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Translational approach to secondary hip fracture prevention in older adults: from basic science to clinical practice

DOCTORAL THESIS BY COMPENDIUM OF PUBLICATIONS

Bernardo Abel Cedeño Veloz

November 2023

Supervisors:

Dr. Nicolas Martínez Velilla, Ph.D

Dr Mikel Izquierdo Redín, Ph.D

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Universidad Pública de Navarra

Departamento de Ciencias de la Salud

Abordaje traslacional en la prevención secundaria de fractura de cadera en el adulto mayor: de la ciencia básica a la práctica clínica

TESIS DOCTORAL POR COMPENDIO DE PUBLICACIONES

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“When I tell someone what I do for a living, they usually have one of two reactions. Either their face contorts as if they’d just smelled something foul, or they offer compliments about my selfless dedication…These apparently opposite responses are actually the same. Both imply that what I’m doing is something no one in their right mind would ever do.”

Louise Aronson, geriatrician

“No hay arma más potente que la verdad en mano de los buenos”

Juan Bosch, ensayista

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List of Abbreviations

ASM	Masa Muscular Esquelética Apendicular
ASMI	Índice de Masa Muscular Esquelética Apendicular
BMD	Densidad mineral ósea
BMI	índice masa corporal
BMT	Marcadores de recambio óseo
CCL-7	Ligando del motivo quimioatrayente (C-C) 7
CIRS-G	Cumulative Illness Rating Scale for Geriatrics
CXCL-8	Ligando del motivo quimioatrayente (C-C) 8
CXCL-12	Ligando del motivo quimioatrayente (C-C) 12
CSF1	Factor estimulante de colonias 1
DXA	Dual-Energy X-ray Absorptiometry
FLS	Fracture Liaison Servicies
FRAX	Herramienta de Evaluación de Riesgo de Fractura
FLT3LG	Ligando de la tirosina quinasa 3 relacionada con Fms
IL-6	Interleucina 6
IL-7	Interleucina 7
LOQ	Límites de cuantificación
LT-α	Linfotoxina-alfa
OA	Osteoartritis
OP	Osteoporosis
PEA	Ensayo de extensión de proximidad
SPPB	Short Physical Performance Battery

Declaration and list of publications

This doctoral thesis is a compilation of **4 articles** that have been published in peer-reviewed international journals, and **1 article** that are under review for publication.

I, Bernardo Abel Cedeño Veloz, declare that this thesis titled, "Translational approach to secondary hip fracture prevention in older adults: from basic science to clinical practice" and the work presented in it are my own. I confirm that:

1. This work was done wholly or mainly while in candidature for a PhD at the Public University of Navarre.
2. No part of this thesis has previously been submitted for a degree or any other qualification at the Public University of Navarre or any other institution.
3. I have acknowledged all main sources of help.
4. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

List of Publications:

1. Cedeno-Veloz BA, Erviti Lopez J, Gutiérrez-Valencia M, Leache Alegría L, Saiz LC, Rodríguez García AM, Sánchez Latorre M, Ramírez Vélez R, Izquierdo M, Martínez-Velilla N. Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J Nutr Health Aging. 2022;26(8):778-785. https://doi.org/10.1007/s12603-022-1825-5.

   Science Citation Index Expanded (SCIE). Category: Nutrition & Dietetics.
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2. Cedeno-Veloz B, Martínez-Velilla N (2023) [Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult]. Rev Esp Geriatr Gerontol 58:101390. https://doi.org/10.1016/j.regg.2023.101390.

   Citiscore. Category: Geriatrics & Gerontology.
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3. Cedeno-Veloz BA, Lozano-Vicario L, Zambom-Ferraresi F, Fernández-Irigoyen J, Santamaría E, Rodríguez-García A, Romero-Ortuno R, Mondragon-Rubio J, Ruiz-Ruiz J, Ramírez-Vélez R, Izquierdo M, Martínez-Velilla N. Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults. Immun Ageing. 2023 Oct 18;20(1):55. https://doi.org/10.1186/s12979-023-00379-z

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4. Cedeno-Veloz B, Lozano-Vicario L, Rodríguez-García A, Zambom-Ferraresi F, Galbete A, Fernández-Irigoyen J, Santamaría E, García-Hermoso A, Calvani R, Ramírez-Vélez R, Izquierdo M, Martínez-Velilla N. Serum biomarkers related to frailty predict negative outcomes in older adults with hip fracture. J Endocrinol Invest. 2023 Aug 21. https://doi.org/10.1007/s40618-023-02181-6

   Science Citation Index Expanded (SCIE). Category: Endocrinology & Metabolism. 2022 Journal Impact Factor 5.4 (Q1)

5. Cedeno-Veloz BA, Irache Casadamon-Munarriz I, Rodríguez García A., Lozano-Vicario L, Zambom-Ferraresi F, Gonzalo Lázaro M, Hidalgo Ovejero MA, Izquierdo M, Martínez-Velilla N. Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail© exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial. Journal of Clinical Medicine. 2023 (Under Review)

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Papers Presented in International Congress.

Poster: "Efficacy of antiresorptive treatment in osteoporotic older adults: results of a systematic review and meta-analysis of randomised clinical trials", WCO-IOF-ESCEO Virtual Congress, August 26-28 2021

Poster: "Association of serum biomarkers in prediction fractures in older adults", WCO-IOF-ESCEO Barcelona 2023 Congress, May 4-7 2023

Poster: "Assessing the predictive capability of serum biomarkers and frailty in hip fracture patients: a prospective cohort study examining short- and long-term outcomes" WCO-IOF-ESCEO Barcelona 2023 Congress, May 4-7 2023

Poster: "Benefit of a Multifactorial Approach with telerehabilitation in older adults after hip fracture: the Active-FLS pragmatic randomized clinical trial protocol", WCO-IOF-ESCEO Virtual Congress, March 24-26 2022

Awards granted to thesis works.

