.110
Normal weight	228 (28.69%)	50 (29.42%)	161 (27.33%)	
Overweight	379 (47.82%)	78 (39.71%)	287 (51.05%)	
Obese	251 (22.68%)	53 (29.26%)	189 (20.94%)	
Lifestyle habits				
Current smoking				
Yes	17 (1.81%)	2 (1.17%)	13 (1.76%)	0.602
No	866 (98.19%)	187 (98.83%)	640 (98.24%)	
Alcohol (3 or more units/day)				
Yes	15 (1.96%)	3 (1.58%)	11 (1.54%)	0.975
No	868 (98.04%)	186 (98.42%)	642 (98.46%)	
Physical activity				
Inactive	524 (81.30%)	110 (86.67%)	390 (81.02%)	0.473
Moderate	24 (4.33%)	4 (2.80%)	17 (3.66%)	
Active	69 (14.37%)	12 (10.53%)	55 (15.32%)	
FRAX				
FRAX Major (mean ± sd)	9.96 ± 9.51	13.56 ± 11.61	8.71 ± 7.67	< 0.001*
FRAX Hip (mean ± sd)	4.42 ± 7.26	6.26 ± 8.81	3.67 ± 5.63	0.004*
Peripheral BMD (g/cm^2)				
Distal (mean ± sd)	0.36 ± 0.12	0.35 ± 0.12	0.37 ± 0.12	0.117
Biochemical assessment				
Vitamin D (nmol/ml)				
< 10	19 (2.03%)	5 (3.26%)	14 (1.87%)	0.607
≥ 10 and < 20	73 (8.52%)	17 (10.14%)	51 (7.76%)	
≥ 20 and < 30	150 (24.03%)	25 (20.85%)	118 (25.55%)	
Normal (≥ 30)	402 (65.42%)	83 (65.74%)	300 (64.82%)	

Table 2 (continued)

	Women ≥ 65 y.o. ($n = 884$) n (%)	Women with prevalent fragility fractures ($n = 189$) n (%)	Women without prevalent fragility fractures ($n = 654$) n (%)	p value
Chronic renal insufficiency (ml/min/1.73 m^2)				
eGFR < 15	7 (0.93%)	1 (0.77%)	6 (1.04%)	0.380
≥ 15 and < 30	9 (1.18%)	3 (2.00%)	6 (1.05%)	
≥ 30 and < 60	194 (27.57%)	37 (26.50%)	148 (27.73%)	
≥ 60 and < 90	360 (56.50%)	86 (62.43%)	260 (55.30%)	
≥ 90	102 (13.82%)	15 (8.30%)	79 (14.87%)	
PTH (pg/ml) (mean ± sd)	50.92 ± 54.62	50.76 ± 5.07	50.67 ± 55.62	0.987
CTX (ng/ml) (mean ± sd)	0.24 ± 0.25	0.26 ± 0.22	0.23 ± 0.27	0.452
P1NP (ng/ml) (mean ± sd)	37.97 ± 30.84	33.03 ± 26.79	39.20 ± 33.12	0.282
Osteocalcin (ng/ml) (mean ± sd)	3.80 ± 3.71	3.56 ± 3.27	3.68 ± 3.80	0.794
Quality of life and physical function				
EQ5D score (mean ± sd)	0.63 ± 0.40	0.55 ± 0.42	0.66 ± 0.40	0.002*
HAQ score (0–3) (mean ± sd)	0.81 ± 1.04	1.04 ± 1.19	0.74 ± 0.99	0.001*

Sample size is not constant due to the following:

Post-menopausal women—ethnicity ($n = 859$), education level ($n = 866$), marital status ($n = 859$), household income ($n = 665$), high blood pressure ($n = 873$), diabetes ($n = 872$), high cholesterol level ($n = 870$), pulmonary disease ($n = 874$), cardiac disease ($n = 866$), gastrointestinal disease (=873), neurologic disease ($n = 875$), neoplastic disease ($n = 879$), thyroid and parathyroid disease ($n = 869$), hypogonadism ($n = 852$), secondary osteoporosis ($n = 883$), glucocorticoids ($n = 883$), parent hip fracture ($n = 883$), body mass index ($n = 865$), current smoking ($n = 883$), alcohol ($n = 883$), physical activity ($n = 617$), N. falls previous 12 months ($n = 840$), FRAX major ($n = 876$), FRAX minor ($n = 876$), bone mineral density wrist ($n = 759$), vitamin D ($n = 644$), eGFR ($n = 672$), PTH ($n = 626$), CTX ($n = 307$), P1NP ($n = 305$), osteocalcin ($n = 308$), EQ5D score ($n = 874$).

With any self-reported fragility fracture—ethnicity ($n = 166$), education level ($n = 181$), marital status ($n = 166$), household income ($n = 146$), high blood pressure ($n = 185$), diabetes ($n = 184$), high cholesterol level ($n = 183$), pulmonary disease ($n = 186$), cardiac disease ($n = 178$), gastrointestinal disease (=185), neurologic disease ($n = 185$), neoplastic disease ($n = 187$), thyroid and parathyroid disease ($n = 185$), hypogonadism ($n = 179$), body mass index ($n = 184$), physical activity ($n = 126$), N. falls previous 12 months ($n = 182$), FRAX major ($n = 187$), FRAX minor ($n = 187$), bone mineral density wrist ($n = 160$), vitamin D ($n = 130$), eGFR ($n = 142$), PTH ($n = 125$), CTX ($n = 65$), P1NP ($n = 64$), osteocalcin ($n = 64$), EQ5D score ($n = 186$).

Without any self-reported fragility fracture—ethnicity ($n = 652$), education level ($n = 646$), marital status ($n = 652$), household income ($n = 500$), high blood pressure ($n = 648$), diabetes ($n = 648$), high cholesterol level ($n = 647$), pulmonary disease ($n = 648$), cardiac disease ($n = 647$), gastrointestinal disease ($n = 648$), neurologic disease ($n = 650$), neoplastic disease ($n = 652$), thyroid and parathyroid disease ($n = 644$), hypogonadism ($n = 634$), secondary osteoporosis ($n = 653$), glucocorticoids ($n = 653$), parent hip fracture ($n = 653$), body mass index ($n = 641$), current smoking ($n = 653$), alcohol ($n = 653$), physical activity ($n = 462$), N. falls previous 12 months ($n = 645$), FRAX major ($n = 649$), FRAX minor ($n = 649$), bone mineral density wrist ($n = 565$), vitamin D ($n = 483$), eGFR ($n = 499$), PTH ($n = 471$), CTX ($n = 224$), P1NP ($n = 223$), osteocalcin ($n = 226$), EQ5D score ($n = 649$)

y.o. years old, % percentage, *sd* standard deviation, *NUTS II* Nomenclature of Territorial Units for Statistics (Norte, Centro, Alentejo, Algarve, Lisboa, Madeira, and the Azores), *EQ5D* European Quality of Life questionnaire five dimensions three levels, *HAQ* Health Assessment Questionnaire, *HADS* Hospital Anxiety and Depression Scale, *eGFR* glomerular filtration rate, *PTH* parathyroid hormone, *CTX*-I cross-linked C-telopeptide of type I collagen, *P1NP* serum amino-terminal pro-peptides of type I procollagen, *BMD* bone mineral density, *ml* milliliters, *ng* nanogram

*p value < 0.05

accounted for more than two thirds of all fragility fractures [5, 6, 32]. The prevalence of fragility fracture found among women older than 65 years in Portugal was lower than in other countries of Northern Europe [33–35], Australia [36], and the USA, but similar to other countries in the Mediterranean region [8].

Regarding treatment rates, we found that a significantly low proportion of senior women who had sustained a fragility fracture were or had ever been treated for osteoporosis. Even when we queried for those that sustained a major osteoporotic fracture (hip, spine, wrist, or humerus), where guidelines [26] recommend osteoporosis treatment, regardless of BMD information and other risk factors, the treatment rates were still low. When we calculated the 10-year risk of a subsequent fragility fracture using the FRAX algorithm without BMD, we found that few women who were eligible for osteoporosis treatment according to Portuguese guidelines [26] were undergoing treatment for osteoporosis. These results highlight the importance of developing strategies to increase the implementation of the osteoporosis treatment guidelines in clinical practice. Moreover, 23.4% of women who had fragility fractures decided not to take prescribed osteoporosis therapeutics, which underscores the need for development of effective osteoporosis and fragility fracture campaigns to increase public awareness and treatment adherence. These awareness campaigns for osteoporosis treatment must take into account the socioeconomic

Table 3 Crude and adjusted analysis for the association between risk factors for fracture and prevalent fragility fracture among Portuguese women 65 years and older

			Self-reported any fragility fractures			
Age	Crude analysis OR [95% CI]	*p* value	Global *p* value	Adjusted[a] analysis OR [95% CI]	Adjusted[a] *p* value	Adjusted global *p* value
70–79 y.o. vs 65–69 y.o.	1.39 [0.81; 2.38]	0.230	0.017*	1.27 [0.68; 2.35]	0.452	0.073
> 80 y.o. vs 65–69 y.o.	2.82 [1.38; 5.79]	0.005*		2.46 [1.11; 5.47]	0.027*	
Body mass index (kg/m^2)						
Obese vs underweight/normal/overweight	1.56 [0.94; 2.61]	0.088		2.05 [1.14; 3.70]	0.017*	
Parent hip fracture (yes vs no)	1.01 [0.45; 2.26]	0.976		1.22 [0.49; 3.04]	0.669	
Current smoking (yes vs no)	0.66 [0.14; 3.10]	0.602		0.65 [0.08; 5.36]	0.691	
Alcohol (3 or more units/day) (yes vs no)	1.02 [0.27; 3.80]	0.975		1.45 [0.34; 6.11]	0.615	
Physical activity (inactive vs active)	1.52 [0.78; 2.99]	0.222		1.23 [0.55; 2.75]	0.619	
Glucocorticoids (yes vs no)	0.79 [0.29; 2.20]	0.658		1.00 [0.34; 2.88]	0.933	
Rheumatoid arthritis (yes vs no)	0.92 [0.28; 3.04]	0.886		1.44 [0.38; 5.45]	0.589	
Spondyloarthritis (yes vs no)	0.56 [0.07; 4.62]	0.590		1.09 [0.12; 10.04]	0.937	
Systemic lupus erythematosus (yes vs no)	NA	NA		NA	NA	
Polymyalgia rheumatica (yes vs no)	NA	NA		NA	NA	
Secondary osteoporosis (yes vs no)	1.08 [0.33; 3.48]	0.899		0.58 [0.13; 2.56]	0.474	
Chronic renal insufficiency						
eGFR < 30 vs eGFR ≥ 30 ml/min/1.73 m^2	1.33 [0.41; 4.34]	0.637		0.98 [0.23; 4.14]	0.976	
Peripheral bone mineral density (g/cm^2)						
Wrist Q1 (< 0.311) vs Q2/Q3/Q4/Q5 (≥ 0.311)	1.96 [1.02; 3.75]	0.044*		2.29 [1.20; 4.35]	0.012*	
Serum markers of bone fragility						
Vitamin D (nmol/ml) (deficiency (< 10) vs normal (≥ 10))	1.77 [0.58; 5.38]	0.315		1.09 [0.14; 8.45]	0.937	
PTH (pg/ml)						
Q2 (between 33.2 and 51.2) vs Q1 (< 33.2)	0.65 [0.32; 1.33]	0.242	0.496	0.60 [0.26; 1.38]	0.225	0.478
Q3 (≥ 51.2) vs Q1 (< 33.2)	0.71 [0.34; 1.48]	0.360		0.66 [0.28; 1.58]	0.355	
CTX (ng/ml)						
Q2 (between 0.167 and 0.26) vs Q1 (< 0.167)	1.55 [0.42; 5.69]	0.511	0.780	2.04 [0.52; 7.95]	0.305	0.568
Q3 (≥ 0.26) vs Q1 (< 0.167)	1.27 [0.50; 3.26]	0.614		1.07 [0.31; 3.66]	0.913	
P1NP (ng/ml)						
Q2 (between 30.3 and 44) vs Q1 (< 30.3)	0.84 [0.26; 2.70]	0.771	0.475	0.54 [0.14; 2.00]	0.354	0.380
Q3 (≥ 44) vs Q1 (< 30.3)	0.55 [0.18; 1.65]	0.284		0.33 [0.07; 1.59]	0.169	
Osteocalcin (ng/ml)						
Q2 (between 2.1 and 3.8) vs Q1 (< 2.1)	0.57 [0.09; 3.47]	0.541	0.697	0.64 [0.11; 3.84]	0.626	0.870
Q3 (≥ 3.8) vs Q1 (< 2.1)	0.77 [0.12; 4.82]	0.778		0.72 [0.09; 5.59]	0.756	

y.o. years old, *%* percentage, *sd* standard deviation, *vs* versus, *eGFR* glomerular filtration rate, *PTH* parathyroid hormone, CTX-I cross-linked C-telopeptide of type I collagen, *P1NP* serum amino-terminal pro-peptides of type I procollagen, *BMD* bone mineral density, *ml* milliliters, *ng* nanogram

[a] Adjusted for age, NUTII (Nomenclature of Territorial Units for Statistics), peripheral bone mineral density (wrist), and body mass index

Except in

Age: NUTII (Nomenclature of Territorial Units for Statistics), peripheral bone mineral density (wrist), and BMI

BMI: Age, NUTII (Nomenclature of Territorial Units for Statistics), and peripheral bone mineral density (wrist)

Peripheral bone mineral density (wrist): NUTII (Nomenclature of Territorial Units for Statistics, and BMI

Vitamin D: Age, season of the year, BMI, and peripheral bone mineral density (wrist)

PTH: Age, peripheral bone mineral density (wrist), and BMI

CTX: Age, peripheral bone mineral density (wrist), and BMI

P1NP: Age, peripheral bone mineral density (wrist), and BMI

Osteocalcin: Age, peripheral bone mineral density (wrist), and BMI

*p value < 0.05

Table 4 Multivariable models for the association between prevalent fragility fracture and quality of life and physical function among Portuguese women 65 years and older

Quality of life and physical function (dependent variables)	Prevalent fragility fracture			
	Crude analysis β [95% CI]	p value	Adjusted[a] analysis β [95% CI]	Adjusted[a] p value
EQ5D	− 0.10 [− 0.17; − 0.04]	0.002*	− 0.06 [− 0.13; 0.01]	0.118
HAQ	0.30 [0.11; 0.49]	0.002*	0.33 [0.13; 0.52]	0.001*

EQ5D European Quality of Life questionnaire five dimensions three levels, *HAQ* Health Assessment Questionnaire

[a] Adjusted for age, NUTSII (Nomenclature of Territorial Units for Statistics), years of education, married/consensual union vs single/widow(er)/divorced, cardiac disease, and categorical BMI

*p value < 0.05

conditions of this population group namely the low literacy and low household income.

Low osteoporosis treatment rates and compliance in high-risk populations are a reality worldwide [37, 38]. There is a large gap between the number of women who are treated compared to the proportion of women eligible for treatment [39]. Hernlund et al. [8] showed that the treatment gap varies between European countries. The largest treatment gaps are described in Bulgaria and the Baltic states, where less than 15% of the population eligible receives osteoporosis treatment; the lowest treatment gap was found in Spain where 75% of the eligible women are potentially undergoing osteoporosis therapy. Moreover, even in patients who sustain a fragility fracture, less than 20% receive treatment in the year following the fracture. Finally, as in our study, it was found that a large number of low-risk women were undergoing osteoporosis treatment.

Considering the risk factors for fracture, we found that in our population, higher age, obesity, and lower distal BMD were independently associated with a prevalent fragility fracture. Age and low distal BMD are known risk factors for fragility fractures, and even for NHNV fractures [35, 40]. It is also well established that low body weight is a risk factor for hip and spine fractures, while obesity is not protective [41, 42]. Recently, obesity was shown as a risk factor for fracture, particularly for NHNV fractures [36, 43], which is in accordance with our data. Indeed, visceral obesity is associated with low BMD, probably due to higher levels of inflammatory cytokines, lower levels of leptin, and higher levels of adiponectin [44]. Moreover, obese people have higher risk of fall and protective response impairment [45].

We did not find any independent association between fragility fractures and other clinical risk factors, namely secondary osteoporosis, smoking, and alcohol intake, probably because very few Portuguese elderly have these risk factors.

Our results show that a prevalent fragility fracture is associated with greater physical disability in Portuguese women older than 65 years of age, which clearly shows that any fragility fracture that occurs after 40 years of age leads to significant disability. Surprisingly, we did not find an independent association between a prevalent fragility fracture and HRQoL. A cross-sectional analysis of the Canadian Multicentre Osteoporosis Study (CaMos) showed that in women, a previous fracture of the hip, sub-clinical spine, or lower body, but not other fragility fracture sites, was negatively associated with HRQoL [6]. These different findings can be explained by the fact that the CaMos study used a different instrument to measure HRQoL than we did in our study. Additionally, the CaMos divided fragility fractures according to facture site, while we analyzed all fragility fractures together. In addition, Roux et al., using the Global Longitudinal Study of Osteoporosis in Women (GLOW) [5], showed that spine, hip, and NHNV fractures had a detrimental effect on women's HRQoL, with the effect greater in the spine and hip. Moreover, they verified that the detrimental effect of the NHNV and spine fractures specifically affected the patients' mobility. This observation is in accordance with our findings that show that a prevalent fragility fracture is associated with greater physical disability.

This study has some important limitations. Considering the cross-sectional nature of the data, it was not possible to establish causal associations between a fragility fracture and risk factors for fracture. Fragility fractures were self-reported, which has been shown as less accurate for clinical vertebral fractures and an underestimation of their prevalence [46]; however, the overall performance of self-reported fragility fractures is acceptable [21–23], which we also demonstrated in this study. Other limitation of our study was the absence of vitamin D supplementation information.

Several important strengths of this current should be acknowledged. Our data came from a large, nationally representative sample of the Portuguese adult population. Our participants were examined by rheumatologists. Different fragility

fractures and health-related measurements were captured, providing relevant information about risk factors, treatment rates, and their impact in Portuguese senior women.

In conclusion, our study demonstrates that fragility fractures are frequently reported among Portuguese senior women and are an important cause of physical disability. The most important risk factors for fractures identified were older age, obesity, and low wrist BMD. The use of osteoporosis treatment in this high-risk group was low due to both underprescribing therapeutics and to patients' non-compliance. This study highlights the need to increase awareness regarding fragility fractures and osteoporosis treatment, targeted not only to healthcare professionals but also to the high-risk population stratum for fragility fractures.

Acknowledgements The EpiReumaPt Study Group acknowledges the invaluable input from Sofia Ramiro, MD, PhD; Pedro Machado, MD, PhD; Henrique de Barros, MD, PhD; João Eurico da Fonseca, MD, PhD; José António Pereira da Silva, MD, PhD; Francisco George, MD; Rui André Santos, MD; Luís Maurício Santos, MD; José Carlos Romeu, MD; Faculdade de Medicina da Universidade de Coimbra, Faculdade de Medicina da Universidade de Lisboa, Faculdade de Medicina da Universidade Porto, Liga Portuguesa Contra as Doenças Reumáticas, Associações de doentes com doenças reumáticas, Administrações Regionais de Saúde (Norte, Centro, Lisboa & Vale do Tejo, Alentejo and Algarve), Governo Regional da Madeira, Governo Regional dos Açores, Associação Nacional de Freguesias, Associação Nacional dos Municípios Portugueses, Câmara Municipal de Lisboa, Centros de Saúde, and Centro Hospitalar do Porto—Hospital de São João.

Funding information The study was supported by unrestricted grants from Direcção-Geral da Saúde, Fundação Calouste Gulbenkian, Fundação Champalimaud, Fundação AstraZeneca, Abbvie, Merck, Sharp & Dohme, Pfizer, Roche, Servier, Bial, D3A Medical Systems, Happybrands, Center de Medicina Laboratorial Germano de Sousa, Clínica Médica da Praia da Vitória, CAL-Clínica, Galp Energia, Açoreana Seguros, and individual rheumatologists.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Cummings SR, Melton LJ (2002) Epidemiology and outcomes of osteoporotic fractures. Lancet 359:1761–1767
2. Johnell O (1997) The socioeconomic burden of fractures: today and in the 21st century. Am J Med 103:20S–25S discussion 25S-26S
3. Stone KL, Seeley DG, Lui LY, Cauley JA, Ensrud K, Browner WS, Nevitt MC, Cummings SR, Osteoporotic Fractures Research G (2003) BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. J Bone Miner Res 18:1947–1954
4. Center JR, Bliuc D, Nguyen TV, Eisman JA (2007) Risk of subsequent fracture after low-trauma fracture in men and women. JAMA 297:387–394
5. Roux C, Wyman A, Hooven FH et al (2012) Burden of non-hip, non-vertebral fractures on quality of life in postmenopausal women: the Global Longitudinal study of Osteoporosis in Women (GLOW). Osteoporos Int 23:2863–2871
6. Adachi JD, Ioannidis G, Pickard L, Berger C, Prior JC, Joseph L, Hanley DA, Olszynski WP, Murray TM, Anastassiades T, Hopman W, Brown JP, Kirkland S, Joyce C, Papaioannou A, Poliquin S, Tenenhouse A, Papadimitropoulos EA (2003) The association between osteoporotic fractures and health-related quality of life as measured by the Health Utilities Index in the Canadian Multicentre Osteoporosis Study (CaMos). Osteoporos Int 14:895–904
7. Pike CT, Birnbaum HG, Schiller M, Swallow E, Burge RT, Edgell ET (2011) Prevalence and costs of osteoporotic patients with subsequent non-vertebral fractures in the US. Osteoporos Int 22:2611–2621
8. Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark J, McCloskey EV, Jonsson B, Kanis JA (2013) Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos 8:136
9. Harvey NC, McCloskey EV, Mitchell PJ, Dawson-Hughes B, Pierroz DD, Reginster JY, Rizzoli R, Cooper C, Kanis JA (2017) Mind the (treatment) gap: a global perspective on current and future strategies for prevention of fragility fractures. Osteoporos Int 28:1507–1529
10. Oden A, McCloskey EV, Kanis JA, Harvey NC, Johansson H (2015) Burden of high fracture probability worldwide: secular increases 2010-2040. Osteoporos Int 26:2243–2248
11. WHO (2016) World health statistics 2016: monitoring health for the SDGs. http://wwwwhoint/gho/publications/world_health_ statistics/2016/Annex_B/en/
12. Eastell R, O'Neill TW, Hofbauer LC, Langdahl B, Reid IR, Gold DT, Cummings SR (2016) Postmenopausal osteoporosis. Nat Rev Dis Primers 2:16069
13. van Staa TP, Dennison EM, Leufkens HG, Cooper C (2001) Epidemiology of fractures in England and Wales. Bone 29:517–522
14. Kanis JA, Hans D, Cooper C et al (2011) Interpretation and use of FRAX in clinical practice. Osteoporos Int 22:2395–2411
15. Rachner TD, Khosla S, Hofbauer LC (2011) Osteoporosis: now and the future. Lancet 377:1276–1287
16. Branco JC, Rodrigues AM, Gouveia N, Eusébio M, Ramiro S, Machado PM, da Costa LP, Mourão AF, Silva I, Laires P, Sepriano A, Araújo F, Gonçalves S, Coelho PS, Tavares V, Cerol J, Mendes JM, Carmona L, Canhão H, on behalf of the EpiReumaPt study group (2016) Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt—a national health survey. RMD Open e000166:2
17. Ramiro S, Canhao H, Branco JC (2010) EpiReumaPt Protocol—Portuguese epidemiologic study of the rheumatic diseases. Acta Reumatol Port 35:384–390
18. Rodrigues AM, Gouveia N, da Costa LP, Eusébio M, Ramiro S, Machado P, Mourão AF, Silva I, Laires P, Sepriano A, Araújo F, Coelho PS, Gonçalves S, Zhao A, Fonseca JE, de Almeida JM, Tavares V, da Silva JA, Barros H, Cerol J, Mendes J, Carmona L, Canhão H, Branco JC (2015) EpiReumaPt—the study of rheumatic and musculoskeletal diseases in Portugal: a detailed view of the methodology. Acta Reumatol Port 40:110–124
19. WHO (1998) Guidelines for preclinical evaluation and clinical trials in osteoporosis. Geneva
20. Melton LJ 3rd, Thamer M, Ray NF, Chan JK, Chesnut CH 3rd, Einhorn TA, Johnston CC, Raisz LG, Silverman SL, Siris ES (1997) Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation. J Bone Miner Res 12:16–23

21. Chen Z, Kooperberg C, Pettinger MB, Bassford T, Cauley JA, LaCroix AZ, Lewis CE, Kipersztok S, Borne C, Jackson RD (2004) Validity of self-report for fractures among a multiethnic cohort of postmenopausal women: results from the Women's Health Initiative observational study and clinical trials. Menopause 11:264–274
22. Ismail AA, O'Neill TW, Cockerill W et al (2000) Validity of self-report of fractures: results from a prospective study in men and women across Europe. EPOS Study Group European Prospective Osteoporosis Study Group Osteoporos Int 11:248–254
23. Honkanen K, Honkanen R, Heikkinen L, Kroger H, Saarikoski S (1999) Validity of self-reports of fractures in perimenopausal women. Am J Epidemiol 150:511–516
24. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M (2007) Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. Psychol Health Med 12:225–235 quiz 235-227
25. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E (2008) FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int 19:385–397
26. Marques A, Rodrigues AM, Romeu JC, Ruano A, Barbosa AP, Simões E, Águas F, Canhão H, Alves JD, Lucas R, Branco JC, Laíns J, Mascarenhas M, Simões S, Tavares V, Lourenço O, da Silva JA (2016) Multidisciplinary Portuguese recommendations on DXA request and indication to treat in the prevention of fragility fractures. Acta Reumatol Port 41:305–321
27. Ferreira LN, Ferreira PL, Pereira LN, Oppe M (2014) EQ-5D Portuguese population norms. Qual Life Res 23:425–430
28. Ferreira LN, Ferreira PL, Pereira LN, Oppe M (2014) The valuation of the EQ-5D in Portugal. Qual Life Res 23:413–423
29. Fries JF, Spitz P, Kraines RG, Holman HR (1980) Measurement of patient outcome in arthritis. Arthritis Rheum 23:137–145
30. Levey AS, Stevens LA, Schmid CH, Zhang Y(L), Castro AF III, Feldman HI, Kusek JW, Eggers P, van Lente F, Greene T, Coresh J, for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150:604–612
31. Gouveia N, Rodrigues AM, Ramiro S, Machado P, da Costa LP, Mourão AF, Silva I, Rego T, Laires P, André R, Mauricio L, Romeu JC, Tavares V, Cerol J, Canhão H, Branco JC (2015) EpiReumaPt: how to perform a national population based study—a practical guide. Acta Reumatol Port 40:128–136
32. Bliuc D, Nguyen TV, Eisman JA, Center JR (2014) The impact of nonhip nonvertebral fractures in elderly women and men. J Clin Endocrinol Metab 99:415–423
33. Ioannidis G, Flahive J, Pickard L et al (2013) Non-hip, non-spine fractures drive healthcare utilization following a fracture: the Global Longitudinal Study of Osteoporosis in Women (GLOW). Osteoporos Int 24:59–67
34. Tran T, Bliuc D, van Geel T, et al. (2017) Population-wide impact of non-hip non-vertebral fractures on mortality. J Bone Miner Res
35. Sornay-Rendu E, Munoz F, Duboeuf F, Delmas PD (2005) Rate of forearm bone loss is associated with an increased risk of fracture independently of bone mass in postmenopausal women: the OFELY study. J Bone Miner Res 20:1929–1935
36. Holloway KL, Henry MJ, Brennan-Olsen SL, Bucki-Smith G, Nicholson GC, Korn S, Sanders KM, Pasco JA, Kotowicz MA (2016) Non-hip and non-vertebral fractures: the neglected fracture sites. Osteoporos Int 27:905–913
37. Kanis JA, Svedbom A, Harvey N, McCloskey EV (2014) The osteoporosis treatment gap. J Bone Miner Res 29:1926–1928
38. Giangregorio L, Papaioannou A, Cranney A, Zytaruk N, Adachi JD (2006) Fragility fractures and the osteoporosis care gap: an international phenomenon. Semin Arthritis Rheum 35:293–305
39. Curtis EM, Moon RJ, Harvey NC, Cooper C (2017) The impact of fragility fracture and approaches to osteoporosis risk assessment worldwide. Bone
40. De Laet C, Kanis JA, Oden A et al (2005) Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int 16:1330–1338
41. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, Pfeilschifter J, Silverman S, Díez-Pérez A, Lindsay R, Saag KG, Netelenbos JC, Gehlbach S, Hooven FH, Flahive J, Adachi JD, Rossini M, Lacroix AZ, Roux C, Sambrook PN, Siris ES, Glow Investigators. (2011) Obesity is not protective against fracture in postmenopausal women: GLOW. Am J Med 124:1043–1050
42. Edwards MH, Jameson K, Denison H, Harvey NC, Sayer AA, Dennison EM, Cooper C (2013) Clinical risk factors, bone density and fall history in the prediction of incident fracture among men and women. Bone 52:541–547
43. Gnudi S, Sitta E, Lisi L (2009) Relationship of body mass index with main limb fragility fractures in postmenopausal women. J Bone Miner Metab 27:479–484
44. Russell M, Mendes N, Miller KK, Rosen CJ, Lee H, Klibanski A, Misra M (2010) Visceral fat is a negative predictor of bone density measures in obese adolescent girls. J Clin Endocrinol Metab 95:1247–1255
45. Finkelstein EA, Chen H, Prabhu M, Trogdon JG, Corso PS (2007) The relationship between obesity and injuries among U.S. adults. Am J Health Promot 21:460–468
46. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ (2002) The accuracy of self-reported fractures in older people. J Clin Epidemiol 55:452–457

SECTION II

CLINICAL RISK FACTORS AND CELLULAR DISTURBANCES ASSOCIATED WITH POOR TRABECULAR MECHANICAL BEHAVIOUR AND WITH HIP FRACTURES

PART 1 - RODRIGUES AM, CAETANO-LOPES J, VALE AC, ET AL. 2012. SMOKING IS A PREDICTOR OF WORSE TRABECULAR MECHANICAL PERFORMANCE IN HIP FRAGILITY FRACTURE PATIENTS. J BONE MINER METAB. 30(6):692-699.

PART 2 – RODRIGUES AM, CAETANO-LOPES J, VALE AC, ET AL. 2012. LOW OSTEOCALCIN/COLLAGEN TYPE I BONE GENE EXPRESSION RATIO IS ASSOCIATED WITH HIP FRAGILITY FRACTURES. BONE. 51:981-989.

J Bone Miner Metab
DOI 10.1007/s00774-012-0370-4

ORIGINAL ARTICLE

Smoking is a predictor of worse trabecular mechanical performance in hip fragility fracture patients

Ana Maria Rodrigues · Joana Caetano-Lopes · Ana Catarina Vale · Inês Aleixo · Ana Sofia Pena · Alexandra Faustino · Alexandre Sepriano · Joaquim Polido-Pereira · Elsa Vieira-Sousa · Raquel Lucas · José Carlos Romeu · Jacinto Monteiro · Maria Fátima Vaz · João Eurico Fonseca · Helena Canhão

Received: 9 November 2011 / Accepted: 21 June 2012

Abstract Clinical risk factors (CRFs) are established predictors of fracture events. However, the influence of individual CRFs on trabecular mechanical fragility is still a subject of debate. In this study, we aimed to assess differences, adjusted for CRFs, between bone macrostructural parameters measured in ex-vivo specimens from hip fragility fracture patients and osteoarthritis patients, and to determine whether individual CRFs could predict trabecular bone mechanical behavior in hip fragility fractures. Additionally, we also looked for associations between the 10-year risk of major and hip fracture calculated by FRAX and trabecular bone mechanical performance. In this case–control study, a group of fragility fracture patients were compared with a group of osteoarthritis patients, both having undergone hip replacement surgery. A clinical protocol was applied in order to collect CRFs [body mass index (BMI), prior fragility fracture, parental history of hip fracture, long-term use of oral glucocorticoids, rheumatoid arthritis, current smoking, alcohol consumption, age and gender]. The 10-year probability of fracture was calculated. Serum bone turnover markers were determined and dual X-ray absorptiometry performed. Femoral head diameter was evaluated and trabecular bone cylinders were drilled for mechanical testing to determine bone strength, stiffness and toughness. We evaluated 40 hip fragility fracture and 52 osteoarthritis patients. Trabecular bone stiffness was significantly lower ($p = 0.042$) in hip fragility fracture patients when compared to osteoarthritic individuals, adjusted for age, gender and BMI. No other macrostructural parameter was statistically different between the groups. In hip fragility fracture patients, smoking habits ($\beta = -0.403$; $p = 0.018$) and female gender ($\beta = -0.416$; $p = 0.008$) were independently associated with lower stiffness. In addition, smoking was also independently associated with worse trabecular strength ($\beta = -0.323$; $p = 0.045$), and toughness ($\beta = -0.403$; $p = 0.018$). In these patients, the 10-year risk of major ($r = -0.550$; $p = 0.012$) and hip fracture ($r = -0.513$; $p = 0.021$) calculated using only CRFs was strongly correlated with femoral neck bone mineral density but not with mechanical performance. Our data showed that among fragility fracture patients active smoking is a predictor of worse intrinsic trabecular mechanical performance, and female gender is also independently associated with lower stiffness. In this population, the 10-year risk of fracture

A. M. Rodrigues · J. Caetano-Lopes · I. Aleixo · A. S. Pena · A. Faustino · A. Sepriano · J. Polido-Pereira · E. Vieira-Sousa · J. E. Fonseca · H. Canhão (✉)
Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, Edifício Egas Moniz, 1649-028 Lisbon, Portugal
e-mail: helenacanhao@netcabo.pt

A. M. Rodrigues · J. Polido-Pereira · E. Vieira-Sousa · J. C. Romeu · J. E. Fonseca · H. Canhão
Rheumatology and Bone Metabolic Diseases Department, Hospital de Santa Maria, Av. Prof. Egas Moniz, 1640-035 Lisbon, Portugal

A. C. Vale · M. F. Vaz
Department of Mechanical Engineering, Instituto Superior Técnico, T.U. Lisbon, Av. Rovisco Pais, 1, 1649-001 Lisbon, Portugal

R. Lucas
Department of Hygiene and Epidemiology, University of Porto Medical School, Porto, Portugal

R. Lucas
Institute of Public Health of University of Porto, Porto, Portugal

J. Monteiro
Ortopaedics Department, Hospital de Santa Maria, Av. Prof. Egas Moniz, 1640-035 Lisbon, Portugal

Published online: 14 August 2012

using CRFs with different weights only reflects bone mass loss but not trabecular mechanical properties.

Keywords Osteoporosis · Fracture risk · FRAX · Mechanical properties

Introduction

Hip fragility fractures are the most severe clinical consequence of osteoporosis. They are characterized by high morbidity and mortality and constitute a major economic burden [1].

A hip fragility fracture is ultimately a mechanical failure of bone structure and it occurs when the load applied exceeds the capacity of the structure to resist it. Although measures of bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2) are strongly correlated with bone mechanical performance, they do not fully explain fracture risk [2–5]. In fact, over half of the subjects who experience a hip fragility fracture do not have a T-score below −2.5 [6]. In addition, it is estimated that 90 % of hip fractures result from a fall but only 5 % of falls result in a hip fracture [7, 8]. Thus, it seems evident that other factors, in addition to falls and aBMD, influence hip fracture risk. Among these other variables, bone intrinsic properties have recently emerged as a major determinant of fragility fractures [9].

The biomechanical performance of excised samples of trabecular bone reflects the net effect of differences in microarchitecture, bone volume fraction and tissue material properties [10]. In fact, compressive mechanical testing represents an established method for evaluating ex vivo the mechanical competence of bone, due to its simplicity and its resemblance to natural deformation [11]. The measures of bone biomechanical performance in compressive tests are stiffness, strength and toughness, which reflect bone macrostructural competence [12]. During ageing, bone resorption on the endocortical, intracortical and trabecular surfaces reduces the amount of bone within the periosteal envelope as trabeculae thin and disappear and cortices thin and become porous. Simultaneously, periosteal bone formation partly offsets removal of bone on the inner surface but is not enough to compensate all the other structural changes, leading to a fragile bone with lower strength, stiffness and toughness [13]. Hip bone strength is determined by the behavior of both cortical and trabecular compartments. However, trabecular bone is metabolically 6–8 times more active than cortical bone and, therefore, it is affected earlier and more severely by age-related changes. This may be particularly important for femoral neck fractures, since this region has a relatively thin cortical shell surrounding a larger volume of trabecular bone that is likely responsible for carrying the majority of loads transmitted across the hip. In fact, using a finite element analysis, Orwoll and colleagues [14] showed that when a load simulating a sideways fall is applied, the region of the femoral neck that fails first is trabecular bone.

In recent years, population-based cohort studies from Europe, North America, Asia and Australia have identified age, gender, body mass index (BMI), prior fragility fracture, parental history of hip fracture, long-term use of oral glucocorticoids (3 months or more), rheumatoid arthritis and other secondary causes of osteoporosis, current smoking [15] and alcohol intake (more than 3 units per day) [16] as clinical risk factors (CRFs) that provided information about fracture risk independently of aBMD [17–21]. A new tool, FRAX, was thereafter developed by the World Health Organization (WHO) in order to assess, in untreated subjects over 40 years old, the 10-year probability of both a major fracture and a hip fracture with or without the use of aBMD. FRAX is an algorithm based on a multivariate model which incorporates, with different weights, independent risk factors for fracture, combined with the corresponding death rate of each country, to compute fracture probability. In fact, when aBMD testing is limited, the use of FRAX with only CRFs might be considered as an approach to define an intervention threshold [22, 23]. Moreover, the FRAX tool using only CRFs has shown a better performance than aBMD by itself in predicting major fracture risk [24]. It is therefore relevant to assess the association of individual CRFs and of the 10-year risk of major and hip fractures with trabecular mechanical competence.

Previous studies demonstrated that CRFs such as age, gender or rheumatoid arthritis interfere with bone composition and with bone mechanical parameters [3, 4, 25–30]. In fact, McCalden et al. have shown in a population aged from 20 to 102 years old that the elderly have a BMD decreased by 60 % in comparison to younger subjects. However, this fact does not fully explain the 92 % reduction in mechanical strength observed in elderly individuals [27]. Interestingly, further emphasizing the influence of CRFs on mechanical properties, Tommasini and colleagues [31] have shown that both genders have a similar age-related degradation of mechanical properties.

Taking into account the current evidence, we hypothesize that CRFs individually are major determinants of intrinsic bone mechanical properties in hip fragility fracture patients. Thus, in this study, we aimed to assess differences, adjusted for CRFs, between bone macrostructural parameters measured in ex-vivo specimens from hip fragility fracture patients compared to osteoarthritis and to determine if individual CRFs could predict trabecular bone mechanical behavior in hip fragility fractures. Additionally, we also looked for associations between the 10-year

risk of major and hip fractures calculated by FRAX and trabecular bone mechanical performance.

Materials and methods

Study population

Patients

In this case–control study we enrolled hip fragility fracture patients undergoing total hip replacement surgery within 8 days post-fracture at the Orthopedic Department of Hospital de Santa Maria in Lisbon, between 2008 and 2009. As controls, we used osteoarthritis patients undergoing total hip replacement surgery and with no prior history of fragility fractures, during the same period.

Consecutive, post-menopausal women and men, older than 50 years, undergoing hip replacement surgery due to hip fragility fracture or to osteoarthritis, who were able to give written consent and clinical information, were recruited for this study. Patients receiving anti-osteoporotic agents, warfarin or anti-epileptic therapies or with secondary causes for osteoporosis, bone metabolic diseases (other than osteoporosis), bone metastasis, primary tumors and osteomyelitis were excluded. Five patients were also excluded because of femoral epiphysis characteristics (size and integrity) that hampered the cylinder extraction for mechanical tests.

Clinical data measurements

A clinical questionnaire was applied in order to collect CRFs—age, gender, BMI, prior fragility fracture (other than the reason/indication for surgery), parental history of hip fracture, long-term use of oral glucocorticoids (≥3 months), rheumatoid arthritis, current smoking, alcohol intake (≥3 units/day) and other secondary causes of osteoporosis, assessed as in the FRAX United Kingdom study [32, 33].

The 10-year probability of fracture was calculated using the FRAX tool available online (http://www.shef.ac.uk/FRAX/), calibrated to the epidemiology of fracture and life expectancy in Spain, since the Portuguese data is not yet available [34]. This algorithm provided two outputs: the 10-year probability of having a major osteoporotic fracture (spine, hip, forearm or humerus) and the 10-year probability of hip fracture alone. To calculate fracture risk we used only CRFs and did not include femoral neck aBMD, in order to avoid the potential confounding of this bone parameter for establishing associations with mechanical tests. Also, in patients undergoing hip arthroplasty due to fragility fracture, the current fracture was not included in the FRAX calculation as a previous fracture.

In a subset of 34 patients, femoral neck aBMD of the contralateral hip was measured by dual X-ray absorptiometry (DXA) scan 4 days after surgery using a Lunar Prodigy densitometer (GE Healthcare, UK).

Written informed consent was obtained from all patients and the study was conducted in accordance with the regulations governing clinical trials such as the Declaration of Helsinki, as amended in Seoul (2008), and was approved by the local Ethics Committee.

Biochemical assessment

Fasting blood samples were collected to assess serum calcium and phosphorus, alkaline phosphatase (ALP), bone-specific ALP (BSALP) and osteocalcin (OC) levels. Cross-linked c-telopeptide of type I collagen (CTX-I) and amino-terminal propeptides of type I procollagen (P1NP) were measured by Elecsys® electrochemiluminescent immunoassay analyzers (Roche Diagnostics, Switzerland).

Bone characteristics

After the orthopedic procedure, the femoral epiphyses were immediately stored at −80 °C. Before testing, they were defrosted at room temperature and measurements of the diameter of the epiphyses were performed in three different axes. Bone cylinders were obtained by drilling through the highest load direction using a perforating drill with a diameter of 15 mm (method adapted from [35]). The cylinders (with only trabecular bone) were de-fatted for 3 h using a chloroform and methanol solution and then hydrated overnight in phosphate-buffered saline solution.

Compressive mechanical testing represents an established method for evaluating ex vivo the mechanical competence of bone, due to its simplicity and its resemblance to natural deformation [11, 36]. Compression tests were performed in an universal testing machine (Instron 5566™, Instron Corporation, Canton, MA, USA) with a load cell of 10 kN and a cross-head rate of 0.1 mm/s. Samples were loaded in the principal stress direction. Stress–strain curves were obtained for each specimen using Bluehill 2 software (Instron). This software has the ability to build stress–strain representations from load–displacement points, normalized for the specimens' dimensions. Analysis of the curves was performed using MatLab 7.1 software (R14 SP3, The Mathworks, Inc.) in order to obtain the following mechanical bone parameters: trabecular strength stiffness and toughness. These measures are obtained by the stress–strain curves from which is calculated the Young's modulus, E (slope of the curve in the straight region), yield stress, σ_y (maximum stress in the

elastic regime) and energy until yield point, W_y (energy absorbed until yield). The stiffness, strength and toughness are determined respectively by the Young's modulus, yield stress and energy until yield point [37].

Statistical analysis

Baseline characteristics are presented as mean ± standard deviation or percentages. Clinical characteristics, markers of bone turnover (ALP, BASLP, CTX, P1NP) and bone macrostructural (trabecular mechanical behavior, femoral neck aBMD and femoral head diameter) parameters were compared between groups with the independent-samples *t* test. Categorical data were tested using chi-squared or Fisher's exact tests.

Bone macrostructural parameters were compared across groups using general linear model (GLM) analysis adjusted for differences in clinical characteristics identified as possible confounders (age, gender and BMI).

Multivariate linear regression models were used to explore the association between bone mechanical behavior (strength, stiffness and toughness) and CRFs (age, gender, BMI, prior fragility fracture, parental history of hip fracture, long-term use of oral glucocorticoids, rheumatoid arthritis, smoking and alcohol intake and other secondary causes of osteoporosis) in hip fragility fracture patients. The CRFs whose prevalence within the group was ≤5 % were excluded to avoid bias of the data. First, the variables were tested by univariate analysis. After that we performed a stepwise linear multivariate regression model using backward selection with the level of significance <0.05. The variables were also analyzed by multivariate linear regression analysis with all variables included.

The relation between FRAX outputs (10-year risk of major and hip fracture) and bone macrostructural parameters in hip fragility fracture patients was assessed by the Pearson correlation coefficient.

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics Software, v.17.0 (SPSS Inc., Chicago, USA) and a 2-tailed *p*-value <0.05 was considered significant.

Results

Patients

We evaluated 40 hip fragility fracture and 52 osteoarthritis patients (Table 1). Fragility fracture patients were older ($p = 0.007$), had lower BMI ($p = 0.007$) and were predominantly women ($p = 0.004$) when compared with the osteoarthritis group. On the other hand, the frequency of patients who reported alcohol intake was significantly increased in osteoarthritis patients ($p = 0.007$). No other CRF was significantly different between the two groups. As expected, the 10-year risk of a major and hip fracture calculated only with CRFs was significantly higher in hip fracture patients ($p < 0.001$) and this difference was maintained even if we added femoral neck aBMD to the calculation.

Regarding serum levels of bone turnover markers, only ALP was significantly increased in the fragility fracture group, but this difference was lost when adjusted for age and gender ($p = 0.114$). No other significant differences were found between the two groups of patients.

Hip fragility fracture patients had lower trabecular stiffness when compared with osteoarthritis patients

Considering bone macrostructure characteristics, hip fragility fracture patients had smaller femoral epiphysis diameter ($p = 0.005$), worse trabecular mechanical behavior (strength $p = 0.046$; stiffness $p = 0.013$) and lower aBMD ($p = 0.002$) than the osteoarthritis group. However, when we adjusted these differences for age, gender and BMI, the only macrostructural bone characteristic that was still significantly different between the groups was trabecular stiffness ($p = 0.042$) (Table 2).

Smoking is a predictor of worse trabecular mechanical performance in patients with hip fragility fractures

The association between trabecular bone mechanical properties and each of the CRFs tested by univariate analysis in hip fragility fracture patients is described in Table 3. The only CRFs associated with lower trabecular strength in univariate analysis was smoking ($\beta = -0.323$; $p = 0.045$). In addition, female gender ($\beta = -0.345$; $p = 0.031$) was significantly associated with lower stiffness. Regarding toughness, no association was identified in the univariate analysis.

Multivariate regression analysis using backward selection demonstrated that in hip fragility fracture patients the only predictor of lower trabecular strength was current smoking ($\beta = -0.323$; $p = 0.045$). However, using multivariate regression analysis with all variables included, female gender was also independently associated with lower strength ($\beta = -0.386$; $p = 0.049$) (data not shown).

Smoking ($\beta = -0.403$; $p = 0.018$) and female gender ($\beta = -0.416$; $p = 0.008$) were also predictors of lower stiffness and this result was demonstrated by the two models of multivariate analysis used. Current smoking was also the only CRF that predicted lower toughness ($\beta = -0.416$; $p = 0.011$) in both models of multivariate regression analysis.