AgNovos-ESCEO Young Investigators Award for "Association of serum biomarkers in prediction fractures in older adults",

Declaration and list of publications

Esta tesis doctoral es un compendio de **4 artículos** que han sido publicados en revistas internacionales revisadas por pares y **1 artículos** que están bajo revisión para su publicación.

Yo, Bernardo Abel Cedeño Veloz, declaro que esta tesis titulada "Abordaje traslacional en la prevención secundaria de fractura de cadera en el adulto mayor: de la ciencia básica a la práctica clínica" y el trabajo presentado en ella son de mi autoría. Confirmo que:

1. Este trabajo se realizó en su totalidad o principalmente durante mi candidatura para un doctorado en la Universidad Pública de Navarra.
2. Ninguna parte de esta tesis ha sido previamente presentada para un título o cualquier otra calificación en la Universidad Pública de Navarra o en cualquier otra institución.
3. He reconocido todas las principales fuentes de ayuda.
4. Cuando la tesis se basa en trabajo realizado conjuntamente con otros, he dejado claro exactamente qué fue hecho por otros y qué he contribuido yo mismo.

Listado de Publicaciones:

1. Cedeno-Veloz BA, Erviti Lopez J, Gutiérrez-Valencia M, Leache Alegría L, Saiz LC, Rodríguez García AM, Sánchez Latorre M, Ramírez Vélez R, Izquierdo M, Martínez-Velilla N. Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J Nutr Health Aging. 2022;26(8):778-785. https://doi.org/10.1007/s12603-022-1825-5.

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   Science Citation Index Expanded (SCIE). Category: Endocrinology & Metabolism. 2022 Journal Impact Factor 5.4 (Q1)

5. Cedeno-Veloz BA, Irache Casadamon-Munarriz I, Rodríguez García A., Lozano-Vicario L, Zambom-Ferraresi F, Gonzalo Lázaro M, Hidalgo Ovejero MA, Izquierdo M, Martínez-Velilla N. Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail© exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial. Journal of Clinical Medicine. 2023 (Under Review)

   Science Citation Index Expanded (SCIE). Category: Medicine, General & Internal. 2022 Journal Impact Factor 3.9 (Q1)

Comunicaciones presentadas en Congresos Internacionales

Poster: "Efficacy of antiresorptive treatment in osteoporotic older adults: results of a systematic review and meta-analysis of randomised clinical trials", WCO-IOF-ESCEO Virtual Congress, August 26-28 2021

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Poster: "Benefit of a Multifactorial Approach with telerehabilitation in older adults after hip fracture: the Active-FLS pragmatic randomized clinical trial protocol", WCO-IOF-ESCEO Virtual Congress, March 24-26 2022

Premios otrogados a trabajos de la tesis doctoral

AgNovos-ESCEO Young Investigators Award por el trabajo "Association of serum biomarkers in prediction fractures in older adults",

Agradecimientos

En el momento de culminar este importante capítulo de mi vida académica y profesional, quiero expresar mi más sincero agradecimiento a las personas e instituciones que hicieron posible la realización de esta tesis doctoral.

En primer lugar, deseo agradecer al Servicio de Geriatría del Hospital Universitario de Navarra y a todos los profesionales (médicos, enfermería, auxiliares, administrativos…). Su compromiso, experiencia y dedicación han sido fundamentales para el desarrollo y la profundidad de este trabajo. Así mismo su afán de innovación, investigación y entusiasmo por el cuidado de las personas son dignos de admiración.

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A mis directores de tesis:

Al Doctor Nicolás Martínez Velilla, como jefe y director de tesis, gracias por empujarme a realizar este fantástico proyecto y permitirme desarrollar estas ideas. Cuando empezó a plantearse esta tesis en la prepandemia de 2020 era un proyecto sencillo con pocas aspiraciones. Hoy es una tesis con artículos publicados en revistas de gran impacto y premios en congresos internacionales. Esto no hubiese sido posible sin los conocimientos y habilidades transmitidos que me han permitido el desarrollo a nivel profesional, académico y personal.

Al Profesor Mikel Izquierdo, como director de tesis y tutor de doctorado, ha sido un mentor y guía. Siendo un investigador internacional muy demandado ha sabido sacar tiempo para transmitirme los métodos y valores que deben guiar a un buen investigador en este complejo mundo tan desconocido para mí al principio. Agradezco profundamente su guía, paciencia y sabiduría durante todo este proceso. Sus consejos y su pasión por la investigación han sido esenciales para el éxito de este proyecto.

Por último, y mucho más importante, agradecer a mi familia y amigos. A mi madre Marcia y a mi padre Bernardo. A mis padres políticos, Pepa y Antonio. Y a mi mujer Alba, a mi hijo Alejandro y a mi futura hija por su amor incondicional, por creer en mí y apoyarme en cada paso de este reto. Siento que este proyecto nos ha quitado tiempo juntos, pero espero que esta nueva etapa nos traiga más amor, viajes y aventuras juntos. Este logro es también suyo.

En definitiva, a todos los que me ayudaron a avanzar y a todas las piedras que me he encontrado en este camino

Gracias

Summary

This doctoral thesis focuses on the relationship between basic scientific discoveries regarding bone health, the current evidence on its management, and its application in the clinical context to prevent secondary fractures in older adults. The risk of fractures in older adults is an emerging public health issue. The prevention of new fractures is of particular concern due to the exponential increase in morbidity and mortality, as well as the associated healthcare costs. Identifying the current issues, understanding specific pathophysiological mechanisms in the older population, and multi-factorial interventions when addressing the problem are essential for a novel and effective strategy for preventing new fractures. This doctoral thesis is based on 5 articles that have been published or are pending in international scientific journals. In the first chapter (Chapter 1), our goal is to analyze the current evidence on the efficacy of pharmacological treatment in preventing hip fractures (as well as other events of interest such as other fractures, bone remodelling markers, side effects, etc.). In the second chapter (Chapter 2), we propose, through an editorial, that the importance of biomarkers in osteoporosis will continue to be fundamental tools for the geriatric medicine of the future by supporting the diagnostic, monitoring, and treatment process. In the third chapter (Chapter 3), we conduct an analysis of biomarkers in patients with and without hip fractures to assess their relationship with fracture risk and their correlation with it. In the fourth chapter (Chapter 4), we relate these same markers to frailty in older adults with hip fractures and their relationship with adverse events (dependency, mortality, gait alteration, etc.) in a 3-month follow-up. In the fifth and final chapter (Chapter 5), we propose detailing and validating a pilot multi-domain intervention system based on telerehabilitation to improve the functional capacity of older patients after a hip fracture.

Below, the methodology and the most relevant results are summarized:

Chapter 1: **Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials**

Objective: Investigate concerns about the benefits of antiresorptive drugs in older adults.

Design: A systematic review and meta-analysis of randomized clinical trials.

Setting and Participants: Older adults aged ≥65 with osteoporosis, with or without a previous fragility fracture.

Method: The primary outcome was hip fracture, and a subgroup analysis (≥75 years, with different types of drugs, and secondary prevention) and a sensitivity analysis using a GRADE assessment were performed. Secondary outcomes were any fracture, vertebral fracture, bone markers, and adverse events.

Results: A total of 12 randomized controlled trials (RCTs) qualified for this meta-analysis, with 36,196 participants. Antiresorptive drugs have a statistically significant effect in preventing hip fractures (RR=0.70; 95%CI 0.60 to 0.81) but with moderate GRADE evidence quality and a high number needed to treat (NNT) of 186. For other outcomes, there is a statistically significant effect, but with low to moderate evidence quality. Antiresorptives did not show a reduction in hip fracture risk in people aged ≥75 years. Results for different types of drugs, secondary prevention, and sensitivity analysis are similar to the main analysis and present the same concerns.

Conclusions and Implications: Antiresorptive drugs have a statistically significant effect in preventing hip fractures but with moderate quality (unclear/high bias risk) and a high NNT (186). This small benefit disappears in those aged ≥75 years but increases in secondary prevention. More RCTs in very older adults with osteoporosis are needed.

Chapter 2: **Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult**

It's crucial to develop a new strategy to understand, predict, and address osteoporosis from a precision medicine standpoint, as treating a patient stratified as high-risk using traditional means would be insufficient to address systemic deterioration in bone microstructure. With analytical aid, these technologies have been providing an increasingly detailed picture of the molecular and cellular alterations underlying osteoporosis and variability among patients at the molecular and cellular levels. Using multi-omics techniques, several proteins associated with bone mineral density and fractures have been identified during human proteome profiling in different populations. Other areas of study within the geroscience of osteoporosis include cellular senescence, microbiome, and genomics. Identifying and developing new biomarkers for osteoporosis, particularly those reflecting the underlying ageing mechanisms, can revolutionize our therapeutic approach to this disease.

Chapter 3: **Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults.**

Objective: Explore the association between serum cytokines and hip fracture status in older adults, and their associations with fracture risk using the reference tool FRAX.

Design: Observational study.

Setting and Participants: 40 participants, including 20 with hip fractures and 20 without fractures.

Method: We compared the population characteristics, functional status, and detailed body composition (determined by densitometry) between groups along with blood biomarker analysis using PEA with Olink.

Results: IL-6, LT-α, FLT3LG, CSF1, and CCL7 were significantly different between patients with and without fractures ($p<0.05$). IL-6 had a moderate correlation with FRAX ($R2=0.409$, $p<0.001$), while CSF1 and CCL7 had weak correlations. LT-α y FLT3LG had showed a negative correlation with hip fracture risk.

Conclusions and Implications: These proteomic tools can identify differentially regulated proteins and might serve as potential markers for estimating fracture risk. However, longitudinal studies will be necessary to validate these results and determine the temporal patterns of changes in cytokine profiles.

Chapter 4: **Serum biomarkers related to frailty predicts negative outcomes in older adults with hip fracture.**

Objective: Investigate the relationships between serum inflammatory biomarkers and frailty in older adults with hip fractures, as well as adverse outcomes one and three months after discharge.

Design: Prospective cohort.

Setting and Participants: 45 patients aged 75 or older who were admitted for a hip fracture.

Method: Comprehensive Geriatric Assessment (CGA), which included a frailty evaluation using the Clinical Frailty Scale (CFS). Blood samples were collected before surgery and analyzed for blood biomarkers using PEA with Olink.

Results: The findings showed that IL-7 (OR 0.66 95% CI 0.46-0.94, $p=0.022$) and CXCL-12 (OR 0.97 95% CI 0.95-0.99, $p=0.011$) were associated with better functional recovery three months after discharge, while CXCL-8 (OR 1.07 95%CI 1.01-1.14, $p=0.019$) was associated with a higher risk of readmission.

Conclusions and Implications: These findings suggest that immunological biomarkers may serve as useful predictors of clinical outcomes in patients with hip fractures.