Table 1 Characteristics of the study population of hip fractures compared with osteoarthritis

	Hip fractures	Osteoarthritis	*p* value
N	40	52	
Clinical risk factors			
Age (years)	80.0 ± 6.9	69.0 ± 8.4	0.007*
Female (%)	80.0	50.0	0.004*
BMI (kg/cm^2)	24.8 ± 4.3	27.5 ± 4.4	0.007*
Previous fractures (%)	87.5	0	NA
Family history of fractures (%)	5.0	5.8	0.894
Secondary causes of osteoporosis (%)	15.0	9.6	0.629
Corticosteroid therapy (%)	15.0	3.8	0.112
Rheumatoid arthritis (%)	12.5	3.8	0.223
Smoking (%)	10.0	19.2	0.240
Alcohol intake (%)	2.5	17.5	0.016*
10-year risk major fracture using only CRFs	15.5 ± 9.3	5.7 ± 5.2	<0.001*
10-year risk major fracture with DXA	14.9 ± 8.7	7.6 ± 6.7	<0.001*
10-year risk hip fracture using only CRFs	8.6 ± 7.4	2.2 ± 3.3	0.013*
10-year risk hip fracture with DXA	7.3 ± 5.5	2.7 ± 3.3	0.011*
Markers of bone turnover			
ALP (IU/L)	94.2 ± 42.0	68.5 ± 31.1	0.004*
BSALP (μg/L)	12.5 ± 6.6	11.6 ± 5.9	0.448
Osteocalcin (ng/mL)	10.9 ± 10.0	10.9 ± 9.6	0.884
P1NP (ng/mL)	57.9 ± 52.5	46.1 ± 29.4	0.223
CTX-I (μg/mL)	67.0 ± 89.6	35.6 ± 19.4	0.055

Values represent mean ± SD. For continuous variables, differences were assessed using independent Student's *t* test. χ^2 or Fisher's exact test was used for proportions. *NA* Not applicable—the exclusion criteria for the osteoarthritis group was the presence of previous fragility fractures

ALP alkaline phosphatase, *BMI* body mass index, *BSALP* bone-specific ALP, *CTX-I* carboxyl-terminal cross-linking telopeptides of type I collagen, *P1NP* amino-terminal propeptides of type I procollagen

* $p < 0.05$

Table 2 Femoral epiphysis characteristics in hip fractures compared with osteoarthritis

	Hip fractures	Osteoarthritis	#*p* value	##*p* value (adjusted for age, gender, BMI)
N	40	52		
Bone macrostructural variables				
Strength (MPa)	6.8 ± 4.1	8.7 ± 4.8	0.046*	0.062
Stiffness (MPa)	324.1 ± 191.8	436.9 ± 236.8	0.013*	0.042*
Toughness (N mm/mm^3)	0.13 ± 0.11	0.19 ± 0.18	0.066	0.101
Femoral neck aBMD (g/cm^2)	0.68 ± 0.09	0.81 ± 0.13	0.002*	0.055
T-score	−2.6 ± 0.74	−1.6 ± 1.0	0.003*	0.069
Diameter (mm)	44.7 ± 3.3	49.3 ± 3.6	0.005*	0.199

Values represent mean and SD

aBMD areal bone mineral density, *BMI* body mass index

p value obtained using independent Student's *t* test

p value obtained using multivariate logistic regression analysis adjusting the differences between hip fractures and controls for age, gender and BMI

* $p < 0.05$

We further investigated the association of smoking with BMD in the subgroup of patients who underwent DXA. We found no significant differences in aBMD ($p = 0.998$) between patients who were currently smoking (mean aBMD 0.672 ± 0.090) from those who did not smoke (mean aBMD 0.685 ± 0.090), adjusted for age, gender and BMI.

Table 3 Univariate analyses and multivariate analysis for the association of CRFs with bone mechanical behavior in hip fracture patients

	Strength (MPa)		Stiffness (MPa)		Toughness (N mm/mm^3)	
	Univariate β estimate (95 %CI) p value	Multivariate β estimate (95 % CI) p value	Univariate β estimate (95 % CI) p value	Multivariate β estimate (95 % CI) p value	Univariate β estimate (95 % CI) p value	Multivariate β estimate (95 % CI) p value
Age	0.280		−0.200		0.307	
	(−0.02–0.36)		(−3.46–14.45)		(0.00–0.01)	
	0.08		0.222		0.057	
Female	−0.200		−0.345	−0.416	−0.080	
	(−5.29–1.29)		[−308.4–(−15.3)]	[−336.1–(−53.9)]	(−0.11–0.07)	
	0.226		0.031*	0.008*	0.629	
BMI	0.073		0.145		−0.010	
	(−0.25–0.39)		(−8.31–21.29)		(−0.009–0.008)	
	0.660		0.380		0.953	
Previous fractures	0.007		0.045		0.085	
	(−3.07–4.67)		(−155.5–204.69)		(−0.08–0.13)	
	0.68		0.78		0.605	
Family history of fractures	NA		NA		NA	
Secondary causes of osteoporosis	−0.016		−0.076		0.013	
	(−4.25–3.86)		(−230.8–145.3)		(−0.10–0.11)	
	0.922		0.648		0.939	
Corticosteroids	−0.130		0.054		−0.228	
	(−5.59–2.46)		(−157.8–218.9)		(−0.18–0.03)	
	0.446		0.744		0.162	
Rheumatoid arthritis	0.074		0.074		−0.186	
	(−5.46–3.47)		(−161.1–253.5)		(−0.18–0.05)	
	0.654		0.654		0.257	
Smoking	−0.323	−0.323	−0.244	−0.403	−0.302	−0.416
	[−8.57–(−0.10)]	[−8.56–(−0.10)]	(−353.0–49.4)	[−457.7−(−45.1)]	(−0.22–0.00)	[−0.26–(−0.36)]
	0.045*	0.045*	0.135	0.018*	0.061	0.011*
Alcohol intake	NA		NA		NA	

Values obtained by linear regression analyses for bone mechanical behavior outcomes. Multivariate analysis by backward selection was performed adjusting for the covariates listed in the table

NA variable frequency ≤5 %

BMI body mass index, *CI* confidence interval

* $p < 0.05$

As expected, there was a significant difference in aBMD ($p = 0.042$) between female (mean aBMD 0.660 ± 0.090) and male (mean aBMD 0.753 ± 0.020) genders when adjusted for age and BMI.

FRAX output using clinical risk factors only reflects lower bone mass but not trabecular mechanical properties

In these patients, the 10-year risk of major ($r = -0.550$; $p = 0.012$) and hip ($r = -0.513$; $p = 0.021$) fracture using only CRFs was strongly correlated with femoral neck aBMD but not with bone trabecular mechanical performance (Table 4).

Discussion

The primary aim of this work was to compare bone macrostructural parameters between hip fragility fracture and osteoarthritis patients. Here we demonstrated that when adjusted for differences in age, gender and BMI the only macrostructural bone characteristic still significantly different between hip fragility fracture and osteoarthritis

Table 4 Relation between FRAX outputs and bone structural parameters in hip fragility fracture patients

	Strength (MPa)	Stiffness (MPa)	Toughness (N mm/mm^3)	Femoral neck aBMD (g/cm^3)
10-year risk of major fracture (%)	$r = -0.045$	$r = -0.172$	$r = -0.097$	$r = -0.550$
	$p = 0.808$	$p = 0.303$	$p = 0.563$	$p = 0.012$*
10-year risk of hip fracture (%)	$r = -0.028$	$r = -0.134$	$r = -0.115$	$R = -0.513$
	$p = 0.866$	$p = 0.417$	$p = 0.485$	$p = 0.021$*

Values obtained by Pearson's correlation coefficient. * $p < 0.05$

aBMD areal bone mineral density

patients is trabecular stiffness, in accordance with several studies comparing trabecular bone mechanical properties between hip fragility fracture and osteoarthritis which found significant differences regarding trabecular strength, stiffness and toughness [37–41]. However, in these studies CRFs were not taken into account. Furthermore, Ciarelli et al. [42] performed a study comparing trabecular mechanical behavior between hip fragility fracture patients and cadavers, which also showed differences. In all these previous studies the differences between groups were lost when adjusted for aBMD but details regarding CRFs were not captured and included in the data analysis. In our study we tried to take a step forward and aimed at determining whether individual CRFs could predict trabecular bone mechanical behavior in hip fragility fracture patients. We documented that, in hip fragility fracture patients, smoking habits and female gender were independently associated with lower stiffness. Furthermore, current smoking was the only predictor of worse trabecular strength and toughness. We did not find significant differences in aBMD between smokers and non-smokers, indicating that the smoking effect on trabecular bone intrinsic properties seems to be independent of aBMD. Corroborating this data, a recent meta-analysis found that low aBMD accounted for only 23 % of the smoking-related risk of hip fracture [17]. In fact, studies in animal models revealed that nicotine had an effect on lowering bone strength but not on aBMD [43].

Finally, we studied the association between the 10-year risk of major and hip fracture and trabecular bone mechanical performance. We demonstrated that in hip fragility fracture patients the 10-year risk of fracture using CRFs with different weights as in the FRAX tool reflects only lower bone mass but not trabecular mechanical properties. It is however necessary to bear in mind that these results were obtained based on an analysis of hip replacement surgery patients due to fragility fracture, who are elderly patients with high a prevalence of previous fragility fractures and are not representative of the general population.

To the best of our knowledge, this is the first report evaluating intrinsic trabecular bone hip properties, using ex-vivo mechanical tests and correlating these results with epidemiological and clinical factors that integrate FRAX in a population of patients with fragility fractures.

In summary, our data showed that among fragility fracture patients current smoking habit is a predictor of worse intrinsic trabecular mechanical performance and that female gender is associated with lower stiffness. Moreover, in this population, the 10-year risk of fracture only reflects bone mass loss but not trabecular mechanical properties.

Acknowledgments This work was supported by unrestricted grants from Institut de Recherches Internacionales Servier (IRIS), Associação Portuguesa de Osteoporose, Merck Sharp and Dome, Faculdade de Medicina de Lisboa/Astrazeneca and Hospital de Santa Maria. A special thanks to the surgeons and nurses from the Orthopaedics Department of Hospital de Santa Maria who collaborated with the collection of samples. Thanks also to nurse L. Narciso from the Rheumatology and Bone Metabolic Diseases Department from Hospital de Santa Maria for the DXA execution and analysis, and IP Perpétuo for the analysis of laboratorial parameters, P.M. Amaral, PhD, was also of great help in the guidance of the mechanical tests set-up.

Conflict of interest No disclosures.

References

1. Cummings SR, Melton LJ (2002) Epidemiology and outcomes of osteoporotic fractures. Lancet 359:1761–1767
2. Abdulghani S, Caetano-Lopes J, Canhao H, Fonseca JE (2009) Biomechanical effects of inflammatory diseases on bone-rheumatoid arthritis as a paradigm. Autoimmun Rev 8:668–671
3. Caetano-Lopes J, Nery AM, Canhao H, Duarte J, Cascao R et al (2010) Chronic arthritis leads to disturbances in the bone collagen network. Arthritis Res Ther 12:R9
4. Caetano-Lopes J, Nery AM, Henriques R, Canhao H, Duarte J et al (2009) Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. Clin Exp Rheumatol 27:475–482
5. Canhao H, Lucas R, Fonseca JE, Costa L, Romeu JC et al (2008) Factors influencing calcaneus quantitative ultrasound measurements in an urban population. Clin Exp Rheumatol 26:67–72
6. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H et al (2004) Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 34:195–202
7. Cummings SR (1985) Are patients with hip fractures more osteoporotic? Review of the evidence. Am J Med 78:487–494

8. Greenspan SL, Myers ER, Maitland LA, Resnick NM, Hayes WC (1994) Fall severity and bone mineral density as risk factors for hip fracture in ambulatory elderly. JAMA 271:128–133
9. Grampp S, Lang P, Jergas M, Gluer CC, Mathur A et al (1995) Assessment of the skeletal status by peripheral quantitative computed tomography of the forearm: short-term precision in vivo and comparison to dual X-ray absorptiometry. J Bone Miner Res 10:1566–1576
10. Carter DR, Hayes WC (1977) The compressive behavior of bone as a two-phase porous structure. J Bone Joint Surg Am 59:954–962
11. Matsuura M, Eckstein F, Lochmuller EM, Zysset PK (2008) The role of fabric in the quasi-static compressive mechanical properties of human trabecular bone from various anatomical locations. Biomech Model Mechanobiol 7:27–42
12. Hernandez CJ, Gupta A, Keaveny TM (2006) A biomechanical analysis of the effects of resorption cavities on cancellous bone strength. J Bone Miner Res 21:1248–1255
13. Seeman E (2002) Pathogenesis of bone fragility in women and men. Lancet 359:1841–1850
14. Orwoll ES, Marshall LM, Nielson CM, Cummings SR, Lapidus J et al (2009) Finite element analysis of the proximal femur and hip fracture risk in older men. J Bone Miner Res 24:475–483
15. Tamaki J, Iki M, Fujita Y, Kouda K, Yura A et al (2011) Impact of smoking on bone mineral density and bone metabolism in elderly men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. Osteoporos Int 22:133–141
16. Soares EV, Favaro WJ, Cagnon VH, Bertran CA, Camilli JA (2010) Effects of alcohol and nicotine on the mechanical resistance of bone and bone neoformation around hydroxyapatite implants. J Bone Miner Metab 28:101–107
17. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C et al (2005) Smoking and fracture risk: a meta-analysis. Osteoporos Int 16:155–162
18. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A et al (2004) A meta-analysis of previous fracture and subsequent fracture risk. Bone 35:375–382
19. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C et al (2004) A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res 19:893–899
20. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C et al (2004) A family history of fracture and fracture risk: a meta-analysis. Bone 35:1029–1037
21. Kanis JA, Johansson H, Johnell O, Oden A, De Laet C et al (2005) Alcohol intake as a risk factor for fracture. Osteoporos Int 16:737–742
22. Kanis JA (2002) Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359:1929–1936
23. Johansson H, Kanis JA, Oden A, Johnell O, McCloskey E (2009) BMD, clinical risk factors and their combination for hip fracture prevention. Osteoporos Int 20:1675–1682
24. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C et al (2007) The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int 18:1033–1046
25. Seeman E (1999) The structural basis of bone fragility in men. Bone 25:143–147
26. Barvencik F, Gebauer M, Beil FT, Vettorazzi E, Mumme M et al (2010) Age- and sex-related changes of humeral head microarchitecture: histomorphometric analysis of 60 human specimens. J Orthop Res 28:18–26
27. McCalden RW, McGeough JA, Court-Brown CM (1997) Age-related changes in the compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. J Bone Joint Surg Am 79:421–427
28. Donnelly E, Boskey AL, Baker SP, van der Meulen MC (2010) Effects of tissue age on bone tissue material composition and nanomechanical properties in the rat cortex. J Biomed Mater Res A 92:1048–1056
29. Yeni YN, Zelman EA, Divine GW, Kim DG, Fyhrie DP (2008) Trabecular shear stress amplification and variability in human vertebral cancellous bone: relationship with age, gender, spine level and trabecular architecture. Bone 42:591–596
30. Akkus O, Adar F, Schaffler MB (2004) Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone 34:443–453
31. Tommasini SM, Nasser P, Jepsen KJ (2007) Sexual dimorphism affects tibia size and shape but not tissue-level mechanical properties. Bone 40:498–505
32. Rodrigues A, Caetano-Lopes J, Nery A, Sousa E, Polido-Pereira J et al (2009) Evaluation of bone mechanical strenght and fracture risk assessment (FRAX) in patients with hip joint replacement surgery. Acta Reumatol Port 34:504–510
33. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E (2008) FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int 19:385–397
34. Canhão HFJ, Queiroz MV (2005) Epidemiology of osteoporosis, mechanisms of bone remodeling and bone protective factors. Acta Reum Port 30:225–240
35. Smith CB, Smith DA (1976) Relations between age, mineral density and mechanical properties of human femoral compacta. Acta Orthop Scand 47:496–502
36. Hernandez CJ, Keaveny TM (2006) A biomechanical perspective on bone quality. Bone 39:1173–1181
37. Fratzl-Zelman N, Roschger P, Gourrier A, Weber M, Misof BM et al (2009) Combination of nanoindentation and quantitative backscattered electron imaging revealed altered bone material properties associated with femoral neck fragility. Calcif Tissue Int 85:335–343
38. Li B, Aspden RM (1997) Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. J Bone Miner Res 12:641–651
39. Li B, Aspden RM (1997) Material properties of bone from the femoral neck and calcar femorale of patients with osteoporosis or osteoarthritis. Osteoporos Int 7:450–456
40. Li B, Aspden RM (1997) Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. Ann Rheum Dis 56:247–254
41. Sun SS, Ma HL, Liu CL, Huang CH, Cheng CK et al. (2008) Difference in femoral head and neck material properties between osteoarthritis and osteoporosis. Clin Biomech (Bristol, Avon) 23: S39–47
42. Ciarelli TE, Fyhrie DP, Schaffler MB, Goldstein SA (2000) Variations in three-dimensional cancellous bone architecture of the proximal femur in female hip fractures and in controls. J Bone Miner Res 15:32–40
43. Akhter MP, Iwaniec UT, Haynatzki GR, Fung YK, Cullen DM et al (2003) Effects of nicotine on bone mass and strength in aged female rats. J Orthop Res 21:14–19

Bone 51 (2012) 981–989

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Low osteocalcin/collagen type I bone gene expression ratio is associated with hip fragility fractures

Ana M. Rodrigues [a,b,1], Joana Caetano-Lopes [a,1], Ana C. Vale [c], Bruno Vidal [a], Ana Lopes [a], Inês Aleixo [a], Joaquim Polido-Pereira [a,b], Alexandre Sepriano [a,d], Inês P. Perpétuo [a], Jacinto Monteiro [e], Maria F. Vaz [c], João E. Fonseca [a,b], Helena Canhão [a,b,*]

[a] Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal
[b] Rheumatology and Bone Metabolic Diseases Department, Hospital de Santa Maria, Av. Prof, Egas Moniz, 1649-035 Lisbon, Portugal
[c] Department of Mechanical Engineering, Instituto Superior Técnico, T.U. Lisbon, Av. Rovisco Pais 1049-001 Lisbon, Portugal
[d] Rheumatology Department, Centro Hospitalar Lisboa Ocidental EPE, Rua da Junqueira, 126, 1349-019 Lisbon, Portugal
[e] Ortopaedics Department, Hospital de Santa Maria, Av. Prof, Egas Moniz, 1649-035 Lisbon, Portugal

ARTICLE INFO

Article history:
Received 24 March 2012
Revised 27 July 2012
Accepted 25 August 2012
Available online 5 September 2012

Edited by: Richard Eastell

Keywords:
Osteocalcin
Apolipoprotein E
Osteoporosis
Bone mechanical behavior
Fragility fractures

ABSTRACT

Introduction: Osteocalcin (OC) is the most abundant non-collagenous bone protein and is determinant for bone mineralization.
We aimed to compare OC bone expression and serum factors related to its carboxylation in hip fragility fracture and osteoarthritis patients. We also aimed to identify which of these factors were associated with worse mechanical behavior and with the hip fracture event.
Methods: In this case-control study, fragility fracture patients submitted to hip replacement surgery were evaluated and compared to a group of osteoarthritis patients submitted to the same procedure. Fasting blood samples were collected to assess apolipoproteinE (apoE) levels, total OC and undercarboxylated osteocalcin (ucOC), vitamin K, LDL cholesterol, triglycerides and bone turnover markers. The frequency of the apoε4 isoform was determined. Femoral epiphyses were collected and trabecular bone cylinders drilled in order to perform compression mechanical tests. Gene expression of bone matrix components was assessed by quantitative RT-PCR analysis.
Results: 64 patients, 25 submitted to hip replacement surgery due to fragility fracture and 39 due to osteoarthritis, were evaluated. Bone OC/collagen expression (OC/COL1A1) ratio was significantly lower in hip fracture compared to osteoarthritis patients ($p<0.017$) adjusted for age, gender and body mass index. Moreover, OC/COL1A1 expression ratio was associated with the hip fracture event (OR ~0; $p=0.003$) independently of the group assigned, or the clinical characteristics. Apoε4 isoform was more frequent in the hip fracture group ($p=0.029$). ucOC levels were higher in the fracture group although not significantly ($p=0.058$). No differences were found regarding total OC ($p=0.602$), apoE ($p=0.467$) and Vitamin K ($p=0.371$).
In hip fracture patients, multivariate analysis, adjusted for clinical characteristics, serum factors related to OC metabolism and gene expression of bone matrix proteins showed that low OC/COL1A1 expression ratio was significantly associated with worse trabecular strength ($\beta=0.607$; $p=0.013$) and stiffness ($\beta=0.693$; $p=0.003$). No association was found between ucOC and bone mechanics. Moreover, in osteoarthritis patients, the multivariate analysis revealed that serum total OC was negatively associated with strength ($\beta=-0.411$; $p=0.030$) and stiffness ($\beta=-0.487$; $p=0.009$).
Conclusion: We demonstrated that low bone OC/COL1A1 expression ratio was an independent predictor of worse trabecular mechanical behavior and of the hip fracture event. These findings suggest that in hip fracture patients the imbalance of bone OC/COL1A1 expression ratio reflects disturbances in osteoblast activity leading to bone fragility.

* Corresponding author. Fax: +351 217999459.
E-mail addresses: anamfrodrigues@gmail.com (A.M. Rodrigues), jribeirolopes@gmail.com (J. Caetano-Lopes), anacvale@hotmail.com (A.C. Vale), bruno_vidal6@hotmail.com (B. Vidal), anafilop@gmail.com (A. Lopes), inesaleixo@hotmail.com (I. Aleixo), polidopereira@gmail.com (J. Polido-Pereira), alexsepriano@gmail.com (A. Sepriano), ines.perpetuo@gmail.com (I.P. Perpétuo), jmmonteiro@netcabo.pt (J. Monteiro), fatima.vaz@ist.utl.pt (M.F. Vaz), jefonseca@netcabo.pt (J.E. Fonseca), helenacanhao@netcabo.pt (H. Canhão).
[1] Contributed equally for this manuscript.

8756-3282/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2012.08.129

Introduction

Hip fractures are the most severe clinical consequence of osteoporosis leading to high morbidity and mortality and constitute a major economic burden [1]. A fracture is ultimately a mechanical failure of bone structure. Therefore, it is crucial to identify factors that are associated with bone biomechanical behavior in osteoporotic individuals in order to find new therapeutic targets.

Mature osteoblasts synthesize and secrete osteocalcin (OC) and other bone matrix components such as type I collagen (COL1A1) and alkaline phosphatase (ALP). Their expression varies according to osteoblast maturation stage and this is influenced by Runt-related transcription factor 2 (RUNX2) and Osterix (OSX) that are essential transcription factors for osteoblast differentiation at an early stage, are involved in the production of bone matrix proteins and are known to inhibit osteoblast late stage differentiation and maturation [2,3].

OC gene expression is restricted to terminally differentiated osteoblasts while collagen is expressed from the pre-osteoblast stage [4]. Therefore, a decreased OC/COL1A1 bone expression ratio indicates disturbances in osteoblast activity [5], as was recently observed by Hopwood et al. [6] in osteoporotic bone. As OC and COL1A1 can influence the organization of the basic constituents of bone, determining both the mineral and organic components of bone, it is speculated that the OC/COL1A1 bone expression ratio could influence bone mechanical properties. In fact, OC is the most abundant non-collagenous protein in bone matrix and together with collagen forms the scaffold for hydroxyapatite crystals deposition [7]. Animal studies have shown that OC interferes directly with matrix mineralization and with bone mechanical behavior, namely with stiffness, which is an indicator of elasticity of bone and reflects bone mineral content and distribution [8,9].

OC is produced by mature osteoblasts [10] and its synthesis is stimulated by 1,25-dihydroxyvitamin D (Fig. 1). In the osteoblast, OC undergoes post-translational modifications such as γ-carboxylation, which requires Vitamin K1 (phylloquinone) as a co-factor, giving rise to carboxylated osteocalcin (cOC), which has high affinity for hydroxyapatite and mineral ions and is determinant for calcium distribution in bone tissue [11]. While the majority of cOC accumulates in the bone matrix, a small fraction is also present in serum and is a sensitive marker of bone formation and a predictor of fragility fractures [12,13]. The molecules of osteocalcin that have not undergone post-translational modifications, the undercarboxylated osteocalcin (ucOC), has recently been the focus of attention due to the findings that it also has biological functions, inhibiting extracellular matrix mineralization [14–20] and having a role on glucose metabolism [21]. Serum levels of ucOC have been associated with the risk of hip fracture [12,13,22–26]. Moreover, OC bone expression and low levels of serum vitamin K1 have been associated with bone fragility and increased risk of fracture [27–29]. Vitamin K1 is the predominant dietary and circulating form of vitamin K and it is mainly transported in the circulation by very-low density lipoproteins (VLDL), low-density lipoproteins (LDL), chylomicrons and triglycerides [30]. Vitamin K1 bone availability is affected by factors influencing lipoprotein metabolism such as apolipoprotein E (apoE) which is a component of lipoproteins and act as a ligand for the uptake of lipoproteins-associated phylloquinone to bone [31]. ApoE polymorphisms are important determinants of vitamin K availability on osteoblasts since apoε4 carriers have less ability to facilitate the vitamin K rich intestinal lipoproteins clearance from circulation to bone [32,33]. Moreover, apoε4 carriers have been shown to accumulate oxidized lipids in the subendothelial space of bone vasculature leading to inhibition of bone formation [34]. Apoε4 was also associated with fracture risk and low bone mineral density (BMD) [35,36], although these results remain controversial [37].

Given the current evidence, we hypothesize that impairment in OC function and/or availability leads to a derangement of the mineralization

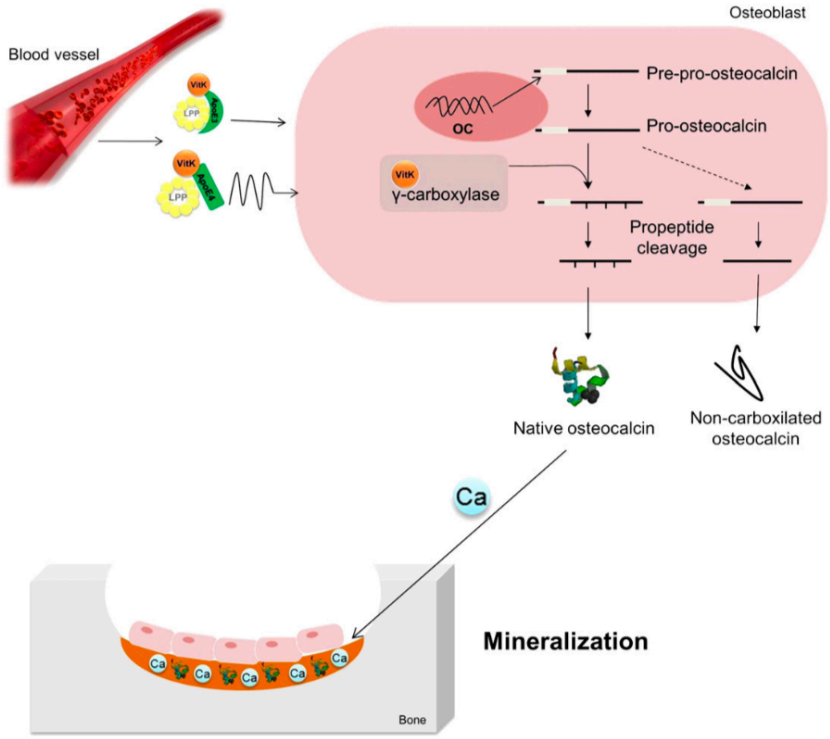

Fig. 1. Osteocalcin γ-carboxylation pathway. Lipoproteins containing apolipoprotein E transport vitamin K to bone where it acts as a co-factor for osteocalcin γ-carboxylation. Carboxylated osteocalcin is responsible for the calcium uptake in bone. The availability of vitamin K on bone depends of apoE affinity to osteoblast. ApoE mutations lead to different affinities for the lipoprotein receptor on osteoblasts, particularly the ε4 codes for the form with lowest affinity. ApoE — apolipoprotein E; Ca^{2+} — calcium; LPP — lipoprotein; OC — osteocalcin; Vit K — vitamin K.

process and influences bone mechanical behavior in the elderly ultimately leading to a hip fracture event. In this work, we aimed to compare serum factors related to OC γ-carboxylation (total OC, ucOC, vitamin K, apoE polymorphisms) and OC bone expression in hip fragility fracture and in osteoarthritis patients. We also aimed to identify which of these factors were associated with worse mechanical behavior and with the hip fracture event.

Materials and methods

Study population

In this case-control study we have enrolled hip fragility fracture patients submitted to total hip replacement surgery within 8 days post-fracture at the Orthopedic department of Hospital de Santa Maria in Lisbon, between 2008 and 2009. As a comparison group, we included osteoarthritis patients submitted to total hip replacement surgery during the same period.

Only patients with more than 50 years and post-menopausal woman able to give clinical information and written informed consent were included in this study. Patients under anti-osteoporotic drugs, warfarin or anti-epileptic drugs or with secondary causes for osteoporosis, bone metabolic diseases (other than osteoporosis), bone metastasis, primary tumors and osteomyelitis were excluded. Five patients were also excluded because of femoral epiphysis characteristics (size and integrity) that hampered the cylinder extraction for mechanical tests.

A clinical questionnaire [38] was applied in order to collect clinical risk factors (CRF) for osteoporotic fractures — age, gender, body mass index (BMI), prior fragility fracture (other than the reason for surgery), parental history of hip fracture, long term use of oral glucocorticoids (≥ 3 months), rheumatoid arthritis, current smoking, alcohol intake (≥ 3 units/day) and other secondary causes of osteoporosis.

The study was conducted in accordance with the regulations governing clinical trials such as the Declaration of Helsinki, as amended in Seoul (2008), and was approved by the local Ethics Committee.

Biochemical assessment

Fasting blood samples were used to assess serum calcium and phosphorus, ALP and bone-specific ALP (BSALP). Serum crosslinked C-telopeptide of type I collagen (CTX-I) and serum amino-terminal propeptides of type I procollagen (P1NP) levels were measured on fully automated Elecsys® electrochemiluminescent immunoassay analyzers (Roche Diagnostics, Switzerland). Concentrations of apoE, LDL-cholesterol and triglycerides were measured in an automated ADVIA 2400 chemistry system (Siemens, USA). Tartrate resistant acid phosphatase 5b (TRAcP5b; Immunodiagnostic systems, USA) levels were determined by enzyme-linked immunoabsorbent assay (ELISA) according to the manufacturer's recommendations.

Vitamin K was measured through high-performance liquid chromatography (HPLC).

Total OC was measured on fully automated Elecsys® electrochemiluminescent immunoassay analyzer (Roche Diagnostics, Switzerland) with a sensitivity of 0.5 ng/ml. This assay measures the N-Mid portion and the fully intact osteocalcin molecule.

ucOC was measured by ELISA (Takara Bio, Japan) with a sensitivity of 0.25 ng/ml. This Undercarboxylated Osteocalcin kit utilizes a set of monoclonal antibodies highly reactive to the ucUC and less reactive to carboxylated at positions 17, 21, 24.

Apolipoprotein E genotyping

Genomic DNA was extracted from a blood sample using a commercial kit (QIAamp DNA blood mini kit, Qiagen, Germany).

Polymerase chain reaction (PCR) was used to amplify a 283-pb sequence of the apoE gene, including two single nucleotide polymorphisms sites at positions rs429358 and rs7412 present in exon 4. The three major isoforms of human apoE (apoε2, apoε3, and apoε4) are coded for by 3 alleles (ε2, ε3, and ε4). The three isoforms differ in amino acid sequence at two sites, residue 112 (position 3937 of exon 4) and residue 158 (position 4075 of exon 4). ε3 is the most frequent isoform [39,40].

Reaction was performed with 50 ng of DNA, 2 mM dNTP, 1 unit of Taq polymerase (Promega, USA). 200 nM of each primer (GTGGCGG AGGAGACGCGGGC and CGCGGATGGCGCTGAGGCCG) were added and the reaction was performed at an annealing temperature of 66 °C. After amplification, PCR products were digested with 2.5 units of AflIII (New England Biolabs, USA) and 5 units of HaeII (New England Biolabs, USA), overnight at 37 °C. The different isoforms were detected by a 3% agarose gel electrophoresis.

Bone gene expression analysis

80 mg of trabecular bone were collected from the distal extremity of the femoral epiphysis and pulverized using a mortar and pestle. RNA was extracted using TRIzol reagent (Invitrogen, UK) with proteinase K (Bioline, UK) digestion [41] to better dissolve extracellular matrix. RNA was cleaned using a commercial kit (RNeasy mini kit, Qiagen, Germany) and genomic DNA contaminants were removed by DNaseI treatment (Qiagen, Germany) (adapted from [42,43]). RNA concentration was determined spectrophotometrically (Nanodrop ND-1000 Spectrophotometer, Thermo Fisher Scientific, USA) and its integrity was assessed by lab-on-a-chip technology (Agilent RNA 6000 Nano Kit, Agilent technologies, USA) according to the manufacturer's instructions. RNA was stored at −80 °C until further use.

Reverse transcription cDNA synthesis was performed on 60 ng of RNA from each sample using the DyNAmo cDNA synthesis kit (Finnzymes, Finland) and 300 ng of random hexamer primers, according to the manufacturer's instructions.

Each cDNA template (3 ng/μl) was amplified in duplicate with DyNAmo Flash SYBR green qPCR kit (Finnzymes, Finland) on a Rotor-Gene thermocycler (Qiagen, Germany) according to the manufacturer's instructions. The reactions were validated by the presence of a single peak in the melt curve analysis.

Primers for the housekeeping and target genes (Supplementary Table) were designed using the software Probefinder (http://qpcr.probefinder.com, Roche, Switzerland) in order to anneal in separate exons preventing amplification of contaminating genomic DNA.

Real time PCR results were analyzed using the standard curve analysis. The conversion of the C_T value in relative expression levels was performed with the slope and the Y intersect extracted from the standard curve and applying the equation $10^{(Y\ intersect - CT/slope)}$ [44,45]. The values obtained were normalized with the housekeeping genes β-2-microglobulin (B2M) and phosphomannomutase 1 (PMM1).

Bone histology and immunohistochemistry

For histological observation, bone samples were fixed in 10% neutral buffered formalin solution and then decalcified in 10% EDTA. After dehydration in increased ethanol concentrations (70%, 96% and 100%), samples were embedded in paraffin, sectioned and stained with hematoxylin and eosin for morphological examination.

Immunohistological were performed using anti-osteocalcin (10 μg/ml) and anti-bone alkaline phosphatase (1:300) (Abcam, UK) antibodies, for osteoblast detection. Tissue sections were incubated with the primary antibody and with EnVision+(Dako, Denmark). Color was developed in a solution containing diaminobenzadine-tetrahydrochloride (Sigma, USA), 0.5% H_2O_2 in phosphate-buffered saline buffer (pH 7.6). Slides were counterstained with hematoxylin and mounted. All images were acquired using a Leica DM 2500 (Leica microsystems, Germany) microscope equipped with a color camera.

Bone structural characteristics

After the orthopedic procedure the femoral epiphyses were immediately stored at −80 °C. Before testing they were defrosted at room temperature. Bone cylinders were obtained by drilling through the highest loading direction (Fig. 2) using a perforating drill with a diameter of 15 mm (adapted from [46]). A cylinder of an aspect ratio (length: diameter) of 2 is the one that provides the most accurate results. However, the length of the cylinder varies between specimens depending on the size of femoral epiphysis, which may give an aspect ratio slightly different. Therefore, a correction was applied to the results when the cylinder did not have the correct length. The cortical shell was cut and the top surfaces were polished. The cylinders (with only trabecular bone) were de-fatted for three hours using a chloroform and methanol solution and were hydrated overnight in saline solution.

Compression tests were performed in a universal testing machine (Instron 5566™, Instron Corporation, Canton, USA) with a load cell of 10 kN and a cross-head rate of 0.1 mm/s. A load cell of 10 kN with precision values of 0.5% was used to measure the forces in the order of 2000 to 4000 N [47]. Samples were loaded in the principal stress direction. Stress–strain curves were obtained for each specimen by the *Bluehill 2 software* (Instron, Copyright 1997–2007). This software has the ability to build stress–strain representations from load-displacement points, normalized for the specimens' dimensions. Analysis of the curves was performed using the *MatLab 7.1 software* (R14 SP3, The Mathworks, Inc., Copyright 1984–2006) in order to obtain the mechanical bone parameters: strength (yield stress), stiffness (Young's modulus) and toughness (energy absorbed until yield).

In a subset of 27 patients, femoral neck areal bone mineral density (aBMD) of the contralateral hip was measured by DXA scan using a Lunar Prodigy densitometer (GE Healthcare, United Kingdom) four days after surgery.

Statistical analysis

Baseline characteristics are presented as median (interquartile range difference) or percentage. All continuous variables were tested for normality with the Kolmogorov–Smirnov test and were compared between groups with Mann–Whitney test. Categorical data were tested using Chi-square or Fisher's exact tests.

Factors related to OC pathway and expression of osteoblast transcription factors RUNX2 and OSX were compared across groups using general linear model (GLM) analysis adjusted for differences in baseline characteristics identified as possible confounders.

To analyze the association between RUNX2 and OSX and bone mechanical properties within groups we used Spearman's rank correlation coefficient.

Multivariate linear regression models were used to explore the association between bone mechanical behavior (strength and stiffness) and factors related to OC pathway within the two groups of patients. Serum levels of total OC and ucOC, serum factors related to OC carboxylation (vitamin K and apoE), bone gene expression of ALP, OC/COL1A1 ratio and the presence of the apoε4 allele were considered potential predictors. The clinical variables that showed significant differences in baseline (age, gender and BMI) were also included in the model. The selection of covariates was stepwise by backward selection, according to the level of significance <0.05.

Multivariate logistic regression was used to explore the association between hip fracture event and gene expression of bone matrix components (OC/COL1A1 and ALP). Other potential predictors of fracture such as baseline clinical characteristics were also included in the model.

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics Software, v.17.0 (SPSS Inc., Chicago, USA) and a two-tailed p-value <0.05 was selected as significant.

Results

Patients, bone turnover markers and bone samples characteristics

64 patients submitted to total hip replacement surgery, 25 due to primary osteoporosis fragility fracture and 39 due to osteoarthritis were evaluated. Fragility fracture patients were older ($p<0.001$), had lower BMI ($p=0.009$) and were predominantly women ($p=0.005$). No other CRF for fracture was significantly different between the two groups (Table 1).

Femoral neck aBMD was significantly lower in hip fractures patients ($p=0.002$). Moreover, in this group of patients, trabecular bone stiffness was significantly lower than that in osteoarthritis ($p=0.019$).

Regarding serum levels of bone turnover markers, CTX-I ($p=0.031$) and ALP ($p=0.008$) were significantly increased in the fragility fracture group. No other significant differences were found between the two groups of patients.

No differences in serum factors related to OC metabolism and of ucOC/total OC ratio were found between groups

There were no significant differences in total OC between groups. Also, there were also no differences regarding serum levels of relevant

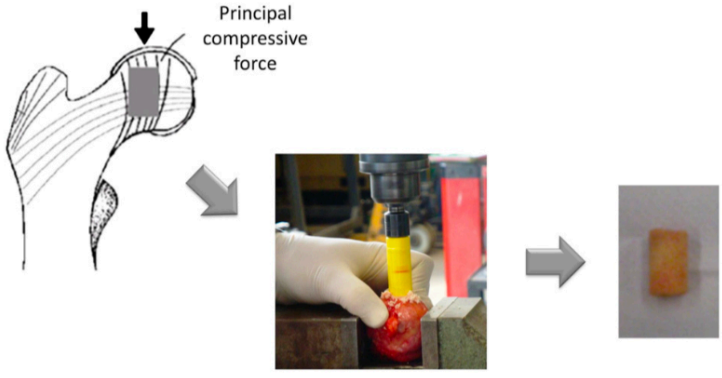

Fig. 2. Set up of the cylinder extraction from the femoral epiphysis for compression mechanical tests. Bone cylinders with an aspect ratio (length: diameter) of 2 were obtained by drilling through the highest loading direction using a perforating drill with a diameter of 15 mm (adapted from [59]).

Table 1
Characterization of hip fracture and osteoarthritis patients.

	Hip fractures	Osteoarthritis	*p*-value
N	25	39	
Clinical characteristics			
Age (years)	82 (8)	70 (10)	<0.001*
Women (%)	88	54	0.005*
Caucasian (%)	100	97	1.000
BMI (kg/cm^2)	24.6 (5.9)	27.4 (5.7)	0.009*
Previous fragility fracture (%)	20	8	0.245
Family history of fractures (%)	8	5	0.640
Smoking (%)	8	18	0.463
Alcohol intake (%)	0	15	0.074
Cholesterol levels			
LDL-C (mg/dl)	73.7 (49.7)	69.3 (44.4)	0.459
Triglycerides (mg/dl)	127 (46)	104 (82)	0.322
Markers of bone turnover			
ALP (UI/l)	84.5 (56)	66 (35)	0.008*
BSALP (μg/l)	11.7 (8.2)	10.4 (7.3)	0.657
P1NP (ng/ml)	44.9 (29.1)	43.0 (36.5)	0.616
CTX-I (μg/ml)	40.0 (36.7)	30.0 (27.0)	0.031*
TRAcP5b	2.4 (1.8)	1.8 (1.7)	0.266
Bone structural characteristics			
Strength (MPa)	6.04 (8.00)	7.98 (7.70)	0.082
Stiffness (MPa)	280.8 (251.0)	447.1 (404.9)	0.019*
Toughness (N mm/mm^3)	0.106 (0.169)	0.136 (0.173)	0.189
Femoral neck aBMD (g/cm^2)	0.67 (0.09)	0.83 (0.19)	0.002*
T score	−2.6 (1.2)	−1.4 (1.4)	0.001*

Values represent median (interquartile range difference). For continuous variables, differences were assessed using non-parametric Mann–Whitney. χ^2 or Fisher's exact test was used for proportions.
ALP — alkaline phosphatase; aBMD — areal bone mineral density; BMI — body mass index; BSALP — bone-specific ALP; CTX-I — carboxyl-terminal cross-linking telopeptides of type I collagen; LDL-C — low-density lipoprotein-cholesterol; P1NP — amino-terminal propeptides of type I procollagen; TRAcP5b — Tartrate-resistant acid phosphatase 5b.
* Unadjusted *p*-value<0.05.

co-factors of OC carboxylation, namely apoE and Vitamin K. All patients had serum vitamin K values within normal range (Table 2). However, ucOC was higher in the fracture group although not reaching a significative difference ($p=0.058$), which is in accordance with the higher bone turnover found in the fragility fracture patients.

The ucOC/total OC ratio, which could reflect the rate of synthesis of OC and not vitamin K levels, was not different between groups.

Table 2
Osteocalcin pathway related factors in hip fragility fracture and osteoarthritis patients.

	Hip fractures	Osteoarthritis	*p*-Value Adjusted for age, gender and BMI
N	25	39	
Serum factors related to osteocalcin metabolism			
total OC (ng/ml)	9.8 (16.1)	10.9 (14.8)	0.602
ucOC (ng/ml)	2.30 (3.70)	1.79 (3.22)	0.058
ApoE (g/l)	0.040 (0.020)	0.040 (0.021)	0.467
Vitamin K (μg/l)	1.54 (2.1)	1.18 (0.9)	0.371
ucOC/total OC	0.27 (0.32)	0.16 (0.23)	0.337
Apolipoprotein E Polymorphisms			
ε4 isoform (%)	36	13	0.029**
Gene expression of bone matrix components			
OC relative expression	0.10 (0.1)	2.16 (4.5)	0.012*
COL1A1 relative expression	11.40 (26.65)	3.13 (15.80)	0.112
ALP relative expression	3.76 (3.2)	1.71 (2.6)	0.336
OC/COL1A1 ratio	0.6 (2.6)	69.3 (43.1)	0.112

Values represent median (Interquatile Range). Serum factors related to osteocalcin metabolism and gene expression of bone matrix components were compared between groups using multivariate regression analysis to adjust the results to age, gender, BMI. ε4 carriers differences were tested using χ^2 or Fisher's exact test.
ALP — alkaline phosphatase; ApoE — apolipoprotein E; COL1A1 — Collagen type 1α1; OC — osteocalcin; total OC — total osteocalcin osteocalcin; ucOC — undercarboxylated osteocalcin.
* *p* value<0.05 adjusted to age, gender and BMI.
** *p* value<0.05 not adjusted.

Apoε4 isoform is more frequent in hip fracture patients

The apoE genotypes distribution at positions rs429358 and rs7412 were in accordance with the Hardy–Weinberg equilibrium. Apoε4 was previously documented as the risk isoform [35] and it differs from the wild-type ε3 by a cysteine-to-arginine change at position 112 [40].

ε4 was present in 16% of all patients. Thirty-six percent of the hip fragility fracture patients were ε4 carriers while only in 13% of osteoarthritis patients ε4 was present ($p=0.029$) (Table 2). Apoε4 carriers had a higher prevalence of previous major fragility fractures ($p=0.040$) and higher levels of bone turnover markers (BSALP ($p=0.005$) and P1NP ($p=0.015$)) independently of the assigned group, age and gender.

OC/COL1A1 bone expression ratio is lower in hip fracture patients

Gene expression of bone matrix proteins was assessed in trabecular bone and compared between hip fracture and osteoarthritis patients, adjusting for age, gender and BMI. OC expression was significantly lower in hip fractures as compared to osteoarthritis patients ($p=0.012$). We also assessed ALP and COL1A1 gene expression and no differences were found between groups. OC/COL1A1 expression ratio was found to be significantly lower in hip fracture patients ($p=0.017$) (Table 2).

Bone expression of RUNX2 and OSX was also determined in both groups as they are the two most important transcription factors that regulate osteoblast commitment from mesenchymal stem cells. No significant differences were found in bone expression of RUNX2 ($p=0.350$) and OSX ($p=0.063$) between the two groups, when adjusted to age, gender or BMI.

Osteoblasts from patients with a fragility fracture show lower staining for OC

We have performed histology and immunohistochemistry in 2 patients with osteoarthritis and 3 patients of fragility fractures. In a qualitative evaluation we found more resorption sites in fragility fractures. We also performed immunohistochemistry for ALP and OC and found that ALP staining in osteoblasts was similar between osteoarthritis and fragility fracture patients. However OC staining is lower in osteoblast from fragility fracture patients (Fig. 3).

OC/COL1A1 bone expression ratio is a predictor of hip fracture event

Using both groups of patients we performed a univariate analysis and found that ε4 presence (OR = 3.825; $p=0.035$), OC relative expression (OR = 0.274; $p=0.013$) and OC/COL1A1 bone expression ratio (OR = 0.865; $p=0.001$) were associated to hip fragility fractures. After a multivariate analysis adjusting for group assignment and clinical variables, only OC/COL1A1 expression ratio remained associated with hip fragility fractures (OR ~0; $p=0.003$).

Factors related to OC pathway are associated with worse mechanical behavior

Univariate analyses were performed in order to assess the relation between bone mechanical behavior and factors related to OC pathway within groups (Table 3).

In hip fracture patients, low OC/COL1A1 expression ratio was associated with worse strength ($\beta=0.549$; $p=0.0012$) and stiffness ($\beta=0.632$; $p=0.002$). Expression of COL1A1 ($\beta=-0.515$; $p=0.020$) and ALP ($\beta=-0.462$; $p=0.040$) were related with worse

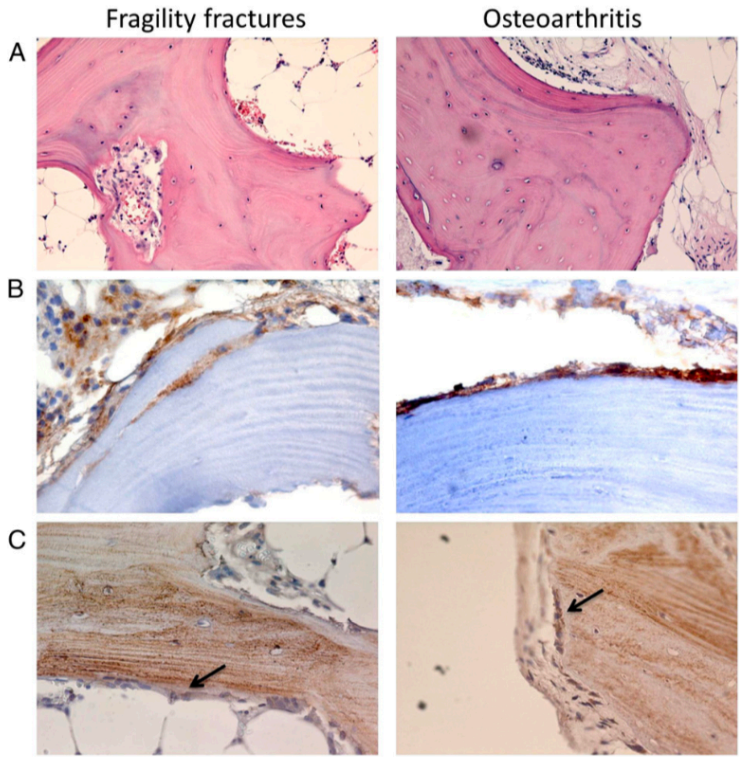

Fig. 3. Histology and immunohistochemistry of trabecular bone samples from fragility fracture and osteoarthritis patients. Histological sections were stained with hematoxylin and eosin (A). Immunohistochemistry was performed for evaluation of ALP (B) OC (C). (A – magnification 200×, B – magnification 600× and C – 400×).

stiffness. In osteoarthritis patients OC relative expression was associated with trabecular strength ($\beta = 0.812$; $p = 0.039$) (Table 3).