Chapter 5: **Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail; exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial.**

The aim of this “under review” article was to present the study protocol to assess the feasibility of implementing a multi-domain intervention based on telerehabilitation to improve the functional capacity of older patients after a hip fracture. This randomized clinical trial will take place at the University Hospital of Navarra, with 174 older adults who have suffered a hip fracture and meet the inclusion criteria will be randomly assigned to the intervention or control group. The intervention group will receive a multicomponent intervention consisting of individualized home exercise using the @ctive hip application for three months, followed by nine months of exercise using

Vivifrail. In addition, the intervention group will receive nutritional intervention, osteoporosis treatment, polypharmacy adjustment, and assessment of the patient's mood, cognitive decline, and fear of falling. The control group will receive standard outpatient care according to local guidelines. The main objective of this study will be to assess the effectiveness of the intervention in modifying primary outcomes, which include changes in functional status during the study period based on the SPPB. The findings of this study will offer valuable insights into the efficacy of a comprehensive approach considering the complexity of frailty in older adults and geriatric syndromes, which are significant factors in individuals at risk of suffering from fragility fractures. These will also have implications for the development of more effective interventions that address the needs of these vulnerable populations.

Resumen

Esta tesis doctoral versa sobre la relación entre los descubrimientos científicos básicos sobre la salud ósea, la evidencia actual que hay sobre su manejo y su aplicación en el contexto clínico para prevenir fracturas secundarias en adultos mayores. El riesgo de fractura en adultos mayores es un problema de salud pública creciente. La prevención de nuevas fracturas es especialmente preocupante debido al aumento exponencial de la morbimortalidad, así como gasto sanitario asociado asociada. La identificación de la problemática actual, el conocimiento de mecanismos fisiopatológicos específicos en la población mayor, así como intervenciones multifactoriales a la hora de abordar el problema son necesarias de cara a una estrategia novedosa y efectiva de prevención de nuevas fracturas. Esta tesis doctoral se basa en 5 artículos que han sido publicados o están pendiente de en revistas científicas internacionales. En el primer capítulo (Capítulo 1) nuestro objetivo es análisis la evidencia actual sobre la eficacia en el tratamiento farmacológico en la prevención de fracturas de cadera (así como otros eventos de intereses como otras fracturas, marcadores de remodelado óseo, efectos secundarios…). En el segundo capítulo (Capítulo 2), planteamos, mediante una editorial, la importancia de los biomarcadores en la osteoporosis continuará siendo herramientas fundamentales para la medicina geriátrica del futuro al poder apoyar en el proceso diagnóstico, monitorización y tratamiento. En el tercer capítulo (Capítulo 3) realizamos un análisis de biomarcadores en paciente con y sin fractura de cadera para valorar su relación con el riesgo de fractura y su correlación con el mismo. En el cuarto capítulo (Capítulo 4) relacionamos estos mismos marcadores con la fragilidad en adultos mayores con fractura de cadera y su relación con eventos adversos (dependencia, mortalidad, alteración marcha…) en un seguimiento a 3 meses. En el quinto y último capítulo (Capítulo 5) se plantea detallar y validar un sistema piloto de intervención multidominio basado en telerrehabilitación para mejorar la capacidad funcional de los pacientes mayores tras la fractura de cadera.

A continuación, se resumen la metodología y los resultados más relevantes:

Capítulo 1: **Eficacia del Tratamiento Antirresortivo en Adultos Mayores con Osteoporosis: Una Revisión Sistemática y Meta-análisis de Ensayos Clínicos Aleatorizados.**

Objetivo: Investigar las preocupaciones acerca de los beneficios de los fármacos antirresortivos en adultos mayores

Diseño: Una revisión sistemática y meta-análisis de ensayos clínicos aleatorizados.

Escenario y Participantes: Adultos mayores de ≥65 años con osteoporosis, con o sin una fractura por fragilidad previa.

Método: el resultado principal fue la fractura de cadera, y se realizó un análisis de subgrupos (≥75 años, con diferentes tipos de medicamentos y prevención secundaria) y un análisis de sensibilidad utilizando una evaluación GRADE. Los resultados secundarios

fueron cualquier tipo de fractura, fractura vertebral, marcadores óseos y eventos adversos.

Resultados: Un total de 12 ensayos controlados aleatorizados (ECA) calificaron para este meta-análisis, con 36,196 participantes. Los fármacos antirresortivos tienen un efecto estadísticamente significativo en la prevención de la fractura de cadera (RR=0.70; 95%CI 0.60 a 0.81), pero con una calidad moderada de evidencia GRADE y un número necesario para tratar (NNT) alto de 186. Para otros resultados, hay un efecto estadísticamente significativo, pero con una calidad de evidencia de baja a moderada. Los antirresortivos no mostraron reducción en el riesgo de fractura de cadera en personas de ≥75 años. Los resultados para diferentes tipos de medicamentos, prevención secundaria y análisis de sensibilidad son similares a los análisis principales y presentan las mismas preocupaciones.

Conclusiones e Implicaciones: Los fármacos antirresortivos tienen un efecto estadísticamente significativo en la prevención de la fractura de cadera, pero con una calidad moderada (riesgo poco claro/alto de sesgo) y un NNT alto (186). Este pequeño beneficio desaparece en aquellos de ≥75 años, pero aumenta en la prevención secundaria. Se necesitan más ECA en adultos muy mayores con osteoporosis.

Capítulo 2: **Importancia de Biomarcadores en la Osteoporosis: Avances en la Gerociencia del Adulto Mayor**

Es crucial desarrollar una nueva estrategia para comprender, predecir y abordar la osteoporosis desde el punto de vista de la medicina de precisión ya que el tratamiento de un paciente estratificado como de alto riesgo con los medios tradicionales resultaría insuficiente para abordar el deterioro sistémico en la microestructura ósea. Con la ayuda analítica, estas tecnologías han estado proporcionando una imagen cada vez más detallada de las alteraciones moleculares y celulares que subyacen a la osteoporosis y la variabilidad entre pacientes a nivel molecular y celular. Mediante el uso de técnicas multi-ómicas, se han identificado varias proteínas asociadas a la densidad mineral ósea y fractura durante el perfilado de proteomas humanos en diferentes poblaciones. Otras áreas de estudio dentro de la gerociencia de la osteoporosis serían senescencia celular, microbioma y genómica. La identificación y desarrollo de nuevos biomarcadores para la osteoporosis, particularmente aquellos que reflejan los mecanismos de envejecimiento subyacentes, pueden revolucionar nuestro abordaje terapéutico de esta enfermedad