In hip fragility patients no association was found between serum OC related factors metabolism and trabecular mechanical behavior. In osteoarthritis patients, high levels of serum total OC were significantly associated with lower strength ($\beta = -0.411$; $p = 0.027$) and stiffness ($\beta = -0.481$; $p = 0.008$).

Reduced trabecular strength ($\beta = -0.333$; $p = 0.038$) and stiffness ($\beta = -0.355$; $p = 0.026$) were also associated to the presence of the ε4 isoform in osteoarthritis group.

In order to identify which of the studied factors were associated with trabecular bone mechanical performance, we performed a multivariate analysis adjusted for patient's clinical characteristics. In hip fracture patients only OC/COL1A1 expression ratio was correlated with trabecular strength ($\beta = 0.607$; $p = 0.013$) and stiffness ($\beta = 0.693$; $p = 0.003$). On the other hand, in osteoarthritis patients, total OC levels remained negatively correlated with strength ($\beta = -0.411$; $p = 0.030$) and stiffness ($\beta = -0.487$; $p = 0.009$).

We also looked for the association between bone expression of RUNX2 and OSX with trabecular bone mechanical behavior within groups. No association was found between RUNX2 and strength ($r = -0.269$; $p = 0.251$) or with stiffness ($r = -0.308$; $p = 0.186$), neither between OSX and strength ($r = -0.321$; $p = 0.226$) or with stiffness ($r = 0.274$; $p = 0.305$) in hip fragility fracture patients. We also did not found any association between RUNX2 and strength ($r^2 = -0.191$; $p = 0.251$) or with stiffness ($r^2 = -0.030$; $p = 0.858$), neither of OSX with strength ($r^2 = -0.003$; $p = 0.987$) or with stiffness ($r^2 = 0.010$; $p = 0.952$) in osteoarthritis patients.

Discussion

In this work, we have compared differences in OC related factors pathway (OC bone gene expression, serum OC, apoE polymorphisms) in hip fragility fracture and osteoarthritis postmenopausal women and men over 50 years old. Moreover, we looked for the factors that were associated with worse trabecular mechanical behavior and with the hip fracture event. We have found that OC relative bone expression and OC/COL1A1 bone expression ratio were significantly lower in hip fracture than in osteoarthritis patients. Consistent with these results, in a subset of patients there were less osteoblasts staining for OC in fragility fracture patients than in osteoarthritis. We also demonstrated that in hip fracture patients low bone OC/COL1A1 expression ratio was associated with worse trabecular mechanical behavior. Moreover, we found no differences of RUNX2 and OSX bone expression between groups and they were not associated with bone mechanical behavior within groups. This fact may indicate that commitment to the osteoblast lineage is not compromised in fragility fractures, but the differentiation of the cells to the final stages are impaired leading to fragility. This final differentiation impairment could be due to the high turnover status seen in these patients.

We also addressed the association of serum markers of OC γ-carboxylation with hip fragility and with bone mechanical behavior. We did not found differences in serum levels of apoE and Vitamin K between hip fracture and osteoarthritis patients. Moreover, there were no significant differences in total OC between groups. However, ucOC, although not significantly, was higher in the fracture group, which is in accordance with the higher bone turnover found in the

Table 3
OC pathway related factors associated with bone mechanical behavior within hip fracture and osteoarthritis groups.

	Strength				Stiffness			
	Hip fracture		Osteoarthritis		Hip fracture		Osteoarthritis	
	Univariate analysis β estimates p-value	Multivariate analysis β estimates p-value	Univariate analysis β estimates p-value	Multivariate analysis β estimates p-value	Univariate analysis β estimates p-value	Multivariate analysis β estimates p-value	Univariate analysis β estimates p-value	Multivariate analysis β estimates p-value
Model fitness (r^2)		0.324		0.237		0.443		0.169
Gene expression of bone matrix components								
OC relative expression	0.092		0.812		0.030		0.160	
	0.700		0.039*		0.900		0.330	
COL1A1 relative expression	−0.419		−0.026		−0.515		0.083	
	0.066		0.874		0.020*		0.615	
ALP relative expression	−0.334		0.102		−0.402		0.282	
	0.150		0.537		0.004*		0.082	
OC/COL1A1	0.549	0.607	0,045		0.632	0.693	0.123	
	0.012*	0.013*	0,785		0.002*	0.003*	0.457	
Serum factors of osteocalcin metabolism								
total OC (ng/ml)	0.150		−0.411	−0.411	0.138		−0.481	−0.487
	0.529		0.027*	0.030*	0.561		0.008*	0.009*
ucOC (ng/ml)	−0.017		−0,285		−0.219		−0.258	
	0.935		0.087		0.293		0.123	
ApoE (g/l)	−0.299		−0.215		−0.237		−0.334	
	0.187		0.221		0.237		0.054	
Vitamin K (μg/l)	−0.170		0.178		−0.292		−0.136	
	0.529		0.495		0.272		0.604	
Apolipoprotein E Polymorphisms								
ε4 isoform	0.149		−0.333		0.098		−0.355	
	0.477		0.038*		0.641		0.026*	

Values obtained by linear regression analyses for bone mechanical behavior outcomes. Multivariate analysis was performed adjusting for clinical characteristics (age, gender, BMI) and the covariates listed in the table.
ALP — alkaline phosphatase; ApoE — apolipoprotein E; COL1A1 — Collagen type 1α1; OC — osteocalcin; total OC — total osteocalcin; ucOC — undercarboxylated osteocalcin.
* p value<0.05.

fragility fracture patients. Although serum levels of total OC, ucOC and vitamin K are reported to be associated with fragility fractures in large population studies [22–24,48] we did not find any differences between hip fractures and osteoarthritis nor any association between serum markers of OC γ-carboxylation and fragility fractures. This homogeneity between the two groups might be explained by the fact that all patients had vitamin K levels within normal range and that our study was underpowered to find these associations.

On the other hand, total OC levels were associated with reduced trabecular strength and stiffness in osteoarthritis patients. In agreement, previous studies have shown that osteoarthritis patients had increased serum biomarkers, such as OC and ALP [49], with increased bone formation and higher aBMD [49]. However, they have also incomplete bone mineralization [50], which might be due to an abnormal osteoblast activity, leading to bone fragility [51]. In fact, Couchourel and co-workers showed that the impaired mineralization observed in osteoarthritis could be due to an abnormally high expression of COL1A1 to COL1A2 ratio [50].

One of the limitations of this work is that the fracture event *per se* could influence the bone gene expression results. However, in a previous work we have demonstrated that in the early post-fracture period no changes occur in gene expression of matrix components namely in OC and COL1A1 expression [43].

Also, serum levels of bone turnover markers might be changed in the early post fracture period [52], which may underestimate the association between total OC and bone mechanical behavior in fragility fracture patients and mask the differences in serum levels of this protein between groups.

Osteoporosis is a complex multifactorial disease which has an important genetic background. Several genes were identified to be responsible for bone fragility and predictors of fractures [53]. Apoε4 isoform has been found to be associated with less vitamin K availability in bone [54] which impairs OC carboxylation. We have depicted that apoε4 was more frequent in the fracture group than in the osteoarthritis group. Interestingly we found an association between the apoε4 isoform with trabecular mechanical behavior, but only in osteoarthritis patients. Several population studies have shown that apoε4 is related to fracture risk [36,55] but a recent meta-analysis did not support this association [37]. In our opinion, apoε4 isoform has a modest effect on bone mechanical fragility, which may not be significantly important among elderly people who have several other risk factors for osteoporosis. On the other hand, apoε4 could have an important impact on osteoarthritis. In fact altered lipid metabolism has been implicated as a critical player in the pathophysiology of osteoarthritis [56,57] and apoε4 carriers have higher cholesterol levels [34,58] which could directly induced cartilage lesion. In addition, apoε4 carriers have an accumulation of oxidized lipids in bone which inhibits osteoblast differentiation and could enhance the mineralization disturbances and bone fragility found in osteoarthritis.

Conclusion

In summary, using a large panel of serum and bone factors that are relevant in osteocalcin pathway, we demonstrated for the first time that in hip fracture patients low bone OC/COL1A1 expression ratio was significantly associated with worse trabecular mechanical behavior and with the hip fracture event. These findings suggest that in hip fracture patients the imbalance of OC/COL1A1 expression ratio reflects disturbances in osteoblast activity, affecting bone metabolism and bone matrix/mineral ratio, ultimately leading to bone fragility.

We anticipate that interfering with the regulators of bone OC/COL1A1 expression ratio might be a possible therapeutic target for osteoporosis.

Conflict of interest statement

The authors declare no conflict of interests.

Authors' contributions

AMR, JPP and AS recruited and evaluated the patients. JCL and AL carried out the molecular genetic studies. IP carried out the immunoassays. ACV and IA participated in the mechanical testing procedures. BV performed the histological and immunohistochemistry techniques. AMR and HC participated in the design of the study and performed the statistical analysis. AMR, JCL, JM, MFV, JEF and HC conceived the study, and participated in its design and coordination and performed draft the manuscript. All authors read and approved the final manuscript.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2012.08.129.

Acknowledgments

This work was supported by unrestricted grants from Institut de Recherches Internacionales Servier (IRIS), Associação Portuguesa de Osteoporose, Merck Sharp and Dome, Faculdade de Medicina de Lisboa/Astrazeneca e Hospital de Santa Maria.

A special thanks to the surgeons and nurses from Orthopaedics department of Hospital de Santa Maria that have collaborated with the collection of samples. Thank you Dr AS Pena, Dr A Faustino, Dr E Vieira-Sousa, nurse L Narciso and Dr. JC Romeu from the Rheumatology and Bone Metabolic Diseases Department from Hospital de Santa Maria for the clinical data collection and DXA performance and analysis. PM Amaral, PhD was also of great help in the advice of the mechanical tests set up.

References

[1] Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet 2002;359:1761-7.
[2] Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997;89:747-54.
[3] Komori T. Regulation of bone development and extracellular matrix protein genes by RUNX2. Cell Tissue Res 2010;339:189-95.
[4] Neve A, Corrado A, Cantatore FP. Osteoblast physiology in normal and pathological conditions. Cell Tissue Res 2011;343:289-302.
[5] Tanaka S, Narusawa K, Onishi H, Miura M, Hijioka A, Kanazawa Y, et al. Lower osteocalcin and osteopontin contents of the femoral head in hip fracture patients than osteoarthritis patients. Osteoporos Int 2011;22:587-97.
[6] Hopwood B, Tsykin A, Findlay DM, Fazzalari NL. Gene expression profile of the bone microenvironment in human fragility fracture bone. Bone 2009;44:87–101.
[7] Caetano-Lopes J, Nery AM, Henriques R, Canhao H, Duarte J, Amaral PM, et al. Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. Clin Exp Rheumatol 2009;27:475-82.
[8] Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, et al. Increased bone formation in osteocalcin-deficient mice. Nature 1996;382:448-52.
[9] Balooch G, Balooch M, Nalla RK, Schilling S, Filvaroff EH, Marshall GW, et al. TGF-beta regulates the mechanical properties and composition of bone matrix. Proc Natl Acad Sci U S A 2005;102:18813-8.
[10] Lian JB, Stein GS, Stein JL, van Wijnen AJ. Regulated expression of the bone-specific osteocalcin gene by vitamins and hormones. Vitam Horm 1999;55:443-509.
[11] Amizuka N, Li M, Kobayashi M, Hara K, Akahane S, Takeuchi K, et al. Vitamin K2, a gamma-carboxylating factor of gla-proteins, normalizes the bone crystal nucleation impaired by Mg-insufficiency. Histol Histopathol 2008;23:1353-66.
[12] Ross PD, Kress BC, Parson RE, Wasnich RD, Armour KA, Mizrahi IA. Serum bone alkaline phosphatase and calcaneus bone density predict fractures: a prospective study. Osteoporos Int 2000;11:76-82.
[13] Garnero P, Dargent-Molina P, Hans D, Schott AM, Breart G, Meunier PJ, et al. Do markers of bone resorption add to bone mineral density and ultrasonographic heel measurement for the prediction of hip fracture in elderly women? The EPIDOS prospective study. Osteoporos Int 1998;8:563-9.
[14] Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. J Cell Biol 2004;165:625-30.
[15] Furie B, Furie BC. The molecular basis of blood coagulation. Cell 1988;53:505-18.
[16] Braam LA, Knapen MH, Geusens P, Brouns F, Hamulyak K, Gerichhausen MJ, et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. Calcif Tissue Int 2003;73:21-6.
[17] Bolton-Smith C, McMurdo ME, Paterson CR, Mole PA, Harvey JM, Fenton ST, et al. Two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women. J Bone Miner Res 2007;22:509-19.
[18] Pan LC, Price PA. The propeptide of rat bone gamma-carboxyglutamic acid protein shares homology with other vitamin K-dependent protein precursors. Proc Natl Acad Sci U S A 1985;82:6109-13.
[19] Watkins SM, Zhu X, Zeisel SH. Phosphatidylethanolamine-N-methyltransferase activity and dietary choline regulate liver-plasma lipid flux and essential fatty acid metabolism in mice. J Nutr 2003;133:3386-91.
[20] Shearer MJ. Role of vitamin K and Gla proteins in the pathophysiology of osteoporosis and vascular calcification. Curr Opin Clin Nutr Metab Care 2000;3:433-8.
[21] Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130:456-69.
[22] Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. Bone 1996;18:487-8.
[23] Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 1997;82:719-24.
[24] Gerdhem P, Ivaska KK, Alatalo SL, Halleen JM, Hellman J, Isaksson A, et al. Biochemical markers of bone metabolism and prediction of fracture in elderly women. J Bone Miner Res 2004;19:386-93.
[25] Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, et al. Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. J Bone Miner Res 1999;14:1404-10.
[26] Brown JP, Delmas PD, Malaval L, Edouard C, Chapuy MC, Meunier PJ. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. Lancet 1984;1:1091-3.
[27] Hart JP, Shearer MJ, Klenerman L, Catterall A, Reeve J, Sambrook PN, et al. Electrochemical detection of depressed circulating levels of vitamin K1 in osteoporosis. J Clin Endocrinol Metab 1985;60:1268-9.
[28] Hodges SJ, Pilkington MJ, Stamp TC, Catterall A, Shearer MJ, Bitensky L, et al. Depressed levels of circulating menaquinones in patients with osteoporotic fractures of the spine and femoral neck. Bone 1991;12:387-9.
[29] Hodges SJ, Akesson K, Vergnaud P, Obrant K, Delmas PD. Circulating levels of vitamins K1 and K2 decreased in elderly women with hip fracture. J Bone Miner Res 1993;8:1241-5.
[30] Lamon-Fava S, Sadowski JA, Davidson KW, O'Brien ME, McNamara JR, Schaefer EJ. Plasma lipoproteins as carriers of phylloquinone (vitamin K1) in humans. Am J Clin Nutr 1998;67:1226-31.
[31] Newman P, Bonello F, Wierzbicki AS, Lumb P, Savidge GF, Shearer MJ. The uptake of lipoprotein-borne phylloquinone (vitamin K1) by osteoblasts and osteoblast-like cells: role of heparan sulfate proteoglycans and apolipoprotein E. J Bone Miner Res 2002;17:426-33.
[32] Saupe J, Shearer MJ, Kohlmeier M. Phylloquinone transport and its influence on gamma-carboxyglutamate residues of osteocalcin in patients on maintenance hemodialysis. Am J Clin Nutr 1993;58:204-8.
[33] Weintraub MS, Eisenberg S, Breslow JL. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 1987;80:1571-7.
[34] Parhami F, Garfinkel A, Demer LL. Role of lipids in osteoporosis. Arterioscler Thromb Vasc Biol 2000;20:2346-8.
[35] Cauley JA, Zmuda JM, Yaffe K, Kuller LH, Ferrell RE, Wisniewski SR, et al. Apolipoprotein E polymorphism: a new genetic marker of hip fracture risk--The Study of Osteoporotic Fractures. J Bone Miner Res 1999;14:1175-81.
[36] Pluijm SM, Dik MG, Jonker C, Deeg DJ, van Kamp GJ, Lips P. Effects of gender and age on the association of apolipoprotein E epsilon4 with bone mineral density, bone turnover and the risk of fractures in older people. Osteoporos Int 2002;13:701-9.
[37] Peter I, Crosier MD, Yoshida M, Booth SL, Cupples LA, Dawson-Hughes B, et al. Associations of APOE gene polymorphisms with bone mineral density and fracture risk: a meta-analysis. Osteoporos Int 2011;22:1199-209.
[38] Rodrigues A, Caetano-Lopes J, Nery A, Sousa E, Polido-Pereira J, Vale M, et al. Evaluation of bone mechanical strength and fracture risk assessment (Frax) in patients with hip joint replacement surgery. Acta Reumatol Port 2009;34:504-10.
[39] Weisgraber KH, Rall Jr SC, Mahley RW. Human E apoprotein heterogeneity. Cysteine–arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 1981;256:9077-83.
[40] Smit M, de Knijff P, van der Kooij-Meijs E, Groenendijk C, van den Maagdenberg AM, Gevers Leuven JA, Stalenhoef AF, et al. Genetic heterogeneity in familial dysbetalipoproteinemia. The E2(lys146-gln) variant results in a dominant mode of inheritance. J Lipid Res 1990;31:45-53.
[41] Egyhazi S, Bjohle J, Skoog L, Huang F, Borg AL, Frostvik Stolt M, et al. Proteinase K added to the extraction procedure markedly increases RNA yield from primary breast tumors for use in microarray studies. Clin Chem 2004;50:975-6.
[42] Ireland D. Analysis of gene expression in bone by quantitative RT-PCR. In: Helfrich MH, Ralston SH, editors. Bone research protocols. New York: Humana Press; 2003. p. 433-40.
[43] Caetano-Lopes J, Lopes A, Rodrigues A, Fernandes D, Perpetuo IP, Monjardino T, et al. Upregulation of inflammatory genes and downregulation of sclerostin gene expression are key elements in the early phase of fragility fracture healing. PLoS One 2011;6:e16947.
[44] Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. Biotechniques 2005;39:75-85.
[45] Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996;6:986-94.
[46] Smith CB, Smith DA. Relations between age, mineral density and mechanical properties of human femoral compacta. Acta Orthop Scand 1976;47:496-502.
[47] Aleixo I, Vale AC, Lúcio M, Amaral PM, Rosa LG, Caetano-Lopes J, et al. A method for the evaluation of femoral head trabecular bone compressive properties. Mater Sci Forum 2013;730-732:3-8 (© (2013) Trans Tech Publications, Switzerland, http://dx.doi:10.4028/www.scientific.net/MSF.730-732.3).
[48] Macdonald HM, McGuigan FE, Lanham-New SA, Fraser WD, Ralston SH, Reid DM. Vitamin K1 intake is associated with higher bone mineral density and reduced bone resorption in early postmenopausal Scottish women: no evidence of gene–

nutrient interaction with apolipoprotein E polymorphisms. Am J Clin Nutr 2008;87:1513-20.
[49] Jiang LS, Zhang ZM, Jiang SD, Chen WH, Dai LY. Differential bone metabolism between postmenopausal women with osteoarthritis and osteoporosis. J Bone Miner Res 2008;23:475-83.
[50] Couchourel D, Aubry I, Delalandre A, Lavigne M, Martel-Pelletier J, Pelletier JP, et al. Altered mineralization of human osteoarthritic osteoblasts is attributable to abnormal type I collagen production. Arthritis Rheum 2009;60: 1438-50.
[51] Li B, Aspden RM. Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. Ann Rheum Dis 1997;56:247-54.
[52] Yu-Yahiro JA, Michael RH, Dubin NH, Fox KM, Sachs M, Hawkes WG, et al. Serum and urine markers of bone metabolism during the year after hip fracture. J Am Geriatr Soc 2001;49:877-83.
[53] Li WF, Hou SX, Yu B, Li MM, Ferec C, Chen JM. Genetics of osteoporosis: accelerating pace in gene identification and validation. Hum Genet 2010;127:249-85.
[54] Kohlmeier M, Saupe J, Schaefer K, Asmus G. Bone fracture history and prospective bone fracture risk of hemodialysis patients are related to apolipoprotein E genotype. Calcif Tissue Int 1998;62:278-81.
[55] Shiraki M, Shiraki Y, Aoki C, Hosoi T, Inoue S, Kaneki M, et al. Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1997;12: 1438-45.
[56] Tsezou A, Iliopoulos D, Malizos KN, Simopoulou T. Impaired expression of genes regulating cholesterol efflux in human osteoarthritic chondrocytes. J Orthop Res 2010;28: 1033-9.
[57] Lippiello L, Walsh T, Fienhold M. The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. Metabolism 1991;40: 571-6.
[58] Mahley RW, Rall Jr SC. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000;1:507-37.
[59] Sun S-S, Ma H-L, Liu C-L, Huang C-H, Cheng C-K, Wei W. Difference in femoral head and neck material properties between osteoarthritis and osteoporosis. Clin Biomech 2008;23:S39-47.

SECTION III

SURROGATE MARKERS OF BONE MINERAL DENSITY AND FRACTURES IN A PORTUGUESE POPULATION BASED LONGITUDINAL COHORT

PART 1 – RODRIGUES AM, EUSÉBIO M, RODRIGUES AB ET AL. LOW SERUM LEVELS OF DKK2 ARE A POTENTIAL SERUM MARKER OF INCIDENT LOW IMPACT FRACTURE RISK IN OLDER WOMEN. (*SUBMITTED TO JBMR PLUS*).

Low Serum Levels of DKK2 are a potential serum marker of Incident Low Impact Fractures Risk in Older Women

Ana Maria Rodrigues, Mónica Eusébio, Ana C Rodrigues, Joana Caetano-Lopes, Inês P Lopes, Ana Lopes, Jorge M Mendes, Pedro Simões Coelho, João Eurico Fonseca, Jaime Branco, Helena Canhão

(Submitted to JBMR Plus)

Abstract

There are currently no robust non-invasive markers of fragility fractures. Secreted frizzled related protein-1 (sFRP-1), dickkopf-related protein 1 (DKK1) and DKK2, and sclerostin (SOST) inhibit Wnt signalling and interfere with osteoblast-mediated bone formation. We evaluated associations of serum levels of sFRP-1, DKK1, DKK2, and SOST with incident low-impact fracture and BMD in 828 women aged ≥65 years from EpiDoC, a longitudinal population-based cohort. A structured questionnaire during a baseline clinical appointment assessed prevalent fragility fractures and clinical risk factors (CRFs) for fracture. Blood was collected to measure serum levels of bone turnover markers and Wnt regulators. Vertebral and hip BMD were determined by DXA scanning. Follow-up assessment was performed through phone call interview; incident fragility fracture was defined by any new self-reported low-impact fracture. Multivariate Cox proportional hazards models were used to analyse fracture risk adjusted for CRFs and BMD. During a mean follow-up of 2.3±1.0 years, 62 low-impact fractures were sustained in 58 women. A low serum DKK2 level (per 1 SD decrease) was associated with a 1.5-fold increase in fracture risk independently of BMD and CRFs. Women in the two lowest DKK2 quartiles had a fracture incidence rate of 32 per 1,000 person-years, whereas women in the two highest quartiles had 14 fragility fractures per 1,000 person-years. A high serum sFRP1 level was associated with a 1.6-fold increase in fracture risk adjusted for CRFs but not independently of BMD. Serum levels of SOST (r=0.191; p=0.0025) and DKK1(r=-0.1725; p=0.011) were correlated with hip BMD but not with incident fragility fracture. These results indicate that serum DKK2 and sFRP1 may predict low-impact fracture and suggest that Wnt pathway regulators should be further studied in other populations as potential non-invasive markers of fragility fracture risk.

Key words: Fracture risk assessment, Screening, Molecular pathways - remodelling, Wnt/β-catenin/LRPs, Aging

Introduction

Osteoporosis is a metabolic skeletal disease characterized by low bone mass and microarchitecture deterioration that frequently affects older adults[1,2]. The clinical consequence of osteoporosis is the occurrence of low-impact fractures, resulting in increased mortality, morbidity, and disability and imposing a major economic burden on European healthcare

systems[3,4]. One strategy for preventing osteoporosis-related fractures is to refine tools for identifying individuals with a high risk of fracture, as almost half of all fractures occur in individuals who are not classified as high risk by DXA scanning [5]. To improve fracture risk assessment, several algorithms have been developed and validated[6-9]. These include clinical risk factors (CRFs) such as age, gender, BMI, prior fragility fracture, parental history of hip fracture, use of oral glucocorticoids, rheumatoid arthritis and other secondary causes of osteoporosis, current smoking and alcohol intake to predict low-impact fracture, independently of BMD [10-14]. Although the performance of these fracture risk prediction tools is good, there is room for improvement in sensitivity and specificity[15-17]. The clinical challenge faced today is the accurate selection of individuals with a high risk of fracture and with indication for treatment to minimize individual and societal costs[1,5].

Serum assays for biochemical markers are important for monitoring alterations in bone formation and resorption, both in normal physiological conditions and in disease. Bone turnover markers (BTMs) reflecting bone remodelling[18] are modestly associated with fracture risk[19-23]. However, uncertainty exists regarding the clinical application of BTMs, as they exhibit short and long-term within-subject variability, and their usefulness for fracture prediction remains to be determined[22,24]. Thus, new biochemical markers of bone metabolism that better predict low-impact fracture are needed.

Recent studies show the importance of Wnt signalling to osteoblast differentiation[25-31]. The most well-studied secreted Wnt antagonists are sclerostin (SOST), dickkopfs (DKKs), and secreted frizzled related proteins (sFRPs), which regulate osteoblast-mediated bone formation[32]. Some Wnt antagonists have been considered not only as treatment targets[33-35] but also as potential markers of bone fragility. Serum levels of DKK1 and SOST increase with age and are associated with loss of bone mass[36,37]. sFRP-1 overexpression decreases bone density and attenuates the bone anabolic effects of PTH[38]. DKK2 can either behave as a Wnt agonist or antagonist, depending on the cellular context[39]. DKK2 inhibits bone formation in the absence of Wnt7b but induces terminal osteoblast differentiation in the presence of high Wnt7b levels[40]. Also, DKK2-null mice are osteopenic with suppressed bone formation[41]. Currently, however, there are conflicting results regarding the association between serum levels of DKK1 and SOST and low-impact fractures[42-46], and, to the best of our knowledge, no studies have addressed the associations of DKK2 and sFRP-1 with fracture.

The aim of the present study was to evaluate the association of serum levels of SOST, DKK1, DKK2, and sFRP-1 with BMD, and the incidence of low-impact fractures in elderly women from a population-based cohort.

Materials and Methods

Participants

This study was conducted as part of the Epidemiology of Chronic Diseases (EpiDoC) cohort initiated in 2011. EpiDoC is a prospective closed cohort study based on a nationally representative sample of adults (≥18 years old) who were non-institutionalized and living in private

households in Portugal Mainland and Islands (Azores and Madeira). The primary aim of the baseline assessment EpiDoC 1 (EpiReumaPt), which occurred between September 2011 and December 2013, was to assess rheumatic and musculoskeletal disease prevalence and burden in Portugal. Multistage random sampling was used for participant selection. Baseline assessment consisted of two phases: the first phase involved a face-to-face interview, and the second phase involved a detailed clinical evaluation of rheumatic and musculoskeletal disease performed by a rheumatologist. All participants enrolled in EpiDoC 1 (n=10,661) were invited to participate in follow-up, of whom 10,153 (95.2%) agreed to participate.

For follow-up waves EpiDoC 2 (2013–2015) and EpiDoC 3 (2015–2016), data were collected using a structured questionnaire through phone call interviews using a computer- assisted personal interview system. In each follow-up interview, research assistants applied a nuclear questionnaire (including questions on new rheumatic disease onset, new fragility fractures, falls, medical treatment, and hospitalisations) and additional questions for each wave depending on its focus.

Necessary sample size was calculated considering the primary aim of EpiDoC 1, which was to determine the prevalence of rheumatoid arthritis with 95% CIs standardized for age and gender according to the total adult population of the studied areas. Assuming an expected prevalence of rheumatoid arthritis of 0.5–1% and a drop-out rate of 50%, a total of 9,000 participants needed to be recruited. We recruited 10,661 participants.

Study population

The population of interest for the present study was women aged ≥65 years who were observed by rheumatologists during the second phase of the baseline EpiDoC 1 assessment and agreed to be followed up in subsequent EpiDoC waves. Full description of this population is provided elsewhere[47]. Women under osteoporosis treatment or diagnosed with bone metastasis or other bone metabolic diseases, such as Paget's disease of bone, were excluded from this study.

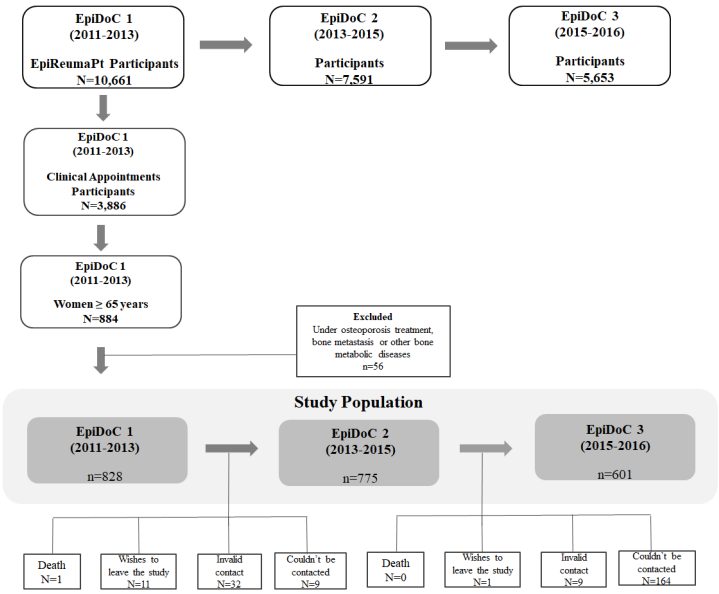

Figure 1. Flowchart of study participants.

Outcome definition and assessment

Fragility fracture events were defined as any self-reported low-impact fracture occurring after 40 years of age, including fractures resulting from a fall from a standing height or sustained fractures in the absence of trauma[48,49]. Self-reports of fragility fractures have been shown to be accurate[50-52]. Incident fractures were defined as self-reported new fractures during the two follow-up waves. The follow-up period was computed as the time from the baseline visit to a report of incident fracture.

Covariate definition and assessment

CRFs for fracture including age, BMI (categorized as underweight: <18.5 kg/m^2, normal weight: 18.5–24.9 kg/m^2, overweight: 25–29.9 kg/m^2, or obese: ≥30 kg/m^2), parental history of hip fracture, long-term use of oral glucocorticoids (≥3 months), rheumatoid arthritis, current smoking, high alcohol intake (≥3 units/day), other secondary causes of osteoporosis, and number of falls in the previous 12 months were collected at baseline. Self-reported previous fragility fractures (i.e., prevalent fragility fractures) were also recorded at baseline. Ten-year probability of major hip fracture was calculated using the Fracture Risk Assessment (FRAX) tool[53] without using hip DXA information.

DXA procedure

All women aged ≥65 years who attended the second phase of the baseline assessment were invited to undergo lumbar and non-dominant hip BMD measurement (g/cm^2) using DXA scanning (Hologic QDR 4500 A, Bedford, MA, USA). Quality control procedures were performed according to the manufacturer´s recommendations.

Biochemical assessment

Blood samples were collected at baseline[54]. Serum was separated by centrifugation (800g for 10 minutes at room temperature) and kept at 4ºC. Serum samples were sent to a central diagnostic laboratory to determine levels of bone remodelling markers, 25-hydroxyvitamin

D_3, intact PTH, and creatinine. The remaining samples were stored at -80ºC at Biobanco-IMM[54,55].

At the central lab, parameters were measured according to manufacturers' instructions. Serum levels of creatinine were measured using the rate-blanked creatinine method (Dimension Vista Intelligent Lab System, Siemens Healthcare, Erlangen, Germany), and glomerular filtration rate was calculated[56]. Serum levels of PTH, osteocalcin, crosslinked C-telopeptide of type I collagen (CTX-I), and amino-terminal propeptides of type I procollagen (P1NP) were measured using a fully automated Immulite 2000® electrochemiluminescent immunoassay analyser (Siemens Healthcare, Germany). Serum levels of 25-hydroxyvitamin D_3 were measured using competitive immunoassay (Liason Analyser, DiaSorin, Saluggia, Italy).

Measurement of Wnt signalling pathway regulators

Levels of Wnt signalling regulators were assessed in serum samples stored at Biobanco-IMM [55]. Baseline serum levels of sFRP-1 (Cloud-Clone Corp., Katy, TX, USA), DKK2 (Elabscience, Wuhan, China), DKK1 (Biomedica Medizinprodukte, Vienna, Austria), and SOST (Biomedica Medizinprodukte) were determined by commercially available ELISA according to the manufacturers' instructions and were analysed using a Tecan Infinite 200 PRO plate reader (Tecan, Männedorf, Switzerland).

Statistical Analysis

Data are presented as mean ± SD or frequency and proportion unless stated otherwise. Baseline characteristics of participants with and without incident fragility fracture were compared using univariable logistic regression analysis. Associations between serum levels of Wnt signalling regulators (sFRP-1, DKK2, DKK1, and SOST) and continuous or T-score categories of axial BMD (lumbar and non-dominant hip) were analysed using Pearson correlations. Associations between serum levels of Wnt signalling regulators and BMD were analysed by univariable linear regression and adjusted for age, BMI, family history of hip fracture, physical activity, and glucocorticoid use. Associations between serum levels of Wnt signalling regulators and incident fragility fracture were analysed using Cox's proportional hazards models with serum levels of Wnt signalling regulators as continuous or standardized (per 1 SD) measures. Fracture risk estimates were adjusted for age, family history of hip fracture, and prevalent fragility fracture. Adjustment for lumbar and hip BMD was performed in a separate Cox regression model.

To further identify high-risk sub-groups of women for incident fragility fracture, serum levels of DKK2 and sFRP-1 were categorized into quartiles. The relationship between DKK2 (ng/mL) quartile and incident fracture rate (per 1,000 person-years) was assessed and adjusted for age, family history of hip fracture, prevalent fragility fracture, and hip BMD. The relationship between sFRP-1 (ng/mL) quartile and incident fracture rate (per 1,000 person-years) was assessed and adjusted for age, family history of hip fracture, and prevalent fragility fracture. Using a risk stratification approach, fracture rate (per 1,000 person-years) was calculated based on the combination of hip BMD (according to quartile distribution) and serum level of DKK2 (lowest two quartiles vs. highest two quartiles). Fracture rate was calculated considering the

presence of CRFs (age, age and presence of prevalent low-impact fracture, age and family history of hip fracture) and serum level of DKK2 (lowest two quartiles vs. highest two quartiles).

Finally, facture rate was calculated considering the 10-year risk of major fracture (<11% vs. ≥11%) without BMD and serum level of DKK2 (according to quartile distribution). The FRAX score cut-off was based on the Portuguese recommendation for fracture risk prediction[57].

Statistical significance was considered as $p<0.05$. All analyses were performed using Stata IC, version 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX, USA: StataCorp LP.).

Ethical approval

The EpiDoC cohort study was approved by the Ethics Committee of NOVA Medical School and the Portuguese Data Protection Authority (Comissão Nacional de Proteção de Dados). Written informed consent in accordance with principles established by the Declaration of Helsinki was obtained from all participants. Further details related to ethical issues are described elsewhere[58].

Results

Of 3,877 participants evaluated by a rheumatologist at baseline, 884 were women aged ≥65 years. After applying exclusion criteria, 828 women were included in this study (Figure 1). During a mean follow-up of 2.3±1.0 years, a total of 62 fragility fractures were sustained in 58 women. Most incident fragility fractures (n=51; 82.3%) were non-hip, non-vertebral (i.e., wrist, lower leg, humerus, rib, clavicle, and elbow). Incident hip or vertebral fractures were reported by 6 (9.7%) and 4 (6.6%) women, respectively. Senior women with incident fragility fractures had significantly more prior fractures and had more frequently a family history of hip fractures. No other CRFs were associated with incident fragility fracture (Table 1).

Table 1. Crude analysis of socio-demographic and economic characteristics, risk factors for fractures, and health status of Portuguese women aged ≥65 years with or without incident fragility fractures.

	All (n=828)	No incident fragility fracture (n=669)	Incident fragility fracture (n=58)	p-value
Age (years)				
65–69	270 (32.61%)	232 (34.68%)	17 (29.31%)	0.2770
70–79	414 (50.00%)	339 (50.67%)	28 (48.28%)	
≥80	144 (17.39%)	98 (14.65%)	13 (22.41%)	
BMI (kg/m²)				
Underweight	7 (0.86%)	5 (0.76%)	0 (0%)	0.8660
Normal	205 (25.31%)	161 (24.51%)	14 (24.14%)	
Overweight	358 (44.20%)	280 (42.62%)	28 (48.28%)	
Obese	240 (29.63%)	211 (32.12%)	16 (27.59%)	
Family history of hip fracture				
Yes	51 (6.17%)	37 (5.54%)	8 (13.79%)	0.016†
No	776 (93.83%)	631 (94.46%)	50 (86.21%)	
Current smoking				
Yes	16 (1.93%)	14 (2.10%)	1 (1.72%)	0.849
No	811 (98.07%)	654 (97.90%)	57 (98.28%)	
High alcohol intake (≥3 units/day)				
Yes	14 (1.69%)	13 (1.95%)	1 (1.72%)	0.906
No	813 (98.31%)	655 (98.05%)	57 (98.28%)	

	All (n=828)	No incident fragility fracture (n=669)	Incident fragility fracture (n=58)	p-value
Physical activity				
Inactive	488 (84.87%)	443 (84.70%)	43 (86.00%)	0.807
Active	87 (15.13%)	80 (15.30%)	7 (14.00%)	
Number of falls in previous 12 months	1.19±3.41	1.03±2.95	1.34±2.27	0.450
Use of glucocorticoids				
Yes	30 (3.63%)	23 (3.44%)	4 (6.90%)	0.192
No	797 (96.37%)	645 (96.56%)	54 (93.10%)	
Rheumatoid arthritis				
Yes	13 (1.57%)	11 (1.65%)	1 (1.72%)	0.965
No	814 (98.43%)	657 (98.35%)	57 (98.28%)	
Secondary osteoporosis				
Yes	25 (3.02%)	20 (2.99%)	3 (5.17%)	0.370
No	802 (96.98%)	648 (97.01%)	55 (94.83%)	
Chronic renal insufficiency (mL/min/1.73 m²)				
eGFR <30	16 (2.51%)	13 (2.50%)	2 (4.76%)	0.392
eGFR ≥30	621 (97.49%)	506 (97.50%)	40 (95.24%)	
Prevalent fragility fracture (self-reported)				
Yes	172 (21.83%)	121 (18.94%)	26 (45.61%)	<0.001[a]
No	616 (78.17%)	518 (81.06%)	31 (54.39%)	
Prevalent fragility fracture site (self-reported)				
Hip	10 (1.27%)	4 (0.63%)	2 (3.51%)	0.046[a]
Vertebral	11 (1.40%)	7 (1.10%)	2 (3.51%)	0.144
Non-hip/non-vertebral	121 (15.94%)	86 (13.85%)	16 (32.00%)	0.001[a]
FRAX score without BMD				
10-year risk of major fracture (mean±SD)	9.62±6.85	9.00±6.04	12.83±10.71	<0.001[a]
10-year risk of hip fracture (mean±SD)	4.24±5.20	3.79±4.29	6.64±9.78	<0.001[a]
Vertebral BMD (g/cm²)				
Vertebral BMD (mean±SD)	0.99±0.21	1.00±0.21	0.97±0.21	0.471
Vertebral BMD (T-score)				
Osteoporosis (≤-2.5)	69 (25.75%)	55 (23.81%)	6 (35.29%)	0.517
Osteopenia (>-2.5 and <-1)	89 (33.21%)	78 (33.77%)	4 (23.53%)	
Normal (≥-1)	110 (41.04%)	98 (42.42%)	7 (41.18%)	
Axial BMD (t-score)				
Osteoporosis (≤-2.5)	74 (27.31%)	59 (25.32%)	7 (38.89%)	0.398
Osteopenia (>-2.5 and <-1)	128 (47.23%)	111(47.64%)	6 (33.33%)	
Normal (≥-1)	69 (25.46%)	63 (27.04%)	5 (27.78%)	
Hip BMD (g/cm²)				
Hip BMD (mean±SD)	0.78±0.14	0.78±0.14	0.79±0.12	0.865
Hip BMD (T-score)				
Osteoporosis (≤-2.5)	27 (9.93%)	21 (8.97%)	3 (16.67%)	0.464
Osteopenia (>-2.5 and <-1)	139 (51.10%)	119 (50.85%)	7 (38.89%)	
Normal (≥-1)	106 (38.97%)	94 (40.17%)	8 (44.44%)	
Vitamin D (mmol/mL)				
Deficiency (<10)	18 (2.96%)	15 (3.02%)	3 (7.50%)	0.341
Insufficiency (≥10 and < 30)	212 (34.81%)	175 (35.28%)	13 (32.50%)	
Normal (≥10)	379 (62.23%)	306 (61.69%)	24 (60.00%)	
BTMs				
CTX-I (ng/mL)	0.25±0.16	0.25±0.16	0.27±0.21	0.612
P1NP (ng/mL)	41.06±20.50	39.75±18.36	41.39±20.06	0.716

	All (n=828)	No incident fragility fracture (n=669)	Incident fragility fracture (n=58)	p-value
Osteocalcin (ng/mL)	3.77±2.46	3.60±2.28	4.05±1.85	0.412
PTH (ng/mL)	49.38±38.69	47.69±36.45	50.24±46.56	0.681
Serum levels of Wnt regulators				
DKK2 (ng/mL)	7.79±2.86	7.75±2.75	6.86±2.39	0.144
sFRP-1 (ng/mL)	2.02±1.37	1.92±1.31	2.56±1.37	0.035 [a]
SOST (pmol/L)	31.89±13.96	32.14±13.80	30.91±15.64	0.700
DKK1 (pmol/L)	132.24±76.48	136.76±76.74	118.16±81.00	0.311

Sample size is not constant. All: BMI (n=810), family history of hip fracture (n=827), current smoking (n=827), high alcohol intake (n=827), physical activity (n=575), number of falls in previous 12 months (n=784), glucocorticoid use (n=827), rheumatoid arthritis (n=827), secondary osteoporosis (n=827), chronic renal insufficiency (n=637), prevalent fragility fracture (n=788), hip (n=788), vertebral (n=788), non-vertebral/non-hip (n=759), prevalent vertebral fracture (n=318), FRAX major (n=820), FRAX hip (n=820), vertebral BMD (n=268), hip BMD (n=271), vitamin D (n=609), CTX-I (n=289), P1NP (n=287), osteocalcin (n=291), PTHi (n=592), SOST (n=321), DKK1 (n=290), DKK2 (n=319), sFRP1 (n=321). No incident fragility fracture: BMI (n=657), family history of hip fracture (n=668), current smoking (n=668), high alcohol intake (n=668), physical activity (n=523), number of falls in previous 12 months (n=634), glucocorticoid use (n=668), rheumatoid arthritis (n=668), secondary osteoporosis (n=668), chronic renal insufficiency (n=519), prevalent fragility fracture (n=639), hip (n=639), vertebral (n=639), non-vertebral/non-hip (n=621), prevalent vertebral fracture (n=276), FRAX major (n=663), FRAX hip (n=663), vertebral BMD (n=231), hip BMD (n=233), vitamin D (n=496), CTX-I (n=241), P1NP (n=241), osteocalcin (n=243), PTHi (n=481), SOST (n=277), DKK1 (n=249), DKK2 (n=275), sFRP-1 (n=276). Incident fragility fracture: physical activity (n=50), number of falls in previous 12 months (n=56), chronic renal insufficiency (n=42), prevalent fragility fracture (n=57), hip (n=57), vertebral (n=57), non-vertebral/non-hip (n=50), prevalent vertebral fracture (n=21), FRAX major (n=57), FRAX hip (n=57), vertebral BMD (n=17), hip BMD (n=18), vitamin D (n=40), CTX-I (n=18), P1NP (n=18), osteocalcin (n=18), PTHi (n=39), SOST (n=20), DKK1 (n=19), DKK2 (n=21), sFRP1 (n=21). [a] $p<0.05$.

Serum levels of sFRP-1, SOST, and DKK1 are associated with BMD

There was no correlation between serum levels of DKK2 and BMD (Table 2). Serum levels of sFRP-1 and SOST were positively correlated with lumbar and hip BMD (Table 2). When BMD was categorized by T-score, the positive correlations for both sFRP-1 and SOST were lost, except for in the normal BMD group. After adjusting for age, BMI, family history of hip fracture, physical activity, and glucocorticoid use, serum level of sFRP-1 was positively correlated with vertebral BMD (β=0.040, $p<0.001$) and hip BMD (β=0.011, p=0.001; Table 3). Using the same adjustment parameters, SOST levels were still positively correlated with vertebral BMD (β=0.001, $p<0.001$) and negatively correlated with hip BMD (β=-0.002, p=0.001; Table 3).

By contrast, serum levels of DKK1 were negatively correlated with hip femoral neck BMD. This association remained significant even after adjusting for CRFs (β=-0.0004, p=0.008; Table 3).

Table 2. Correlations between serum levels of DKK2, SOST, DKK1, and sFRP-1, and BMD stratified by t-score groups.

	Vertebral BMD (g/cm²)				Hip BMD (g/cm²)			
	Continuous (g/cm²)	Osteoporosis (t-score ≤-2.5)	Osteopenia (t-score >-2.5 and <-1)	Normal (t-score ≥-1)	Continuous (g/cm²)	Osteoporosis (t-score ≤-2.5)	Osteopenia (t-score >-2.5 and <-1)	Normal (t-score ≥-1)
DKK2 (ng/mL)	-0.0537	-0.1415	0.0774	0.0042	-0.0279	0.0081	0.0194	-0.0034
sFRP-1 (ng/mL)	0.2603[c]	0.0652	0.1864	0.4066[c]	0.1621[a]	0.0223	0.1912	0.1172
SOST (pmol/L)	0.2944[c]	-0.1569	0.0591	0.2099[a]	0.1917[b]	0.0244	0.1781	0.0053
DKK1 (pmol/L)	-0.0162	0.1610	-0.2916[a]	-0.0385	-0.1725[b]	-0.1662	-0.2235[a]	-0.2083

Sample size is not constant. Vertebral BMD: SOST (n=245), DKK1 (n=220), DKK2 (n=243), sFRP-1 (n=244). Hip BMD: SOST (n=247), DKK1 (n=218), DKK2 (n=245), sFRP1 (n=246). [a] p<0.05, [b] p<0.01, [c] p<0.001.

Table 3. Crude and adjusted analysis of associations between serum levels of DKK2, SOST, DKK1, and sFRP-1, and BMD.

	Vertebral BMD (g/cm²)				Hip BMD (g/cm²)			
	Crude		Adjusted		Crude		Adjusted	
	β	p	β	p	β	p	β	p
DKK2 (ng/mL)	-0.004	0.404	-0.003	0.536	-0.002	0.664	-0.002	0.613
sFRP-1 (ng/mL)	0.040	<0.001[a]	0.043	<0.001[a]	0.016	0.011[a]	0.016	0.020[a]
SOST (pmol/L)	0.005	<0.001[a]	0.005	<0.001[a]	0.002	0.002[a]	-0.002	0.001[a]
DKK1 (pmol/L)	-0.000	0.811	-0.000	0.867	-0.0003	0.011[a]	-0.0004	0.008[a]

Sample size is not constant. Crude vertebral BMD: SOST (n=245), DKK1 (n=220), DKK2 (n=243), sFRP1 (n=244). Crude hip BMD: SOST (n=247), DKK1 (n=218), DKK2 (n=245), sFRP1 (n=246). Adjusted vertebral BMD: SOST (n=178), DKK1 (n=163), DKK2 (n=177), sFRP1 (n=176). Adjusted hip BMD: SOST (n=182), DKK1 (n=164), DKK2 (n=181), sFRP1 (n=180). Adjusted for age, BMI, family history of hip fracture, physical activity, and glucocorticoid use. [a] p<0.05.

Serum levels of DKK2 and sFRP-1 are independently associated with incident low-impact fracture

Low serum level of DKK2 was associated with an increased risk of low-impact fracture in Cox proportional hazards models (Table 4). This association remained significant after adjusting for independent CRFs for low-impact fracture identified in this population: age, family history of hip fracture, and prevalent fragility fracture [HR (95% CI) per 1 SD increase, 0.61 (0.39; 0.98)]. The HR also remained significant after adjusting for vertebral BMD [HR (95% CI) per 1 SD increase, 0.47 (0.27; 0.82)] and hip BMD [HR (95% CI) per 1 SD increase, 0.53 (0.32; 0.88); Table 4]. Women in the two highest DKK2 quartiles had a fracture incidence rate of 14 per 1,000 person-years, whereas women in the two lowest DKK2 quartiles had a fracture incidence rate of 32 per 1,000 person-years (Figure 2).

High serum level of sFRP-1 was associated with an increased risk of low-impact fracture [HR (95% CI) per 1 SD increase, 1.45 (1.01; 2.09)]. This association was independent of CRFs [HR (95% CI) per 1 SD increase, 1.62 (1.09; 2.42)], but dependent on BMD (Table 4). Women in the two lowest sFRP-1 quartiles had a fracture incidence rate of 11 per 1,000 person-years, whereas women in the two highest sFRP1 quartiles had a fracture incidence rate of 31 per 1,000 person-years (Figure 2).

Cox proportional hazards models showed no association between serum level of SOST or DKK1 and fracture risk in senior women. Also, no associations were found between BTMs and low-impact fracture incidence (Table 4).

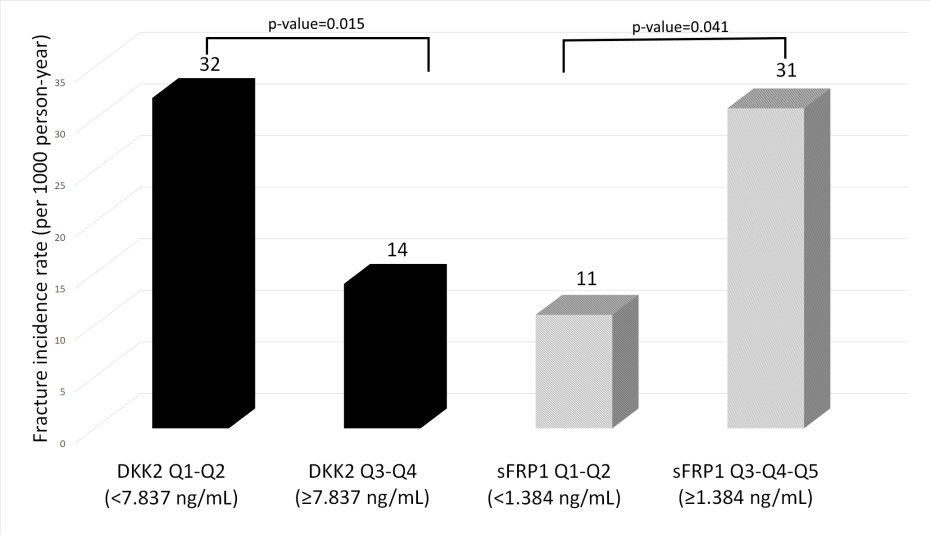

Figure 2. Association between DKK2 and sFRP-1 quartiles with incident fracture rate (per 1,000 person-years). Women in the two highest DKK2 quartiles had a significantly lower fracture incidence than women in two lowest DKK2 quartiles.

Table 4. Crude and adjusted analysis of associations between serum levels of Wnt regulators and BTMs and incident fragility fracture.

Incidence fracture	**Continuous**		**Per 1 SD increase**	
WNT regulators				
DKK2 (ng/mL)	**HR**	**p**	**HR**	**p**
Crude	0.861 (0.738; 1.006)	0.059	0.655 (0.423; 1.016)	0.059
CRFs adjusted[1]	0.842 (0.714; 0.993)	0.041†	0.615 (0.386; 0.979)	0.041[a]
CRFs+vertebral BMD adjusted[2]	0.767 (0.630; 0.933)	0.008†	0.471 (0.271; 0.821)	0.008[a]
CRFs+hip BMD adjusted[3]	0.798 (0.665; 0.958)	0.015†	0.529 (0.316; 0.885)	0.015[a]
sFRP-1 (ng/mL)	**HR**	**p**	**HR**	**p**
Crude	1.318 (1.008; 1.722)	0.043†	1.453 (1.011; 2.087)	0.043[a]
CRFs adjusted[1]	1.431 (1.067; 1.918)	0.017†	1.624 (1.092; 2.416)	0.017[a]
CRFs+vertebral BMD adjusted[2]	1.265 (0.891; 1.796)	0.188	1.375 (0.856; 2.209)	0.188
CRFs+hip BMD adjusted[3]	1.329 (0.971; 1.819)	0.075	1.470 (0.961; 2.248)	0.075
SOST (pmol/L)	**HR**	**p**	**HR**	**p**
Crude	1.001 (0.970; 1.033)	0.939	1.017 (0.661; 1.564)	0.939
CRFs adjusted[1]	1.007 (0.975; 1.039)	0.677	1.097 (0.709; 1.696)	0.677
CRFs+vertebral BMD adjusted[2]	0.999 (0.957; 1.043)	0.956	0.983 (0.545; 1.774)	0.956
CRFs+hip BMD adjusted[3]	0.989 (0.949; 1.030)	0.578	0.853 (0.488; 1.491)	0.578
DKK1 (pmol/L)	**HR**	**p**	**HR**	**p**
Crude	0.998 (0.990; 1.003)	0.512	0.845 (0.511; 1.396)	0.512
CRFs Adjusted[1]	0.996 (0.989; 1.004)	0.307	0.743 (0.421; 1.313)	0.307
CRFs+vertebral BMD adjusted[2]	0.993 (0.983; 1,003)	0.151	0.588 (0.285; 1.214)	0.151
CRFs+hip BMD adjusted[3]	0.991 (0.982; 1.000)	0.078	0.510 (0.241; 1.079)	0.078
Bone Turnover Markers				
CTX-I (ng/mL)	**HR**	**p**	**HR**	**p**
Crude	2.076 (0.197; 21.862)	0.543	1.126 (0.768; 1.652)	0.543
CRFs adjusted[1]	2.687 (0.241; 29.973)	0.422	1.175 (0.793; 1.740)	0.422
CRFs+vertebral BMD adjusted[2]	1.469 (0.001; 3849.7)	0.924	1.065 (0.295; 3.836)	0.924
CRFs+hip BMD adjusted[3]	1.024 (0.001; 1544.9)	0.995	1.004 (0.305; 3.306)	0.995
P1NP (ng/mL)	**HR**	**p**	**HR**	**p**
Crude	1.005 (0.981; 1.030)	0.679	1.111 (0.679; 1.813)	0.679
CRFs adjusted[1]	1.010 (0.985; 1.035)	0.434	1.217 (0.744; 1.992)	0.434

Incidence fracture	Continuous		Per 1 SD increase	
CRFs+vertebral BMD adjusted[2]	1.032 (0.965; 1.104)	0.361	1.885 (0.484; 7.346)	0.361
CRFs+hip BMD adjusted[3]	1.025 (0.966; 1.088)	0.413	1.654 (0.496; 5.521)	0.413
Osteocalcin (ng/mL)	**HR**	**p**	**HR**	**p**
Crude	1.075 (0.925; 1.250)	0.346	1.191 (0.828; 1.713)	0.346
CRFs adjusted[1]	1.091 (0.936; 1.272)	0.266	1.234 (0.852; 1.787)	0.266
CRFs+vertebral BMD adjusted[2]	1.299 (0.963; 1.751)	0.087	1.879 (0.913; 3.868)	0.913
CRFs+hip BMD adjusted[3]	1.123 (0.870; 1.450)	0.373	1.324 (0.714; 2.452)	0.373

[1]Adjusted for age, family history of hip fracture, and prevalent fragility fracture (self-reported). [2]Adjusted for age, family history of hip fracture, prevalent fragility fracture (self-reported), and vertebral BMD. [3]Adjusted for age, family history of hip fracture, prevalent fragility fracture (self-reported), and hip BMD. Sample size is not constant. SOST: crude (n=529), adjusted[1] (n=496), adjusted[2] (n=368), adjusted[3] (n=371). DKK1: crude (n=476), adjusted[1] (n=443), adjusted[2] (n=323), adjusted[3] (n=320). DKK2: crude (n=527), adjusted[1] (n=494), adjusted[2] (n=366), adjusted[3] (n=369). sFRP-1: crude (n=529), adjusted[1] (n=496), adjusted[2] (n=366), adjusted[3] (n=369). CTX-I: crude (n=450), adjusted[1] (n=420), adjusted[2] (n=138), adjusted[3] (n=138). P1NP: crude (n=451), adjusted[1] (n=421), adjusted[2] (n=138); adjusted[3] (n=140). Osteocalcin: crude (n=455), adjusted[1] (n=425), adjusted[2] (n=138), adjusted[3] (n=140). [a] $p<0.05$.

Serum level of DKK2 improves fracture risk prediction independently of BMD, CRFs, and FRAX score

Using a risk-stratified approach, the fracture incidence rate (per 1,000 person-years) was calculated based on the combination of hip BMD, CRFs, baseline 10-year risk of major fracture using FRAX score (without BMD), and serum level of DKK2 (Figure 3). Women in the lowest hip BMD quartile and the two lowest DKK2 quartiles had the highest fracture incidence rate (41 per 1,000 person-years; Figure 3A). Among women with a history of low-impact fracture, women in the two lowest DKK2 quartiles had a higher fracture rate (96 per 1,000 person-years) than women in the two highest DKK2 quartiles (33 per 1,000 person-years), although this difference did not reach statistical significance, presumably due to the low incidence of events (p=0.08; Figure 3B). Serum level of DKK2 did not discriminate among women with a family history of hip fracture (Figure 3B).

Women with a 10-year risk of major fracture <11% (18 per 1,000 person-years) had a lower fracture incidence rate than women with a 10-year risk of major fracture ≥11% [41 per 1,000 person-years, $p<0.001$; HR (95% CI) per 1 SD increase, 2.37 (1.40; 4.00)]. When we calculated fracture rate, based on a combination of FRAX score and DKK2 serum level, women in the two lowest DKK2 quartiles had the highest fracture incidence rate independently of 10-year risk of major fracture (Figure 3C).

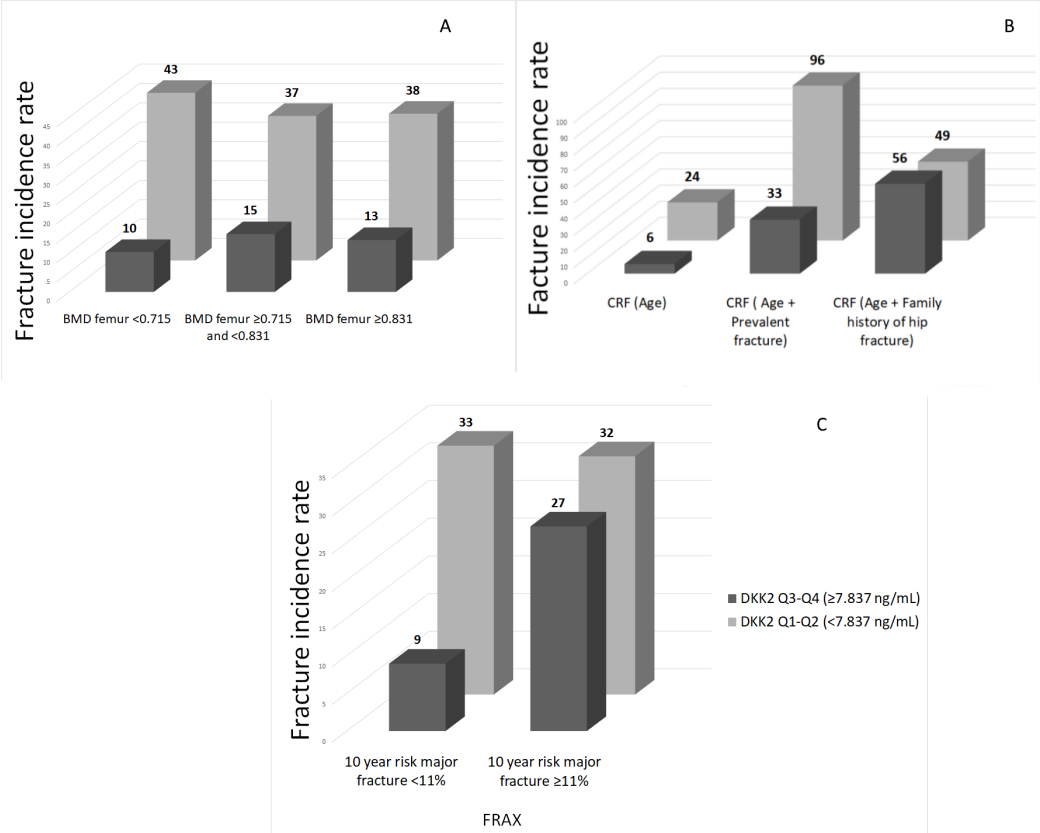

Figure 3. Relationship between DKK2 quartiles and hip BMD terciles (A), CRF (B), and FRAX score with incident fracture rate (per 1,000 person-years). Women in the lowest hip BMD tercile and the two lowest DKK2 quartiles had the highest fracture incidence rate (A). Among women with a history of low-impact fracture, women in the two lowest DKK2 quartiles had a higher fracture rate (B). Women in the two lowest DKK2 quartiles had the highest fracture incidence rate independently of being under or above the cut-off for 10-year major fracture risk (i.e., 11%) (C).

Discussion

The present study, conducted in a population-based cohort of senior women, showed that low serum level of DKK2 predicted low-impact fractures, independently of BMD and CRFs for fracture. For every 1 SD decrease in DKK2, fracture risk increased by approximately 1.5-fold. Serum levels of DKK2 were not associated with vertebral or hip BMD. Our results suggest a possible interaction among BMD, FRAX score without BMD, and serum DKK2 in the assessment of fracture risk, which would need to be further investigated in a larger study with a longer follow-up period.

DKK2 inhibits Wnt-β-catenin signalling by binding to low-density lipoprotein receptor-related proteins 5 and 6[59] and acts as a fine-tuning regulator of osteoblast differentiation and function[40]. DKK2 is upregulated in osteoarthritis subchondral bone (with local high bone mass), and *in vitro* upregulation of DKK2 in osteoblasts increases their ability to form mineralized nodules[60]. By contrast, DKK2 deficiency *in vivo* leads not only to mineralization disturbances and bone fragility but also to a moderate reduction in bone mass[40]. These results are in line with our present findings showing that decreased serum level of DKK2

was associated with bone fragility fracture but not with vertebral and hip BMD. This may be due to the fact that osteoblast mineralization disturbances, signalled by low levels of DKK2, lead to bone nanoarchitecture disorganization and fragility, independently of bone mass loss[61-63].

Overexpression of sFRP-1 in human osteoblasts accelerates the rate of cell death and, thus, inhibits bone formation[38,64]. Concordantly, we found that high levels of sFRP-1 was associated with incident low-impact fracture, independent of CRFs for fracture. However, this association was not independent of BMD. Surprisingly, in our study, high serum levels of sFRP-1 and SOST were significantly associated with high vertebral and hip BMD. Given that sFRP-1 and SOST inhibit osteoblast proliferation and maturation, we would have expected a negative correlation with BMD. Other studies also found the same paradoxical results[36,43,65]. Of interest, when we analysed the association between serum levels of SOST and sFRP-1 and BMD categorized by T-score, we confirmed the existence of correlations in women with normal BMD but not with osteopenia or osteoporosis. One possible explanation is that, in healthy individuals, normal BMD is maintained through local downregulation of Wnt inhibitors in association with high systemic serum levels of SOST and sFRP-1. In agreement with this possibility, serum level of SOST is higher in men (who have a lower global fracture risk) than in women[66]. However, in pathological situations, as in individuals with bone fragility and a high risk of fracture, this regulation system is disrupted, and serum levels of Wnt regulators are associated with osteoblast dysfunction, bone fragility, and fracture, as our results showed.

In our study, serum levels of SOST were not significantly associated with incident low-impact fracture. Similarly, the OFELY study followed postmenopausal women for 6 years and reported no association between serum level of SOST and incident fracture[42]. Amrein et al. also found no association between SOST serum level and fragility fractures in institutionalized elderly women[67]. By contrast, the Center of Excellence for Osteoporosis Research Study followed 707 postmenopausal women and showed that a high serum SOST level is associated with an increased risk of fracture[44].

Although we observed a negative correlation between serum levels of DKK1 and BMD, similar to what was previously reported[37,68], we found no association between DKK1 serum levels and incident fracture. In a cross-sectional study in Sweden, serum levels of DKK1 were increased in patients with a fresh hip fracture when compared with healthy volunteers[69]. In contrast, a study from Korea did not found an association of serum levels of DKK1 and prevalent osteoporotic fractures[45].

We also found no associations between serum levels of BTMs (P1NP, CTX-I, and osteocalcin) and fragility fractures. Although several studies have proposed BTMs as fracture risk predictors, their results are not conclusive[19,23,70-72]. A recent meta-analysis reports a modest association between CTX-I and fragility fracture, although this association is not independent of BMD[23]. These ambiguous study results probably contribute to the low acceptance and utility of BTMs in clinical practice and re-enforce the need to find alternative markers of fragility fracture risk.

This study has some limitations. First, fragility fractures were self-reported, which is less accurate than clinically verified vertebral fractures, leading to underestimation of their prevalence[73]; however, the overall performance of self-reported fragility fractures is respectable[50-52]. Second, the number of incident fractures was relatively low because of a short follow-up duration (2.3±1.0 years). Hence, these results must be confirmed in other cohorts with more participants and longer follow-up periods. Nevertheless, several strengths of this study should also be acknowledged. Our data came from a large, representative sample of the Portuguese adult population and participants were examined by rheumatologists at baseline. Furthermore, different fragility fractures and health-related measurements were captured, providing relevant information about risk factors.

In conclusion, we report that low serum levels of DKK2 predicts risk of low-impact fractures, independently of BMD and CRFs and thus should be explored as a potential non-invasive marker of fragility fracture risk. High serum level of sFRP-1 were significantly associated with fracture, although this association was not independent of BMD. Both SOST and DKK1 were associated with BMD but not with incident fracture, although the number of new fractures recorded may not have allowed the detection of these latter associations. These results indicate that serum DKK2 and sFRP1 may predict low-impact fracture and suggest that Wnt pathway inhibitors should be further studied in other populations as potential non-invasive markers of fragility fracture risk.

References

1. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet. May 18 2002;359(9319):1761-7.
2. Avendano M, Glymour MM, Banks J, Mackenbach JP. Health disadvantage in US adults aged 50 to 74 years: a comparison of the health of rich and poor Americans with that of Europeans. Am J Public Health. Mar 2009;99(3):540-8. Epub 2009/01/20.
3. Johnell O. The socioeconomic burden of fractures: today and in the 21st century. Am J Med. Aug 18 1997;103(2A):20S-5S; discussion 5S-6S.
4. Looker AC, Orwoll ES, Johnston CC, Jr., Lindsay RL, Wahner HW, Dunn WL, et al. Prevalence of low femoral bone density in older U.S. adults from NHANES III. J Bone Miner Res. Nov 1997;12(11):1761-8. Epub 1998/02/07.
5. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. Jan 2004;34(1):195-202. Epub 2004/01/31.
6. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. Aug 2007;18(8):1033-46. Epub 2007/02/27.
7. Hippisley-Cox J, Coupland C. Predicting risk of osteoporotic fracture in men and women in England and Wales: prospective derivation and validation of QFractureScores. BMJ. Nov 19 2009;339:b4229. Epub 2009/11/21.
8. Nguyen ND, Frost SA, Center JR, Eisman JA, Nguyen TV. Development of a nomogram for individualizing hip fracture risk in men and women.

Osteoporos Int. Aug 2007;18(8):1109-17. Epub 2007/03/21.

9. Bolland MJ, Siu AT, Mason BH, Horne AM, Ames RW, Grey AB, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. J Bone Miner Res. Feb 2011;26(2):420-7. Epub 2010/08/20.
10. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. Osteoporos Int. Feb 2005;16(2):155-62. Epub 2004/06/04.
11. 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. Aug 2004;35(2):375-82. Epub 2004/07/23.
12. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton IL, et al. A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res. Jun 2004;19(6):893-9. Epub 2004/05/06.
13. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, et al. A family history of fracture and fracture risk: a meta-analysis. Bone. Nov 2004;35(5):1029-37. Epub 2004/11/16.
14. Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, et al. Alcohol intake as a risk factor for fracture. Osteoporos Int. Jul 2005;16(7):737-42. Epub 2004/09/30.
15. Rubin KH, Friis-Holmberg T, Hermann AP, Abrahamsen B, Brixen K. Risk assessment tools to identify women with increased risk of osteoporotic fracture: complexity or simplicity? A systematic review. J Bone Miner Res. Aug 2013;28(8):1701-17. Epub 2013/04/18.
16. Marques A, Ferreira RJ, Santos E, Loza E, Carmona L, da Silva JA. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Ann Rheum Dis. Nov 2015;74(11):1958-67. Epub 2015/08/08.
17. Shepstone L, Lenaghan E, Cooper C, Clarke S, Fong-Soe-Khioe R, Fordham R, et al. Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial. Lancet. Feb 24 2018;391(10122):741-7. Epub 2017/12/20.
18. Naylor K, Eastell R. Bone turnover markers: use in osteoporosis. Nat Rev Rheumatol. Jun 5 2012;8(7):379-89. Epub 2012/06/06.
19. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res. Oct 1996;11(10):1531-8. Epub 1996/10/01.
20. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res. Aug 2000;15(8):1526-36. Epub 2000/08/10.
21. Ivaska KK, Gerdhem P, Vaananen HK, Akesson K, Obrant KJ. Bone turnover markers and prediction of fracture: a prospective follow-up study of 1040 elderly women for a mean of 9 years. J Bone Miner Res. Feb 2010;25(2):393-403. Epub 2009/12/08.
22. Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. Feb 2011;22(2):391-420. Epub 2010/12/25.
23. Johansson H, Oden A, Kanis JA, McCloskey EV, Morris HA, Cooper C, et al. A meta-analysis of reference markers

of bone turnover for prediction of fracture. Calcif Tissue Int. May 2014;94(5):560-7. Epub 2014/03/05.

24. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. Mar 2014;51(Pt 2):189-202. Epub 2014/01/09.
25. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, et al. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med. May 16 2002;346(20):1513-21. Epub 2002/05/17.
26. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. Nov 16 2001;107(4):513-23. Epub 2001/11/24.
27. Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, et al. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet. Jan 2002;70(1):11-9. Epub 2001/12/13.
28. Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C, et al. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet. Feb 2002;39(2):91-7. Epub 2002/02/12.
29. Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, et al. Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet. Mar 2001;68(3):577-89. Epub 2001/02/17.
30. Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, et al. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science. Mar 2 2007;315(5816):1278-82. Epub 2007/03/03.
31. Wergedal JE, Veskovic K, Hellan M, Nyght C, Balemans W, Libanati C, et al. Patients with Van Buchem disease, an osteosclerotic genetic disease, have elevated bone formation markers, higher bone density, and greater derived polar moment of inertia than normal. J Clin Endocrinol Metab. Dec 2003;88(12):5778-83. Epub 2003/12/13.
32. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. Gene. Jan 15 2012;492(1):1-18. Epub 2011/11/15.
33. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. N Engl J Med. Oct 12 2017;377(15):1417-27. Epub 2017/09/12.
34. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. N Engl J Med. Oct 20 2016;375(16):1532-43. Epub 2016/11/01.
35. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med. Feb 2007;13(2):156-63. Epub 2007/01/24.
36. Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res. Feb 2011;26(2):373-9. Epub 2010/08/20.
37. Butler JS, Murray DW, Hurson CJ, O'Brien J, Doran PP, O'Byrne JM. The role of Dkk1

in bone mass regulation: correlating serum Dkk1 expression with bone mineral density. J Orthop Res. Mar 2011;29(3):414-8. Epub 2010/10/13.

38. Yao W, Cheng Z, Shahnazari M, Dai W, Johnson ML, Lane NE. Overexpression of secreted frizzled-related protein 1 inhibits bone formation and attenuates parathyroid hormone bone anabolic effects. J Bone Miner Res. Feb 2010;25(2):190-9. Epub 2009/07/15.
39. Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, et al. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature. Jun 6 2002;417(6889):664-7. Epub 2002/06/07.
40. Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, et al. Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. Nat Genet. Sep 2005;37(9):945-52. Epub 2005/08/02.
41. Li L, Mao J, Sun L, Liu W, Wu D. Second cysteine-rich domain of Dickkopf-2 activates canonical Wnt signaling pathway via LRP-6 independently of dishevelled. J Biol Chem. Feb 22 2002;277(8):5977-81. Epub 2001/12/14.
42. Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. Osteoporos Int. Feb 2013;24(2):489-94. Epub 2012/04/25.
43. Arasu A, Cawthon PM, Lui LY, Do TP, Arora PS, Cauley JA, et al. Serum sclerostin and risk of hip fracture in older Caucasian women. J Clin Endocrinol Metab. Jun 2012;97(6):2027-32. Epub 2012/04/03.
44. Ardawi MS, Rouzi AA, Al-Sibiani SA, Al-Senani NS, Qari MH, Mousa SA. High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the Center of Excellence for Osteoporosis Research Study. J Bone Miner Res. Dec 2012;27(12):2592-602. Epub 2012/07/28.
45. Lim Y, Kim CH, Lee SY, Kim H, Ahn SH, Lee SH, et al. Decreased Plasma Levels of Sclerostin But Not Dickkopf-1 are Associated with an Increased Prevalence of Osteoporotic Fracture and Lower Bone Mineral Density in Postmenopausal Korean Women. Calcif Tissue Int. Oct 2016;99(4):350-9. Epub 2016/06/13.
46. Rossini M, Viapiana O, Idolazzi L, Ghellere F, Fracassi E, Troplini S, et al. Higher Level of Dickkopf-1 is Associated with Low Bone Mineral Density and Higher Prevalence of Vertebral Fractures in Patients with Ankylosing Spondylitis. Calcif Tissue Int. May 2016;98(5):438-45. Epub 2015/12/10.
47. Rodrigues AM, Eusebio M, Santos MJ, Gouveia N, Tavares V, Coelho PS, et al. The burden and undertreatment of fragility fractures among senior women. Arch Osteoporos. Mar 7 2018;13(1):22. Epub 2018/03/09.
48. WHO. Guidelines for preclinical evaluation and clinical trials in osteoporosis. Geneva. 1998.
49. Melton LJ, 3rd, Thamer M, Ray NF, Chan JK, Chesnut CH, 3rd, Einhorn TA, et al. Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation. J Bone Miner Res. Jan 1997;12(1):16-23.
50. Chen Z, Kooperberg C, Pettinger MB, Bassford T, Cauley JA, LaCroix AZ, et al. Validity of self-report for fractures

among a multiethnic cohort of postmenopausal women: results from the Women's Health Initiative observational study and clinical trials. Menopause. May-Jun 2004;11(3):264-74.

51. Ismail AA, O'Neill TW, Cockerill W, Finn JD, Cannata JB, Hoszowski K, et al. Validity of self-report of fractures: results from a prospective study in men and women across Europe. EPOS Study Group. European Prospective Osteoporosis Study Group. Osteoporos Int. 2000;11(3):248-54.
52. Honkanen K, Honkanen R, Heikkinen L, Kroger H, Saarikoski S. Validity of self-reports of fractures in perimenopausal women. Am J Epidemiol. Sep 01 1999;150(5):511-6.
53. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int. Apr 2008;19(4):385-97. Epub 2008/02/23.
54. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M. Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. Psychol Health Med. Mar 2007;12(2):225-35; quiz 35-7.
55. Fonseca JE. Biobanco-IMM, Lisbon Academic Medical Centre: a case study. Acta Med Port. Jul-Aug 2013;26(4):312-4. Epub 2013/09/11.
56. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. May 05 2009;150(9):604-12.
57. Rodrigues AM, Canhao H, Marques A, Ambrosio C, Borges J, Coelho P, et al. Portuguese recommendations for the prevention, diagnosis and management of primary osteoporosis - 2018 update. Acta Reumatol Port. Jan-Mar 2018;43(1):10-31. Epub 2018/03/31.
58. Gouveia N, Rodrigues AM, Ramiro S, Machado P, da Costa LP, Mourao AF, et al. EpiReumaPt: how to perform a national population based study - a practical guide. Acta Reumatol Port. Apr-Jun 2015;40(2):128-36.
59. Bao J, Zheng JJ, Wu D. The structural basis of DKK-mediated inhibition of Wnt/LRP signaling. Sci Signal. May 15 2012;5(224):pe22. Epub 2012/05/17.
60. Chan TF, Couchourel D, Abed E, Delalandre A, Duval N, Lajeunesse D. Elevated Dickkopf-2 levels contribute to the abnormal phenotype of human osteoarthritic osteoblasts. J Bone Miner Res. Jul 2011;26(7):1399-410. Epub 2011/02/12.
61. Rodrigues AM, Caetano-Lopes J, Vale AC, Vidal B, Lopes A, Aleixo I, et al. Low osteocalcin/collagen type I bone gene expression ratio is associated with hip fragility fractures. Bone. Dec 2012;51(6):981-9. Epub 2012/09/15.
62. Boivin G, Bala Y, Doublier A, Farlay D, Ste-Marie LG, Meunier PJ, et al. The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. Bone. Sep 2008;43(3):532-8. Epub 2008/07/05.
63. Roschger P, Paschalis EP, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. Bone. Mar 2008;42(3):456-66. Epub 2007/12/22.
64. Bodine PV, Billiard J, Moran RA, Ponce-de-Leon H, McLarney S, Mangine A, et al. The Wnt antagonist secreted frizzled-related protein-1 controls osteoblast and osteocyte apoptosis. J Cell Biochem. Dec 15 2005;96(6):1212-30. Epub 2005/09/09.

65. Polyzos SA, Anastasilakis AD, Bratengeier C, Woloszczuk W, Papatheodorou A, Terpos E. Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women--the six-month effect of risedronate and teriparatide. Osteoporos Int. Mar 2012;23(3):1171-6. Epub 2011/02/10.
66. Durosier C, van Lierop A, Ferrari S, Chevalley T, Papapoulos S, Rizzoli R. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. J Clin Endocrinol Metab. Sep 2013;98(9):3873-83. Epub 2013/07/19.
67. Amrein K, Dobnig H, Wagner D, Piswanger-Solkner C, Pieber TR, Pilz S, et al. Sclerostin in institutionalized elderly women: associations with quantitative bone ultrasound, bone turnover, fractures, and mortality. J Am Geriatr Soc. Jun 2014;62(6):1023-9. Epub 2014/05/02.
68. Anastasilakis AD, Polyzos SA, Avramidis A, Toulis KA, Papatheodorou A, Terpos E. The effect of teriparatide on serum Dickkopf-1 levels in postmenopausal women with established osteoporosis. Clin Endocrinol (Oxf). Jun 2010;72(6):752-7. Epub 2009/10/17.
69. Wanby P, Nobin R, Von SP, Brudin L, Carlsson M. Serum levels of the bone turnover markers dickkopf-1, sclerostin, osteoprotegerin, osteopontin, osteocalcin and 25-hydroxyvitamin D in Swedish geriatric patients aged 75 years or older with a fresh hip fracture and in healthy controls. J Endocrinol Invest. Aug 2016;39(8):855-63. Epub 2016/02/07.
70. Johnell O, Oden A, De Laet C, Garnero P, Delmas PD, Kanis JA. Biochemical indices of bone turnover and the assessment of fracture probability. Osteoporos Int. Jul 2002;13(7):523-6. Epub 2002/07/12.
71. Chubb SA, Byrnes E, Manning L, Beilby JP, Ebeling PR, Vasikaran SD, et al. Reference intervals for bone turnover markers and their association with incident hip fractures in older men: the Health in Men study. J Clin Endocrinol Metab. Jan 2015;100(1):90-9. Epub 2014/10/17.
72. Shigdel R, Osima M, Ahmed LA, Joakimsen RM, Eriksen EF, Zebaze R, et al. Bone turnover markers are associated with higher cortical porosity, thinner cortices, and larger size of the proximal femur and non-vertebral fractures. Bone. Dec 2015;81:1-6. Epub 2015/06/27.
73. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ. The accuracy of self-reported fractures in older people. J Clin Epidemiol. May 2002;55(5):452-7.

SECTION IV

STRATEGIES TO REDUCE NEW FRAGILITY FRACTURES IN PORTUGUESE POPULATION

PART 1 – MARQUES A, RODRIGUES AM, ROMEU JC, ET AL. 2016. MULTIDISCIPLINARY PORTUGUESE RECOMMENDATIONS ON DXA REQUEST AND INDICATION TO TREAT IN THE PREVENTION OF FRAGILITY FRACTURES. ACTA REUMATOL PORT. 41: 305-321.

PART 2 – RODRIGUES AM, CANHÃO H, MARQUES A, ET AL. 2018. PORTUGUESE RECOMMENDATIONS FOR THE PREVENTION, DIAGNOSIS AND MANAGEMENT OF PRIMARY OSTEOPOROSIS – 2018 UPDATE. ACTA REUMATOL PORT. 43:10-31.

PRÁTICA CLINICA

Multidisciplinary Portuguese recommendations on DXA request and indication to treat in the prevention of fragility fractures

Marques A[1], Rodrigues AM[2], Romeu JC[3], Ruano A[4], Barbosa AP[5], Simões E[5], Águas F[6], Canhão H[7], Alves JD[8], Lucas R[9], Branco JC[10], Laíns J[11], Mascarenhas M[12], Simões S[13], Tavares V[14], Lourenço O[15], da Silva JA[16]

ACTA REUMATOL PORT. 2016;41:305-321

INTRODUCTION

Osteoporosis (OP) is a metabolic skeletal disease characterized by low bone mass and microarchitecture deterioration leading to increased bone fragility and susceptibility to fracture. In Portugal, the annual hip fragility fracture incidence is estimated to be between 154 to 572 per 100.000 women and 77 to 232 per 100.000 men, depending on age[1]. More than 10.000 patients are admitted every year to the Portuguese National Health Service due to hip fragility fractures, justifying annual total health care expenditures of over 220 million euro[2]. This corresponded to 1.4% of the total national health care expenditure in 2013, including private and public services, according to Portuguese Health Statistics[3]. The total expense with fragility fractures is much higher, as hip fractures only account for about 39.1% of the total number of fragility fractures observed in Portugal according to a recent study[4].

Altogether, osteoporotic fractures currently represent an enormous social and economic burden in Portugal, despite the fact that this country has one of the lowest incidences of fragility fractures in Western Europe[1]. The size of the problem will tend to increase relentlessly due to the increasing ageing of the population and other societal changes[5], unless effective preventive measures are put in place.

This paper reports on the work of an Expert Committee convened to foster such measures, by providing physicians with practical and valid recommendations regarding the initiation of pharmacological treatment for osteoporosis and/or the request of DXA evaluation, in order to optimize the efficiency of interventions and minimize the costs and risks for individuals and society.

Since the last publication of recommendations for the diagnosis and treatment of osteoporosis in Portugal in 2007[6], the FRAX® tool has been incorporated in the clinical guidelines for OP of several countries[5,7-12]. In fact, over half of the subjects who experience a fragility fracture do not have OP as defined by BMD[13]. FRAX® integrates a set of well-proven clinical risk factors for fracture, independent of BMD: age, gender, body mass

1. Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal. Health Sciences Research Unit: Nursing (UICISA:E), Coimbra, Portugal
2. Representing the Portuguese Society of Rheumatology – SPR;
3. Rheumatology Department, Hospital de Santa Maria, Lisboa, Portugal
4. Representing Portuguese Society of Orthopaedics and Traumatology- SPOT
5. Representing the Portuguese Society of Osteoporosis and Metabolic Bone Diseases - SPODOM
6. Representing the Portuguese Society of Gynecology - SPG
7. EpiDoC Unit, CEDOC, NOVA Medical School, Universidade Nova de Lisboa, Lisboa, Portugal
8. Serviço Medicina 4, Hospital Prof Doutor Fernando Fonseca; CEDOC/NOVA Medical School; Representing the Portuguese Society for Internal Medicine - SPMI
9. EPIUnit - Institute of Public Health, University of Porto. Representing the National Observatory for Rheumatic Diseases – ONDOR
10. Centro Hospitalar de Lisboa Ocidental & CEDOC. Nova Medical School/Faculdade de Ciências Médicas da Universidade Nova de Lisboa
11. Representing the Portuguese Society of Physical and Rehabilitation Medicine – SPMFR
12. Representing the Portuguese Society of Endocrinology, Diabetes and Metabolism – SPDM
13. Representing the Portuguese Association of General Practitioners – APMGF
14. Representing the National Association against Osteoporosis - APOROS
15. Centro de Estudos e Investigação em Saúde da Universidade de Coimbra, -CEISUC, Faculdade de Economia da Universidade de Coimbra. Coimbra, Portugal
16. Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal. Clínica Universitária de Reumatologia, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

index, prior fragility fracture, parental history of hip fracture, long-term use of oral glucocorticoids, rheumatoid arthritis and other secondary causes of osteoporosis, current smoking and alcohol intake, with or without BMD. It provides an estimate of the risk of major osteoporotic fracture (hip, clinical spine, humerus or wrist fracture) and of hip fracture in the subsequent 10 years[14,15]. FRAX® provides valid predictions without BMD values[16,17], although its accuracy increases when BMD is also considered[18]. This algorithm is applied upon the fracture epidemiology and death rates of each country, to provide locally optimized estimates of fracture probability. The FRAX® was derived from population-based cohort studies from Europe, North America, Asia and Australia and has been validated in 62 countries and adopted by many as the key basis for decisions on whom to treat.

With this in mind, we have recently validated the FRAX model for the estimation of osteoporotic fracture probability in the portuguese population – FRAX®Port[15] (http://www.shef.ac.uk/FRAX/tool.aspx?country=53). Through systematic literature review and meta-analysis[19], as well as consensus discussion we have decided that FRAX® is the most appropriate instrument to achieve similar purposes in Portugal. Among its advantages lies the possibility of using it even in the absence of BMD, allowing its output to decide if and when DXA is needed.

We have also performed a nation-wide careful evaluation of the costs of hip fractures and their impact upon quality of life and mortality[2]. The fracture risk probabilities above which the different interventions become cost-effective, in the actual Portuguese settings, were defined based on matured economic methodology, assisted by internationally renowned experts[2].

These developments laid the optimal ground for a timely review of the Portuguese recommendations regarding the risk threshold for DXA investigation and pharmacological treatment of osteoporosis.

On these bases, we now recommend that decisions regarding the performance of dual X-ray absorptiometry (DXA) or the initiation of treatment are based on estimates of the actual risk of fracture and the economic implications of fractures and the different preventive strategies.

This report does not cover all possible management options and is not intended to override the individual physician's responsibility towards the patient or the personal choice of each patient. The authors wish to emphasize that formal guidance for every specific situation or co-morbidity cannot be provided due to lack of appropriate evidence. Judicious clinical judgment is required in such conditions.

This work, as well the series of supporting studies already published or under publication, have been funded by the Portuguese Government through the Direcção Geral da Saúde – DGS (Portuguese Health Directorate) following a proposal presented by Associação Nacional Contra a Osteoporose – APOROS (National Association Against Osteoporosis) and by an unrestricted grant from Amgen. None of the financial providers had any involvement in the design of the studies, interpretation of their results or the content of derived reports and recommendations.

A total of 10 recommendations were produced (Table I).

METHODS

DEVELOPMENT OF GUIDELINES

A number of national experts on osteoporosis and all the relevant Portuguese scientific societies were invited and accepted to participate in the development of these recommendations: Rheumatology; Orthopaedics and Traumatology; Endocrinology, Diabetes and Metabolism; Gynaecology; Internal Medicine; Physical and Rehabilitation Medicine; Family Medicine, National Observatory for Rheumatic Diseases and Portuguese Society for Osteoporosis and Metabolic Bone Diseases. The only national patient organization active in the field of osteoporosis, Associação Portuguesa Contra a Osteoporose – APOROS, also participated in the Committee. Altogether, the Committee had 17 voting members, all of whom are co-authors of this report.

Relevant questions to be addressed by the recommendations were defined by consensus in a first round of e-mail consultations upon a draft prepared by the Principal Investigator (JAPS) and the research fellow (AM). A thorough literature review was performed in order to address each question (AM and JAPS) and made available to the committee members prior to the meeting. The electronic search was performed in PubMed MEDLINE (2006- January 15th 2015). The search strategies included the following medical descriptors: "Osteoporosis", "Osteoporotic fractures", "Risk Assessment", "Algorithms," "Recommendations",

TABLE I. SUMMARY OF RECOMMENDATIONS ON DXA REQUEST AND INDICATION TO TREAT IN THE PREVENTION OF FRAGILITY FRACTURES

Recommendation	Votes	Average agreement
1 The implementation of general, non-pharmacological, preventive measures for osteoporosis, such as diet, vitamin D supplementation, exercise, falls prevention and monitoring the use of any bone active drug should apply to all ages, whenever correctable risk factors are identified, irrespective of FRAX® and BMD.	Approved 17/17 favorable votes	97.0% (75-100)
2 Pharmacological treatment for osteoporosis should be recommended, unless contraindicated, in all subjects over the age of 50, who have previously experienced either: A. ≥1 fragility fracture of the hip or ≥ 1 symptomatic vertebral fragility fracture or B. ≥2 fragility fractures, independently of the site of fracture or the absence of symptoms (e.g. two asymptomatic vertebral fractures).	Approved 17/17 favorable votes	95.6% (70-100)
3 All Portuguese women and men over the age 50 should have their ten-year risk of osteoporotic fracture estimated with the FRAX®Port tool, with or without DXA.	Approved 17/17 favorable votes	95.9 % (80-100)
4 For FRAX®Port estimates, without DXA, between 7% and 11% for major osteoporotic fracture AND between 2.0% and 3% for hip fracture, BMD of the femoral neck should be obtained and entered into a new FRAX®Port ten-year risk estimation (see Figure 2). DXA may be justified in additional special conditions, as described in text.	Approved 16 favorable votes and one abstention	90.9% (60-100)
5 A. In men and women with a fracture risk estimate (without BMD) below 7% for major osteoporotic fractures AND 2% for hip fracture a decision not to treat with pharmacological agents may be warranted, without the need to perform DXA. Applicable general preventive measures should be applied.	Approved 16 favorable votes and one abstention	95.0% (50-100)
5 B. In such cases, FRAX®Port estimates should be repeated with a frequency that depends on how close the previous estimate is to lower limit of indication to DXA and also on the occurrence of significant changes in clinical risk factors. (see Figure 2A).	Approved 16 favorable and 1 abstention	93.8% (60-100)
6 In men and women with a fracture risk estimate, without DXA, above, 11% for major osteoporotic fracture OR 3% for hip fracture, pharmacological treatment with generic alendronate is cost-effective and should be advised (unless contra-indicated), without the need to perform DXA. (see figure 2A).	Approved 16 favorable votes and one abstention	95.3% (80-100)
7 In men and women with a FRAX®Port ten-year risk estimate, including DXA, at or above 9% for major osteoporotic or 2.5% for hip fractures pharmacological treatment for osteoporosis with generic alendronate is cost-effective and should be advised (unless contra-indicated). (see Table I and Figure 2B).	Approved 17/17 favorable votes	93.2% (60-100)
8 The decision to start anti-osteoporotic treatment with agents other than generic alendronate should be informed by their respective cost-effectiveness thresholds (see Table III).	Approved 16 favorable votes and one against	88.1% (0-100)
9 A. In men and women with a FRAX®Port ten-year risk estimate, including DXA, below 9% for major osteoporotic AND below 2.5% for hip fractures, pharmacological agents are not cost-effective and a decision not to use them may be warranted. Applicable general preventive measures should be applied.	Approved 17/17 favorable votes	96.5% (80-100)
9 B. In such patients, DXA and FRAX®Port assessments should be repeated every 2 years or whenever clinical risk factors change significantly (see figure 2). DXA may not be needed in case the previous BMD values are reassuring.	Approved 16 favorable votes and one abstention	92.8% (75-100)
10 While using FRAX®Port for the sake of these recommendations, health professionals should be aware of several limitations of this tool and considerer judicious adjustments of the risk estimates provide by this tool in specific circumstances, described below.	Approved 17/17 favorable votes	97.6% (70-100)

"Guidelines", "Treatment", "Cost-effectiveness", "Bone Mineral Density" and "DXA". Original articles, reviews and guidelines regarding threshold for treatment initiation and DXA request were included in this review. References cited in published Systematic Reviews or in original articles were also checked.

Possible alternative answers to the elected questions, according to the collected evidence, were drafted by the principal investigator and submitted, together with the respective evidence, to the Expert Committee in a second round of emails. Committee members were asked to appraise the supportive evidence and alternative recommendations or to propose additional ones. All alternatives were circulated in a third round of e-mails, prior to the final face-to-face meeting.

This meeting was held on the 13th March 2015 to discuss the generated evidence, vote on the possible answers and thus generate a set of recommendations. The meeting was recorded for documentation and future clarification of doubts. The votes of individual representatives and degree of agreement regarding each recommendation were registered. Portuguese data on the cost-effectiveness of interventions according to different fracture risk thresholds were disclosed to the panel, for the first time, only after all the guiding principles, presented below, had been irrevocably established. They were only known to three of the members, who performed the study (AM, OL, JAPS). This strategy was adopted to guarantee that the cost-effectiveness basis for the decision to intervene was based on the grounds of guiding principles and not contaminated by considerations of the percentage of the population eligible for intervention, its overall costs, or the (dis)similarity of our intervention thresholds *vis-a-vis* other published guidance.

A final round of e-mails was conducted to refine some recommendations.

Finally, this paper was drafted and circulated among the committee members until a final version was reached and submitted to the individual societies' and associations' approval and endorsement.

UNDERLYING CONCEPTUAL DEFINITIONS: GUIDING PRINCIPLES

As a preparatory phase for the definition of the recommendations, the Committee planned and developed a detailed discussion dedicated to the establishment of a number of guiding principles and concepts. These are presented below:

GUIDING PRINCIPLE 1

Risk factors for osteoporosis, as those related with diet, exercise, sun exposure, medications, should be assessed by health professionals and patients throughout life, and corrected when appropriate

This guiding principle was approved by all committee members 17/17 votes.

Many risk factors for osteoporosis influence bone health from the earliest phases and throughout life, even if the consequences of osteoporosis only become apparent later in life. This is the case, for example, of diet (calcium, protein), exercise, vitamin D status, and medications such as glucocorticoids. All these conditions have health implications far beyond the limits of bone health and should, therefore, be considered as a medical routine. The correction of these risk factors is an integral part of osteoporosis management, usually referred to as "General Measures".