Capítulo 3: **Efecto de los biomarcadores inmunológicos asociados con la fractura de cadera y el riesgo de fractura en adultos mayores.**

Objetivo: explorar la asociación entre las citocinas séricas y el estado de fractura de cadera en adultos mayores, y sus asociaciones con el riesgo de fractura utilizando la herramienta de referencia FRAX

Diseño: estudio observacional

Escenario y Participantes: 40 participantes, incluyendo 20 con fractura de cadera y 20 sin fractura

Método: Comparamos las características de la población, el estado funcional y la composición corporal detallada (determinada mediante densitometría) entre grupos junto con el análisis de biomarcadores sanguíneos mediante PEA con Olink

Resultados: IL-6, LT-α, FLT3LG, CSF1 y CCL7 eran significativamente diferentes entre los pacientes con y sin fractura ($p<0.05$). IL-6 tuvo una correlación moderada con FRAX ($R2=0.409$, $p<0.001$), mientras que CSF1 y CCL7 tuvieron correlaciones débiles con FRAX. LT-α y FLT3LG mostraron una correlación negativa con el riesgo de fractura

Conclusiones e Implicaciones: las herramientas proteómicas dirigidas tienen la capacidad de identificar proteínas reguladas de manera diferencial y pueden servir como posibles marcadores para estimar el riesgo de fractura. Sin embargo, se necesitarán estudios longitudinales para validar estos resultados y determinar los patrones temporales de cambios en los perfiles de citocinas.

Capítulo 4: **Marcadores séricos relacionados con la fragilidad predicen resultados negativos en adultos mayores con fractura de cadera.**

Objetivo: investigar las relaciones entre los biomarcadores inflamatorios séricos y la fragilidad en adultos mayores con fractura de cadera, así como resultados adversos a uno y tres meses después del alta

Diseño: cohorte prospectiva

Escenario y Participantes: 45 pacientes de 75 años o más que fueron admitidos por fractura de cadera

Método: Evaluación Geriátrica Integral (CGA), que incluyó una evaluación de la fragilidad utilizando la Escala Clínica de Fragilidad (CFS). Se recogieron muestras de sangre antes de la cirugía con análisis de biomarcadores sanguíneos mediante PEA con Olink

Resultados: Los resultados mostraron que IL-7 (OR 0.66 95% CI 0.46-0.94, $p=0.022$) y CXCL-12 (OR 0.97 95% CI 0.95-0.99, $p=0.011$) se asociaron con una mejor recuperación funcional a los tres meses después del alta, mientras que CXCL-8 (OR 1.07 95%CI 1.01-1.14, $p=0.019$) se asoció con un mayor riesgo de reingreso.

Conclusiones e Implicaciones: Estos hallazgos sugieren que los biomarcadores inmunológicos pueden representar predictores útiles de los resultados clínicos en pacientes con fractura de cadera.

Capítulo 5: **Efecto de una intervención multicomponente con tele-rehabilitación y el programa de ejercicios Vivifrail sobre la capacidad funcional tras una fractura de cadera: Protocolo de estudio para el ensayo controlado aleatorizado ActiveFLS.**

El objetivo de este artículo “en revisión” fue presentar el protocolo de estudio para evaluar la viabilidad de la implementación de una intervención multidominio basado en telerrehabilitación para mejorar la capacidad funcional de los pacientes mayores tras la fractura de cadera. Este ensayo clínico aleatorizado se llevará a cabo en el Hospital Universitario de Navarra con 174 adultos mayores que han sufrido una fractura de

cadera y que cumplan con los criterios de inclusión serán asignados aleatoriamente al grupo de intervención o control. El grupo de intervención recibirá una intervención multicomponente consistente en ejercicio individualizado en casa utilizando la aplicación @ctive hip durante tres meses, seguido de nueve meses de ejercicio utilizando Vivifrail. Además, el grupo de intervención recibirá intervención nutricional, tratamiento de osteoporosis, ajuste de polifarmacia y evaluación del estado de ánimo del paciente, deterioro cognitivo y miedo a caer. El grupo de control recibirá atención ambulatoria estándar según las guías locales. El objetivo principal de este estudio será evaluar la efectividad de la intervención en la modificación de los resultados primarios, que incluyen cambios en el estado funcional durante el período de estudio basado en la SPPB. Los hallazgos de este estudio ofrecerán valiosos conocimientos sobre la eficacia de un enfoque integral que considera la complejidad de la fragilidad en adultos mayores y síndromes geriátricos, que son factores importantes en individuos en riesgo de sufrir fracturas por fragilidad. Así mismo tendrán implicaciones para el desarrollo de intervenciones más efectivas que aborden las necesidades de estas poblaciones vulnerables.

General Background

Aging involves various changes in body composition, such as the loss of bone mass. Bone mass starts to decline by 0.5% beginning at the age of 30, with a rapid point decrease in postmenopausal women, while this decrease remains stable in men [1]. These changes, along with multiple contributing factors such as sedentary lifestyle, malnutrition, chronic diseases, and some pharmacological treatments (e.g., corticosteroids), ultimately lead to osteoporosis[2]. Osteoporosis is an age-related syndrome that has been associated with poor outcomes such as disability, an increased risk of falls and fractures, loss of independence, high costs to healthcare systems, and an increased risk of premature death[3].