GUIDING PRINCIPLE 2

The decision to institute pharmacological treatment in osteoporosis should be based on the individual's ten-year risk of subsequent osteoporotic fracture as estimated by the FRAX®Port tool

This guiding principle was approved by all committee members 17/17 votes

FRAX® is an algorithm developed by the Centre for Metabolic Bone Diseases, University of Sheffield, UK, which allows the estimation of the individual risk of osteoporotic fractures over the subsequent 10 years on the basis of 11 clinical risk factors (CRFs) that have been shown, through individual studies and meta-analyses, to influence the risk of fracture, independently of BMD. They are all easily available in clinical practice: age, weight, height, prior fragility fracture, parental history of hip fracture, current tobacco smoking, ≥ 3 months glucocorticoids use, rheumatoid arthritis, causes of secondary osteoporosis (type I diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition, or malabsorption and chronic liver disease) and alcohol consumption. FRAX® can be used with or without BMD (Figure 1).

When calculated using only CRFs, i.e, without considering BMD, FRAX® has been shown to have a better performance than BMD alone in predicting major fracture risk[20]. The development of this tool was based on excellent methodology[14] and its validity has been ex-

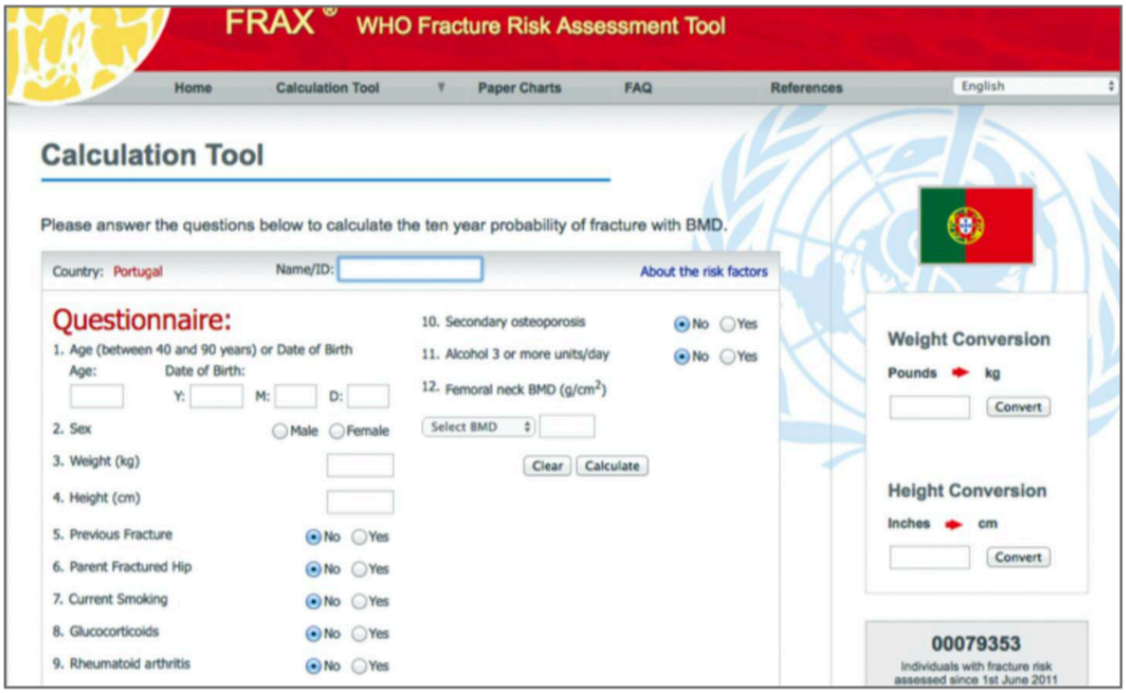

FIGURE 1. Screen page for input of data and risk estimation in the Portuguese version of the FRAX® tool (Portuguese model, version 3.9. http://www.shef.ac.uk/FRAX/tool.aspx?country=53)
[With permission of the Centre for Metabolic Bone Diseases, University of Sheffield Medical School, UK]

ternally confirmed, up until now, by twenty-six studies performed in different countries and cohorts[14,21-43]. A total of 62 countries and/or ethnic models, are currently available and several others are being developed[5].

A recent systematic literature review and meta-analysis performed by some of the Committee members[19] clearly demonstrated that FRAX is the most robust and accessible tool available to predict the risk of osteoporotic fractures. Its accuracy is well established and demonstrated by area under the curve (AUCs) from receiver operating characteristic (ROC) analysis for fracture prediction that range from 0.71 to 0.79 in meta-analysis. This performance in only surpassed by the QFracture tool[19], but this instrument requires the consideration of 31 clinical risk factors and has only been validated for the UK and Ireland.

The FRAX®Port tool is the Portuguese version of FRAX®, developed to incorporate the actual epidemiology of hip fractures and mortality in the general Portuguese population[15]. The methodology and results of this adaptation have been endorsed by the Sheffield University department responsible for FRAX® and by all Portuguese scientific societies and patients' organizations related to osteoporosis. It is readily available online.

GUIDING PRINCIPLE 3

The presence of previous fragility fractures justifies the consideration of pharmacological treatment, irrespective of the risk-estimate by the FRAX®Port tool

This guiding principle was approved by 15 favorable votes, one against and one abstention.

Several studies support the conclusion that it is cost-effective to treat individuals with a prior hip or vertebral fragility fracture[8,9,44]. Vertebral fractures, for example, are a very strong risk factor for subsequent hip and vertebral fracture[45,46], whereas forearm fractures predict future vertebral and hip fractures[47].

The vote against was justified on the basis that previous fractures are already accounted for in FRAX®.

The time elapsed since the last previous fracture is

also relevant: the risk of further fractures is greatest during the first 2–3 years but remains significantly elevated for up to 10–15 years (most notably for proximal femoral fractures, vertebral fractures, and humeral fractures) [48,49].

GUIDING PRINCIPLE 4

Physicians should be aware of the limitations of FRAX® and of DXA, and make judicious informed adaptations of the fracture risk estimate when such limitations apply

This guiding principle was approved by all committee members 17/17 favorable votes.

GUIDING PRINCIPLE 5

Portuguese intervention thresholds should be based on a similar FRAX® ten-year risk estimate for all ages. This principle should only be overruled if the health-economics evaluations demonstrated that the intervention threshold for any given age&gender group differs more than 50% from the value recommended on the basis of the overall population

This guiding principle was approved by 10 of the 17 committee members, 4 voted against and three abstained.

This was one of the most controversial points in the consensus meeting. The final recommendation is similar to the guidelines adopted by the National Osteoporosis Foundation – USA[12] and Canada[11]. In both these cases, the threshold for intervention was defined as the level of risk above which the cost per QALY gained was within the national acceptable limits. In both these guidelines, a similar value of estimated risk of fracture was adopted as the threshold for intervention for all ages and both genders, despite there being small age- and gender-related differences in the levels of risk that defined cost-effectiveness.

The recommendations issued by the United Kingdom's Royal College of Physicians[44], the Swiss association Against Osteoporosis[9] and the French National Authority for Health[7] adopted a different conceptual drive: Treatment is recommended for all people whose 10--year FRAX® estimated risk is equal or superior to that of a female patient of similar age, who has already suffered a fragility fracture. This concept is based on the fact that treatment in people with a previous fragility fracture has been shown to be cost-effective. Given that the risk of fracture increases with age, all other things being equal, this approach determines that the intervention threshold increases substantially with age. As an example, according to the UK guidance referred above, treatment will be recommended for a 50 year old whose 10-year risk of fracture is 7.5% but not for a 70 year-old whose ten-year estimated risk is 24%. The majority of our committee refused this philosophical approach. This was based mainly on the argument that the gain of one Quality-adjusted life year (QALY) should be considered of the same value for all ages. It was emphasized that age, as well as mortality are already considered in FRAX® and thus influence the fracture risk estimate. Overall, the majority of the committee decided to stand by the concept that, for the sake of equity, similar gains in health, as measured by QALYs, should justify similar financial efforts by society, irrespective of age.

GUIDING PRINCIPLE 6

The Portuguese intervention thresholds should be based on cost-effectiveness data

This guiding principle was approved by all committee members 17/17 votes.

By doing this, the Committee decided to accept that the threshold for intervention, at a population level, should be informed by economic considerations, rather than on a «political» perspective of a level of risk that would justify intervention, irrespective of its costs and societal willingness to pay. The committee thus acknowledges that the cost of intervention and the societal willingness to pay needs to be taken into account in decisions to treat or not to treat.

This principle implies that decisions to treat should have a similar foundation in all realms of medicine in our country – the impact of interventions in terms of QALYs gained should be calculated, the cost per QALY gained (or Incremental Cost-Effectiveness Ratio – ICER) determined and, naturally, a similar willingness to pay for a QALY should be applied, whatever the disease and intervention under consideration.

GUIDING PRINCIPLE 7

The intervention thresholds should be based on data reflecting the Portuguese reality on fractures, mortality, costs and treatment efficacy

This guiding principle was approved by all committee members 17/17 votes.

Recommendations on the level of fracture risk above which pharmacological intervention become cost-effective are inextricably dependent on dimensions that vary enormously at a national level, such as: epidemio-

logy of fractures, general mortality, mortality associated with fractures, medical interventions used in fracture cases, costs of caring for fractures, costs of preventive interventions, health policies, cost per QALY gained (ICER), economic status of the country and willingness to pay. This imposes the need to consider national data when making such decisions, and requires that intervention-threshold recommendations for Portugal had to wait until such data became available.

GUIDING PRINCIPLE 8

The threshold for pharmacological treatment of osteoporosis shall be established at ten-year risk estimates that correspond to a Willingness to Pay per QALY gained of €32,000.
The cheapest of all pharmacological interventions should be taken as the basis to decide on the actual intervention threshold for the Portuguese population.

This guiding principle was approved by 16 committee members and one abstention.

Cost-effectiveness of a given intervention can only be established by comparing its impact to a set value of willingness to pay for a QALY gained[50]. There is no established Portuguese national policy establishing Willingness to Pay for QALYs. So, the panel decided to endorse the recommendations issued by WHO, that this should be set at 2 fold the National Gross Product per capita[51] – 32.000€ is a rounding up of 2 × 16.400€, the Portuguese Gross domestic product (GDP) for year 2014[52].

The choice for the cheapest intervention as a reference is based on the fact that the costs as well as the effectiveness of each of the available alternatives are taken into account while establishing the respective Cost per QALY (ICER).

All the above decisions were made before the actual cost-effectiveness studies for Portugal were presented to the Committee.

GUIDING PRINCIPLE 9

DXA should be performed when it has a reasonable probability of changing the decision to treat/not to treat that can be taken on the basis of the FRAX®Port risk estimation made without DXA

This guiding principle was approved by 16 favorable votes and one abstention.

Adding DXA to CRFs in FRAX® results, according to our meta-analysis, in the improvement of the AUC from 0.74 to 0.79[19]. DXA may also assist the clinician in gauging the probability of secondary osteoporosis, in quantifying response to therapy and motivating the patient to treatment. The Committee considered that performing one DXA examination, at the time of deciding whether to treat, represents a relatively minor cost in view of the overall burden of the disease, which is compensated by the benefits than can be derived from that exam. This perspective led to a less stringent recommendation on when to perform DXA.

Based on this guiding principle the following concepts were defined for the purposes of these recommendations:

- **Intervention threshold:** A FRAX®Port ten-year risk-estimate value, with BMD, above which pharmacological treatment is warranted.
- **Range of fracture risk indicating the need for DXA:** A range of FRAX®Port ten-year risk-estimate, without BMD, within which DXA is justified, because it holds a reasonable probability of changing the decision to treat or not-to-treat.

Ideally, the lower and upper threshold for DXA evaluation would be based on real life Portuguese data establishing the probability of BMD inducing a change in the decision to treat/not to treat, around the intervention threshold. In the absence of such data, and taking into account the issues described above, the Committee consensually decided to establish these values at 2% and 0.5% above and below the intervention threshold for major osteoporotic and for hip fractures respectively.

COST-EFFECTIVENESS ANALYSIS

Once the above Guiding Principles were adopted, the Portuguese cost-effectiveness analysis with generic alendronate (the less expensive intervention) versus no treatment was presented to the Committee (Table II).

A detailed study in a representative sample of Portuguese patients with hip fractures was performed to establish the impact of osteoporotic fractures in terms of resource consumption (direct and indirect costs), mortality and quality of life. A societal perspective was adopted, i.e. all costs were considered irrespective of the payer being the patient or the security system[2].

These data were incorporated in a previously validated Markov economic model[53] which synthetized relevant available data, such as the incidence of fractures and their age distribution, the general population mortality, the cost, effectiveness and risk of adverse events

TABLE II. COST-EFFECTIVE INTERVENTION THRESHOLDS EXPRESSED AS THE 10-YEAR PROBABILITY OF A MAJOR /HIP FRACTURE (%) AT WHICH INTERVENTION WITH GENERIC ALENDRONATE BECOMES COST-EFFECTIVE IN COMPARISON TO NO TREATMENT, ADOPTING A WILLINGNESS TO PAY OF €32,000.00/QALY

Age	10-year probability of a major fracture (%)	10-year probability of a hip fracture (%)
50	8.6	2.6
55	8.7	2.4
60	10.4	3.0
65	9.2	2.3
70	8.6	2.3
75	8.1	2.1
80	7.1	1.7
85	5.9	1.3
All ages	8.8	2.5

The intervention threshold for "All ages" is not the arithmetic mean of the individual age-groups values but the result of QALY calculations including the overall population. Adapted from [54]

of the different medications, need for co-medications and control procedures and drop-out rates. This model allows the estimation of Incremental Cost-Effectiveness Ratio – ICER, for each intervention, a concept that can be understood as the cost paid for each QALY gained, in comparison to no treatment. The results were used to establish the levels of estimated risk of fracture at which each given intervention becomes cost effective, i.e. results in costs per QALY within the established willingness to pay.

Based on the published results[54], the Committee decided to adopt the FRAX®Port risk estimates of 9% for major osteoporotic fractures and 2.5% for hip fractures as the intervention thresholds for generic alendronate, in Portugal – Table II. The values for assessment threshold were established as 2% and 0.5% above and below the threshold of intervention for major osteoporotic or hip fractures, respectively.

RECOMMENDATIONS

RECOMMENDATION 1

The implementation of general, non-pharmacological, preventive measures for osteoporosis, such as diet, vitamin D supplementation, exercise, falls prevention and monitoring the use of any bone active drug should apply to all ages, whenever correctable risk factors are identified, irrespective of FRAX® and BMD

This recommendation was approved by all committee members the 17/17 votes and an average agreement of 97 % (mín.-máx.= 75-100) .

RECOMMENDATION 2

Pharmacological treatment for osteoporosis should be recommended, unless contraindicated, in all subjects over the age of 50 who have previously experienced either:

A. ≥ 1 fragility fracture of the hip or ≥1 symptomatic vertebral fragility fracture or

B. ≥ 2 fragility fractures, independently of the site of fracture or the absence of symptoms (e.g. two asymptomatic vertebral fractures).

This recommendation was approved by all committee members the 17/17 votes and an average agreement of 95.6 % (70-100).

Specifications to Recommendation 2

For this purpose, a fragility fracture is defined as a fracture occurring spontaneously or following minor trauma, i.e similar or inferior to that of a fall from body height, after exclusion of pathological local causes of fracture such as neoplasia.

This recommendation implies that the presence of such fractures overrides the FRAX®Port, i.e treatment should be considered in these patients irrespective of FRAX®Port risk-estimate or DXA measurements. This does not imply that FRAX® or DXA should not be performed, as they may provide useful information to guide further investigation and choice of therapeutic interventions.

Recommending treatment for people who have al-

ready endured a fragility fracture, irrespective of FRAX® is common to all of the abovementioned recommendations: NOF-USA[8] Canada[11], France[7] and Switzerland[9]. This concept is inherent to the NOGG/ /UK recommendations[44]. The exact definition varies between documents. No evidence was found to propose the inclusion of ≥ 2 fragility fractures (other than hip or clinical vertebral) for treatment without further assessment. This was a consensus recommendation, based on the authors' opinion and experience.

RECOMMENDATION 3

All Portuguese women and men over the age 50 should have their ten-year risk of osteoporotic fracture estimated with the FRAX®Port tool, with or without DXA

This recommendation was approved by all committee members 17/17 votes and an agreement of 95.9 % (80--100).

Specifications to recommendation 3

The decision to perform DXA should, ideally, be based on this initial FRAX®Port without BMD, as described below. However, if a recent BMD is already available, its value should be entered in the FRAX®Port calculation. The decision process for treatment should, in such case, be based on Recommendations 7, 8 and 9. DXA values can be acceptable for this purpose for up two years, unless significant events for bone metabolism take place meanwhile.

Physicians are strongly recommended to strictly adhere to the definitions of clinical risk factors as described in the FRAX® website.

RECOMMENDATION 4

For FRAX®Port estimates, without DXA, between 7% and 11% for major osteoporotic fracture and between 2% and 3% for hip fracture, BMD of the proximal femur, and, if possible and indicated, the spine should be assessed and the results of femoral neck T-score entered into FRAX®Port. (Figure 2). DXA may be justified in additional special conditions, as described below.

This recommendation was approved by 16 favorable votes and one abstention with an average agreement of 90.9 % (60-100).

Specifications to recommendation 4

For the purposes of this recommendation, BMD should be assessed by dual x-ray absorptiometry (DXA).

The spine and proximal femur, are the sites recommended for DXA evaluation[55]. Spine DXA is prone to overestimate BMD in the presence of osteoarthritis, vertebral fractures and other calcifying changes overlaying the sites of interest.

The T score value for the femoral neck should be used for FRAX®Port.

In the context of decision to/not-to treat, DXA results must be considered in the context of FRAX®Port and not in isolation. This principle implies that the diagnosis of osteoporosis or osteopenia based on densitometry does not, *per se*, warrant the initiation of pharmacological treatment for osteoporosis.

The use of DXA for monitoring therapy is controversial, it is rarely justifiable at intervals of less than 2-3 years and may be dispensable altogether if the

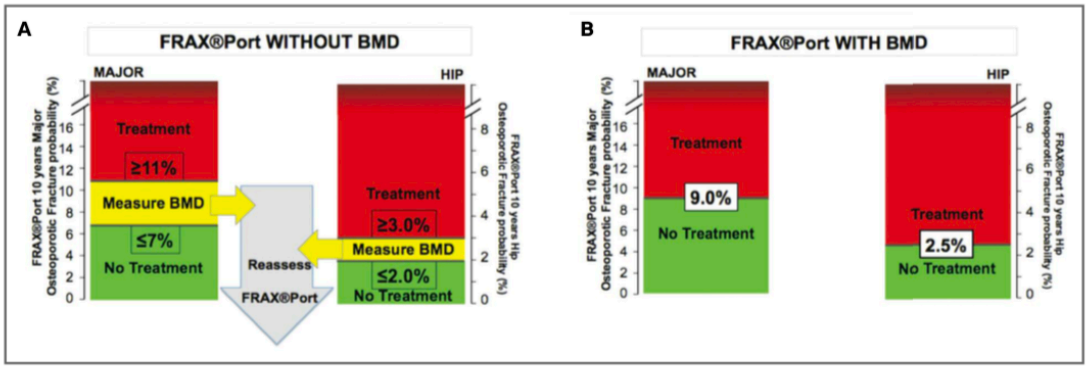

FIGURE 2. Use of FRAX®Port ten-year estimated risk of major osteoporotic and hip fractures to decide on request of DXA and on initiation of pharmacologic treatment for osteoporosis. A: Estimates without BMD. B. Estimates with BMD.

adherence to effective therapy is guaranteed (for more info on the appropriate use and interpretation of DXA see references[18,56,57]).

The committee considers that performing DXA may occasionally be justified outside these FRAX boundaries or irrespective of them, including in the conditions described in Table III.

Other conditions, with less well-established relationship with osteoporosis, may also justify the performance of DXA as part of the diagnostic work-up. These include Cystic fibrosis; Ehlers-Danlos; Gaucher's disease; Glycogen storage diseases; Hemochromatosis; Homocystinuria; Hypophosphatasia; Marfan syndrome; Menkes steely hair syndrome; Porphyria; Riley-Day syndrome; Athletic amenorrhea; Hyperprolactinemia; Panhypopituitarism; Turner's and Klinefelter's syndromes; Cushing's syndrome; Thyrotoxicosis; Gastric bypass; Gastrointestinal surgery; Pancreatic disease; Primary biliary cirrhosis; Hemophilia; Leukemia; Lymphomas; Monoclonal gammopathies; Multiple myeloma; Sickle cell disease; Systemic mastocytosis; Thalassemia; Ankylosing spondylitis; Systemic lupus erythematosus; Amyloidosis; Chronic metabolic acidosis; Chronic obstructive lung disease; Congestive heart failure; Depression; End-stage renal disease; Hypercalciuria; Idiopathic scoliosis; Post-transplant bone disease; Sarcoidosis; type I diabetes mellitus.

Some medications with less well-established relationship with osteoporosis, may also justify the performance of DXA in special cases. These include: Aluminum (in antacids); Anticoagulants (heparin); Barbiturates; Cancer chemotherapeutic drugs; Depomedroxyprogesterone; Lithium; Cyclosporine A and tacrolimus; Methotrexate; Parental nutrition; Proton pump inhibitors; Selective serotonin reuptake inhibitors; Tamoxifen®; Thiazolidinediones (such as Actos®); Thyroid hormones (in excess).

TABLE III. CONDITIONS/DISEASES AND TREATMENTS WITH IMPACT UPON BMD, AS ESTABLISHED BY SYSTEMATIC LITERATURE REVIEWS AND/OR META-ANALYSIS

Patients with the following conditions/diseases	Patients starting or under the following medications
Fragility fracture age ≤50 years (58)	Androgen deprivation therapy (59-61)
Prolonged immobilization and paralysis(62, 63)	Glucocorticoids (64)
Falls history (5, 6, 8, 11, 18)	Anticonvulsants (65)
Anorexia nervosa (66, 67)	Gonadotropin-releasing hormone analogues (GnRH) (68-70)
Calcium and vitamin D deficiency (5, 8, 71, 72)	Aromatase inhibitors (73-77)
Intestinal malabsorption (8, 78)	Antiretroviral therapy (72, 79)
Rheumatoid arthritis (80)	
Hyperparathyroidism (81, 82)	

RECOMMENDATION 5

A. In men and women with a fracture risk estimate (without BMD) below 7% for major osteoporotic fractures and 2% for hip fracture a decision not to treat with pharmacological agents may be warranted, without the need to perform DXA. Applicable general preventive measures should be applied

This recommendation was approved by 16 favorable votes and one abstention with an average agreement of 95 % (50-100).

B. In such cases, FRAX®Port estimates should be repeated with a frequency that depends on how close the previous estimate is to lower limit of indication to DXA and also on the occurrence of significant changes in clinical risk factors. (Figure 2a)

This recommendation was approved by 16 favorable votes and one abstention with an average agreement of 93.8 % (60-100).

Regarding recommendation 5B the Committee presumes that FRAX®Port reassessments will, on average, in such cases, be justified every 5 years from age 50 to 70 and every two to three years thereafter, in the absence of relevant intercurrences.

RECOMMENDATION 6

In men and women with a fracture risk estimate, without DXA, above, 11% for major osteoporotic fracture or 3% for hip fracture, pharmacological treatment with generic alendronate is cost-effective and should be advised (unless contra-indicated), without the need to perform DXA. (Figure 2a)

This recommendation was approved by 16 favorable

votes and one abstention and an average agreement of 95.3 % (80-100).

RECOMMENDATION 7

In men and women with a FRAX®Port ten-year risk-estimate, including DXA, at or above 9% for major osteoporotic or 2.5% for hip fractures pharmacological treatment for osteoporosis with generic alendronate is cost-effective and should be advised (unless contra-indicated).
(See Table II and Figure 2B)
This recommendation was approved by all committee members 17/17 votes with an average agreement of 93.2 % (60-100).

RECOMMENDATION 8

The decision to start anti-osteoporotic treatment with agents other than generic alendronate should be informed by their respective cost-effectiveness thresholds (Table IV)
This recommendation was approved by 16 favorable votes and one against with an average agreement of 88.1 % (0-100).

Specifications to recommendation 8
This recommendation does not preclude the decision to prescribe these medications at lower risk-estimates, based on clinical grounds, such as formal-contraindication to less expensive alternatives, or conditions making the selected choice especially appropriate. The individual physician may also decide to adopt a different willingness to pay.

This specification was approved by 16 favorable votes and one against and an average agreement of 99.3% (90-100).

The cost per QALY associated with different medications is affected by their cost and effectiveness in different clinical settings. IV presents the risk-estimate levels at which treatment with zoledronic acid, denosumab and teriparatide become cost-effective in comparison to no-treatment and may, thus, be recommended on cost-effectiveness grounds, as described by Marques et al[54].

The authors want to highlight that no national data is available on cost-effectiveness thresholds for other drugs. The only alternative is to extrapolate based on indicators of effectiveness, persistence and cost of those alternative drugs compared to the studied options.

RECOMMENDATION 9

A. In men and women with a FRAX®Port ten-year risk estimate, including DXA, below 9% for major osteoporotic and below 2.5% for hip fractures, pharmacological agents are not cost-effective and a decision not to use them may be warranted. Applicable general preventive measures should be applied
This recommendation was approved by all committee members the 17/17 votes and an average agreement of 96.5 % (80-100).

B. In such patients, DXA and FRAX®Port assessments should be repeated every 2 years or whenever clinical risk factors change significantly (Figure 2). DXA may not be needed in case the previous BMD values are reassuring
This recommendation was approved by 16 favorable votes one abstention and an agreement of 92.8% (75--100).

TABLE IV. COST-EFFECTIVENESS THRESHOLDS FOR SEVERAL MEDICATIONS, BASED ON THE FRAX®PORT TEN-YEAR OSTEOPOROTIC FRACTURE RISK ESTIMATE, BASED ON A WILLINGNESS TO PAY OF 32.000€/QALY AND CURRENT COST OF MEDICATION. ADAPTED FROM [54]

		Without DXA		With DXA	
	Cost basis/year (€)	Major %	Hip %	Major %	Hip %
Generic alendronate	99	11	3	9	2.5
Zoledronic acid	347	22	12	20	10
Denosumab	552	37	25	35	23
Teriparatide	4234	80	65	78	63

RECOMMENDATION 10

While using FRAX®Port for the sake of these recommendations, health professionals should be aware of several limitations of this tool and considerer judicious adjustments of the risk estimates provide by this tool in specific circumstances, described below

This recommendation was approved by all committee members the 17/17 favorable votes with an average agreement of 97.6 % (70-100).

Specifications of recommendation 10

1. The limitations of FRAX®Port are the same as those of FRAX®. Some of these may be resolved in future revisions of the tool;
2. FRAX® does not take into account the number of prior fragility fractures[18], but this limitation is overcome by the Committees decision to recommend previous fragility fracture as an independent criterion to start treatment.
3. FRAX® has not been validated to be used in patients under osteoporotic treatment or for monitoring the effects of treatment[18].

   This specification was approved by 16 favorable votes, one abstention and an average agreement of 100%.
4. Falls are an important clinical risk factor for fractures and are not included in the FRAX® tool[9]. No formal recommendation can be made for this purpose, due to lack of appropriate scientific evidence. The best reference values that we can be provided are based on calculations performed with the QFracture®2013[83], a validated and accurate fracture risk estimation tool, which considers falls. In this context, the presence of a "history of falls", multiplies by a factor of around 1.5, the 10-year fracture risk estimate made in its absence.

   This specification was approved by 17 favorable votes and an average agreement of 92.1 % (0-100).
5. The FRAX tool does not take into account the corticosteroid dose above 5mg Prednisolone equivalent for three months. The Committee recommends that the 10-year probabilities of a hip fracture or a major osteoporotic fracture be adjusted according to the dose of glucocorticoids as described in Table V. No adjustments regarding duration of treatment can be proposed, due to lack of appropriate evidence.

   This specific recommendation was approved by 16 favorable votes, one abstention and an average agreement of 87.5% (50-100).
6. FRAX® algorithm uses T-score for femoral neck BMD and does not take into account the lumbar spine BMD. However, when there is a large discordance (> 1SD) in the T-score of femoral neck and lumbar spine, it is proposed that the clinician may increase/decrease FRAX® estimate for major osteoporotic fractures by 10% for each rounded T-score difference between the lumbar spine and femoral neck[5,85].

   For example if T-score femoral neck = -1.5 and T-score lumbar spine = -2.8, the FRAX® estimate for major osteoporotic fractures should be increased by 10% percent (for example from 7% to 7.7%). If the values were -1.5 and -1.9 respectively, no changes should be made (difference <0.5 T). If femoral neck T score = -2.3 and lumbar spine T score = -3.9, the difference (1.6) is rounded to 2 T score and the major osteoporotic fractures risk estimate should be increase by 20% (for example from 8% to 9.6%, justifying medication according to the present recommendations).
   As in all other circumstances, it is important to guarantee the quality and validity of lumbar spine DXA.

   This specification was approved by 17 favorable votes and an agreement of 91.5% (75-100).

In Figure 3 we present a simplified integrated flow chart of decisions on treatment and DXA assessment according to the current recommendations. Take into account that the intervention thresholds are based on calculations for generic alendronate. Please refer to recommendation 8 to adapt for other medications.

TABLE V. RECOMMENDED ADJUSTMENT OF 10-YEAR PROBABILITIES FOR MAJOR OSTEOPOROTIC FRACTURE OR HIP FRACTURE FOR ALL AGES, ACCORDING TO DAILY DOSE OF GLUCOCORTICOIDS. ADAPTED FROM [5,84]. MULTIPLY THE FRAX®PORT FRACTURE RISK ESTIMATE BY THE PROVIDED ADJUSTMENT FACTOR

	Adjustment factor for ten year-probability estimates (for all ages)	
Prednisolone equivalent (mg/day)	Major osteoporotic fracture	Hip fracture
<2.5	0.8	0.65
2.5–7.5	No adjustment	
≥7.5	1.15	1.20

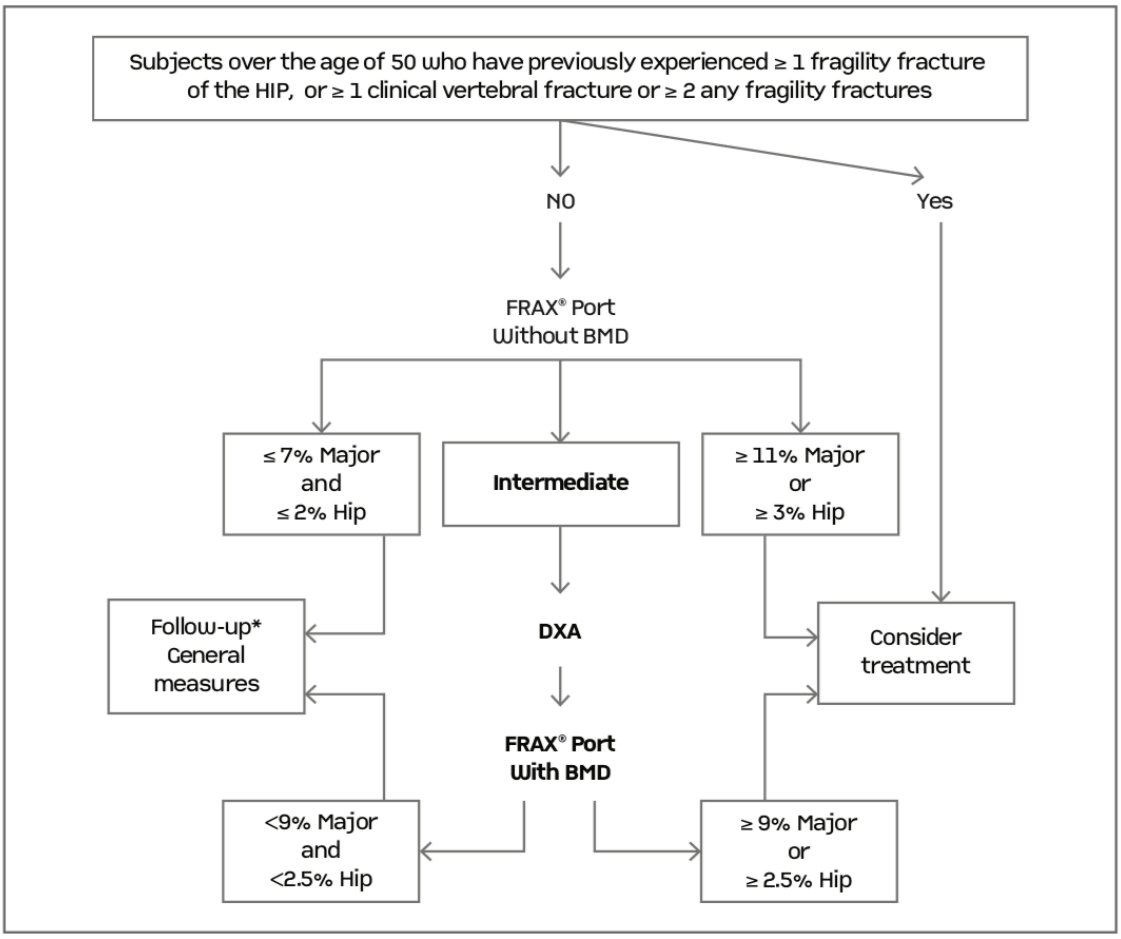

FIGURE 3. Integrated approach of osteoporosis intervention thresholds and DXA request for Portuguese patients according to the current recommendations. Intervention thresholds described in this figure are appropriate for generic alendronate. Consider recommendation 8 (Table IV) for other agents.
BMD = bone mineral density; DXA= Dual-energy X-ray absorptiometry; *Follow up – Repeat assessments as suggested in recommendations 5B and 9B

DISCUSSION AND FINAL REMARKS

Ten recommendations regarding who to treat for osteoporosis and who to examine with DXA in daily clinical practice have been developed for Portuguese patients, based on consensualized guiding principles and updated epidemiologic and economic evaluations in the Portuguese setting (Table I). The recommendations are practical, evidence-based and supported by a panel of experts and representatives of all Portuguese scientific societies and patients' associations with an interest in Osteoporosis.

Evidence was used as the basis for recommendations as much as possible and this was supplemented by collegial decisions of the experts when decisive evidence was lacking. Considerable effort was put in to trying to keep the recommendations as simple, but also comprehensive, i.e capable of responding to most of the practicing clinicians needs.

These recommendations provide a much more robust and rationale basis for treatment decisions than considering solely the bone mineral density (BMD) or asking clinicians to base decisions on a subjective weighting of clinical risk factors. FRAX® allows the in-

tegration of a large number of clinical risk factors for fractures, whose relevance has been proven by evidence and whose impact has been estimated by meta-analysis. Moreover, the Portuguese version of FRAX incorporates the actual epidemiology of fragility fractures and mortality in the target population. The consideration of cost-effectiveness analyses of interventions in our actual epidemiologic and economic context, responds to the responsibility of making judicious use of the limited resources available for health care. These calculations were performed using state-of-the-art economic models and prestiged economic counseling. The adopted willingness to pay follows international recommendations.

A certain degree of arbitrariness was used in establishing the same cost-effective intervention threshold for all ages, despite there being considerable variability between the age groups. The same applies to the amount adopted as willingness to pay (WTP): some practitioners may have a different view and the WTP may change according to GDP and national health policies. Expert users may wish to produce a more precise definition of cost-effective threshold for specific individual cases, taking into account the patient's age, the medication being considered or a WTP of their own choice. This can be achieved through the use of a dedicated tool made available by Marques et al[54] https://dl.dropboxusercontent.com/u/4287154/OsteoporoseThrCalc/ThreshComputationPortugalFINAL. xlsm.

These recommendations represent an important paradigm shift, which was made possible by the development of FRAX®, its Portuguese adaptation and the economic evaluations described above. We believe that the potential of this change towards supporting a more efficient use of human and financial resources in the combat to the ever-growing epidemics of osteoporotic fractures is truly enormous. However, it all depends on the use that health professionals, both individually and as a community, make of these new tools. It is expected that the endorsement of these recommendations by all the experts and societies represented will increase their dissemination and implementation into national clinical practice, thus expanding their potential to foster progress on the current standard of osteoporosis management in our country.

We will be greatly indebted to all health professionals who may be willing to share their views and experiences on using these recommendations and offer suggestions on how to improve their reach on behalf of public health (reuma@huc.min-saude.pt)

This article has been copublished in the issue no. 6 of Revista Portuguesa de Medicina Geral Familiar (Rev Port Med Geral Fam 2016; 32:425-441).

CORRESPONDENCE TO
José António Pereira da Silva
Serviço de Reumatologia, 7º piso,
Centro Hospitalar e Universitário de Coimbra
Coimbra, Portugal
E-mail: jdasilva@chuc.min-saude.pt

REFERENCES

1. de Pina MF, Alves SM, Barbosa M, Barros H. Hip fractures cluster in space: an epidemiological analysis in Portugal. Osteoporosis international 2008;19(12): 1797-1804.
2. Marques A, Lourenco O, da Silva JA. The burden of osteoporotic hip fractures in Portugal: costs, health related quality of life and mortality. Osteoporosis international 2015;26(11):2623-2630.
3. Instituto Nacional de Estatística. Conta Satélite da Saúde 2000-2012. INE IPL, Portugal. 2013.
4. Rodrigues V. Epidemiologia de Fraturas Osteoporóticas no Serviço de Urgência dos CHUC: Faculdade de Medicina, Universidade de Coimbra; 2015.
5. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporosis international 2013;24(1):23-57.
6. Tavares V, Canhao H, Gomes JA, Simoes E, Romeu JC, Coelho P, et al. [Recommendations for the diagnosis and management of osteoporosis]. Acta reumatologica portuguesa 2007;32(1): 49-59.
7. Briot K, Cortet B, Thomas T, Audran M, Blain H, Breuil V, et al. 2012 update of French guidelines for the pharmacological treatment of postmenopausal osteoporosis. Joint, bone, spine: revue du rhumatisme. 2012;79(3):304-313.
8. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporosis international 2014;25(10):2359-2381.
9. Lippuner K, Johansson H, Borgstrom F, Kanis JA, Rizzoli R. Cost-effective intervention thresholds against osteoporotic fractures based on FRAX(R) in Switzerland. Osteoporosis international 2012;23(11):2579-2589.
10. Makras P, Athanasakis K, Boubouchairopoulou N, Rizou S, Anastasilakis AD, Kyriopoulos J, et al. Cost-effective osteoporosis treatment thresholds in Greece. Osteoporosis international 2015 Jul;26(7):1949-1957.
11. Papaioannou A, Morin S, Cheung AM, Atkinson S, Brown JP, Feldman S, et al. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2010;182(17):1864-1873.
12. Tosteson AN, Melton LJ, 3rd, Dawson-Hughes B, Baim S, Favus MJ, Khosla S, et al. Cost-effective osteoporosis treatment thresholds: the United States perspective. Osteoporosis international 2008;19(4):437-447.
13. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004;34(1):195-202.

14. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporosis International. 2007;18(8):1033--1046.
15. Marques A, Mota A, Canhao H, Romeu JC, Machado P, Ruano A, et al. A FRAX model for the estimation of osteoporotic fracture probability in Portugal. Acta reumatologica portuguesa. 2013;38(2):104-112.
16. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002;359(9321):1929-1936.
17. Johansson H, Kanis JA, Oden A, Johnell O, McCloskey E. BMD, clinical risk factors and their combination for hip fracture prevention. Osteoporosis international. 2009;20(10):1675-1682.
18. Kanis JA, Hans D, Cooper C, Baim S, Bilezikian JP, Binkley N, et al. Interpretation and use of FRAX in clinical practice. Osteoporosis international. 2011;22(9):2395-2411.
19. Marques A, Ferreira RJ, Santos E, Loza E, Carmona L, da Silva JA. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Annals of the rheumatic diseases. 2015;74(11):1958-1967.
20. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18(8):1033-1046.
21. Azagra R, Roca G, Martin-Sanchez JC, Casado E, Encabo G, Zwart M, et al. FRAX(registered trademark) thresholds to identify people with high or low risk of osteoporotic fracture in Spanish female population. Medicina clinica 2015 Jan 6;144(1):1--8.
22. Bolland MJ, Siu AT, Mason BH, Horne AM, Ames RW, Grey AB, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. Journal of bone and mineral research. 2011;26(2):420-427.
23. Brennan SL, Leslie Wd Fau - Lix LM, Lix Lm Fau - Johansson H, Johansson H Fau - Oden A, Oden A Fau - McCloskey E, McCloskey E Fau - Kanis JA, et al. FRAX provides robust fracture prediction regardless of socioeconomic status. (1433-2965 (Electronic).
24. Cheung EYN, Bow CH, Cheung CL, Soong C, Yeung S, Loong C, et al. Discriminative value of FRAX for fracture prediction in a cohort of Chinese postmenopausal women. Osteoporosis International. 2012;23(3):871-878.
25. Cummins NM, Poku EK, Towler MR, O'Driscoll OM, Ralston SH. clinical risk factors for osteoporosis in Ireland and the UK: a comparison of FRAX and QFractureScores. Calcified tissue international. 2011;89(2):172-177.
26. Donaldson MG, Palermo L, Schousboe JT, Ensrud KE, Hochberg MC, Cummings SR. FRAX and risk of vertebral fractures: the fracture intervention trial. Journal of bone and mineral research. 2009;24(11):1793-1799.
27. Ensrud KE, Lui LY, Taylor BC, Schousboe JT, Donaldson MG, Fink HA, et al. A comparison of prediction models for fractures in older women: Is more better? Archives of internal medicine. 2009;169(22):2087-2094.
28. Ettinger B, Ensrud KE, Blackwell T, Curtis JR, Lapidus JA, Orwoll ES. Performance of FRAX in a cohort of community-dwelling, ambulatory older men: the Osteoporotic Fractures in Men (MrOS) study. Osteoporosis international. 2013;24(4):1185--1193.
29. Fraser LA, Langsetmo L, Berger C, Ioannidis G, Goltzman D, Adachi JD, et al. Fracture prediction and calibration of a Canadian FRAX(R) tool: a population-based report from CaMos. Osteoporosis international. 2011;22(3):829-837.
30. Friis-Holmberg T, Rubin KH, Brixen K, Tolstrup JS, Bech M. Fracture risk prediction using phalangeal bone mineral density or FRAX(registered trademark)? A danish cohort study on men and women. Journal of Clinical Densitometry. 2014;17(1):7-15.
31. Gonzalez-Macias J, Marin F, Vila J, Diez-Perez A. Probability of fractures predicted by FRAX(registered trademark) and observed incidence in the Spanish ECOSAP Study cohort. Bone. 2012;50(1):373-377.
32. Hillier TA, Cauley Ja Fau - Rizzo JH, Rizzo Jh Fau - Pedula KL, Pedula Kl Fau - Ensrud KE, Ensrud Ke Fau - Bauer DC, Bauer Dc Fau - Lui L-Y, et al. WHO absolute fracture risk models (FRAX): do clinical risk factors improve fracture prediction in older women without osteoporosis? 2011;26(8):1774-1782.
33. Langsetmo L, Nguyen TV, Nguyen ND, Kovacs CS, Prior JC, Center JR, et al. Independent external validation of nomograms for predicting risk of low-trauma fracture and hip fracture. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2011;183(2):E107-114.
34. Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E, Kanis JA. Independent clinical validation of a Canadian FRAX tool: fracture prediction and model calibration. Journal of bone and mineral research. 2010;25(11):2350-2358.
35. Premaor M, Parker RA, Cummings S, Ensrud K, Cauley JA, Lui LY, et al. Predictive value of FRAX for fracture in obese older women. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2013;28(1):188-195.
36. Pressman AR, Lo JC, Chandra M, Ettinger B. Methods for assessing fracture risk prediction models: experience with FRAX in a large integrated health care delivery system. Journal of clinical densitometry. 2011;14(4):407-415.
37. Sambrook PN, Flahive J, Hooven FH, Boonen S, Chapurlat R, Lindsay R, et al. Predicting fractures in an international cohort using risk factor algorithms without BMD. Journal of bone and mineral research. 2011;26(11):2770-2777.
38. Sandhu SK, Nguyen ND, Center JR, Pocock NA, Eisman JA, Nguyen TV. Prognosis of fracture: evaluation of predictive accuracy of the FRAX algorithm and Garvan nomogram. Osteoporosis international. 2010;21(5): 863-871.
39. Sornay-Rendu E, Munoz F, Delmas PD, Chapurlat RD. The FRAX tool in French women: How well does it describe the real incidence of fracture in the OFELY cohort. Journal of Bone and Mineral Research. 2010;25(10):2101-2107.
40. Sund R, Honkanen R Fau - Johansson H, Johansson H Fau - Oden A, Oden A Fau - McCloskey E, McCloskey E Fau - Kanis J, Kanis J Fau - Kroger H, et al. Evaluation of the FRAX model for hip fracture predictions in the population-based Kuopio Osteoporosis Risk Factor and Prevention Study (OSTPRE). (1432--0827 (Electronic)).
41. Tamaki J, Iki M, Kadowaki E, Sato Y, Kajita E, Kagamimori S, et al. Fracture risk prediction using FRAX(R): a 10-year follow-up survey of the Japanese Population-Based Osteoporosis (JPOS) Cohort Study. Osteoporosis international 2011;22(12):3037--3045.
42. Tebe Cordomi C, del Rio LM, Di Gregorio S, Casas L, Estrada MD, Kotzeva A, et al. Validation of the FRAX predictive model for major osteoporotic fracture in a historical cohort of spanish

women. Journal of Clinical Densitometry. 2013;16(2):231-237.
43. Tremollieres FA, Pouilles JM, Drewniak N, Laparra J, Ribot CA, Dargent-Molina P. Fracture risk prediction using BMD and clinical risk factors in early postmenopausal women: sensitivity of the WHO FRAX tool. Journal of bone and mineral research 2010;25(5):1002-1009.
44. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A. Case finding for the management of osteoporosis with FRAX—assessment and intervention thresholds for the UK. Osteoporosis international 2008;19(10):1395-1408.
45. Johnell O, Oden A, Caulin F, Kanis JA. Acute and long-term increase in fracture risk after hospitalization for vertebral fracture. Osteoporosis international 2001;12(3):207-214.
46. Klotzbuecher CM, Ross PD, Landsman PB, Abbott TA, 3rd, Berger M. Patients with prior fractures have an increased risk of future fractures: a summary of the literature and statistical synthesis. Journal of bone and mineral research. 2000;15(4):721--739.
47. Cuddihy MT, Gabriel SE, Crowson CS, O'Fallon WM, Melton LJ, 3rd. Forearm fractures as predictors of subsequent osteoporotic fractures. Osteoporosis international. 1999;9(6):469-475.
48. van Geel TA, van Helden S, Geusens PP, Winkens B, Dinant GJ. Clinical subsequent fractures cluster in time after first fractures. Annals of the rheumatic diseases. 2009;68(1):99-102.
49. Giangregorio LM, Leslie WD. Time since prior fracture is a risk modifier for 10-year osteoporotic fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(6):1400-1405.
50. Borgstrom F, Johnell O, Kanis JA, Jonsson B, Rehnberg C. At what hip fracture risk is it cost-effective to treat? International intervention thresholds for the treatment of osteoporosis. Osteoporosis international. 2006;17(10):1459-1471.
51. Marseille E, Larson, B., Kazi, D. S., Kahnd, J. G., & Rosenb, S. Thresholds for the cost–effectiveness of interventions: alternative approaches. Bulletin of the World Health Organization 2015;93:118-124.
52. European Commission Economic and Financial Affairs. http://ec.europa.eu/economy_finance/ameco/user/serie/ResultSerie.cfm.Accessed 7 Feb 2015.
53. Borgstrom F, Strom O, Kleman M, McCloskey E, Johansson H, Oden A, et al. Cost-effectiveness of bazedoxifene incorporating the FRAX(R) algorithm in a European perspective. Osteoporosis international. 2011;22(3):955-965.
54. Marques A, Lourenco O, Ortsater G, et al. Cost-Effectiveness of Intervention Thresholds for the Treatment of Osteoporosis Based on FRAX® in Portugal. Calcif Tissue Int 2016;99(2):131--141.
55. Blake GM, Fogelman I. The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. Postgraduate medical journal. 2007;83(982):509-517.
56. Schousboe JT, Shepherd JA, Bilezikian JP, Baim S. Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. Journal of clinical densitometry 2013;16(4):455-466.
57. Watts NB. Fundamentals and pitfalls of bone densitometry using dual-energy X-ray absorptiometry (DXA). Osteoporosis international. 2004;15(11):847-854.
58. Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Archives of osteoporosis. 2013;8(1-2):136.
59. Ding H, Yang L, Du W, Teng Y, Fu SJ, Tao Y, et al. Bisphosphonates for osteoporosis in nonmetastatic prostate cancer patients receiving androgen-deprivation therapy: a systematic review and meta-analysis. Asian Pacific journal of cancer prevention : APJCP. 2013;14(5):3337-3343.
60. Serpa Neto A, Tobias-Machado M, Esteves MA, Senra MD, Wroclawski ML, Fonseca FL, et al. A systematic review and meta-analysis of bone metabolism in prostate adenocarcinoma. BMC Urol. 2010;10:9.
61. Lattouf JB, Saad F. Bone complications of androgen deprivation therapy: screening, prevention, and treatment. Current opinion in urology. 2010;20(3):247-252.
62. Charmetant C, Phaner V, Condemine A, Calmels P. Diagnosis and treatment of osteoporosis in spinal cord injury patients: A literature review. Annals of physical and rehabilitation medicine. 2010;53(10):655-668.
63. Smith E, Carroll A. Bone mineral density in adults disabled through acquired neurological conditions: a review. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry. 2011;14(2):85-94.
64. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton IL, et al. A meta-analysis of prior corticosteroid use and fracture risk. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2004;19(6):893-899.
65. Lee RH, Lyles KW, Colon-Emeric C. A review of the effect of anticonvulsant medications on bone mineral density and fracture risk. The American journal of geriatric pharmacotherapy. 2010;8(1):34-46.
66. Kueper J, Beyth S, Liebergall M, Kaplan L, Schroeder JE. Evidence for the adverse effect of starvation on bone quality: a review of the literature. Int J Endocrinol. 2015;2015:628740.
67. Fazeli PK, Klibanski A. Anorexia nervosa and bone metabolism. Bone. 2014;66:39-45.
68. Sagsveen M, Farmer JE, Prentice A, Breeze A. Gonadotrophin-releasing hormone analogues for endometriosis: bone mineral density. The Cochrane database of systematic reviews. 2003(4): CD001297.
69. Wu D, Hu M, Hong L, Hong S, Ding W, Min J, et al. Clinical efficacy of add-back therapy in treatment of endometriosis: a meta-analysis. Archives of gynecology and obstetrics. 2014; 290(3):513-23.
70. Fernandez H, Lucas C, Hedon B, Meyer JL, Mayenga JM, Roux C. One year comparison between two add-back therapies in patients treated with a GnRH agonist for symptomatic endometriosis: a randomized double-blind trial. Human reproduction (Oxford, England). 2004;19(6):1465-1471.
71. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. 2014;383(9912):146-155.
72. Boonen S, Bischoff-Ferrari HA, Cooper C, Lips P, Ljunggren O, Meunier PJ, et al. Addressing the musculoskeletal components of fracture risk with calcium and vitamin D: a review of the evidence. Calcified tissue international. 2006;78(5):257-270.
73. Bauer M, Bryce J, Hadji P. Aromatase inhibitor-associated bone loss and its management with bisphosphonates in patients with breast cancer. Breast cancer (Dove Medical Press). 2012;4:91--101.

74. Hadji P, Aapro MS, Body JJ, Bundred NJ, Brufsky A, Coleman RE, et al. Management of aromatase inhibitor-associated bone loss in postmenopausal women with breast cancer: practical guidance for prevention and treatment. Annals of oncology 2011;22(12):2546-2555.
75. Ligibel JA, O'Malley AJ, Fisher M, Daniel GW, Winer EP, Keating NL. Patterns of bone density evaluation in a community population treated with aromatase inhibitors. Breast cancer research and treatment. 2012;134(3):1305-1313.
76. Pavone ME, Bulun SE. Aromatase inhibitors for the treatment of endometriosis. Fertil Steril. 2012;98(6):1370-1379.
77. Usluogullari B, Duvan C, Usluogullari C. Use of aromatase inhibitors in practice of gynecology. Journal of ovarian research. 2015;8(1):4.
78. Vestergaard P. Bone loss associated with gastrointestinal disease: prevalence and pathogenesis. European journal of gastroenterology & hepatology. 2003;15(8):851-856.
79. Brown TT, Ruppe MD, Kassner R, Kumar P, Kehoe T, Dobs AS, et al. Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. The Journal of clinical endocrinology and metabolism. 2004;89(3):1200-1206.
80. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, et al. Assessment of fracture risk. Osteoporosis international. 2005;16(6):581-589.
81. Khan A, Grey A, Shoback D. Medical management of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. The Journal of clinical endocrinology and metabolism. 2009;94(2):373-381.
82. Marcocci C, Bollerslev J, Khan AA, Shoback DM. Medical management of primary hyperparathyroidism: proceedings of the fourth International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism. The Journal of clinical endocrinology and metabolism. 2014;99(10):3607-3618.
83. Hippisley-Cox J, Coupland C. Derivation and validation of updated QFracture algorithm to predict risk of osteoporotic fracture in primary care in the United Kingdom: prospective open cohort study. BMJ. 2012;344:e3427.
84. Kanis JA, Johansson H, Oden A, McCloskey EV. Guidance for the adjustment of FRAX according to the dose of glucocorticoids. Osteoporosis international. 2011;22(3):809-816.
85. Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E, Kanis JA. Spine-hip discordance and fracture risk assessment: a physician-friendly FRAX enhancement. Osteoporosis international 2011;22(3):839-847.

PRÁTICA CLINICA

Portuguese recommendations for the prevention, diagnosis and management of primary osteoporosis – 2018 update

Rodrigues AM [1,2,3], Canhão H[1,4], Marques A[5,6], Ambrósio C[7], Borges J[8], Coelho P[8], Costa L[9], Fernandes S[8], Gonçalves I[10], Gonçalves MJ[11,12], Guerra M[13], Marques ML[5], Pimenta S[9], Pinto P[13], Sequeira G[14], Simões E[8], Teixeira L[15], Vaz C[9], Vieira-Sousa E[11,12], Vieira R[13], Alvarenga F[10], Araújo F[16], Barcelos A[7,17], Barcelos F[8], Barros R[11,12], Bernardes M[9], Canas da Silva J[15], Cordeiro A[15], Costa M[18], Cunha-Miranda L[8], Cruz M[10], Duarte AC[13], Duarte C[5], Faustino A[8], Figueiredo G[19], Fonseca JE [11,12] Furtado C[19], Gomes J[18], Lopes C[18], Mourão AF[18,20], Oliveira M[21], Pimentel-Santos FM[18,20], Ribeiro A[22], Sampaio da Nóvoa T[19], Santiago M[5], Silva C[8], Silva-Dinis [11,12], Sousa S[15], Tavares-Costa J[23],Terroso G[9], Vilar A[8], Branco JC[18,20], Tavares V[15], Romeu JC[11], da Silva JAP[5]
on behalf of the Portuguese Society of Rheumatology

ACTA REUMATOL PORT. 2018;43:10-31

ABSTRACT

Background: Advances in osteoporosis (OP)case definition, treatment options, optimal therapy duration and pharmacoeconomic evidence in the national context motivated the Portuguese Society of Rheumatology (SPR) to update the Portuguese recommendations for the diagnosis and management of osteoporosis published in 2007.

Methods: SPR bone diseases' working group organized meetings involving 55 participants (rheumatologists, rheumatology fellows and one OP specialist nurse) to debate and develop the document. First, the working group selected 11 pertinent clinical questions for the diagnosis and management of osteoporosis in standard clinical practice. Then, each question was investigated through literature review and draft recommendations were built through consensus. When insufficient evidence was available, recommendations were based on experts' opinion and on good clinical practice. At two national meetings, the recommendations were discussed and updated. A draft of the recommendations full text was submitted to critical review among the working

1. EpiDoC Unit, Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
2. Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
3. Rheumatology Unit, Hospital do Santo Espírito da Ilha Terceira, Açores, Portugal
4. Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Lisboa Portugal
5. Rheumatology Department, Centro Hospitalar e Universitário de Coimbra, Clínica Universitária de Reumatologia, University of Coimbra, Coimbra, Portugal.
6. Coimbra Nursing School, Esenfc, Health Sciences Research Unit: Nursing (UICiSA:E), Coimbra, Portugal.
7. Rheumatology Department, Centro Hospitalar do Baixo Vouga, E.P.E. Aveiro, Portugal
8. Instituto Português de Reumatologia, Lisboa, Portugal
9. Rheumatology Department, Centro Hospitalar de S. João, Porto, Portugal.
10. Consultório Privado de Reumatologia, Portugal
11. Serviço de Reumatologia e Doenças Ósseas Metabólicas, Hospital de Santa Maria, CHLN, Centro Académico de Medicina de Lisboa, Portugal
12. Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Centro Académico de Medicina de Lisboa, Portugal
13. Rheumatology Department, Centro Hospitalar Vila Nova de Gaia/Espinho, Vila Nova de Gaia, Portugal
14. Rheumatology Department, Centro Hospitalar Universitário do Algarve, Algarve, Portugal
15. Rheumatology Department, Hospital Garcia de Orta, Almada, Portugal
16. Rheumatology Department, Hospital de Sant´ Ana
17. Institute of Biomedicina- iBiMED, Aveiro, Portugal
18. Rheumatology Department, Centro Hospitalar Lisboa Ocidental (CHLO- E.P.E.) - Hospital Egas Moniz, Lisboa, Portugal
19 Rheumatology Department, Hospital do Divino Espírito Santo, Ponta Delgada, Açores, Portugal
20. Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School, Universidade Nova de Lisboa (NMS/UNL), Lisboa, Portugal
21. Rheumatology Department, Centro Hospitalar Cova da Beira, Covilhã, Portugal
22. Rheumatology Department, Hospital de Braga, Braga, Portugal
23. Rheumatology Department, Unidade Local de saúde do Alto Minho, Hospital Conde de Bertiandos, Ponte de Lima, Portugal

group and suggestions were incorporated. A final version was circulated among all Portuguese rheumatologists before publication and the level of agreement was anonymously assessed using an online survey.

Results: The 2018 SPR recommendations provide comprehensive guidance on osteoporosis prevention, diagnosis, fracture risk assessment, pharmacological treatment initiation, therapy options and duration of treatment, based on the best available evidence. They attained desirable agreement among Portuguese rheumatologists. As more evidence becomes available, periodic revisions will be performed.

Target audience and patient population: The target audience for these guidelines includes all clinicians. The target patient population includes adult Portuguese people.

Intended use: These recommendations provide general guidance for typical cases. They may not be appropriate in all situations - clinicians are encouraged to consider this information together with updated evidence and their best clinical judgment in individual cases.

Keywords: Portugal; Fragility fracture; Osteoporosis; Recommendations.

INTRODUCTION

Osteoporosis (OP) is characterized by reduced bone mass and micro-architectural deterioration which results in increased bone fragility and propensity to fracture[1]. With the progressive ageing of the population, OP has become one of the most common human diseases worldwide, and a major public health concern. Most individuals are at risk of suffering from OP during their lifetime[2]. Fragility fractures, the main consequence of OP, results in increased morbidity and mortality and represent a major and growing economic burden on health-care systems worldwide[3,4]. European health authorities estimated, in 2011, that 22 million women and 5.5 million men in the European Union had osteoporosis and that 3.5 million suffered new fragility fractures every year, comprising 610.000 hip fractures, 520,000 vertebral fractures, 560,000 forearm fractures and 1,800,000 other fractures[5]. There is considerable international variability in fracture incidence rate, which has been attributed to age, socioeconomic status and other factors, frequently obscure, related to geography, as some regions have 3 times higher rates than apparently other similar ones[6,7].

In Portugal in 2011-2013, the prevalence of OP in people aged 18+, was estimated at 10.2% (17.0% in women and 2.6% in men)[8]. Altogether, 40,000 osteoporotic fractures are estimated to occur annually in Portugal[9], including over 10,000 hip fractures, the only type of fractures with truly reliable data in Portugal.[10] This number has been increasing steadily in Portugal (5,600 in 1989; 6,718 in 1994; 8,500 in 2000; 9,523 in 2006; 10,124 in 2011) and this is, most probably, accompanied by a proportional increase in other osteoporotic fractures (vertebral, forearm and humerus)[10-12]. Expanding life expectancy is the suggested underlying cause[13]. The incidence of hip fragility fractures in Portugal has been estimated at 154 to 572 per 100,000 women/year and 77 to 232 per 100,000 men[12], one of the lowest in Europe[13]. The social and economic burden imposed by osteoporotic fractures is enormous. The societal cost per each hip fracture in Portugal was estimated at 13,434 euros in the first and 5,985 euros in the second year, following fracture, totalling 216 million euros, taking the incidence and costs of the year 2011. Hip fractures are associated with an absolute excess mortality of 12% in the first year and a sharp drop in quality of life[14]. This individual, social and economic load is bound to increase exponentially over the years to come, unless effective preventive measures are put in place.

Over the last decade, several new therapeutic options that effectively decrease the risk of fracture have become available[15], and new evidence has been gathered regarding treatment duration[16]. The most relevant current clinical challenge consists in accurately identifying and selecting the individuals that will benefit the most from pharmacological treatment: ie, those whose high risk of fracture can be reduced, in order to minimize individual and societal costs. In fact, the need to base the decision to treat on the estimate of absolute fracture risk is now widely accepted[17,18]. Several countries have included validated tools for fracture risk assessment in their OP recommendations[19-24]. The knowledge-based necessary to allow the Portuguese adherence to these modern trends has dramatically increased over recent years: the Portuguese version of the Fracture Risk Assessment Tool (FRAX®) was established[12] and

fully validated to the Portuguese population[14,25]. Furthermore the cost of fractures was studied[14], the cost-effectiveness thresholds for intervention were calculated[26] and multidisciplinary recommendations for dual-energy x-ray absorptiometry (DXA) request and indication to treat and prevent fragility fractures were issued[27]. In light of this new knowledge, the SPR decided to update the 2007 recommendations for the treatment of OP[28], covering the diagnosis, prevention and management of osteoporosis in the adult population.

These recommendations may not be appropriate in all situations and we encourage clinicians to combine this information, with updated knowledge and their best clinical judgment in individual cases.

CORE BACKGROUND CONCEPTS

Some of the major conceptual changes observed in the field of OP in the last decade reside in: 1. The sedimentation of the notion that the sole aim of treating OP is to prevent fragility fractures; 2. The recognition that the risk of fractures is influenced by numerous clinical and environmental risk factors beyond bone mineral density[29-31]. The majority of these factors have been captured in risk prediction tools that are easily accessible and reliable for use in current practice, with emphasis on FRAX®, the most widely validated and adopted fracture risk prediction tool worldwide[32].

This has led to the distinction between two concepts: the diagnostic threshold and the intervention threshold. The diagnosis of OP remains unchanged, based on the threshold of bone mineral density (BMD) T score ≤-2,5, as established by World Health Organization (WHO)[1,33,34]. This, however, does not coincide with the intervention threshold, which should now be based on the absolute risk of fracture, as estimated by the composite consideration of its several determinants, i.e. by the use of fracture risk prediction tools[17,35].

METHODS

To develop these recommendations a working group of 55 participants including rheumatologists and rheumatology fellows and one OP specialized nurse was formed. First, the working group selected pertinent clinical questions for the diagnosis, prevention and management of osteoporosis in clinical practice. A thorough literature review was then performed to address each question. The electronic search was performed in PubMed MEDLINE (2006--2017). The search strategies included the following medical descriptors: "Osteoporosis", "Fragility fractures", "Risk assessment", "Recommendations", "Guidelines", "Treatment", "Bone mineral density", "DXA", "Bone turnover markers" and "Biochemical markers of bone remodelling". Guidelines and systematic literature reviews regarding the diagnosis and management of OP were also scrutinized and their reference lists were checked to assure completeness. After the literature review, the working group elaborated proposals for recommendations that were presented, discussed and revised in two national meetings, using the nominal group technique, and refined through electronic consultation.

A draft document presenting the proposed recommendations and their respective supporting evidence was circulated to the working group of Portuguese rheumatologists, rheumatology fellows and one OP specialized nurse and modifications of format and content were made. Finally, the document circulated among all Portuguese rheumatologists, rheumatology fellows and OP specialized nurse, who anonymously voted online on the level of agreement with each recommendation (total of 88 participants). Agreement was measured on a 10-point numerical rating scale (1=no agreement, 10=full agreement).

RESULTS

To Guide Readers, recommendations are structured around eleven clinically relevant questions:

- *Question 1. When should clinicians think of osteoporosis?*
- *Question 2. How shall clinicians assess the fracture risk of individual patients?*
- *Question 3. When and how should bone mineral density be measured?*
- *Question 4. When and how should secondary osteoporosis be suspected and investigated in adults?*
- *Question 5. Who should be pharmacologically treated for osteoporosis?*
- *Question 6. How should primary osteoporosis be treated?*
- *Question 7. How should osteoporosis in men and secondary osteoporosis be managed?*
- *Question 8. How should the efficacy of osteoporosis*

treatment be monitored?
- *Question 9. When should drug holiday and therapeutic switch be considered?*
- *Question 10. Which are the best strategies to prevent osteoporosis in the general population?*
- *Question 11. When should an osteoporotic patient be referred to a rheumatologist?*

Eleven recommendations were formulated, reaching a high level of agreement among Portuguese rheumatologists (Table I).

RECOMMENDATIONS

QUESTION 1. WHEN SHOULD CLINICIANS THINK OF OSTEOPOROSIS?

- **Recommendation 1a. Clinical risk factors for osteoporosis and fragility fractures should be identified and corrected, if possible, throughout life.**
- **Recommendation 1b. The risk of fracture should be regularly assessed and managed in all women and men over the age of 50.**
- **Recommendation 1c. The risk of fracture does not need to be assessed in people <50 years, unless relevant clinical risk factors are present.**

Although osteoporotic fractures typically occur over the age of 55 (wrist) or 75 (hip, humerus), the underlying OP has its roots, as back in life as, the early childhood. In fact, bone health throughout life can be decisively influenced by events affecting bone mass accrual during infancy and adolescence. Peak bone mass, achieved at 18-25 years of age is a major determinant of bone mineral density and bone fragility later in life. It is largely determined by genetic factors, and also by nutrition, physical activity, endocrine status, health status and medication[36]. The rate of bone mass loss that follows early adulthood and especially the menopause is also influenced by a variety of health and lifestyle dimensions. These clinical risk factors (CRF) have been shown to influence the risk of fracture, independent of the bone mineral density (BMD)[37].

Because OP progresses asymptomatically until a fragility fracture (low trauma fracture) occurs, all modifiable clinical risk factors for low bone mass peak, fast bone loss and fractures should be kept under clinical scrutiny, especially in those with a family history of OP.

The clinical risk factors for fracture include (but are not limited to):
- Age (>65 years)
- Female gender
- Low body mass index (<18.5Kg/m^2)
- Prior fragility fracture
- Parental history of hip fracture
- Long term use of oral glucocorticoids (>5mg of prednisolone per day or equivalent for longer than 3 months)
- Current smoking
- Alcohol intake >3 units/day
- Rheumatoid arthritis and other secondary causes of OP (*diabetes mellitus*, hypogonadism, anorexia nervosa, inflammatory bowel disease, calcium/vitamin D deficiency, hyperparathyroidism), prolonged immobilization and paralysis, medications (anticonvulsants, anticoagulants, proton pump inhibitor and antiretroviral therapy)[19,20,27,38]
- Frequent falls[20,39]

Clinical algorithms for fracture risk estimation, such as the FRAX®, integrate most or all these risk factors, with or without BMD, providing a very convenient and reliable tool to stratify individuals according to risk of fracture and, therefore, to the need of pharmacological intervention[40]. They have only been validated for people age 40+. The typically low fracture risk in generally healthy individuals before the age 50 justifies the age limit indicated in the recommendation for risk fracture assessment.

QUESTION 2. HOW SHALL CLINICIANS ASSESS THE FRACTURE RISK OF INDIVIDUAL PATIENTS?

- **Recommendation 2. Fracture risk assessment for Portuguese individuals should be preferentially based on the use of the FRAX® algorithm, as validated for the Portuguese population.**

A FRAX® algorithm has been established for the Portuguese population and internationally recognized by – FRAX®Port https://www.shef.ac.uk/FRAX/tool.jsp?lang=pt). A recent large-scale population-based study demonstrated that this tool has a high validity and predictive value regarding the subsequent occurrence of fragility fractures in the Portuguese population[25,26]. Evaluation of the clinical risk factors included in FRAX®, should strictly respect the definitions provided by the tool and available at its website[40]. This algorithm is validated for the general

TABLE I. AGREEMENT RATES OF 2017 OP RECOMMENDATION PORTUGUESE SOCIETY OF RHEUMATOLOGY AMONG RHEUMATOLOGISTS

Recommendation	Votes	Agreement Mean (SD) % score ≥ 8
Recommendation 1 1a. Clinical risk factors for osteoporosis and fragility fractures should be identified and corrected, if possible, throughout life 1b. The risk of fracture should be regularly assessed and managed in all women and men over the age of 50 1c. The risk of fracture does not need to be assessed in people <50 years, unless relevant clinical risk factors are present.	88	8.9 (1.3) 90%
Recommendation 2 Fracture risk assessment for Portuguese individuals should be preferentially based on the use of FRAX® algorithm, as validated for the Portuguese population.	88	8.4 (1.8) 75%
Recommendation 3 3a. Bone Mineral Density should be assessed, for clinical purposes, by dual X-ray absorptiometry (DXA) 3b. The decision to perform DXA should be primarily based on the risk of fracture as estimated by clinical risk factors, which can be provided by FRAX®Port. 3c. DXA is warranted in Portugal when FRAX®Port estimates, without DXA, are between 7% and 11% for major osteoporotic fracture AND between 2% AND 3% for hip fracture. 3d. DXA may be, otherwise, justified to evaluate patients with risk factors for osteoporosis not included in FRAX®, to study secondary osteoporosis (table 2) or to evaluate the efficacy of interventions.	88	8.6 (1,2) 85%
Recommendation 4 4a. Secondary Osteoporosis should be suspected in the presence of – conditions known to induce osteoporosis (Table 2) – fragility fractures occurring before the age of 70 for men or before menopause for women – low Z scores in DXA (≤-2.0) 4b. Suspected secondary osteoporosis justifies thorough clinical evaluation and appropriate hypothesis-driven investigations.	88	8.8 (1.7) 86%
Recommendation 5 Pharmacological treatment for osteoporosis should be initiated, unless contraindicated, in all subjects over the age of 50 who satisfy one or more of the following criteria: – ≥ 1 fragility fracture of the hip or ≥ 1 symptomatic vertebral fragility fracture. – ≥ 2 fragility fractures, independently of the site of fracture or the absence of symptoms (e.g. two asymptomatic vertebral fractures). – Estimates of FRAX®Port, without DXA, ≥ 11% for major osteoporotic fracture OR ≥ 3% for hip fracture – Estimates of FRAX®Port, with DXA, ≥ 9% for major osteoporotic fracture OR ≥ 2.5% for hip fracture	88	8.3 (1.7) 79%
Recommendation 6 6.a. Non-pharmacological preventive measures for osteoporosis, designed to correct modifiable relevant clinical risk factors should always be implemented. These include the promotion of healthy diet, regular weight-bearing exercise, adequate calcium intake and sun exposure or supplementation with vitamin D, as well as the prevention of falls, and avoidance of excessive alcohol intake and smoking.	88	8.9 (1.4) 84%

continues on the next page

TABLE I. CONTINUATION

Recommendation	Votes	Agreement Mean (SD) % score ≥ 8
6b. Based on cost-effectiveness considerations, the first line treatment for osteoporosis is oral bisphoshonates (namely oral alendronate). 6c. Intravenous zoledronic acid and subcutaneous denosumab should be considered in case of oral intolerance, malabsorption, dementia and non-compliance. Denosumab can also be preferred in case of renal insufficiency. Teriparatide is an option in patients with very high risk of subsequent fracture.		
Recommendation 7 7a. Osteoporosis in men is more often due to comorbidities: special attention should be paid to secondary causes of OP. 7b. Fracture risk assessment and treatment of male primary osteoporosis is similar to that described in women, except for hormone-based medications.	83	9.0 (1.1) 90%
Recommendation 8 8a. Clinical risk factors, occurrence of fractures, body height, and the adherence to lifestyle interventions and medication should be reassessed annually. Vertebral imaging may be performed if necessary. 8b. DXA assessment should not be repeated within less than 2 years, unless clinical risk factors significantly change. Biochemical markers have little role in evaluating the treatment response/adherence in individual patients. 8c. The absence of a new low trauma fracture, the stability or improvement of BMD over >2 years, and a guaranteed adherence to therapy are consistent with a satisfactory course of treatment.	83	8.7 (1.8) 85%
Recommendation 9 9a. Drug holidays should only be considered for bisphosphonates. An interruption of therapy with these agents, for 2 to 3 years, may be considered if the three following conditions are simultaneously verified – The treatment has been strictly adhered to for at least 5 years with oral or 3 years with intravenous bisphosphonates – No fragility fractures have been observed under treatment – Femoral BMD T Score is >-2.5 9b. Switching anti-osteoporotic therapy should be considered whenever significant adverse events occur or comorbidity emerges that advises reconsideration of the agent being used (eg: newly established renal failure in patients under bisphosphonates). 9c. Stopping anti-osteoporotic therapy should be considered if – it is verified that the criteria to recommend its introduction are not met – significant toxicity contraindicates continuation	83	8.7 (1.2) 85%
Recommendation 10 Healthy diets, adequate sun exposure and regular weight-bearing exercise should be promoted, for bone health, in every stage of life in the general population.	83	8.7 (1.2) 85%
Recommendation 11 A referral to rheumatology should be considered in case of unclear fracture risk assessment, doubts regarding treatment strategies, secondary osteoporosis, inadequate response to therapy or unremitting pain after fracture.	83	9.1 (1.6) 92%

population from 40 to 90 years old who are treatment – naïve for OP.

FRAX® has several limitations, which should be considered for clinical decision in individual cases. Among these, we highlight that FRAX®: 1. Does not take into account the occurrence of falls as a clinical risk factor; 2. Does not consider vertebral bone mineral density; 3. Does not take into account the dose-dependent and time exposure relationships of clinical risk factors (eg: glucocorticoid dose and duration, number of previous fractures) and fractures.[40] In addition, the discriminatory value of the FRAX® algorithm among some sub groups of patients with high risk of fracture, such as those with chronic kidney disease[41], diabetes[42], cancer, mental disorders and related medications[43] is limited.

QUESTION 3. WHEN AND HOW SHOULD BONE MINERAL DENSITY BE MEASURED?

- **Recommendation 3.a. Bone mineral density should be assessed, for clinical purposes, by dual X-ray absorptiometry (DXA)**
- **Recommendation 3.b. The decision to perform DXA should be primarily based on the risk of fracture as estimated by clinical risk factors, which can be provided by FRAX®Port.**
- **Recommendation 3.c. DXA is warranted in Portugal when FRAX®Port estimates, without DXA, are between 7% and 11% for major osteoporotic fracture AND between 2% and 3% for hip fracture.**
- **Recommendation 3.d. DXA may be, otherwise, justified to evaluate patients with risk factors for osteoporosis not included in FRAX®, to study secondary osteoporosis (Table II) or to evaluate the efficacy of interventions.**

These recommendations are rooted on the overarching principles that the decision to make investigations in clinical practice should be based on: 1. The probability that the result will be abnormal; 2. That the result might change subsequent decisions, the decision being, in this case - to treat or not to treat. Prospective studies with DXA have showed that, particularly in old adult women, the risk of fractures approximately doubles for each reduction of one standard deviation (SD) in BMD[44,45]. However, the diagnostic threshold of a T-score ≤ -2.5, defined by WHO in 1994, fails to identify a significant number of those who actually suffer a fragility fracture. BMD values below the osteoporosis diagnostic threshold have high specificity but low sensitivity[34,44,46]. Clinical risk factors for fractures, which are statistically significant independently of BMD, have been identified[47,48]. Considered individually, each clinical risk factor also has poor specificity and sensitivity in predicting fracture risk[47] but combined, they have a performance that is similar to BMD[19,29,46,49,50]. In fact, the validation study of FRAX®-Port[25] demonstrated that the accuracy of this tool was very similar, with and without BMD, at group level.

Taken together, the available evidence suggests that, the most efficient way of screening individuals at risk of a fragility fracture, resides in using FRAX tool without BMD[25,50]. BMD measurement may be justified when the risk estimate is in the vicinity of the lower cost-effective intervention thresholds previously calculated for Portugal (9% for major and 2,5% for hip fractures)[26] because, in such cases, the dichotomous decision to treat/not to treat may be changed by consideration of DXA values. For this reason, the Portuguese multidisciplinary recommendations[27] endorsed by the SPR, established an uncertainty margin of 2% and 0.5 % around the stated intervention threshold, for major fracture and hip fractures, respectively, which demands the performance of DXA to support the final decision to initiate treatment. It is estimated that the probability that the decision to treat/not to treat, will be changed by DXA, in patients whose prior estimated fracture risk is either above or below the uncertainty margin, is too small to make DXA warranted for these purposes. The width of this uncertainty margin was, however, based solely on expert opinion (Figure 1).

BMD should also be assessed to determine the individual risk of fracture in cases of suspected secondary OP, in the presence of risk factors not included in FRAX tool, and in patients treated with anti-osteoporotic drugs (Table II and Figure 1)[22,51,52].

QUESTION 4. WHEN AND HOW SHOULD SECONDARY OSTEOPOROSIS BE SUSPECTED AND INVESTIGATED IN ADULTS?

- **Recommendation 4.A. Secondary osteoporosis should be suspected in the presence of conditions known to induce osteoporosis (Table II) fragility fractures occurring before the age of 70 for men or before menopause for women low Z scores in DXA (≤-2.0)**

TABLE II. RISK FACTORS FOR BONE FRAGILITY AND SECONDARY CAUSES OF OSTEOPOROSIS

Inflammatory conditions
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Ankylosing spondylitis
- Crohn's disease, ulcerative colitis
- Sarcoidosis
- HIV infection

Endocrinopathies or metabolic causes
- Hypercortisolaemia (Cushing's syndrome)
- Hyperthyroidism
- Primary hyperparathyroidism
- Hyperprolactinaemia
- Premature menopause (auto-immune, surgical, drugs)
- Male hypogonadism
- Acromegaly
- Growth hormone defficiency
- Diabetes mellitus type I and II
- Porphyria
- Hypophosphatasia
- Pregnancy

Liver and GI conditions/Nutrition
- Chronic liver disease
- Primary biliary cirrhosis
- Gastrointestinal resection or bypass
- Celiac disease
- Malabsorption
- Lactose intolerance
- Pancreatic insufficiency
- Total parental nutrition
- Alcoholism
- Anorexia Nervosa
- Calcium deficiency

Haematological conditions
- Multiple myeloma and monoclonal gammopathy of unknown significance
- Myeloproliferative disorders
- Systemic mastocytosis
- Thalassemia
- Hemophilia
- Sickle cell anaemia

Kidney diseases
- Chronic kidney disease
- Kidney transplantation
- Idiopathic renal hypercalciuria
- Renal tubular acidosis

Genetic disorders
- Osteogenesis imperfecta
- Marfan's syndrome
- Ehlers–Danlos syndrome
- Homocystinuria
- Pseudoxanthoma elasticum
- Gaucher disease
- Hypophosphatasia
- Haemochromatosis

Drugs
- *Glucocorticoids*
- *Antiepileptics:*
- *Hypoglycaemiants* (thiazolidinediones)
- *Lipase inhibitors*
- *Selective serotonin reuptake inhibitors*
- *Excess thyroxine supplementation*
- *Aromatase inhibitors*
- *Gonadotropin-releasing hormone agonists*
- *Depot medroxyprogesterone acetate*
- *Tamoxifen*
- *Chemotherapy*
- *Immunosuppressants:* cyclosporine, tacrolimus
- *Furosemide*
- *Lithium*
- *Heparin*
- *Proton pump inhibitors*
- *Aluminium-containing antacids*
- *Antipsychotics*
- *Anti-retroviral drugs*

Adapted from Sheu A *et al*, Hofbauer LC and Camacho[22,51,52]

- **Recommendation 4.B. Suspected secondary osteoporosis justifies thorough clinical evaluation and appropriate hypothesis-driven investigations.**

The reader should be aware that most European and American guidelines for the management of postmenopausal osteoporosis recommend that secondary causes and contributory factors to OP should be

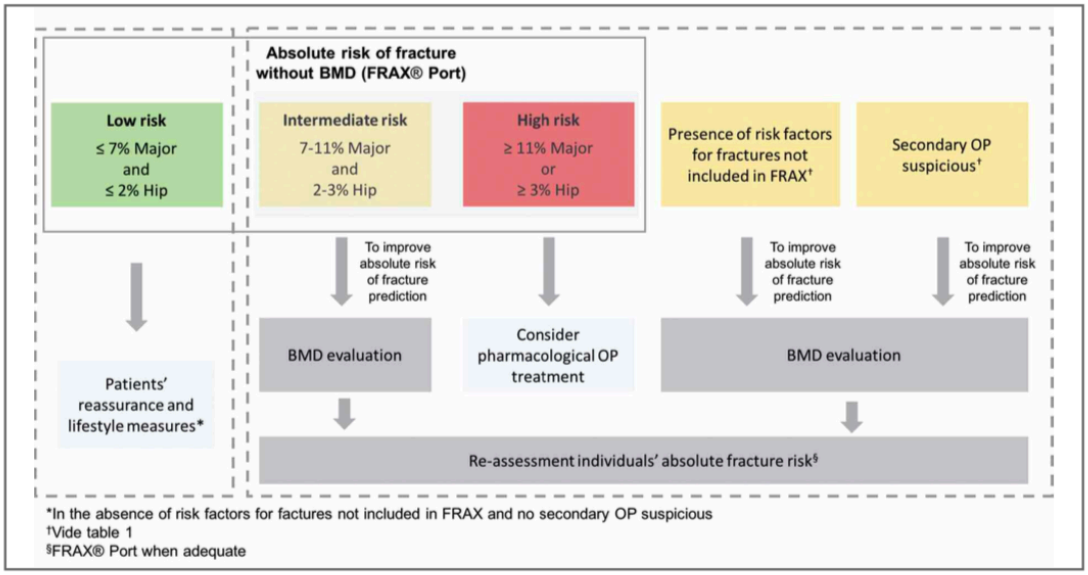

FIGURE 1. Flowchart of fracture risk assessment

searched in every patient with OP, irrespective of the presence or absence of fragility fractures[19,20,24,53]. Some scenarios are highly suspicious for secondary OP, like fragility fractures occurring in men with less than <70 years old[54], or in premenopausal women without obvious risk factors for osteoporosis; or multiple low-impact fractures, very low bone mineral density, Z-score ≤–2.0, atypical fractures or occurrence of fractures despite anti-osteoporotic therapy[51,52].

The causes of secondary OP are numerous, (Table II) but the prevalence of undiagnosed secondary causes of osteoporosis is not well established[55]. In an observational retrospective study from a Fracture Clinic, secondary causes were found to be infrequent (17/499, 3.4%)[56]. The clinical evaluation is aimed to exclude diseases that can mimic osteoporosis (eg osteomalacia) and to elucidate potential causes of OP that may influence management[19]. A complete medical history should be collected focusing on endocrine, metabolic and inflammatory disorders associated with altered bone metabolism (including malabsorption syndromes), personal habits (diet, exercise patterns, sun exposure, tobacco and alcohol consumption) and past and present medications capable of interfering with bone metabolism. A family history of bone fragility provides a hint for genetic contributions towards OP. The clinical factors included in FRAX® provide a general, although not exhaustive, guide for these explorations[40]. Special attention should be given to common medications whose association with OP and fragility fractures is frequently ignored, such as proton pump inhibitors, selective serotonin reuptake inhibitors, anticonvulsants, thiazolidinediones (diabetes), aromatase inhibitors, tamoxifen, luteinizing hormone releasing hormone (LHRH) analogues (breast cancer) and gonadotropin-releasing hormone (GnRH) agonists and antiandrogens (prostate cancer).

Physical examination should pay special attention to low height and/or low body mass index (<18.5 Kg/m^2), signs of hypogonadism and presence of kyphosis, joint inflammation, blue sclera and poor dentition.

A basic lab screening for secondary causes of OP should include serum calcium, phosphate, protein electrophoresis, alkaline phosphatase, creatinine, full blood counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT)), fasting glucose, thyroid (thyroid-stimulating hormone (TSH)) and parathyroid (parathyroid hormone (PTH) function tests. Depending on clinical findings or previous

investigations results, other laboratory tests can be considered with emphasis on serum 25(OH)vitamin D, 24-hour urine calcium , total and free testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) (suspected hypogonadism in men), cortisol levels, and anti-transglutaminase (suspected malabsorption).

Primary hyperparathyroidism in one of the most common causes of secondary OP. The diagnosis is primarily biochemical, based on the finding of hypercalcemia together with PTH levels that are high or inappropriately normal relative to serum calcium levels. The clinician should keep in mind that near-normal calcium levels may be found in mild primary hyperparathyroidism: calcium levels should be measured several times and corrected for albumin.[57]

QUESTION 5. WHO SHOULD BE PHARMACOLOGICALLY TREATED FOR OSTEOPOROSIS?

- **Recommendation 5. Pharmacological treatment for osteoporosis should be initiated, unless contraindicated, in all subjects over the age of 50 who satisfy one or more of the following criteria:**
  - **≥1 fragility fracture of the hip or ≥1 symptomatic vertebral fragility fracture.**
  - **≥2 fragility fractures, independently of the site of fracture or the absence of symptoms (e.g. two asymptomatic vertebral fractures).**
  - **Estimates of FRAX®Port, without DXA, ≥ 11% for major osteoporotic fracture OR ≥ 3% for hip fracture**
  - **Estimates of FRAX®Port, with DXA, ≥ 9% for major osteoporotic fracture OR ≥ 2.5% for hip fracture**

The decision to (not) prescribe anti-osteoporotic medications should be based on the individual's ten-year risk of subsequent osteoporotic fracture as estimated by the FRAX®Port tool. The risk-based thresholds for intervention indicated above are based on cost-effectiveness analysis and are applicable to the most affordable treatment scheme: generic alendronate (Figure 2). More expensive medications have higher cost-effective thresholds of intervention (Table III)[26]. Patients with prior fragility fractures (particularly hip) will have a significantly cost-effective reduction on the risk of subsequent fragility fracture with pharmacologic therapy, independently of their BMD[58-66]. It also noteworthy that some international recommendations advice that treatment should be started in the presence of a vertebral deformity grade 2 (ie height loss >25-40%) even if asymptomatic[67]. The reader is made aware that many international recommendations indicate that patients with a DXA T score ≤ -2.5 should also be treated, irrespective of FRAX® and age[19-23]. These recommendations were based on the principle that the elevated risk of fracture associated with a T score of -2.5 or less at femoral neck or lumbar spine has showed to be reduced with pharmacological treatment [61,63,64,66,68-77].The SPR, in accordance with the

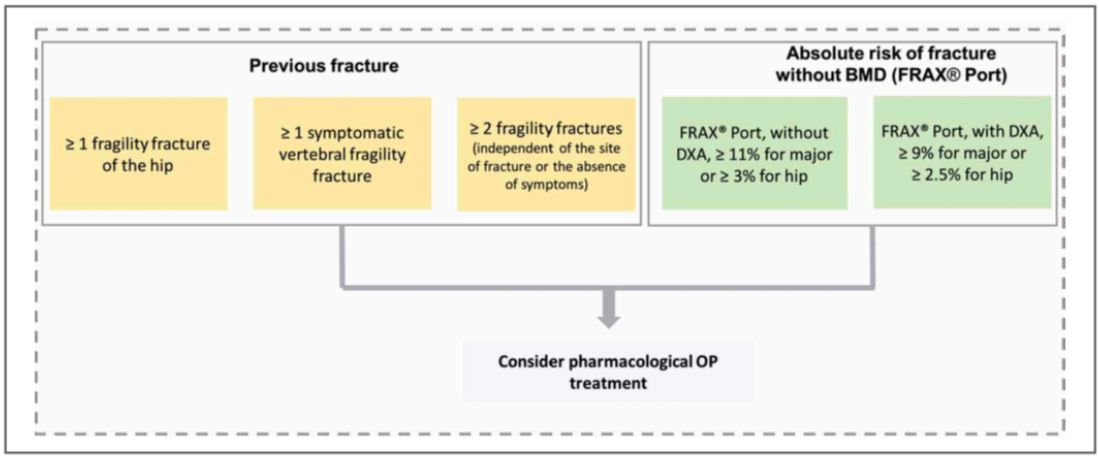

FIGURE 2. Criteria for pharmacological OP treatment

TABLE III. COST-EFFECTIVENESS THRESHOLDS FOR INTERVENTION WITH SEVERAL MEDICATIONS IN PORTUGAL, BASED ON THE FRAX®PORT TEN-YEAR OSTEOPOROTIC FRACTURE RISK ESTIMATE, FOR DIFFERENT MEDICATIONS, BASED ON A WILLINGNESS TO PAY OF 32.000€/QALY AND CURRENT COST OF MEDICATION

		Without DXA		With DXA	
	Cost basis/year (€)	Major %	Hip %	Major %	Hip %
Generic alendronate	99	11	3	9	2.5
Zoledronic acid	347	22	12	20	10
Denosumab	552	37	25	35	23
Teriparatide	4234	80	65	78	63

Adapted from Marques *et al*[26].

Portuguese Multidisciplinary Recommendations, does not endorse this policy, because a low BMD is not necessarily associated with a significant risk of fracture, especially in young people[45,46] .

QUESTION 6. HOW SHOULD PRIMARY OSTEOPOROSIS BE TREATED?

- **Recommendation 6a. Non-pharmacological preventive measures for osteoporosis, designed to correct modifiable relevant clinical risk factors should always be implemented. These include the promotion of a healthy diet, regular weight-bearing exercise, adequate calcium intake and sun exposure or supplementation with vitamin D, as well as the prevention of falls, and avoidance of excessive alcohol intake and smoking.**

Adequate nutrition with a well-balanced diet, sufficient sun exposure and regular weight-bearing exercise are important measures that promote bone health, not only in the general population, but especially in patients with osteoporosis[79]. Several studies have shown that excessive alcohol intake and smoking are deleterious for bone[80-83] and increase the risk of fragility fractures[48,49]. If adequate intake of calcium cannot be assured through diet, supplementation is indicated up to the recommended daily intake of 1000-1200 mg/day[20]. The side effects of calcium supplementation include kidney stones and gastrointestinal symptoms. The cardiovascular risk increase due to calcium supplementation is controversial and is considered negligible if associated to vitamin D within the recommended doses[84-88]. Adequate vitamin D status must be assured in patients with OP and serum 25 (OH) vitamin D should be measured in patients considered at risk of severe vitamin D deficiency: advanced age, obesity, renal insufficiency, malabsorption, chronic liver failure and exposure to medications that increase breakdown of vitamin D (anticonvulsants, highly active antiretroviral therapy (HAART) and glucocorticoids)[89].Vitamin D supplementation (800-2000UI/day or equivalent) should be considered in patients with serum 25(OH)Vitamin D levels below 30ng/ml[90,91]. All clinical trials with pharmacological therapies for OP were performed while guaranteeing adequate calcium and vitamin D levels through diet, sun exposure or supplementation[61,63,64,66,68-77].

- **Recommendation 6b. Based on cost-effectiveness considerations, the first line treatment for osteoporosis is oral bisphoshonates (namely generic oral alendronate).**
- **Recommendation 6c. Intravenous zoledronic acid and subcutaneous denosumab should be considered in case of oral intolerance, malabsorption, dementia and non-compliance. Denosumab can also be preferred in case of renal insufficiency. Teriparatide is an option in patients with very high risk of subsequent fracture.**

The current evidence does not allow a clear distinction between available treatments in terms of their relative efficacy in the prevention of fractures, as demonstrated by network meta-analyses designed to overcome the lack of head-to-head comparisons[92,93].

Bisphosphonates are considered the first line of therapy for osteoporosis in several countries[19,20,23,94,95]. In Portugal, generic oral alendronate is the most cost-

-effective drug available (Table III). The decision to start an anti-osteoporotic treatment with agents other than generic alendronate should be informed by their respective cost-effectiveness thresholds in Portugal (see Table III)[26]. Alendronate[58] and risedronate[60] are oral bisphosphonates that have demonstrated a broad anti-fracture efficacy (for vertebral, non-vertebral and hip fractures), generic alendronate being the less expensive in Portugal. The other available oral bisphosphonate, ibandronate, reduces the incidence of vertebral fractures but its ability to reduce the rate of nonvertebral fractures has not been robustly documented[59]. Annual intravenous infusions of zoledronic acid have also been shown to significantly reduce the incidence of vertebral, non-vertebral and hip fractures[74]. Moreover, zoledronic acid has also been demonstrated to prevent new fractures and decrease mortality after a recent hip fracture[65].

Denosumab, a monoclonal anti-RANKL antibody, has proven efficacy in the prevention of vertebral, non-vertebral and hip fractures when administered as 6-montly subcutaneous injections. Unlike bisphosphonates, denosumab has no renal excretion and its use in chronic renal disease seems to be safe and effective [96-99].The use of bisphosphonates in osteoporosis patients does not seem to have renal toxicity, but their use in chronic renal insufficiency should be cautious[100].In fact, there is insufficient data about the efficacy of bisphosphonates, raloxifene and teriparatide in preventing fractures in patients with renal insufficiency [101-104]. Osteonecrosis of the jaw and atypical femoral fractures are extremely rare with the usual doses of bisphosphonates and denosumab[100,105,106].

Teriparatide, the N-terminal 34 aminoacids of PTH, stimulates bone formation and is administrated subcutaneously, on a daily basis, for 18 to 24 months. The efficacy of teriparatide in reducing the incidence of vertebral and non-vertebral fractures is well established but not in hip fractures[107]. Overlapping teriparatide with bisphosphonates or denosumab and continuing an antiresorptive agent after teriparatide therapy seems to optimize the increase of BMD[108-111]. Due to its high cost and daily subcutaneous administration, teriparatide is usually reserved for subjects at very high risk of fragility fractures, namely with several previous fractures[112]. Unlike the bisphosphonates, both denosumab and teraparatide are followed by an abrupt and rapid bone loss when discontinued, thus requiring careful management of long-term therapy[113,114].

Raloxifene is a selective oestrogen receptor modulator that reduces the incidence of vertebral fractures but not hip or non-vertebral fractures. It has been demonstrated to reduce the risk of invasive breast cancer in postmenopausal women but to increase the risk of stroke and venous thromboembolism[115-118]. The recent recommendations of the American College of Physicians explicitly recommend against the use of hormone replacement therapy or raloxifene for the treatment of osteoporosis [119].

QUESTION 7. HOW SHOULD WE MANAGE OSTEOPOROSIS IN MEN AND SECONDARY OSTEOPOROSIS?

- **Recommendation 7a. Osteoporosis in men is more often due to comorbidities: special attention should be given to secondary causes of OP.**
- **Recommendation 7b. Fracture risk assessment and treatment of male primary osteoporosis is similar to that described in women, except for hormone-based medications.**

Osteoporosis in men is more often secondary than in women, approximately two thirds of all cases of male osteoporosis, according to some studies [120]. The most common secondary causes of OP in men include hypogonadism, alcohol abuse, multiple myeloma, hyperparathyroidism, malabsorption and glucocorticoid use[120]. For this reason, investigation of secondary causes of osteoporosis is especially warranted in males, as they may significantly influence the treatment strategy.

The overall management strategy for primary osteoporosis in men does not differ from that recommended for women: all risks factors for osteoporosis, fractures and falls should be corrected, as described above. The decision to start anti-osteoporotic medications is based on the same criteria and cost--effectiveness thresholds. Regarding the choice of treatment, data that specifically apply to men are scarce and expectations are extrapolated from studies in females, as the efficacy is expected to be similar in men and women[121]. One study demonstrated that treatment with zoledronic acid reduced vertebral fractures in osteoporotic men[122].