The direct cost of incident fractures in Spain[4] in 2019 was €1,813 million. Added to this was the ongoing cost in 2019 from fractures that occurred before 2019, which amounted to €2,198 million (long-term disability). The cost of pharmacological intervention (assessment and treatment) was €303 million. Thus, the total direct cost (excluding the value of QALYs lost) amounted to €4.3 billion in 2019. The cost of osteoporotic fractures in Spain accounted for approximately 3.8% of healthcare spending (i.e. €4.3 billion out of €104.3 billion in 2019), somewhat more than the EU27+2 average of 3.5% and ranked Spain 11th amongst the EU27+2 countries. These numbers indicate a substantial impact of fragility fractures on the healthcare budget.

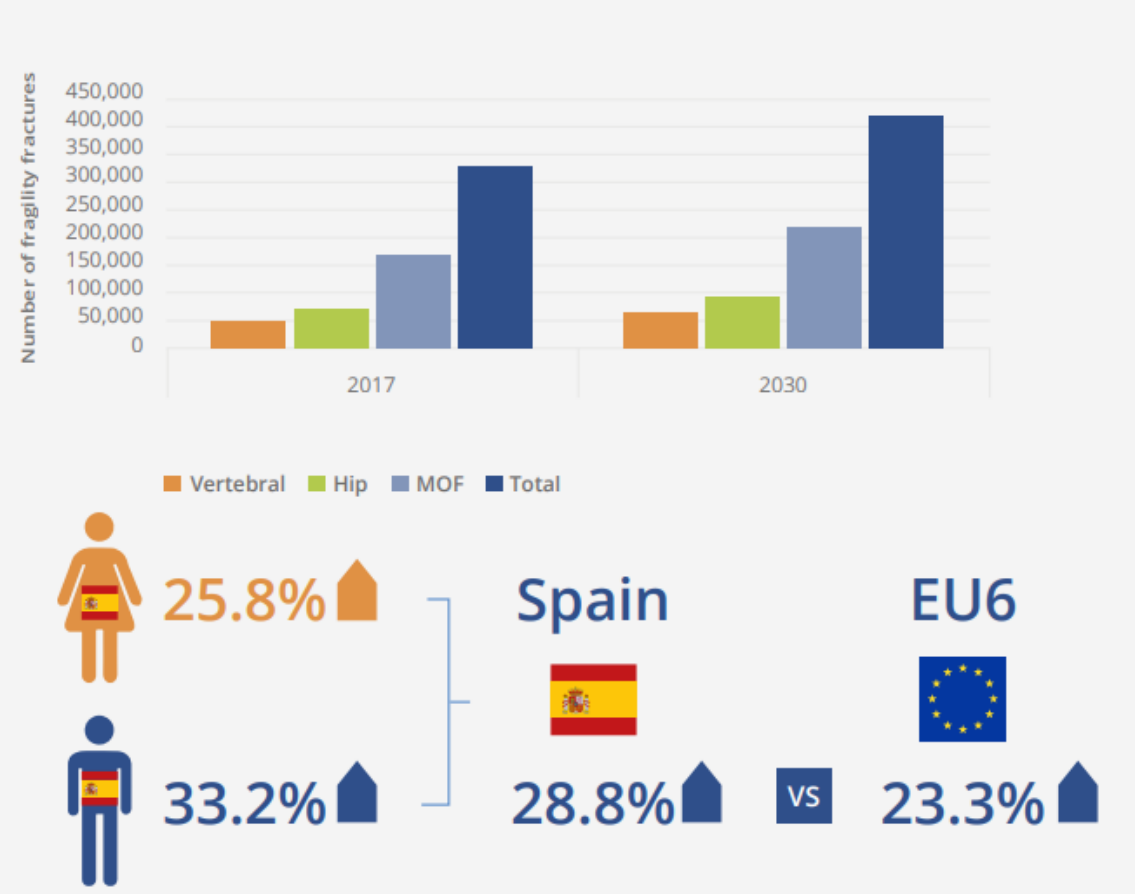


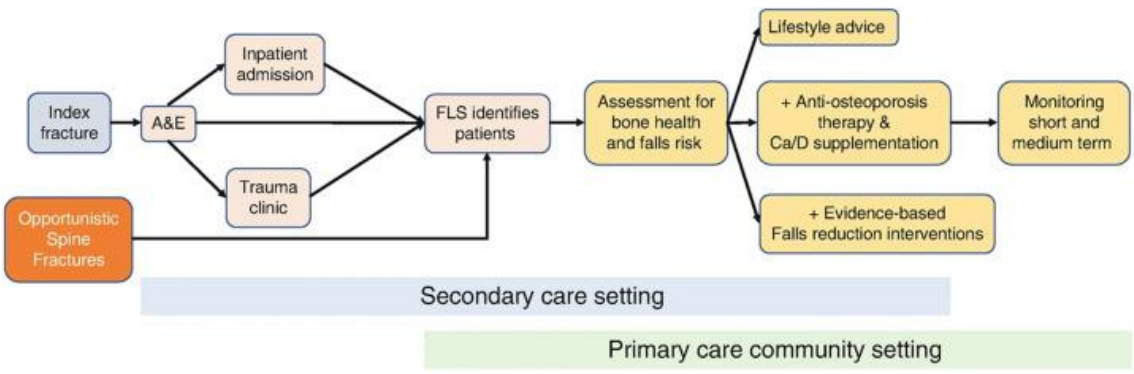
Figure 1: Estimated number of fragility fractures by fracture category for Spain in 2017 and 2030 (C. Sing et al 2023)[5].

Despite efforts to curb the increasing incidence of fractures, it remains a "silent epidemic" [3] affecting populations worldwide. The declining incidence of fractures in many countries in recent years is insufficient to offset the impact of the growing aging population[5]. Consequently, the number of fractures is projected to nearly double over the next 20 to 30 years. Interventions are needed to prevent hip fractures, improve the treatment gap, and provide post-hip fracture care to achieve better patient outcomes and fewer future hip fractures. Only by coordinating the many approaches already in place and expanding secondary prevention to under-serviced and under-resourced countries and regions can this tsunami of future fractures be averted[6].