Treatment of secondary osteoporosis largely exceeds the scope of these recommendations, given the variety of conditions and nuances that need to be considered. Interested readers are advised to consult

the most relevant literature to the case at hand[123] . The recent Italian Guidelines for the diagnosis, prevention and management of osteoporosis[23] provide a wide scope review of numerous conditions. The prevention and treatment of glucocorticoid induced osteoporosis are the object of several dedicated recommendations[124,125].

QUESTION 8. HOW SHOULD THE EFFICACY OF OP TREATMENT BE MONITORED?

- **Recommendation 8a. Clinical risk factors, occurrence of fractures, body height, and the adherence to lifestyle interventions and medication should be reassessed annually. Vertebral imaging may be performed if necessary.**
- **Recommendation 8b. DXA assessment should not be repeated within less than 2 years, unless clinical risk factors significantly change. Biochemical markers have little role in evaluating the treatment response/adherence in individual patients.**

Periodic follow-up is important to ensure the adherence to treatment and life-style interventions, monitor adverse events and evaluate the response to treatment[112,126]. OP patients have a low/moderate adherence to anti-osteoporotic drugs, which leads to a loss of efficacy in fracture prevention[127,128]. Regular clinical evaluations have demonstrated to increase treatment adherence[129]. During clinical appointment, patients should also be inquired regarding new clinical risk factors, new onset of secondary OP and adverse events related to OP drugs, which may require adjustment of the treatment plan[20]. To evaluate treatment efficacy, subjects should be asked regarding the occurrence of new fragility fractures. Vertebral imaging should be performed if a new vertebral fracture is suspected[20,126].

DXA testing can be advocated to monitor OP treatment efficacy. In fact, pilot studies with anti-osteoporotic drugs have shown a small to moderate relationship between the increase of BMD and the reduction of fracture risk in different trials. However, several studies demonstrate that women treated with bisphosphonates, raloxifene, and teriparatide benefited from reduced rate of fractures even if the BMD did not increase[130-132]. Accordingly, many experts consider that medication can be expected to be efficient and that the most important task of the clinician in this respect resides in guaranteeing adherence to evidence-based treatment. The recent recommendations of the American College of Physicians explicitly recommend against bone mineral density monitoring during pharmacologic treatment in women[119]. In any case, the time interval to repeat DXA must be sufficiently long to allow for detectable changes, which means that DXA assessment should not be repeated within less than 2 years[19,20,112].

Bone turnover markers (BTM), namely serum levels of procollagen I N-terminal extension peptide (P1NP) and C-telopeptide break (CTX) are typically reduced after 3-6 months of anti-resorptive therapy and increase after 1-3 months of anabolic therapy[19,20,112,126,133,134]. Studies have showed that short--term decrease in markers of bone turnover is associated with gains in BMD and with a reduction in the rate of fragility fractures[135-140]. The International Osteoporosis Foundation and the European Calcified Tissue Society[141] proposed that BTM should be used as a screening strategy to detect a lack of adherence to bisphosphonates based on the Trio study results[142]. However, the serum levels of these markers are extremely variable, depending on several factors not related to bone metabolism, such as diet, time of the day and of the year, concomitant medications, etc. This strongly reduces their value in individual patients, despite the sensitivity to change at the group level. Altogether, we consider that their use in clinical practice is rarely justifiable in agreement with the recent Italian Guidelines explicitly state that "bone markers cannot be used for routine clinical evaluations at present"[23].

- **Recommendation 8c. The absence of new low trauma fractures, the stability or improvement of BMD over >2 years, and a guaranteed adherence to therapy are consistent with a satisfactory course of treatment.**

The available evidence does not support a clear definition of the success or failure of OP treatment. Even the occurrence of a new fragility fracture cannot be taken as a demonstration of treatment failure: another one may have been prevented, as no medication has been shown to prevent all fractures. Despite this, treatment failure was defined by the International Osteoporosis foundation (IOF), based on expert opinion, as the occurrence of an incident fracture after at least 6 months of anti-osteoporotic treatment and/or a decrease in BMD greater than the least

significant change (approximately 5 % at the spine 4% at the femoral neck) over 2 years of treatment[133].

QUESTION 9. WHEN SHOULD DRUG HOLIDAY AND THERAPEUTIC SWITCH BE CONSIDERED?

- **Recommendation 9a. Drug holidays should only be considered for bisphosphonates. An interruption of therapy with these agents, for 2 to 3 years, may be considered if the three following conditions are simultaneously verified**
  - **The treatment has been strictly adhered to for at least 5 years with oral or 3 years with intravenous bisphosphonates**
  - **No fragility fractures have been observed under treatment**
  - **Femoral BMD T Score is >-2.5**

This recommendation is similar to that of the American Society for Bone and Mineral Research, which proposes that, in patients who have received bisphosphonates for ≥5 years if oral or for ≥3 years if intravenous, treatment with bisphosphonates or alternative therapy should be continued for up to ten years in those with hip, spine or multiple other osteoporotic fracture before or during therapy, a hip T-score ≤-2.5 or FRAX fracture risk score that is above country specific thresholds.[16]

Evidence for additional benefit of long-term bisphosphonates is provided by extensions of pivotal studies with alendronate (FLEX study)[68] and zoledronate (HORIZON extension study) [143].These studies verified that an additional 5 years treatment with alendronate or additional 3 years with zoledronate was associated with, respectively, fewer clinical vertebral fractures and fewer morphometric spine fractures. The risk of atypical femoral fracture is increased with prolonged therapy, but these events remain rare and are clearly outweighed by vertebral fracture risk reduction in high-risk patients[144]. On the other hand, the effects of bisphosphonates on bone persist for at least 2 years after discontinuation of long-term therapy. This allows for the consideration of bisphosphonate holiday in individuals not at high risk[68,143,145-149].

Teriparatide is not licensed to use for longer than 24 months, due to fears of osteosarcoma [110].

- **Recommendation 9b - Switching anti-osteoporotic therapy should be considered whenever significant adverse events occur or comorbidity emerge that advises reconsideration of the agent being used (eg: newly established renal failure in patients under bisphosphonates).**
- **Recommendation 9c -Interruption of anti-osteoporotic therapy should be considered if**
  - **it is verified that the criteria to recommend its introduction are not met**
  - **significant toxicity contraindicates continuation**

Evidence supporting the switch from bisphosphonate to teriparatide or denosumab is limited to the effect on BMD and bone turnover markers, there being no evidence regarding fracture incidence[110,150]. Teriparatide should be stopped after 18 to 24 months of treatment[110] and should be followed by bisphosphonate or denosumab[109,111,151]. Age, is not a reason to stop anti-osteoporotic therapy given that the risk of fractures steadily increases with age[2].

QUESTION 10. WHAT ARE THE BEST STRATEGIES TO PREVENT OSTEOPOROSIS IN THE GENERAL POPULATION?

- **Recommendation 10. Healthy diet, adequate sun exposure and regular weight-bearing exercise should be promoted, for bone health, in every stage of life, in the general population.**

Genetic factors account for 60 to 80% of the peak bone mass, but there is evidence that lifestyle factors, like adequate nutrition and regular weight-bearing exercise, are essential to achieve the genetic potential and have a positive effect in bone mass accrual in childhood and adolescence[36]. A 10% increase in peak bone mass has been predicted to delay the development of osteoporosis by 13 years[36,152]. The same lifestyle factors are advocated to prevent premature or accelerated bone mass in adults and old adults, although the evidence that these interventions will reduce fracture risk at any age is limited[152].

A well-balanced diet should provide adequate amounts of calcium, vitamin D and proteins, as well as other elements that are important for bone health (e.g. zinc, manganese, vitamin A, vitamin C, vitamin K, complex B vitamin, potassium and sodium)[152].

Recommended dietary allowances for calcium and vitamin D vary according to age group, gender and special situations. National recommendations for a healthy nutrition have been issued by the Direc-

torate-General of Health of the European Union and should be followed[9,153]. Dairy products are the main dietary source of calcium due to their high calcium content and bioavailability, providing also other important nutrients. Three servings of dairy products per day (milk, cheese or yogurt) deliver most of the recommended calcium intake for the general population[9]. Bioavailability of calcium provided by non-dairy sources is reduced and it may be impossible to meet recommendations in a dairy-free diet[9]. Calcium supplements may be an alternative if dietary intake is insufficient. Head-to-head studies have shown that increments in bone mass are higher with dietary calcium than with supplements[9]. There is an ongoing debate over the negative role of calcium (dietary or supplements) in cardiovascular diseases, hypertension, kidney stones and prostate cancer, as well as its positive effect in hypertension, colorectal cancer, preeclampsia and weight management [84]. For these reasons, we recommend that calcium intake should be mostly dietary and within recommended allowances. Supplements should only be considered for patients with OP under pharmacologically treatment or subjects unable to have an adequate calcium intake through diet.

Vitamin D is essential for bone development and maintenance throughout life, and it also has an important role in muscle, improving strength and function [89]. Vitamin D is obtained primarily from sun exposure, as the relevant dietary sources are very few (fresh or canned oily fish, cod liver oil, egg yolk)[89]. Skin mediated production varies greatly with age, skin type, latitude, time of day and season and use of sunscreen products. Supplementation of vitamin D may be considered in special situations (namely OP subjects under pharmacological treatment) and is recommended by the Directorate General of Health for those over 65 years of age[9,90]. The currently recommended intake of vitamin D in adults varies from 600 to 6000 UI/day, according to age, gender and body mass index[89,154].

There is strong evidence that exercise begun early in life contributes to higher peak bone mass. The importance of physical exercise in adults lies not only in the potential to reduce bone loss and improve muscle strength, but also in helping to prevent falls by enhancing coordination, balance and posture. Resistance training and weight-bearing exercises are the most beneficial for bone mass (ie, dancing, jogging, climbing stairs)[155,156].

Finally, excessive alcohol intake (more than 3 units/day for men and 2 units /day for women) and smoking are deleterious for bone and considered clinical risk factors for fractures. Excessive alcohol intake and smoking should be avoided in order to prevent osteoporosis[40,155].

QUESTION 11. WHEN SHOULD AN OSTEOPOROTIC PATIENT BE REFERRED TO A RHEUMATOLOGIST?

- **Recommendation 11.** ***A referral to rheumatology should be considered in case of unclear fracture risk assessment, doubts regarding treatment strategies, secondary osteoporosis, inadequate response to therapy or unremitting pain after fracture.***

Rheumatologists provide care for patients with OP in a cost-efficient, evidence-based and patient centered approach. The main aim in the treatment of an OP patient is to prevent a fragility fracture, improve quality of life and prevent disability. Rheumatologists work in a variety of settings in the hospital, namely outpatient office, infusion center and inpatient clinic. In addition, they are intensively trained and experienced in the diagnosis and management of complex cases of osteoporosis. OP patients should be referred to a rheumatologist when there is an inadequate response to therapy, which is indicated by significant loss of BMD or occurrence of fragility fracture in patients with good compliance to appropriate therapy, as defined in recommendation 8c.

In selected cases, referral may also be indicated if the caring physician is uncertain about the absolute risk of fracture, about the secondary nature of osteoporosis or the most appropriate treatment. This may also be justified to reassure patients who feel anxious or disturbed by the diagnosis or its management.

Referral should be based on appropriate information, including a clear expression of the questions to be addressed and all clinically pertinent information, such as current and previous medications, FRAX® estimates and relevant medical history, imaging and lab results.

AREAS WHERE EVIDENCE IS LACKING

In the present OP recommendations, the SPR recommends FRAX® algorithm to evaluate individuals absolute risk of fracture. A recent randomized controlled trial revealed that FRAX® algo-

rithm is a feasible and effective screening tool in reducing hip fractures[157]. However, it is important to note that evidence linking FRAX® scores to treatment efficacy is lacking[158]. In addition, comparative effectiveness trials evaluating pharmacologic treatments for low bone density or osteoporosis and high risk of fracture patients are also lacking[119].

CONCLUSION

This article presents the 2018 update of the Portuguese recommendations for diagnosis and management of OP in adults. They are meant to provide a valid guide on OP diagnosis, fracture risk assessment, pharmacological treatment decision, therapeutic options and duration, informed by national evidence and circumstances. These recommendations may not be appropriate in all situations and we encourage clinicians to use this information together with their best clinical judgment in the individual case.

CONFLICTS OF INTEREST

None of the authors report conflicts of interest

CORRESPONDENCE TO

José António Pereira da Silva
Reumatologia
Centro Hospitalar e Universitário de Coimbra
3000-075 Coimbra
jdasilva@chuc.min-saude.pt

REFERENCES

1. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8): 1137-1141.
2. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002;359(9321):1929-1936.
3. Bliuc D, Nguyen ND, Nguyen TV, Eisman JA, Center JR. Compound risk of high mortality following osteoporotic fracture and refracture in elderly women and men. J Bone Miner Res. 2013;28(11):2317-2324.
4. Frost SA, Nguyen ND, Center JR, Eisman JA, Nguyen TV. Excess mortality attributable to hip-fracture: a relative survival analysis. Bone. 2013;56(1):23-29.
5. Hernlund E, Svedbom A, Ivergard M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Archives of osteoporosis. 2013;8(1-2):136.
6. de Pina MF, Alves SM, Barbosa M, Barros H. Hip fractures cluster in space: an epidemiological analysis in Portugal. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2008;19(12):1797-1804.
7. Oliveira CM, Alves SM, Pina MF. Marked socioeconomic inequalities in hip fracture incidence rates during the Bone and Joint Decade (2000-2010) in Portugal: age and sex temporal trends in a population based study. Journal of epidemiology and community health. 2016;70(8):755-763.
8. Branco JC, Rodrigues AM, Gouveia N, et al. Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt-a national health survey. RMD Open. 2016;2(1): e000166.
9. Circular Normativa Direcção Geral da Saúde - Orientação técnica sobre suplemento de Cálcio e Vitamina D em pessoas idosas. Nº: 13/DSCS/DPCD/ DSQC Ad.
10. Marques A, Lourenco O, da Silva JA. The burden of osteoporotic hip fractures in Portugal: costs, health related quality of life and mortality. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2015.
11. Branco JC, Felicissimo P, Monteiro J. [Epidemiology of hip fractures and its social and economic impact. A revision of severe osteoporosis current standard of care]. Acta reumatologica portuguesa. 2009;34(3):475-485.
12. Marques A, Mota A, Canhao H, et al. A FRAX model for the estimation of osteoporotic fracture probability in Portugal. Acta Reumatol Port. 2013;38(2):104-112.
13. Kanis JA, Oden A, McCloskey EV, et al. A systematic review of hip fracture incidence and probability of fracture worldwide. Osteoporos Int. 2012;23(9):2239-2256.
14. Marques A, Lourenco O, da Silva JA, Portuguese Working Group for the Study of the Burden of Hip Fractures in P. The burden of osteoporotic hip fractures in Portugal: costs, health related quality of life and mortality. Osteoporos Int. 2015;26(11):2623-2630.
15. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet. 2011;377(9773):1276-1287.
16. Adler RA, El-Hajj Fuleihan G, Bauer DC, 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.
17. Rubin KH, Abrahamsen B, Friis-Holmberg T, et al. Comparison of different screening tools (FRAX(R), OST, ORAI, OSIRIS, SCORE and age alone) to identify women

with increased risk of fracture. A population-based prospective study. Bone. 2013;56(1):16-22.
18. Wright NC, Saag KG, Dawson-Hughes B, Khosla S, Siris ES. The impact of the new National Bone Health Alliance (NBHA) diagnostic criteria on the prevalence of osteoporosis in the United States: supplementary presentation. Osteoporos Int. 2017;28(11):3283-3284.
19. Kanis JA, McCloskey EV, Johansson H, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2013;24(1):23-57.
20. Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporos Int. 2014;25(10):2359-2381.
21. Papaioannou A, Morin S, Cheung AM, et al. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. CMAJ. 2010;182(17):1864-1873.
22. Camacho PM, Petak SM, Binkley N, et al. American Association of Clinical Endocrinologists and American College of Endocrinology Clinical Practice Guidelines for the Diagnosis and Treatment of Postmenopausal Osteoporosis - 2016. Endocr Pract. 2016;22(Suppl 4):1--42.
23. Rossini M, Adami S, Bertoldo F, et al. Guidelines for the diagnosis, prevention and management of osteoporosis. Reumatismo. 2016;68(1):1-39.
24. Tarantino U, Iolascon G, Cianferotti L, et al. Clinical guidelines for the prevention and treatment of osteoporosis: summary statements and recommendations from the Italian Society for Orthopaedics and Traumatology. J Orthop Traumatol. 2017;18(Suppl 1):3-36.
25. Marques A, Lucas R, Simoes E, Verstappen SMM, Jacobs JWG, da Silva JAP. Do we need bone mineral density to estimate osteoporotic fracture risk? A 10-year prospective multicentre validation study. RMD Open. 2017;3(2): e000509.
26. Marques A, Lourenco O, Ortsater G, Borgstrom F, Kanis JA, da Silva JA. Cost-Effectiveness of Intervention Thresholds for the Treatment of Osteoporosis Based on FRAX((R)) in Portugal. Calcif Tissue Int. 2016;99(2): 131-141.
27. Marques A, Rodrigues AM, Romeu JC, et al. Multidisciplinary Portuguese recommendations on DXA request and indication to treat in the prevention of fragility fractures. Acta Reumatol Port. 2016;41(4):305-321.
28. Tavares V, Canhao H, Gomes JA, et al. [Recommendations for the diagnosis and management of osteoporosis]. Acta Reumatol Port. 2007;32(1):49-59.
29. Kanis JA, Oden A, Johnell O, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18(8):1033-1046.
30. Hippisley-Cox J, Coupland C. Predicting risk of osteoporotic fracture in men and women in England and Wales: prospective derivation and validation of QFractureScores. BMJ. 2009;339:b4229.
31. Schuit SC, van der Klift M, Weel AE, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004;34(1):195-202.
32. Marques A, Ferreira RJ, Santos E, Loza E, Carmona L, da Silva JA. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(11):1958-1967.
33. Genant HK, Cooper C, Poor G, et al. Interim report and recommendations of the World Health Organization Task-Force for Osteoporosis. Osteoporos Int. 1999; 10(4):259-264.
34. Organization WH. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Report Series 1994;843.
35. Rubin KH, Friis-Holmberg T, Hermann AP, Abrahamsen B, Brixen K. Risk assessment tools to identify women with increased risk of osteoporotic fracture: complexity or simplicity? A systematic review. J Bone Miner Res. 2013;28(8):1701-1717.
36. Farr JN, Khosla S. Skeletal changes through the lifespan—from growth to senescence. Nat Rev Endocrinol. 2015;11(9):513-521.
37. Kanis JA, Adachi JD, Cooper C, et al. Standardising the descriptive epidemiology of osteoporosis: recommendations from the Epidemiology and Quality of Life Working Group of IOF. Osteoporos Int. 2013;24(11):2763--2764.
38. Lentle B, Cheung AM, Hanley DA, et al. Osteoporosis Canada 2010 guidelines for the assessment of fracture risk. Can Assoc Radiol J. 2011;62(4):243-250.
39. Blain H, Masud T, Dargent-Molina P, et al. A comprehensive fracture prevention strategy in older adults: the European Union Geriatric Medicine Society (EUGMS) statement. Aging Clin Exp Res. 2016;28(4):797-803.
40. Kanis JA, Hans D, Cooper C, et al. Interpretation and use of FRAX in clinical practice. Osteoporos Int. 2011; 22(9):2395-2411.
41. Jamal SA, West SL, Nickolas TL. The clinical utility of FRAX to discriminate fracture status in men and women with chronic kidney disease. Osteoporos Int. 2014;25 (1):71-76.
42. Majumdar SR, Leslie WD, Lix LM, et al. Longer Duration of Diabetes Strongly Impacts Fracture Risk Assessment: The Manitoba BMD Cohort. J Clin Endocrinol Metab. 2016;101(11):4489-4496.
43. Bolton JM, Morin SN, Majumdar SR, et al. Association of Mental Disorders and Related Medication Use With Risk for Major Osteoporotic Fractures. JAMA Psychiatry. 2017;74(6):641-648.
44. Johnell O, Kanis JA, Oden A, et al. Predictive value of

BMD for hip and other fractures. J Bone Miner Res. 2005;20(7):1185-1194.
45. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ. 1996;312(7041): 1254-1259.
46. Johansson H, Kanis JA, Oden A, Johnell O, McCloskey E. BMD, clinical risk factors and their combination for hip fracture prevention. Osteoporos Int. 2009;20(10): 1675-1682.
47. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med. 1995;332(12):767-773.
48. Kanis JA, Johansson H, Johnell O, et al. Alcohol intake as a risk factor for fracture. Osteoporos Int. 2005;16(7): 737-742.
49. Kanis JA, Johnell O, Oden A, et al. Smoking and fracture risk: a meta-analysis. Osteoporos Int. 2005;16(2): 155-162.
50. Leslie WD, Morin S, Lix LM, et al. Fracture risk assessment without bone density measurement in routine clinical practice. Osteoporos Int. 2012;23(1):75-85.
51. Hofbauer LC, Hamann C, Ebeling PR. Approach to the patient with secondary osteoporosis. Eur J Endocrinol. 2010;162(6):1009-1020.
52. Sheu A, Diamond T. Secondary osteoporosis. Aust Prescr. 2016;39(3):85-87.
53. Compston J, Cooper A, Cooper C, et al. Guidelines for the diagnosis and management of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. Maturitas. 2009;62(2):105-108.
54. Watts NB, Adler RA, Bilezikian JP, et al. Osteoporosis in men: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(6):1802-1822.
55. Painter SE, Kleerekoper M, Camacho PM. Secondary osteoporosis: a review of the recent evidence. Endocr Pract. 2006;12(4):436-445.
56. van Veenendaal LM, de Klerk G, van der Velde D. A painful finger as first sign of a malignancy. Geriatr Orthop Surg Rehabil. 2014;5(1):18-20.
57. Bilezikian JP, Brandi ML, Eastell R, et al. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the Fourth International Workshop. J Clin Endocrinol Metab. 2014;99 (10):3561-3569.
58. Black DM, Cummings SR, Karpf DB, 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--1541.
59. Chesnut CH, 3rd, Skag A, Christiansen C, et al. Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. J Bone Miner Res. 2004;19(8):1241-1249.
60. Harris ST, Watts NB, Genant HK, et al. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy With Risedronate Therapy (VERT) Study Group. JAMA. 1999;282 (14):1344-1352.
61. Reginster J, Minne HW, Sorensen OH, et al. Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. Vertebral Efficacy with Risedronate Therapy (VERT) Study Group. Osteoporos Int. 2000;11(1):83--91.
62. Kanis JA, Barton IP, Johnell O. Risedronate decreases fracture risk in patients selected solely on the basis of prior vertebral fracture. Osteoporos Int. 2005;16(5): 475-482.
63. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009;361(8): 756-765.
64. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med. 2001;344(19):1434-1441.
65. Lyles KW, Colon-Emeric CS, Magaziner JS, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357(18):1799-1809.
66. Black DM, Thompson DE, Bauer DC, et al. Fracture risk reduction with alendronate in women with osteoporosis: the Fracture Intervention Trial. FIT Research Group. J Clin Endocrinol Metab. 2000;85(11):4118-4124.
67. Johansson H, Oden A, McCloskey EV, Kanis JA. Mild morphometric vertebral fractures predict vertebral fractures but not non-vertebral fractures. Osteoporos Int. 2014;25(1):235-241.
68. Black DM, Schwartz AV, Ensrud KE, 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): 2927-2938.
69. Bone HG, Hosking D, Devogelaer JP, et al. Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med. 2004;350(12):1189--1199.
70. Miller PD, McClung MR, Macovei L, et al. Monthly oral ibandronate therapy in postmenopausal osteoporosis: 1-year results from the MOBILE study. J Bone Miner Res. 2005;20(8):1315-1322.
71. Reginster JY, Adami S, Lakatos P, et al. Efficacy and tolerability of once-monthly oral ibandronate in postmenopausal osteoporosis: 2 year results from the MOBILE study. Ann Rheum Dis. 2006;65(5):654-661.
72. Eisman JA, Civitelli R, Adami S, et al. Efficacy and tole-

rability of intravenous ibandronate injections in postmenopausal osteoporosis: 2-year results from the DIVA study. J Rheumatol. 2008;35(3):488-497.
73. McClung MR, Geusens P, Miller PD, et al. Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. N Engl J Med. 2001;344(5):333-340.
74. Black DM, Delmas PD, Eastell R, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med. 2007;356(18):1809-1822.
75. Sorensen OH, Crawford GM, Mulder H, et al. Long-term efficacy of risedronate: a 5-year placebo-controlled clinical experience. Bone. 2003;32(2):120-126.
76. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-333.
77. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA. 2004;291(14):1701--1712.
78. Cummings SR, Black DM, Thompson DE, et al. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. JAMA. 1998;280(24):2077--2082.
79. Abrahamsen B, Brask-Lindemann D, Rubin KH, Schwarz P. A review of lifestyle, smoking and other modifiable risk factors for osteoporotic fractures. Bonekey Rep. 2014;3:574.
80. Rodrigues AM, Caetano-Lopes J, Vale AC, et al. Smoking is a predictor of worse trabecular mechanical performance in hip fragility fracture patients. J Bone Miner Metab. 2012;30(6):692-699.
81. Maddalozzo GF, Turner RT, Edwards CH, et al. Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. Osteoporos Int. 2009;20(9):1529-1538.
82. Maurel DB, Boisseau N, Benhamou CL, Jaffre C. Alcohol and bone: review of dose effects and mechanisms. Osteoporos Int. 2012;23(1):1-16.
83. Yoon V, Maalouf NM, Sakhaee K. The effects of smoking on bone metabolism. Osteoporos Int. 2012;23(8):2081--2092.
84. Harvey NC, Biver E, Kaufman JM, et al. The role of calcium supplementation in healthy musculoskeletal ageing : An expert consensus meeting of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Foundation for Osteoporosis (IOF). Osteoporos Int. 2017;28(2):447-462.
85. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ. 2011;342:d2040.
86. Bolland MJ, Barber PA, Doughty RN, et al. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. BMJ. 2008;336(7638):262-266.
87. Lewis JR, Zhu K, Prince RL. Adverse events from calcium supplementation: relationship to errors in myocardial infarction self-reporting in randomized controlled trials of calcium supplementation. J Bone Miner Res. 2012;27(3):719-722.
88. Lewis JR, Radavelli-Bagatini S, Rejnmark L, et al. The effects of calcium supplementation on verified coronary heart disease hospitalization and death in postmenopausal women: a collaborative meta-analysis of randomized controlled trials. J Bone Miner Res. 2015;30(1):165-175.
89. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281.
90. Rizzoli R, Boonen S, Brandi ML, et al. Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Curr Med Res Opin. 2013;29(4):305-313.
91. LeBlanc ES, Chou R, Pappas M. Screening for vitamin D deficiency. Ann Intern Med. 2015;162(10):738.
92. Murad MH, Drake MT, Mullan RJ, et al. Clinical review. Comparative effectiveness of drug treatments to prevent fragility fractures: a systematic review and network meta-analysis. J Clin Endocrinol Metab. 2012;97(6):1871-1880.
93. Freemantle N, Cooper C, Diez-Perez A, et al. Results of indirect and mixed treatment comparison of fracture efficacy for osteoporosis treatments: a meta-analysis. Osteoporos Int. 2013;24(1):209-217.
94. Perez Edo L, Alonso Ruiz A, Roig Vilaseca D, et al. [2011 Up-date of the consensus statement of the Spanish Society of Rheumatology on osteoporosis]. Reumatol Clin. 2011;7(6):357-379.
95. Lems WF, Dreinhofer KE, Bischoff-Ferrari H, et al. EULAR/EFORT recommendations for management of patients older than 50 years with a fragility fracture and prevention of subsequent fractures. Ann Rheum Dis. 2017;76(5):802-810.
96. Jamal SA, Ljunggren O, Stehman-Breen C, et al. Effects of denosumab on fracture and bone mineral density by level of kidney function. J Bone Miner Res. 2011;26(8):1829-1835.
97. Hiramatsu R, Ubara Y, Sawa N, et al. Denosumab for low bone mass in hemodialysis patients: a noncontrolled trial. Am J Kidney Dis. 2015;66(1):175-177.

98. Festuccia F, Jafari MT, Moioli A, et al. Safety and efficacy of denosumab in osteoporotic hemodialysed patients. J Nephrol. 2017;30(2):271-279.
99. Schipper LG, Fleuren HW, van den Bergh JP, Meinardi JR, Veldman BA, Kramers C. Treatment of osteoporosis in renal insufficiency. Clin Rheumatol. 2015;34(8): 1341-1345.
100. Boonen S, Sellmeyer DE, Lippuner K, et al. Renal safety of annual zoledronic acid infusions in osteoporotic postmenopausal women. Kidney Int. 2008;74(5):641--648.
101. Warriner AH, Outman RC, Saag KG, et al. Management of osteoporosis among home health and long-term care patients with a prior fracture. South Med J. 2009;102 (4):397-404.
102. Jamal SA, Bauer DC, Ensrud KE, et al. Alendronate treatment in women with normal to severely impaired renal function: an analysis of the fracture intervention trial. J Bone Miner Res. 2007;22(4):503-508.
103. Ishani A, Blackwell T, Jamal SA, Cummings SR, Ensrud KE, Investigators M. The effect of raloxifene treatment in postmenopausal women with CKD. J Am Soc Nephrol. 2008;19(7):1430-1438.
104. Miller PD, Schwartz EN, Chen P, Misurski DA, Krege JH. Teriparatide in postmenopausal women with osteoporosis and mild or moderate renal impairment. Osteoporos Int. 2007;18(1):59-68.
105. FDA U.S. Food and Drug Administration: protecting and promoting your health. Update of safety review follow-up to the October 1, 2007 early communication about the ongoing safety review of bisphosphonates. In. Silver Spring (MD): FDA U.S. Food and Drug Administration; 2008.
106. Kyrgidis A, Toulis KA. Denosumab-related osteonecrosis of the jaws. Osteoporos Int. 2011;22(1):369-370.
107. Abrahamsen B, Jorgensen HL, Laulund AS, et al. The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. J Bone Miner Res. 2015;30(5):898-905.
108. Muschitz C, Kocijan R, Fahrleitner-Pammer A, et al. Overlapping and continued alendronate or raloxifene administration in patients on teriparatide: effects on areal and volumetric bone mineral density—the CONFORS Study. J Bone Miner Res. 2014;29(8):1777-1785.
109. Cosman F, Eriksen EF, Recknor C, et al. Effects of intravenous zoledronic acid plus subcutaneous teriparatide [rhPTH(1-34)] in postmenopausal osteoporosis. J Bone Miner Res. 2011;26(3):503-511.
110. Cosman F, Wermers RA, Recknor C, et al. Effects of teriparatide in postmenopausal women with osteoporosis on prior alendronate or raloxifene: differences between stopping and continuing the antiresorptive agent. J Clin Endocrinol Metab. 2009;94(10):3772-3780.
111. Leder BZ, Tsai JN, Uihlein AV, et al. Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised controlled trial. Lancet. 2015;386(9999):1147-1155.
112. Briot K, Cortet B, Thomas T, et al. 2012 update of French guidelines for the pharmacological treatment of postmenopausal osteoporosis. Joint Bone Spine. 2012;79(3):304-313.
113. Leder BZ, Neer RM, Wyland JJ, Lee HW, Burnett-Bowie SM, Finkelstein JS. Effects of teriparatide treatment and discontinuation in postmenopausal women and eugonadal men with osteoporosis. J Clin Endocrinol Metab. 2009;94(8):2915-2921.
114. Miller PD, Bolognese MA, Lewiecki EM, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. Bone. 2008;43(2):222-229.
115. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA. 1999;281(23):2189-2197.
116. Martino S, Cauley JA, Barrett-Connor E, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst. 2004;96(23):1751-1761.
117. Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA. 2006;295(23):2727-2741.
118. Barrett-Connor E, Mosca L, Collins P, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355(2): 125-137.
119. Qaseem A, Forciea MA, McLean RM, Denberg TD, Clinical Guidelines Committee of the American College of P. Treatment of Low Bone Density or Osteoporosis to Prevent Fractures in Men and Women: A Clinical Practice Guideline Update From the American College of Physicians. Ann Intern Med. 2017;166(11):818-839.
120. Adler RA. Osteoporosis in men: a review. Bone Res. 2014;2:14001.
121. Watts NB, Adler RA, Bilezikian JP, et al. Osteoporosis in men: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(6):1802-1822.
122. Boonen S, Reginster JY, Kaufman JM, et al. Fracture risk and zoledronic acid therapy in men with osteoporosis. N Engl J Med. 2012;367(18):1714-1723.
123. Tremollieres FA, Ceausu I, Depypere H, et al. Osteopo-

rosis management in patients with breast cancer: EMAS position statement. Maturitas. 2017;95:65-71.
124. Buckley L, Guyatt G, Fink HA, et al. 2017 American College of Rheumatology Guideline for the Prevention and Treatment of Glucocorticoid-Induced Osteoporosis. Arthritis Rheumatol. 2017;69(8):1521-1537.
125. Briot K, Cortet B, Roux C, et al. 2014 update of recommendations on the prevention and treatment of glucocorticoid-induced osteoporosis. Joint Bone Spine. 2014;81(6):493-501.
126. Maraka S, Kennel KA. Bisphosphonates for the prevention and treatment of osteoporosis. BMJ. 2015;351: h3783.
127. Kothawala P, Badamgarav E, Ryu S, Miller RM, Halbert RJ. Systematic review and meta-analysis of real-world adherence to drug therapy for osteoporosis. Mayo Clin Proc. 2007;82(12):1493-1501.
128. Imaz I, Zegarra P, Gonzalez-Enriquez J, Rubio B, Alcazar R, Amate JM. Poor bisphosphonate adherence for treatment of osteoporosis increases fracture risk: systematic review and meta-analysis. Osteoporos Int. 2010;21(11):1943-1951.
129. McCloskey EV, Beneton M, Charlesworth D, et al. Clodronate reduces the incidence of fractures in community-dwelling elderly women unselected for osteoporosis: results of a double-blind, placebo-controlled randomized study. J Bone Miner Res. 2007;22(1):135-141.
130. Cummings SR, Karpf DB, Harris F, et al. Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. Am J Med. 2002;112(4):281-289.
131. Chen P, Miller PD, Delmas PD, Misurski DA, Krege JH. Change in lumbar spine BMD and vertebral fracture risk reduction in teriparatide-treated postmenopausal women with osteoporosis. J Bone Miner Res. 2006;21 (11):1785-1790.
132. Austin M, Yang YC, Vittinghoff E, et al. Relationship between bone mineral density changes with denosumab treatment and risk reduction for vertebral and nonvertebral fractures. J Bone Miner Res. 2012;27(3):687--693.
133. Diez-Perez A, Adachi JD, Agnusdei D, et al. Treatment failure in osteoporosis. Osteoporos Int. 2012;23(12): 2769-2774.
134. Lewiecki EM, Cummings SR, Cosman F. Treat-to-target for osteoporosis: is now the time? J Clin Endocrinol Metab. 2013;98(3):946-953.
135. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. 2011;22(2): 391-420.
136. Ravn P, Hosking D, Thompson D, et al. Monitoring of alendronate treatment and prediction of effect on bone mass by biochemical markers in the early postmenopausal intervention cohort study. J Clin Endocrinol Metab. 1999;84(7):2363-2368.
137. Bauer DC, Black DM, Garnero P, et al. Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. J Bone Miner Res. 2004;19(8):1250-1258.
138. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD. Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res. 2003;18(6):1051-1056.
139. Eastell R, Krege JH, Chen P, Glass EV, Reginster JY. Development of an algorithm for using PINP to monitor treatment of patients with teriparatide. Curr Med Res Opin. 2006;22(1):61-66.
140. Eastell R, Christiansen C, Grauer A, et al. Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. J Bone Miner Res. 2011;26(3):530--537.
141. Diez-Perez A, Naylor KE, Abrahamsen B, et al. International Osteoporosis Foundation and European Calcified Tissue Society Working Group. Recommendations for the screening of adherence to oral bisphosphonates. Osteoporos Int. 2017;28(3):767-774.
142. Naylor KE, Jacques RM, Paggiosi M, et al. Response of bone turnover markers to three oral bisphosphonate therapies in postmenopausal osteoporosis: the TRIO study. Osteoporos Int. 2016;27(1):21-31.
143. Black DM, Reid IR, Boonen S, 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--254.
144. Dell RM, Adams AL, Greene DF, et al. Incidence of atypical nontraumatic diaphyseal fractures of the femur. J Bone Miner Res. 2012;27(12):2544-2550.
145. Ensrud KE, Barrett-Connor EL, Schwartz A, et al. Randomized trial of effect of alendronate continuation versus discontinuation in women with low BMD: results from the Fracture Intervention Trial long-term extension. J Bone Miner Res. 2004;19(8):1259-1269.
146. Ravn P, Christensen JO, Baumann M, Clemmesen B. Changes in biochemical markers and bone mass after withdrawal of ibandronate treatment: prediction of bone mass changes during treatment. Bone. 1998;22 (5):559-564.
147. Watts NB, Chines A, Olszynski WP, et al. Fracture risk remains reduced one year after discontinuation of risedronate. Osteoporos Int. 2008;19(3):365-372.
148. Russell RG. Bisphosphonates: the first 40 years. Bone. 2011;49(1):2-19.
149. Compston J, Bowring C, Cooper A, et al. Diagnosis and management of osteoporosis in postmenopausal women and older men in the UK: National Osteoporosis

Guideline Group (NOGG) update 2013. Maturitas. 2013;75(4):392-396.
150. Reid IR, Miller PD, Brown JP, et al. Effects of denosumab on bone histomorphometry: the FREEDOM and STAND studies. J Bone Miner Res. 2010;25(10):2256--2265.
151. Leder BZ, Tsai JN, Jiang LA, Lee H. Importance of prompt antiresorptive therapy in postmenopausal women discontinuing teriparatide or denosumab: The Denosumab and Teriparatide Follow-up study (DATA-Follow-up). Bone. 2017;98:54-58.
152. Weaver CM, Gordon CM, Janz KF, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016;27(4):1281-1386.
153. Graça P, Camolas J, Gregório MJ, Sousa S. Programa Nacional para a Promoção da Alimentação Saudável In. Lisboa: Direção-Geral da Saúde; 2017.
154. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-1930.
155. Weaver CM, Alexander DD, Boushey CJ, et al. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. Osteoporos Int. 2016;27(1):367-376.
156. Zhao R, Zhao M, Xu Z. The effects of differing resistance training modes on the preservation of bone mineral density in postmenopausal women: a meta-analysis. Osteoporos Int. 2015;26(5):1605-1618.
157. Shepstone L, Lenaghan E, Cooper C, et al. Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial. Lancet. 2017.
158. Kanis JA, Johansson H, Oden A, McCloskey EV. A meta-analysis of the efficacy of raloxifene on all clinical and vertebral fractures and its dependency on FRAX. Bone. 2010;47(4):729-735.

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

The work presented in this thesis focuses on fragility fracture prevention, from assessment of an individual patient´s risk to driving changes in health policies. It encompasses five major contributions to fragility fracture prevention and management: 1) we determined the relative importance of osteoporosis and fragility fractures in Portugal and identified population strata at highest risk of fractures to establish priorities for research and public health actions; 2) we contributed to increasing knowledge about the clinical risk factors for fragility fractures associated with bone biomechanics; 3) we provided further insight into understanding the mechanisms promoting fragility fractures by examining the relationships between osteoblast differentiation markers and bone intrinsic mechanical properties; 4) we identified new serum markers (among osteoblast differentiation regulators) of bone fragility and fractures, which may be used to improve risk stratification; and 5) we contributed to the improvement of individual clinical assessment through the development of national clinical consensus recommendations regarding fragility fracture risk assessment, monitoring, and management.

The studies presented in this thesis crossed two main areas of clinical research: patient-oriented mechanistic research and epidemiological research. This interaction between laboratory-based research and population-based research enabled us to contribute in a robust manner to the scientific understanding of fragility fractures.

We began by addressing the prevalence and burden of osteoporosis in Portugal (Chapter IV, section I, part 1) under the scope of EpiReumaPt study. The results of the study showed that 10.2% of Portuguese adults have osteoporosis. In the study, osteoporosis was determined clinically by a rheumatologist based on pre-determined criteria. Osteoporosis prevalence was higher in women (17%) than in men (2.6%) and increased with age. Almost half (40%) of Portuguese adults 75 years of age or older have osteoporosis, and a diagnosis of osteoporosis was associated with substantial physical functional impairment but not with anxiety or depression symptoms. Contrary to

previous studies, we verified that osteoporosis is not less frequent in Portugal than in northern European countries (30, 31). Osteoporosis is highly prevalent in Portugal, with a prevalence rate similar to that reported in other countries (149-158).

The second study of this thesis, presented in Chapter IV, Section I, part 2, focused on the stratum most vulnerable to osteoporosis and to fragility fractures: senior women (women aged ≥65 years old). We evaluated the prevalence of and risk factors for fragility fractures, as well as osteoporosis treatment rates, in these women and verified that self-reported fragility fractures were highly prevalent (20.7%). However, the high prevalence of fragility fractures was in stark contrast with the low rate of osteoporosis treatment (13.9%). NHNV (lower leg, wrist, humerus, rib, clavicle, and elbow) fractures accounted for the majority of fragility fractures, and the clinical risk factors independently associated with prevalent fragility fractures were increased age, obesity, and a lower distal BMD. The prevalence of fragility fractures among senior women in Portugal was similar to that of other Mediterranean countries (2), but slightly lower than the rate in other countries of northern Europe (159-161), Australia (162), and the United States of America. The finding that NHNV fractures were the most prevalent fracture sites is consistent with the results of other studies reporting that NHNV fractures accounted for more than two-thirds of all fragility fractures (7, 8, 163). The results of our analysis of clinical risk factors for prevalent fragility fractures can be added to those of previous studies performed in other populations, especially our findings of age and low distal BMD as risk factors (161, 164). The association between obesity and fragility fractures (especially NHNV fractures) was reported in other studies (162, 165). It is interesting to speculate on the mechanisms by which obesity may confer an increased risk of fracture. Recent studies showed that visceral obesity is associated with a low BMD, probably because of higher levels of inflammatory cytokines, lower levels of leptin, and higher levels of adiponectin (166). Moreover, obese people have a higher risk of falls and impaired protective responses (167).

Worldwide, osteoporosis treatments are not appropriately provided to high-risk patients, such as those who have sustained a previous fragility fracture (146, 168). There is a large gap between the number of women who are treated and the number of

women eligible for treatment (169). The largest treatment gaps were previously described in Bulgaria and the Baltic States, where less than 15% of the eligible population receives osteoporosis treatment; in Spain, 75% of eligible women do not receive osteoporosis treatment (2). Moreover, even in patients who sustain a fragility fracture, less than 20% receive treatment in the year following the fracture. Our study showed that treatment rates in Portugal are even lower, as only 13.9% of senior women who sustained a fragility fracture ever received osteoporosis treatment. Osteoporosis treatment was never prescribed in 54.7% of women with a fracture and 23.4% with a fracture were offered a prescription but refused treatment. When we calculated the 10-year risk of a subsequent fragility fracture using the FRAX algorithm without BMD, we found that a low proportion of women who were eligible for osteoporosis treatment according to Portuguese guidelines (20) were actually receiving this treatment. These results highlight the importance of updating osteoporosis treatment guidelines in Portugal and ensuring their correct implementation in clinical practice.

Low treatment rates are not the only reason for the high prevalence of fragility fractures in the elderly, and better risk stratification approaches and screening strategies are required. Assessment of fracture risk should include the most relevant factors that contribute to fragility fractures. The most widely tools for risk assessment are measurement of BMD, determination of serum levels of bone turnover markers, and the use of algorithms for fracture risk prediction that include weighted clinical risk factors for fractures and BMD, such as the FRAX tool (91, 92). The fracture risk prediction algorithms, in particular, have improved the identification of individuals with a high risk of fractures, but they still fail to identify a substantial number of individuals who will have a fragility fracture, especially non-hip fractures (93-95).

The challenge to better identify seniors at high risk for a fragility fracture led us to search for novel noninvasive biomarkers of bone fragility and fractures. To achieve this goal, we conducted patient-oriented mechanistic research, looking for associations between cellular mechanism dysfunction and bone fragility among the elderly. In the third thesis study, presented in Chapter IV, section II, part 1, we compared patients undergoing hip replacement surgery because of a fragility fracture with patients undergoing the same

surgery because of osteoarthritis. First, we compared bone macrostructural parameters between hip fragility fracture and osteoarthritis patients. We demonstrated that when adjusted for differences in age, sex, and BMI, the only macrostructural bone characteristic still significantly different between the hip fragility fracture and osteoarthritis groups was trabecular stiffness. Specifically, it was lower in patients with fragility fractures. We also demonstrated that smoking and female sex were independently associated with lower stiffness in patients with a hip fragility fracture. We did not find significant differences in areal BMD between smokers and non-smokers, suggesting that the effects of smoking on trabecular bone's intrinsic properties are independent of areal BMD. Corroborating this, a recent meta-analysis found that a low areal BMD accounted for only 23% of the smoking-related risk of hip fracture (116). In fact, animal model studies revealed that nicotine reduced bone strength but did not affect areal BMD (170). It is interesting to speculate on the mechanisms by which smoking is associated with poor bone quality. The changes in bone metabolism induced by smoking are not completely clear; however, they may be related to altered calciotropic hormone metabolism (171). Smoking may alter the hepatic metabolism of vitamin D by influencing 25-hydroxylase in the liver and lowering serum levels of 25-hydroxyvitamin D, similar to smoking's ability to enhance hepatic degradation of oestrogen (172). Smoking also affects the production and binding of oestradiol (173, 174). Furthermore, nicotine has direct effects on bone cells: it inhibits osteoblast bone formation (175-177) and stimulates osteoclast bone resorption (178).

This third study was particularly original because it evaluated intrinsic trabecular bone hip properties using *ex vivo* mechanical tests and correlated these results with epidemiological and clinical factors in the elderly. Furthermore, it showed that patients with hip fractures had significantly lower bone stiffness than those with osteoporosis. Disturbances in this intrinsic mechanical property are linked with mineralization disturbances (179), and Fratzl-Zelman and colleagues have shown that under-mineralized bone matrix is associated with fragility fractures (180).

Considering that osteoblasts are responsible for producing mineralized bone (57, 58), we hypothesized that with ageing, bone mineralization is impaired because of

osteoblast dysfunction, leading to fragility fractures. Osteoblast differentiation from mesenchymal progenitors initially requires the transcription factors RUNX2 and OSX and later requires canonical Wnt signalling (181-184). Differentiated osteoblasts synthesize COL1a1 and OCL, and the ratio of OCL/COL1a1 expression in bone reflects the relationship between osteoblast terminal differentiation and mineralization impairment.

In Chapter IV, Section II, Part 2, we presented the fourth study of this thesis, which was conducted using data from patients undergoing hip replacement surgery at Hospital de Santa Maria and a large panel of bone factors that are markers of osteoblast commitment and differentiation. We evaluated whether osteoblast differentiation dysfunction was associated with bone fragility and fractures among elderly patients. We have found that OCL relative bone expression and the bone OCL/COL1A1 expression ratio (markers of osteoblast terminal differentiation) were significantly lower in patients with hip fractures than in those with osteoarthritis. Likewise, in a subset of these patients, fewer osteoblasts stained positively for OC in patients with fragility fractures than in those with osteoarthritis**.** We also demonstrated that in patients with hip fractures, a low bone OCL/COL1A1 expression ratio was associated with worse trabecular mechanical behaviour. Moreover, we found no differences in RUNX2 and OSX bone expression between groups, and expression of these transcription factors was not associated with bone mechanical behaviour within the groups. These observations indicate that commitment to the osteoblast lineage is not compromised in fragility fractures, but differentiation of cells to the final stage is impaired, leading to fragility. These findings suggest that in patients with hip fractures, imbalance of the OC/COL1A1 expression ratio reflects disturbances in osteoblast activity, affecting bone metabolism and bone matrix/mineral ratio, ultimately leading to bone fragility. In conclusion, this work reinforced the importance of osteoblast dysfunction in bone intrinsic properties and fractures among elderly.