Although the most common osteoporotic fractures are those of the vertebra, hip, and wrist, the **hip fracture** is the most serious. It leads to high morbidity and mortality both in- [5,7,8] and outclinic [5,8,9]. Up to 20% of patients with hip fractures will develop a postoperative complication, with chest infections (9%) and heart failure (5%) being the most common[5,10]. The mortality rate is 10% at one month and 30% at one year. Likewise, aside from the high healthcare costs (ranging between 7031€ and 12,321€ depending on the region of Spain) mainly due to high health resource utilization during the first hospitalisation[11], only 50% return to their previous level of mobility, and 10 to 20% of patients are discharged to a residential or nursing care placement[12], with a significant decline in their quality of life in the physical, psychological, and social domain[13].

To approach this health epidemic, a large number of resources have been dedicated over the past 50 years to basic research on bone metabolism, epidemiological studies, pharmacological treatments, and overall management of osteoporosis. Risk factors for developing the condition have been identified, tools have been developed for assessing the risk of fractures, and we have non-invasive techniques for diagnosing the quantity and quality of the bone [14]. In response to this global phenomenon, **Fracture Liaison Services (FLS)** have been established around the world to fill this treatment gap[15]. The concept of the "FLS" – as a service led by an interdisciplinary team with a coordinator at its head – was specifically developed for the secondary prevention of fractures (figure 2).

Figure 2: An example of a fracture liaison service model (Paccou et al 2023)[15]



However, many FLS focus mainly on secondary prevention, bone metabolism treatments, therapeutic adherence, and mortality[15,16]. Concerns surrounding antiresorptive treatment relate to the fact that studies have focused on surrogate variables, such as bone mineral density (BMD), bone turnover markers (BTM) and non-vertebral fractures[17], and the translation of these variables into clinical relevance, such as fracture prevention, is controversial[18]. These drugs have been shown to increase BMD (especially denosumab[19]), change BTM and reduce fragility fractures in osteoporotic patients[20,21]; however, hip fractures have a much greater clinical impact in terms of ability, function, quality of life and accommodation[22], and cause more morbidity and mortality compared to other fractures[23]. Antiresorptive drugs are usually the first-line treatment in older adults (≥65 years)[20]; however, there is no clear evidence of their usefulness in this older population[18], and there are some concerns surrounding the benefits: firstly, a study based on screening for osteoporosis in older women did not reduce the incidence of osteoporosis-related fractures[24]; secondly, according to a recent meta-analysis, no significant association was found between all drug treatments for osteoporosis and the overall mortality rate[25]; finally, nonspecific exclusion criteria (comorbidities, severe illness, low life expectancy…) in many studies[26] mean that older adults were misrepresented in the studies, especially those over ≥75 years.

Given the complexity of conducting new clinical trials due to their financial cost and the difficulty in recruitment, **meta-analyses and cohort studies** emerge as an option for studying the efficacy of these drugs in preventing fractures[27]. These studies have been published periodically and have yielded both favourable [28–30]and unfavourable [18,31,32] results for their use in the prevention following hip fractures. For all these reasons, this remains an unresolved issue.

Adding to this issue with pharmacological management, historically, the prediction of fracture risk related to osteoporosis has been suboptimal. In the assessment of this risk, the most commonly studied factors have been Bone Mineral Density (BMD), markers of bone turnover (MRO), and the Fracture Risk Assessment Tool (FRAX®). The calculation of risk using these tools is a matter of debate, as they are considered to have a series of limitations, especially in older adults[33]. **FRAX**, despite its widespread usage as a simple and primary care-applicable tool for estimating fracture risk, has a limitation in that it does not accommodate dose-response considerations for diverse risk factors [34,35], potentially **underestimating fracture risk**[36], and is **unsuitable for adults aged over 90**[37]. While FRAX advances fracture prognostication beyond the capabilities of BMD measurements alone, the accuracy of its fracture risk prediction displays variation across distinct study populations[38]. This problem has been linked to the fact that osteoporosis management in older adults has been conducted without considering other crucial factors that affect older adults such as functional and cognitive impairment, frailty, sarcopenia, falls, pain, malnutrition, and comorbidities[39–43]. Presently, a revised version of FRAX is under development[44], new scales are in development[45] and more advance prediction models are in study[46], intending to address the aforementioned limitations.

Insufficient understanding of the pathophysiological and molecular mechanisms of OP and other chronic bone conditions has led to the lack of mechanism-based diagnoses [47]. However, proteomic approaches that examine changes in biomarkers show promise in developing minimally invasive diagnostic biomarkers for OP. Unfortunately, data from older adults are scarce, emphasizing the need to identify valid biomarkers for both diagnosing and evaluating treatments and interventions. The complex pathophysiology of osteoporosis, sarcopenia, osteosarcopenia, frailty, and hip fractures hampers the identification of biomarkers, especially proinflammatory cytokines [48–50]. Therefore, identifying high-risk populations and exploring potential biomarkers associated related to bone changes is crucial for effective health promotion[51]. With analytical aid, these technologies have been providing an increasingly detailed picture of the molecular and cellular alterations underlying osteoporosis and variability among patients at the molecular and cellular level[52] for predicting fractures beyond FRAX and predicting outcomes in frail older adults with hip fractures[53–55]. Using **multi-omics techniques**, several proteins associated with bone mineral density and fractures have been identified during human proteome profiling in different populations[56].