In the fifth study of this thesis, presented in Chapter IV, section III, part 1, we implemented rigorous research to refine fracture risk assessment in senior women through the identification of new serum markers for bone fragility and fractures. The

candidate osteoblast-mediated bone formation regulators were the inhibitors of Wnt signalling. We decided to use these candidate proteins not only because this pathway is a major regulator of osteoblast terminal differentiation but also because prior studies identified the importance of Wnt signalling in post-menopausal osteoporosis. It was previously shown that serum DKK1 and SOST levels increase with age and are associated with loss of bone mass (77-79). Mirza and colleagues found that serum SOST levels were significantly higher in post-menopausal women and were inversely associated with the free oestrogen index (80). In addition, treatment with either anti-SOST or anti-DKK1 antibodies in post-menopausal osteoporosis increased bone formation, bone mass, and bone strength and were effective in preventing fracture (81, 82, 115). In this study, we used a subpopulation of the Nationwide cohort, the EpiDoC cohort, to evaluate whether serum levels of Wnt regulators (DKK1, DKK2, SOST, and sFRP-1) were associated with bone fragility and fractures and could constitute new markers of fragility fractures. Our study showed that low serum levels of DKK2 predicted low-impact fractures independently of BMD and clinical risk factors for fractures. For every 1 SD decrease in DKK2, fracture risk increased approximately 1.5-fold. Serum levels of DKK2 were not associated with vertebral or hip BMD. This may be attributed to the fact that osteoblast mineralization disturbances, represented by low levels of DKK2, lead to bone nanoarchitecture disorganization and fragility, independent of loss of bone mass (185-187). We also found that high levels of sFRP-1 were associated with incident low-impact fractures, independent of clinical risk factors for fracture. However, this association was not independent of BMD. Furthermore, serum levels of SOST were not significantly associated with incident low-impact fractures. Similarly, the OFELY study followed post-menopausal women for 6 years and reported no association between serum levels of SOST and incident fractures (188). Amrein et al. also found no association between SOST serum levels and fragility fractures in institutionalized elderly women (189). By contrast, the Centre of Excellence for Osteoporosis Research Study, which followed 707 post-menopausal women, reported that a high serum SOST level was associated with an increased risk of fractures (190). Although we observed a negative correlation between serum levels of DKK1 and BMD, similar to the results of previous reports (79, 191), we found no association between DKK1 serum levels and incident fractures. In a cross-sectional study in Sweden, serum levels of DKK1 were increased in patients with a recent

hip fracture when compared with healthy volunteers (192). In contrast, a study from Korea found no association between serum DKK1 levels and osteoporotic fractures (193). In conclusion, we found that low serum levels of DKK2 predict an increased risk of low-impact fractures, independent of BMD and clinical risk factors, and should thus be explored as a potential noninvasive marker of fragility fracture risk.

The final work of this thesis, presented in Chapter IV, Section IV, involved the development of national clinical consensus recommendations for osteoporosis diagnosis and treatment to reduce the incidence of fragility fractures. This work was motivated by the observation, which we verified, that among a high-risk population (senior women), osteoporosis treatment rates are lower in Portugal than elsewhere in Europe. We thereby considered that it was of upmost importance to generate clinical consensus recommendations in our country and implement a uniform risk stratification approach, a unique screening strategy, and an effective treatment plan for the Portuguese adult population with a moderate to high fracture risk.

Previous recommendations from the Portuguese Society of Rheumatology on osteoporosis management were published in 2007 (194). In these recommendations, fracture risk assessment was based mostly on BMD in the lumbar spine and hip, as determined by DXA. Pharmacological treatment was recommended for patients meeting these indications: 1) WHO definition of osteoporosis; 2) osteopenic patients with clinical risk factors for fragility fractures; and 3) fragility fracture patients with osteopenia and /or other clinical risks.

Osteoporosis treatment recommendations for post-menopausal women from the National Health Directorate, published in 2011 (195), acknowledged that treatment decisions should be based on an individual's absolute risk of fracture. It was recognized that an individual's fracture risk is influenced by several clinical risk factors beyond BMD (93, 196, 197). However, because our country did not have valid tools to evaluate individual fracture risk, treatment decision recommendations were based on the existence of a previous fragility fracture or DXA results plus clinical risk factors (195).

The work presented in Chapter IV, Section IV of this thesis, aimed to shift fracture risk assessment in Portuguese clinical practice from assessment based mostly on BMD to an absolute individual fracture risk estimation. It is well established in the scientific literature that screening based on BMD alone fails to predict almost 50% of fragility fractures (93, 98). Because of that, several fracture risk prediction algorithms were developed, with the FRAX algorithm being the most widely used tool. Absolute fracture risk calculation using FRAX (clinical risk factors with or without BMD information) increased the prediction of fragility fracture, particularly hip fractures (197). In fact, a recent randomized controlled trial, comparing a FRAX-based screening program with usual management in women aged 70-85 years in the United Kingdom showed that the FRAX screening program effectively reduced the 5-year risk of a hip fracture by 28%, although no improvement was found in the prediction of other fragility fractures (94). This study, concluded that the FRAX algorithm is a valid tool for fracture risk assessment in the community. Regardless, there is still room for improvement in predicting the risk of fragility fracture. Future studies should continue to look for new tools that enhance fracture risk prediction, such as the serum biomarkers we studied in Chapter IV, Section III, part 1.

The FRAX tool has some caveats (198); however, it is currently the best and most studied tool available for fracture risk prediction (25, 199). In fact, a meta-analysis evaluating the accuracy of osteoporotic fracture risk prediction tools showed that FRAX has the largest number of externally validated and independent studies with satisfactory accuracy (area under the curve $>$ 0.709), which was higher than BMD (25). The FRAX tool was validated in Portugal by Marques et al. (33), and cost-effectiveness analysis were performed to define national treatment thresholds for osteoporosis pharmacological treatment (126). To implement this new knowledge in Portugal, clinical consensus recommendations regarding fracture risk assessment and treatment plans were needed.

The first consensus recommendations in which the PhD student participated were the multidisciplinary recommendations for DXA requests and indications for the treatment and prevention of fragility fractures. When generating these recommendations, a panel

of 17 persons composed of osteoporosis experts and members of relevant Portuguese scientific societies (Rheumatology, Orthopaedics, Endocrinology, Gynaecology, and Internal Medicine) defined the questions that needed to be addressed in these recommendations; 2 researchers reviewed the literature; the principal investigator created draft recommendations; and the 17 experts reformulated and voted on the recommendations during two meetings. The consensus recommendations established among representatives from different specialities were a very important step in improving patient fracture risk stratification. In these recommendations, it was stated for the first time in Portugal that "all subjects over age of 50 years should have their 10-year risk of fracture estimated using FRAX tool with or without DEXA result." Also, the threshold of osteoporosis pharmacological treatment for people with a high risk of fractures was based on a cost-effectiveness analysis performed in Portugal.

In the second study related to consensus recommendations, presented in Chapter IV, Section IV, part 2, 55 rheumatologists and rheumatology fellows and one specialized rheumatology nurse were involved in the "2018 Update of the Portuguese Recommendations for the Prevention, Diagnosis, and Management of Primary Osteoporosis." At the first meeting, relevant questions were defined by the working group, and a thorough review of the literature was conducted by research fellows (including the PhD student) to address each question. After the literature review, the working group prepared proposals for the recommendations, which were presented, discussed, and revised in two national meetings and refined through electronic consultation. The draft recommendations proposal, which included summaries of the supporting evidence, was prepared by the PhD student and principal investigator. The draft proposal was reviewed again by the working group. Finally, the document was circulated among all Portuguese rheumatologists and rheumatology fellows (N=226), who anonymously voted via an online survey on their level of agreement with each recommendation. In total, 88 participants (69.8% of the Portuguese rheumatologists and rheumatology fellows) voted, and the mean agreement for the recommendations was 8.7 on a 10-point numerical rating scale (1 = no agreement, 10 = full agreement). Each recommendation was based on the best available evidence and were made as "good practice recommendations," defined as the working group believing the benefits

of following the recommendation far outweighed the harms, although the supporting evidence was indirect or weak.

The final recommendations of the Update contain several new and updated recommendations. First, a clear statement is made regarding the benefits of assessing clinical risk factors for fragility fractures throughout life. The recommendations also state (as in the multidisciplinary recommendations) that absolute fracture risk should be assessed in all adults after the age of 50 years using the FRAX tool validated for the Portuguese population. Pharmacological treatment should be started if an individual has a previous fragility fracture or is above the FRAX thresholds previously defined in the multidisciplinary recommendations. Furthermore, the final recommendations updated the treatment plan, considering efficacy data, costs, benefits, and harms of each drug. Overall, the Update provides a useful guide for fracture risk assessment, treatment strategies, and patient monitoring, which was generated by the approval of a large number of Portuguese rheumatologists and rheumatology fellows.

Conclusions

In conclusion, this thesis provided rigorous epidemiological data regarding the prevalence of osteoporosis and fragility fractures in Portugal. It contributed to refining fracture risk assessment through the identification of new serum markers (among regulators of osteoblast-mediated bone formation) associated with bone fragility and fractures. The identification of these markers arose from mechanistic clinical research on bone biomechanics and osteoblast dysfunction in patients with hip fractures. These studies showed that fragility fractures are associated with reduced bone stiffness, reflecting mineralization disturbances. Reduced osteoblast terminal differentiation is also associated with poor bone mechanics and fragility fractures. This thesis also describes the formulation of national consensus recommendations regarding fracture risk assessment of individuals, as well as osteoporosis clinical management and treatment, with the goal of changing clinical practice and reducing the incidence of fragility fractures in Portugal.

Future Perspectives and Our Future Research Work

Many unmet research needs in osteoporosis and fragility fracture field remain to be addressed. One item on the agenda for future research is to provide evidence for the benefits of a screening strategy based on absolute fracture risk over standard of care, particularly to validate the benefits of the treatment thresholds based on cost-effectiveness analyses over usual care. Fracture risk assessment can be refined, and new fragility fracture markers (such as serum biomarkers) that independently increase fracture risk can and should be incorporated into the FRAX tool. In our future research, the new biomarker for fragility fracture (serum level of DKK2) identified in this PhD thesis can be tested over a longer follow-up period in another cohort of Portuguese subjects to determine the reproducibility of our findings. If the same results are obtained, it will be important to conduct research to test these findings in other countries as well. Other interesting future questions are how to incorporate serum biomarkers into an algorithm of fracture prediction for testing in long-term observational studies and randomized controlled trials and whether we can establish biomarker cut-off values for fragility fracture risk prediction.

Furthermore, fragility fractures must be adequately addressed in older people to reduce new fracture events, physical disability, and premature death. We hypothesize that a patient-centred solution for the community setting will effectively reduce the incidence of fragility fractures among the elderly. We conceptualize healthcare as a continuum, provided at the community level, including at home, to promote healthy active ageing instead of a reactive system that simply treats acute events and uncontrolled chronic diseases. This could be achieved through the development of innovative treatment approaches to reduce fracture risk. In our future research, we plan to explore the use an interactive online tool composed of a comprehensive multi-module protocol with clinical data and a customized intervention strategy. This intervention tool is an online streaming platform that creates a visual intervention program protocol of lifestyles and treatment adherence. Exercise videos, tips, and simple questionnaires can be uploaded into the program. It has a short message service (SMS) notification system and an

interactive chat room. All contents can be uploaded by the user and customized according to the needs of the individual. This platform will be validated and tested to assist seniors living at home who have a high risk of fragility fracture. It will provide assistance with falls prevention, long-term self-management, motivation to adhere to osteoporosis treatment, and lifestyle improvements. This research concept is based on a previous project conducted by our research team that has already shown promising results, which involved a television program designed to improve living conditions in the elderly; the program is available through all Portuguese television providers (200). Every day, individuals were given tasks and tips to improve their physical condition, diet, and lifestyle. All content for the proposed projects has already been developed. The new challenge will be to integrate the television app with an online interactive platform that will send notifications to individuals and allow them to communicate with health professionals, who can answer questions and discuss other issues; respond to questionnaires; and record measures they have or have not taken. This platform will boost home-based intervention programs in a population at high-risk for fragility fracture, in which effective and accessible treatment strategies are clearly needed.

Finally, we propose to create a national network of coordinator-based systems led by rheumatologists for the management of health issues related to fragility fractures. The costs and effectiveness in fracture risk reduction of these coordinator-based systems should be tested and compared with standard of care (using non-adherent health units as comparators). At present, patients with a fragility fracture receive fragmented care in Portugal. They typically present with their first fracture to the emergency department or an orthopaedic surgeon. Those health care providers focus on repairing the broken bones and medically managing acute complications. The identification and management of patients with an increased risk of future fragility fracture is another dimension that typically occurs in hospital outpatient clinics (such as orthopaedics, rheumatology, or endocrinology clinics) or the primary care setting. However, no clear pathway is created to ensure that all patients with a fragility fracture are evaluated for future fracture risk, osteoporosis, and the need for treatment to prevent future fractures. This proposed national network of coordinator-based systems will develop and implement an effective referral strategy for patients with a recent fragility fracture (within the past 12 months), and a personalized multidisciplinary treatment approach will be created in accordance

with Portuguese guidelines to prevent new fragility fractures, disability, and early death. This national network coordinator-based integrated system—composed of healthcare professionals (using the best evidence in clinical practice), patients, and families—will contribute to the attainment of our ultimate goal: preventing fractures in all Portuguese seniors.

CHAPTER VI

REFERENCES

1. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137-41.
2. Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8:136.
3. Sornay-Rendu E, Munoz F, Garnero P, Duboeuf F, Delmas PD. Identification of osteopenic women at high risk of fracture: the OFELY study. J Bone Miner Res. 2005;20(10):1813-9.
4. Sheu A, Diamond T. Secondary osteoporosis. Aust Prescr. 2016;39(3):85-7.
5. Samelson EJ, Hannan MT, Zhang Y, Genant HK, Felson DT, Kiel DP. Incidence and risk factors for vertebral fracture in women and men: 25-year follow-up results from the population-based Framingham study. J Bone Miner Res. 2006;21(8):1207-14.
6. Kanis JA, Borgstrom F, Compston J, Dreinhofer K, Nolte E, Jonsson L, et al. SCOPE: a scorecard for osteoporosis in Europe. Arch Osteoporos. 2013;8:144.
7. Roux C, Wyman A, Hooven FH, Gehlbach SH, Adachi JD, Chapurlat RD, et al. Burden of non-hip, non-vertebral fractures on quality of life in postmenopausal women: the Global Longitudinal study of Osteoporosis in Women (GLOW). Osteoporos Int. 2012;23(12):2863-71.
8. Adachi JD, Ioannidis G, Pickard L, Berger C, Prior JC, Joseph L, et al. The association between osteoporotic fractures and health-related quality of life as measured by the Health Utilities Index in the Canadian Multicentre Osteoporosis Study (CaMos). Osteoporos Int. 2003;14(11):895-904.
9. Pike CT, Birnbaum HG, Schiller M, Swallow E, Burge RT, Edgell ET. Prevalence and costs of osteoporotic patients with subsequent non-vertebral fractures in the US. Osteoporos Int. 2011;22(10):2611-21.
10. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res. 2005;20(7):1185-94.
11. Fiorano-Charlier C, Ostertag A, Aquino JP, de Vernejoul MC, Baudoin C. Reduced bone mineral density in postmenopausal women self-reporting premenopausal wrist fractures. Bone. 2002;31(1):102-6.
12. Melton LJ, 3rd, Atkinson EJ, O'Fallon WM, Wahner HW, Riggs BL. Long-term fracture prediction by bone mineral assessed at different skeletal sites. J Bone Miner Res. 1993;8(10):1227-33.
13. Stone KL, Seeley DG, Lui LY, Cauley JA, Ensrud K, Browner WS, et al. BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. J Bone Miner Res. 2003;18(11):1947-54.
14. Center JR, Bliuc D, Nguyen TV, Eisman JA. Risk of subsequent fracture after low-trauma fracture in men and women. JAMA. 2007;297(4):387-94.
15. Oden A, McCloskey EV, Kanis JA, Harvey NC, Johansson H. Burden of high fracture probability worldwide: secular increases 2010-2040. Osteoporos Int. 2015;26(9):2243-8.

16. WHO. World health statistics 2016: monitoring health for the SDGs. http://wwwwhoint/gho/publications/world_health_ statistics/2016/Annex_B/en/ 2016.
17. Harvey NC, McCloskey EV, Mitchell PJ, Dawson-Hughes B, Pierroz DD, Reginster JY, et al. Mind the (treatment) gap: a global perspective on current and future strategies for prevention of fragility fractures. Osteoporos Int. 2017;28(5):1507-29.
18. Compston JE, Rosen CJ. Epidemiology. In: Compston JE, Rosen CJ, editors. Osteoporosis. 2nd edition ed. Oxford: Health Press; 1999.
19. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002;359(9321):1929-36.
20. Marques A, Rodrigues AM, Romeu JC, Ruano A, Barbosa AP, Simoes E, et al. Multidisciplinary Portuguese recommendations on DXA request and indication to treat in the prevention of fragility fractures. Acta Reumatol Port. 2016;41(4):305-21.
21. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporos Int. 2014;25(10):2359-81.
22. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster JY, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2013;24(1):23-57.
23. Lentle B, Cheung AM, Hanley DA, Leslie WD, Lyons D, Papaioannou A, et al. Osteoporosis Canada 2010 guidelines for the assessment of fracture risk. Can Assoc Radiol J. 2011;62(4):243-50.
24. Blain H, Masud T, Dargent-Molina P, Martin FC, Rosendahl E, van der Velde N, et al. A comprehensive fracture prevention strategy in older adults: the European Union Geriatric Medicine Society (EUGMS) statement. Aging Clin Exp Res. 2016;28(4):797-803.
25. Marques A, Ferreira RJ, Santos E, Loza E, Carmona L, da Silva JA. The accuracy of osteoporotic fracture risk prediction tools: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(11):1958-67.
26. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17(12):1726-33.
27. Oden A, McCloskey EV, Johansson H, Kanis JA. Assessing the impact of osteoporosis on the burden of hip fractures. Calcif Tissue Int. 2013;92(1):42-9.
28. Bliuc D, Nguyen ND, Milch VE, Nguyen TV, Eisman JA, Center JR. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. JAMA. 2009;301(5):513-21.
29. Papadimitriou N, Tsilidis KK, Orfanos P, Benetou V, Ntzani EE, Soerjomataram I, et al. Burden of hip fracture using disability-adjusted life-years: a pooled analysis of prospective cohorts in the CHANCES consortium. Lancet Public Health. 2017;2(5):e239-e46.
30. Elffors I, Allander E, Kanis JA, Gullberg B, Johnell O, Dequeker J, et al. The variable incidence of hip fracture in southern Europe: the MEDOS Study. Osteoporos Int. 1994;4:253-63.
31. Falch JA, Ilebekk A, Slungaard U. Epidemiology of hip fractures in Norway. Acta Orthop Scand. 1985;56:12-6.
32. de Pina MF, Alves SM, Barbosa M, Barros H. Hip fractures cluster in space: an epidemiological analysis in Portugal. Osteoporos Int. 2008;19(12):1797-804.

33. Marques A, Mota A, Canhao H, Romeu JC, Machado P, Ruano A, et al. A FRAX model for the estimation of osteoporotic fracture probability in Portugal. Acta Reumatol Port. 2013;38(2):104-12.
34. Marques A, Lourenco O, da Silva JA. The burden of osteoporotic hip fractures in Portugal: costs, health related quality of life and mortality. Osteoporos Int. 2015;26(11):2623-30.
35. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. N Engl J Med. 2006;354(21):2250-61.
36. Herring GM. A comparison of bone matrix and tendon with particular reference to glycoprotein content. The Biochemical journal. 1976;159(3):749-55.
37. Currey JD. The mechanical consequences of variation in the mineral content of bone. J Biomech. 1969;2(1):1-11.
38. Vashishth D, Gibson GJ, Khoury JI, Schaffler MB, Kimura J, Fyhrie DP. Influence of nonenzymatic glycation on biomechanical properties of cortical bone. Bone. 2001;28(2):195-201.
39. Recker RR. Skeletal fragility and bone quality. J Musculoskelet Neuronal Interact. 2007;7(1):54-5.
40. Roschger P, Rinnerthaler S, Yates J, Rodan GA, Fratzl P, Klaushofer K. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. Bone. 2001;29(2):185-91.
41. Durchschlag E, Paschalis EP, Zoehrer R, Roschger P, Fratzl P, Recker R, et al. Bone material properties in trabecular bone from human iliac crest biopsies after 3- and 5-year treatment with risedronate. J Bone Miner Res. 2006;21(10):1581-90.
42. Brouwers JE, van Rietbergen B, Huiskes R, Ito K. Effects of PTH treatment on tibial bone of ovariectomized rats assessed by in vivo micro-CT. Osteoporos Int. 2009;20(11):1823-35.
43. Boivin G, Farlay D, Khebbab MT, Jaurand X, Delmas PD, Meunier PJ. In osteoporotic women treated with strontium ranelate, strontium is located in bone formed during treatment with a maintained degree of mineralization. Osteoporos Int. 2010;21(4):667-77.
44. Black DM, Thompson DE, Bauer DC, Ensrud K, Musliner T, Hochberg MC, et al. Fracture risk reduction with alendronate in women with osteoporosis: the Fracture Intervention Trial. FIT Research Group. J Clin Endocrinol Metab. 2000;85(11):4118-24.
45. Zoehrer R, Roschger P, Paschalis EP, Hofstaetter JG, Durchschlag E, Fratzl P, et al. Effects of 3- and 5-year treatment with risedronate on bone mineralization density distribution in triple biopsies of the iliac crest in postmenopausal women. J Bone Miner Res. 2006;21(7):1106-12.
46. Mirzaali MJ, Schwiedrzik JJ, Thaiwichai S, Best JP, Michler J, Zysset PK, et al. Mechanical properties of cortical bone and their relationships with age, gender, composition and microindentation properties in the elderly. Bone. 2016;93:196-211.
47. Zhu K, Briffa K, Smith A, Mountain J, Briggs AM, Lye S, et al. Gender differences in the relationships between lean body mass, fat mass and peak bone mass in young adults. Osteoporos Int. 2014;25(5):1563-70.
48. Nelson DA, Pettifor JM, Barondess DA, Cody DD, Uusi-Rasi K, Beck TJ. Comparison of cross-sectional geometry of the proximal femur in white and black women from Detroit and Johannesburg. J Bone Miner Res. 2004;19(4):560-5.

49. Cardoso L, Herman BC, Verborgt O, Laudier D, Majeska RJ, Schaffler MB. Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. J Bone Miner Res. 2009;24(4):597-605.
50. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE. Peak trabecular vertebral density: a comparison of adolescent and adult females. Calcif Tissue Int. 1988;43(4):260-2.
51. Martin TJ, Seeman E. Bone remodelling: its local regulation and the emergence of bone fragility. Best Pract Res Clin Endocrinol Metab. 2008;22(5):701-22.
52. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89(2):309-19.
53. Shevde NK, Bendixen AC, Dienger KM, Pike JW. Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci U S A. 2000;97(14):7829-34.
54. Frenkel B, Hong A, Baniwal SK, Coetzee GA, Ohlsson C, Khalid O, et al. Regulation of adult bone turnover by sex steroids. J Cell Physiol. 2010;224(2):305-10.
55. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001;22(4):477-501.
56. Takayanagi H. Osteoimmunology and the effects of the immune system on bone. Nat Rev Rheumatol. 2009;5(12):667-76.
57. Komori T. Regulation of osteoblast differentiation by transcription factors. J Cell Biochem. 2006;99(5):1233-9.
58. Raisz LG, Kream BE. Regulation of bone formation (second of two parts). N Engl J Med. 1983;309(2):83-9.
59. Deng ZL, Sharff KA, Tang N, Song WX, Luo J, Luo X, et al. Regulation of osteogenic differentiation during skeletal development. Front Biosci. 2008;13:2001-21.
60. Krishnan V, Moore TL, Ma YL, Helvering LM, Frolik CA, Valasek KM, et al. Parathyroid hormone bone anabolic action requires Cbfa1/Runx2-dependent signaling. Mol Endocrinol. 2003;17(3):423-35.
61. Yang X, Tare RS, Partridge KA, Roach HI, Clarke NM, Howdle SM, et al. Induction of human osteoprogenitor chemotaxis, proliferation, differentiation, and bone formation by osteoblast stimulating factor-1/pleiotrophin: osteoconductive biomimetic scaffolds for tissue engineering. J Bone Miner Res. 2003;18(1):47-57.
62. Montero A, Okada Y, Tomita M, Ito M, Tsurukami H, Nakamura T, et al. Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. J Clin Invest. 2000;105(8):1085-93.
63. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, et al. Circulating levels of IGF-1 directly regulate bone growth and density. J Clin Invest. 2002;110(6):771-81.
64. Goldberg D, Polly P, Eisman JA, Morrison NA. Identification of an osteocalcin gene promoter sequence that binds AP1. J Cell Biochem. 1996;60(4):447-57.
65. Neve A, Corrado A, Cantatore FP. Osteoblast physiology in normal and pathological conditions. Cell Tissue Res. 2011;343(2):289-302.
66. Baron R, Rawadi G. Targeting the Wnt/beta-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology. 2007;148(6):2635-43.

67. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest. 2006;116(5):1202-9.
68. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127(3):469-80.
69. Cadigan KM, Liu YI. Wnt signaling: complexity at the surface. J Cell Sci. 2006;119(Pt 3):395-402.
70. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. Gene. 2004;341:19-39.
71. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 1997;16(13):3797-804.
72. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol. 1998;14:59-88.
73. Niziolek PJ, Farmer TL, Cui Y, Turner CH, Warman ML, Robling AG. High-bone-mass-producing mutations in the Wnt signaling pathway result in distinct skeletal phenotypes. Bone. 2011;49(5):1010-9.
74. Leupin O, Piters E, Halleux C, Hu S, Kramer I, Morvan F, et al. Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. J Biol Chem. 2011;286(22):19489-500.
75. Choi HY, Dieckmann M, Herz J, Niemeier A. Lrp4, a novel receptor for Dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover in vivo. PLoS One. 2009;4(11):e7930.
76. Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, et al. Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. Nat Genet. 2005;37(9):945-52.
77. Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res. 2011;26(2):373-9.
78. Modder UI, Clowes JA, Hoey K, Peterson JM, McCready L, Oursler MJ, et al. Regulation of circulating sclerostin levels by sex steroids in women and in men. J Bone Miner Res. 2011;26(1):27-34.
79. Butler JS, Murray DW, Hurson CJ, O'Brien J, Doran PP, O'Byrne JM. The role of Dkk1 in bone mass regulation: correlating serum Dkk1 expression with bone mineral density. J Orthop Res. 2011;29(3):414-8.
80. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. J Clin Endocrinol Metab. 2010;95(4):1991-7.
81. Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res. 2009;24(4):578-88.
82. Glantschnig H, Scott K, Hampton R, Wei N, McCracken P, Nantermet P, et al. A rate-limiting role for Dickkopf-1 in bone formation and the remediation of bone loss in mouse and primate models of postmenopausal osteoporosis by an experimental therapeutic antibody. J Pharmacol Exp Ther. 2011;338(2):568-78.
83. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev. 1989;69(3):990-1047.
84. Lian JB, Stein GS, Bortell R, Owen TA. Phenotype suppression: a postulated molecular mechanism for mediating the relationship of proliferation and differentiation

by Fos/Jun interactions at AP-1 sites in steroid responsive promoter elements of tissue-specific genes. J Cell Biochem. 1991;45(1):9-14.
85. de Pollak C, Arnaud E, Renier D, Marie PJ. Age-related changes in bone formation, osteoblastic cell proliferation, and differentiation during postnatal osteogenesis in human calvaria. J Cell Biochem. 1997;64(1):128-39.
86. Gerstenfeld LC, Chipman SD, Kelly CM, Hodgens KJ, Lee DD, Landis WJ. Collagen expression, ultrastructural assembly, and mineralization in cultures of chicken embryo osteoblasts. J Cell Biol. 1988;106(3):979-89.
87. Ernst M, Heath JK, Rodan GA. Estradiol effects on proliferation, messenger ribonucleic acid for collagen and insulin-like growth factor-I, and parathyroid hormone-stimulated adenylate cyclase activity in osteoblastic cells from calvariae and long bones. Endocrinology. 1989;125(2):825-33.
88. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med. 1994;331(16):1056-61.
89. Almeida M, Martin-Millan M, Ambrogini E, Bradsher R, 3rd, Han L, Chen XD, et al. Estrogens attenuate oxidative stress and the differentiation and apoptosis of osteoblasts by DNA-binding-independent actions of the ERalpha. J Bone Miner Res. 2010;25(4):769-81.
90. Okazaki R, Inoue D, Shibata M, Saika M, Kido S, Ooka H, et al. Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) alpha or beta. Endocrinology. 2002;143(6):2349-56.
91. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int. 2008;19:385-97.
92. Kanis JA, Harvey NC, Cooper C, Johansson H, Oden A, McCloskey EV, et al. A systematic review of intervention thresholds based on FRAX : A report prepared for the National Osteoporosis Guideline Group and the International Osteoporosis Foundation. Arch Osteoporos. 2016;11(1):25.
93. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004;34(1):195-202.
94. Shepstone L, Lenaghan E, Cooper C, Clarke S, Fong-Soe-Khioe R, Fordham R, et al. Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial. Lancet. 2018;391(10122):741-7.
95. Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. 2011;22(2):391-420.
96. Kanis JA, Melton LJ, Christiansen C, Johnston C, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9:1137-41.
97. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ. 1996;312(7041):1254-9.
98. Kanis JA, Adachi JD, Cooper C, Clark P, Cummings SR, Diaz-Curiel M, et al. Standardising the descriptive epidemiology of osteoporosis: recommendations from the

Epidemiology and Quality of Life Working Group of IOF. Osteoporos Int. 2013;24(11):2763-4.
99. Duboeuf F, Bauer DC, Chapurlat RD, Dinten JM, Delmas P. Assessment of vertebral fracture using densitometric morphometry. J Clin Densitom. 2005;8(3):362-8.
100. Hans D, Barthe N, Boutroy S, Pothuaud L, Winzenrieth R, Krieg MA. Correlations between trabecular bone score, measured using anteroposterior dual-energy X-ray absorptiometry acquisition, and 3-dimensional parameters of bone microarchitecture: an experimental study on human cadaver vertebrae. J Clin Densitom. 2011;14(3):302-12.
101. Roux JP, Wegrzyn J, Boutroy S, Bouxsein ML, Hans D, Chapurlat R. The predictive value of trabecular bone score (TBS) on whole lumbar vertebrae mechanics: an ex vivo study. Osteoporos Int. 2013;24(9):2455-60.
102. Leslie WD, Aubry-Rozier B, Lix LM, Morin SN, Majumdar SR, Hans D. Spine bone texture assessed by trabecular bone score (TBS) predicts osteoporotic fractures in men: the Manitoba Bone Density Program. Bone. 2014;67:10-4.
103. McCloskey EV, Oden A, Harvey NC, Leslie WD, Hans D, Johansson H, et al. A Meta-Analysis of Trabecular Bone Score in Fracture Risk Prediction and Its Relationship to FRAX. J Bone Miner Res. 2016;31(5):940-8.
104. Harvey NC, Gluer CC, Binkley N, McCloskey EV, Brandi ML, Cooper C, et al. Trabecular bone score (TBS) as a new complementary approach for osteoporosis evaluation in clinical practice. Bone. 2015;78:216-24.
105. Szulc P, Delmas PD. Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. Osteoporos Int. 2008;19(12):1683-704.
106. Naylor K, Eastell R. Bone turnover markers: use in osteoporosis. Nat Rev Rheumatol. 2012;8(7):379-89.
107. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res. 1996;11(10):1531-8.
108. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res. 2000;15(8):1526-36.
109. Ivaska KK, Gerdhem P, Vaananen HK, Akesson K, Obrant KJ. Bone turnover markers and prediction of fracture: a prospective follow-up study of 1040 elderly women for a mean of 9 years. J Bone Miner Res. 2010;25(2):393-403.
110. Johansson H, Oden A, Kanis JA, McCloskey EV, Morris HA, Cooper C, et al. A meta-analysis of reference markers of bone turnover for prediction of fracture. Calcif Tissue Int. 2014;94(5):560-7.
111. Johnell O, Oden A, De Laet C, Garnero P, Delmas PD, Kanis JA. Biochemical indices of bone turnover and the assessment of fracture probability. Osteoporos Int. 2002;13(7):523-6.
112. Chubb SA, Byrnes E, Manning L, Beilby JP, Ebeling PR, Vasikaran SD, et al. Reference intervals for bone turnover markers and their association with incident hip fractures in older men: the Health in Men study. J Clin Endocrinol Metab. 2015;100(1):90-9.

113. Shigdel R, Osima M, Ahmed LA, Joakimsen RM, Eriksen EF, Zebaze R, et al. Bone turnover markers are associated with higher cortical porosity, thinner cortices, and larger size of the proximal femur and non-vertebral fractures. Bone. 2015;81:1-6.
114. Recker RR, Benson CT, Matsumoto T, Bolognese MA, Robins DA, Alam J, et al. A randomized, double-blind phase 2 clinical trial of blosozumab, a sclerostin antibody, in postmenopausal women with low bone mineral density. J Bone Miner Res. 2015;30(2):216-24.
115. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. N Engl J Med. 2016;375(16):1532-43.
116. Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. Osteoporos Int. 2005;16(2):155-62.
117. 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.
118. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton IL, et al. A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res. 2004;19(6):893-9.
119. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, et al. A family history of fracture and fracture risk: a meta-analysis. Bone. 2004;35(5):1029-37.
120. Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, et al. Alcohol intake as a risk factor for fracture. Osteoporos Int. 2005;16(7):737-42.
121. Dargent-Molina P, Douchin MN, Cormier C, Meunier PJ, Breart G, Group ES. Use of clinical risk factors in elderly women with low bone mineral density to identify women at higher risk of hip fracture: The EPIDOS prospective study. Osteoporos Int. 2002;13(7):593-9.
122. Johansson H, Kanis JA, Oden A, Johnell O, McCloskey E. BMD, clinical risk factors and their combination for hip fracture prevention. Osteoporos Int. 2009;20(10):1675-82.
123. Shepstone L, Lenaghan E, Cooper C, Clarke S, Fong-Soe-Khioe R, Fordham R, et al. Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial. Lancet. 2017.
124. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A, et al. Case finding for the management of osteoporosis with FRAX--assessment and intervention thresholds for the UK. Osteoporos Int. 2008;19(10):1395-408.
125. Tosteson AN, Melton LJ, 3rd, Dawson-Hughes B, Baim S, Favus MJ, Khosla S, et al. Cost-effective osteoporosis treatment thresholds: the United States perspective. Osteoporos Int. 2008;19(4):437-47.
126. Marques A, Lourenco O, Ortsater G, Borgstrom F, Kanis JA, da Silva JA. Cost-Effectiveness of Intervention Thresholds for the Treatment of Osteoporosis Based on FRAX((R)) in Portugal. Calcif Tissue Int. 2016;99(2):131-41.
127. Rodrigues AM, Caetano-Lopes J, Vale AC, Aleixo I, Pena AS, Faustino A, et al. Smoking is a predictor of worse trabecular mechanical performance in hip fragility fracture patients. J Bone Miner Metab. 2012;30(6):692-9.
128. Maddalozzo GF, Turner RT, Edwards CH, Howe KS, Widrick JJ, Rosen CJ, et al. Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. Osteoporos Int. 2009;20(9):1529-38.

129. Maurel DB, Boisseau N, Benhamou CL, Jaffre C. Alcohol and bone: review of dose effects and mechanisms. Osteoporos Int. 2012;23(1):1-16.
130. Yoon V, Maalouf NM, Sakhaee K. The effects of smoking on bone metabolism. Osteoporos Int. 2012;23(8):2081-92.
131. Abrahamsen B, Brask-Lindemann D, Rubin KH, Schwarz P. A review of lifestyle, smoking and other modifiable risk factors for osteoporotic fractures. Bonekey Rep. 2014;3:574.
132. 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.
133. Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, et al. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy With Risedronate Therapy (VERT) Study Group. JAMA. 1999;282(14):1344-52.
134. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med. 2007;356(18):1809-22.
135. Lyles KW, Colon-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357(18):1799-809.
136. Abrahamsen B, Jorgensen HL, Laulund AS, Nybo M, Bauer DC, Brix TH, et al. The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. J Bone Miner Res. 2015;30(5):898-905.
137. Briot K, Cortet B, Thomas T, Audran M, Blain H, Breuil V, et al. 2012 update of French guidelines for the pharmacological treatment of postmenopausal osteoporosis. Joint Bone Spine. 2012;79(3):304-13.
138. Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA. 1999;281(23):2189-97.
139. Martino S, Cauley JA, Barrett-Connor E, Powles TJ, Mershon J, Disch D, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst. 2004;96(23):1751-61.
140. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA. 2006;295(23):2727-41.
141. Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355(2):125-37.
142. Jamal SA, Ljunggren O, Stehman-Breen C, Cummings SR, McClung MR, Goemaere S, et al. Effects of denosumab on fracture and bone mineral density by level of kidney function. J Bone Miner Res. 2011;26(8):1829-35.

143. Hiramatsu R, Ubara Y, Sawa N, Hoshino J, Hasegawa E, Kawada M, et al. Denosumab for low bone mass in hemodialysis patients: a noncontrolled trial. Am J Kidney Dis. 2015;66(1):175-7.
144. Festuccia F, Jafari MT, Moioli A, Fofi C, Barberi S, Amendola S, et al. Safety and efficacy of denosumab in osteoporotic hemodialysed patients. J Nephrol. 2017;30(2):271-9.
145. Schipper LG, Fleuren HW, van den Bergh JP, Meinardi JR, Veldman BA, Kramers C. Treatment of osteoporosis in renal insufficiency. Clin Rheumatol. 2015;34(8):1341-5.
146. Kanis JA, Svedbom A, Harvey N, McCloskey EV. The osteoporosis treatment gap. J Bone Miner Res. 2014;29(9):1926-8.
147. Qaseem A, Forciea MA, McLean RM, Denberg TD. Treatment of Low Bone Density or Osteoporosis to Prevent Fractures in Men and Women: A Clinical Practice Guideline Update From the American College of Physicians. Annals of internal medicine. 2017;166(11):818-39.
148. 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.
149. Hoy D, March L, Brooks P, Blyth F, Woolf A, Bain C, et al. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. Annals of the rheumatic diseases. 2014;73(6):968-74.
150. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014;73(7):1316-22.
151. Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014;73(7):1323-30.
152. Smith E, Hoy DG, Cross M, Vos T, Naghavi M, Buchbinder R, et al. The global burden of other musculoskeletal disorders: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73(8):1462-9.
153. Sanchez-Riera L, Carnahan E, Vos T, Veerman L, Norman R, Lim SS, et al. The global burden attributable to low bone mineral density. Annals of the rheumatic diseases. 2014;73(9):1635-45.
154. Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthritis research & therapy. 2009;11(3):229.
155. Pelaez-Ballestas I, Sanin LH, Moreno-Montoya J, Alvarez-Nemegyei J, Burgos-Vargas R, Garza-Elizondo M, et al. Epidemiology of the rheumatic diseases in Mexico. A study of 5 regions based on the COPCORD methodology. The Journal of rheumatology Supplement. 2011;86:3-8.
156. Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis and rheumatism. 2008;58(1):15-25.
157. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis and rheumatism. 2008;58(1):26-35.

158. Carmona L, Ballina J, Gabriel R, Laffon A, Group ES. The burden of musculoskeletal diseases in the general population of Spain: results from a national survey. Annals of the rheumatic diseases. 2001;60(11):1040-5.
159. Ioannidis G, Flahive J, Pickard L, Papaioannou A, Chapurlat RD, Saag KG, et al. Non-hip, non-spine fractures drive healthcare utilization following a fracture: the Global Longitudinal Study of Osteoporosis in Women (GLOW). Osteoporos Int. 2013;24(1):59-67.
160. Tran T, Bliuc D, van Geel T, Adachi JD, Berger C, van den Bergh J, et al. Population-Wide Impact of Non-Hip Non-Vertebral Fractures on Mortality. J Bone Miner Res. 2017.
161. Sornay-Rendu E, Munoz F, Duboeuf F, Delmas PD. Rate of forearm bone loss is associated with an increased risk of fracture independently of bone mass in postmenopausal women: the OFELY study. J Bone Miner Res. 2005;20(11):1929-35.
162. Holloway KL, Henry MJ, Brennan-Olsen SL, Bucki-Smith G, Nicholson GC, Korn S, et al. Non-hip and non-vertebral fractures: the neglected fracture sites. Osteoporos Int. 2016;27(3):905-13.
163. Bliuc D, Nguyen TV, Eisman JA, Center JR. The impact of nonhip nonvertebral fractures in elderly women and men. J Clin Endocrinol Metab. 2014;99(2):415-23.
164. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005;16(11):1330-8.
165. Gnudi S, Sitta E, Lisi L. Relationship of body mass index with main limb fragility fractures in postmenopausal women. J Bone Miner Metab. 2009;27(4):479-84.
166. Russell M, Mendes N, Miller KK, Rosen CJ, Lee H, Klibanski A, et al. Visceral fat is a negative predictor of bone density measures in obese adolescent girls. J Clin Endocrinol Metab. 2010;95(3):1247-55.
167. Finkelstein EA, Chen H, Prabhu M, Trogdon JG, Corso PS. The relationship between obesity and injuries among U.S. adults. Am J Health Promot. 2007;21(5):460-8.
168. Giangregorio L, Papaioannou A, Cranney A, Zytaruk N, Adachi JD. Fragility fractures and the osteoporosis care gap: an international phenomenon. Semin Arthritis Rheum. 2006;35(5):293-305.
169. Curtis EM, Moon RJ, Harvey NC, Cooper C. The impact of fragility fracture and approaches to osteoporosis risk assessment worldwide. Bone. 2017.
170. Akhter MP, Iwaniec UT, Haynatzki GR, Fung YK, Cullen DM, Recker RR. Effects of nicotine on bone mass and strength in aged female rats. J Orthop Res. 2003;21(1):14-9.
171. Krall EA, Dawson-Hughes B. Smoking increases bone loss and decreases intestinal calcium absorption. J Bone Miner Res. 1999;14(2):215-20.
172. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med. 1986;315(21):1305-9.
173. Barbieri RL, Gochberg J, Ryan KJ. Nicotine, cotinine, and anabasine inhibit aromatase in human trophoblast in vitro. J Clin Invest. 1986;77(6):1727-33.
174. Cassidenti DL, Vijod AG, Vijod MA, Stanczyk FZ, Lobo RA. Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women. Am J Obstet Gynecol. 1990;163(6 Pt 1):1953-60.

175. Walker LM, Preston MR, Magnay JL, Thomas PB, El Haj AJ. Nicotinic regulation of c-fos and osteopontin expression in human-derived osteoblast-like cells and human trabecular bone organ culture. Bone. 2001;28(6):603-8.
176. Liu XD, Zhu YK, Umino T, Spurzem JR, Romberger DJ, Wang H, et al. Cigarette smoke inhibits osteogenic differentiation and proliferation of human osteoprogenitor cells in monolayer and three-dimensional collagen gel culture. J Lab Clin Med. 2001;137(3):208-19.
177. Liu X, Kohyama T, Kobayashi T, Abe S, Kim HJ, Reed EC, et al. Cigarette smoke extract inhibits chemotaxis and collagen gel contraction mediated by human bone marrow osteoprogenitor cells and osteoblast-like cells. Osteoporos Int. 2003;14(3):235-42.
178. Tanaka H, Tanabe N, Shoji M, Suzuki N, Katono T, Sato S, et al. Nicotine and lipopolysaccharide stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts. Life Sci. 2006;78(15):1733-40.
179. Chavassieux P, Seeman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease. Endocr Rev. 2007;28(2):151-64.
180. Fratzl-Zelman N, Roschger P, Misof BM, Nawrot-Wawrzyniak K, Potter-Lang S, Muschitz C, et al. Fragility fractures in men with idiopathic osteoporosis are associated with undermineralization of the bone matrix without evidence of increased bone turnover. Calcif Tissue Int. 2011;88(5):378-87.
181. Long F. Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol. 2011;13(1):27-38.
182. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell. 1997;89(5):755-64.
183. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 2002;108(1):17-29.
184. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005;8(5):739-50.
185. Rodrigues AM, Caetano-Lopes J, Vale AC, Vidal B, Lopes A, Aleixo I, et al. Low osteocalcin/collagen type I bone gene expression ratio is associated with hip fragility fractures. Bone. 2012;51(6):981-9.
186. Boivin G, Bala Y, Doublier A, Farlay D, Ste-Marie LG, Meunier PJ, et al. The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. Bone. 2008;43(3):532-8.
187. Roschger P, Paschalis EP, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. Bone. 2008;42(3):456-66.
188. Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. Osteoporos Int. 2013;24(2):489-94.

189. Amrein K, Dobnig H, Wagner D, Piswanger-Solkner C, Pieber TR, Pilz S, et al. Sclerostin in institutionalized elderly women: associations with quantitative bone ultrasound, bone turnover, fractures, and mortality. J Am Geriatr Soc. 2014;62(6):1023-9.
190. Ardawi MS, Rouzi AA, Al-Sibiani SA, Al-Senani NS, Qari MH, Mousa SA. High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the Center of Excellence for Osteoporosis Research Study. J Bone Miner Res. 2012;27(12):2592-602.
191. Anastasilakis AD, Polyzos SA, Avramidis A, Toulis KA, Papatheodorou A, Terpos E. The effect of teriparatide on serum Dickkopf-1 levels in postmenopausal women with established osteoporosis. Clin Endocrinol (Oxf). 2010;72(6):752-7.
192. Wanby P, Nobin R, Von SP, Brudin L, Carlsson M. Serum levels of the bone turnover markers dickkopf-1, sclerostin, osteoprotegerin, osteopontin, osteocalcin and 25-hydroxyvitamin D in Swedish geriatric patients aged 75 years or older with a fresh hip fracture and in healthy controls. J Endocrinol Invest. 2016;39(8):855-63.
193. Lim Y, Kim CH, Lee SY, Kim H, Ahn SH, Lee SH, et al. Decreased Plasma Levels of Sclerostin But Not Dickkopf-1 are Associated with an Increased Prevalence of Osteoporotic Fracture and Lower Bone Mineral Density in Postmenopausal Korean Women. Calcif Tissue Int. 2016;99(4):350-9.
194. Tavares V, Canhao H, Gomes JA, Simoes E, Romeu JC, Coelho P, et al. [Recommendations for the diagnosis and management of osteoporosis]. Acta Reumatol Port. 2007;32(1):49-59.
195. Direção-Geral da Saúde. Norma da Direção-Geral da Saúde - Tratamento Farmacológico da Osteoporose Pós-menopausica. 2011.
196. Hippisley-Cox J, Coupland C, Robson J, Sheikh A, Brindle P. Predicting risk of type 2 diabetes in England and Wales: prospective derivation and validation of QDScore. BMJ. 2009;338:b880.
197. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18(8):1033-46.
198. Jarvinen TL, Jokihaara J, Guy P, Alonso-Coello P, Collins GS, Michaelsson K, et al. Conflicts at the heart of the FRAX tool. CMAJ. 2014;186(3):165-7.
199. Leslie WD, Majumdar SR, Morin SN, Lix LM, Schousboe JT, Ensrud KE, et al. Performance of FRAX in clinical practice according to sex and osteoporosis definitions: the Manitoba BMD registry. Osteoporos Int. 2018;29(3):759-67.
200. Rodrigues AM, Gregorio MJ, Gein P, Eusebio M, Santos MJ, de Sousa RD, et al. Home-Based Intervention Program to Reduce Food Insecurity in Elderly Populations Using a TV App: Study Protocol of the Randomized Controlled Trial Saude.Come Senior. JMIR Res Protoc. 2017;6(3):e40.