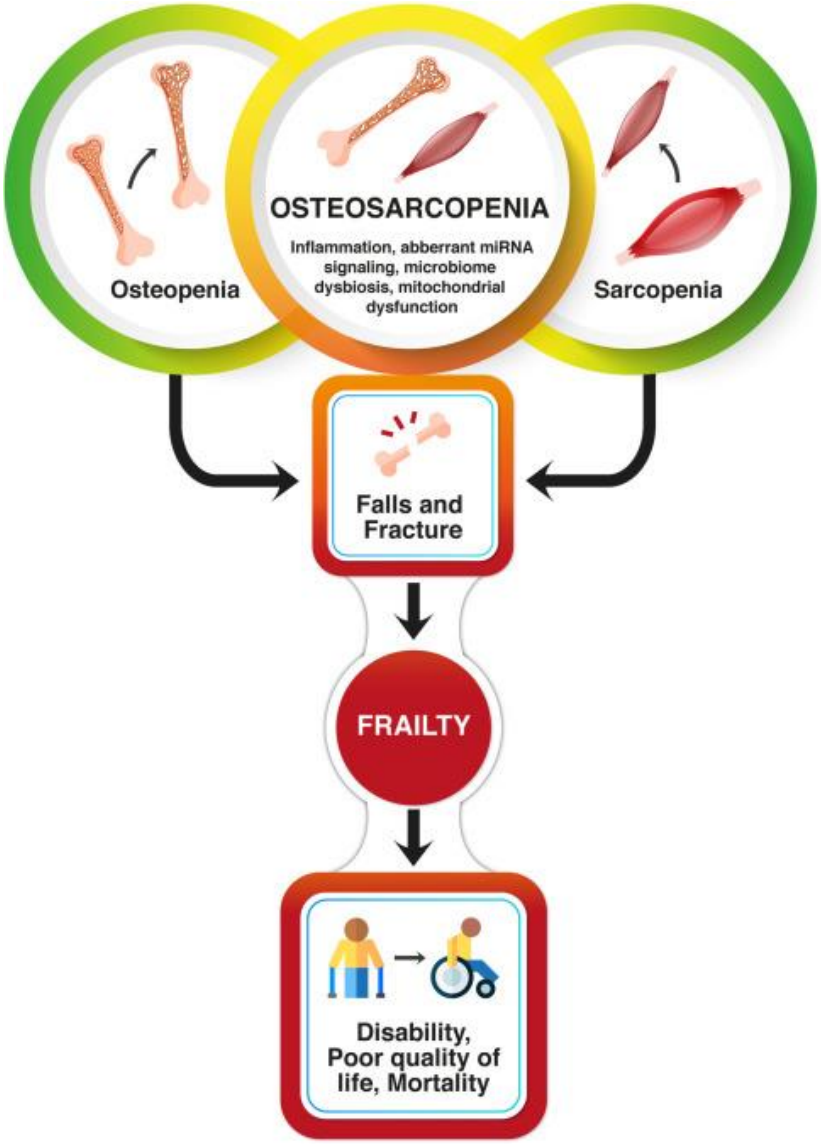


Figure 3: The interrelationship and continuum of the complex pathophysiology of osteoporosis, sarcopenia, osteosarcopenia, frailty, and fractures (Seldeen et al 2022) [50]

All of the above is added to the circumstance that most health systems are **fragmented** and cannot ensure the adequate management of frail and complex individuals at risk of or experiencing fragility fractures[57]. Consequently, these patients require a particular approach that has not yet been studied in FLS [58,59]. Moreover, despite increasing prescription proportions, medical treatment for secondary fracture prevention remains low in some FLS. In addition, it is more common to be prescribed vitamin D or calcium than osteoporosis medication after a fragility fracture, contrary to current guidelines[60]. In this context, it's crucial to develop a new strategy to understand, predict, and address osteoporosis and hip fracture from a precision medicine standpoint, as treating a patient stratified as high risk using traditional means would be insufficient to address systemic deterioration in bone microstructure[61]. Unfortunately, data from older adults are scarce, emphasizing the need to identify valid biomarkers for both diagnosing and evaluating treatments and interventions.

This thesis describes the rationale, design, methodologies, and results of different studies to take a translational approach to secondary hip fracture prevention in older adults. From the current evidence on the efficacy of pharmacological treatment in preventing hip fractures (chapter 1) and in real-world databases (chapter 2), this thesis talk about the importance of biomarkers in osteoporosis (chapter 3) and, after that, analyses we conduct an analysis of biomarkers related to fracture risk (chapter 4), frailty and adverse events (chapter 5). In the final chapter, we write a proposal for a multi-domain intervention system based on telerehabilitation to improve the functional capacity of older patients after a hip fracture (chapter 6). We hypothesized that an individualized multi-domain approach would result in greater improvements in functional capacity compared to usual clinical care.

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Aims and layouts of the thesis.

Chapter 1: **Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials.**

Research aim: To analyse the effectiveness of antiresorptive treatment compared to placebo on hip fracture prevention in older adults.

Hypothesis: antiresorptive drugs may have a marginal effect in preventing hip fractures in older adults.

Chapter 2: **Effectiveness of antiresorptives in preventing hip fractures in older women (≥ 75 years) with osteoporosis: a nested case-control study within a BIFAP cohort.**

Research aim: To determine the real-world effectiveness of antiresorptive treatment in preventing hip fractures in older women with osteoporosis.

Hypothesis: antiresorptive drugs may have a marginal effect in preventing hip fractures in older women with osteoporosis.

Chapter 3: **Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult.**

Research aim: To review new biomarkers for osteoporosis, particularly those reflecting the underlying aging and frailty mechanisms.

Hypothesis: It's crucial to develop a new strategy to understand, predict, and address osteoporosis from a precision medicine standpoint.

Chapter 4: **Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults.**

Research aim: To explore the association between serum cytokines and hip fracture status in older adults, and their associations with fracture risk using the reference tool FRAX.

Hypothesis: biomarkers might serve as potential markers for estimating fracture risk.

Chapter 5: **Serum biomarkers related to frailty predicts negative outcomes in older adults with hip fracture.**

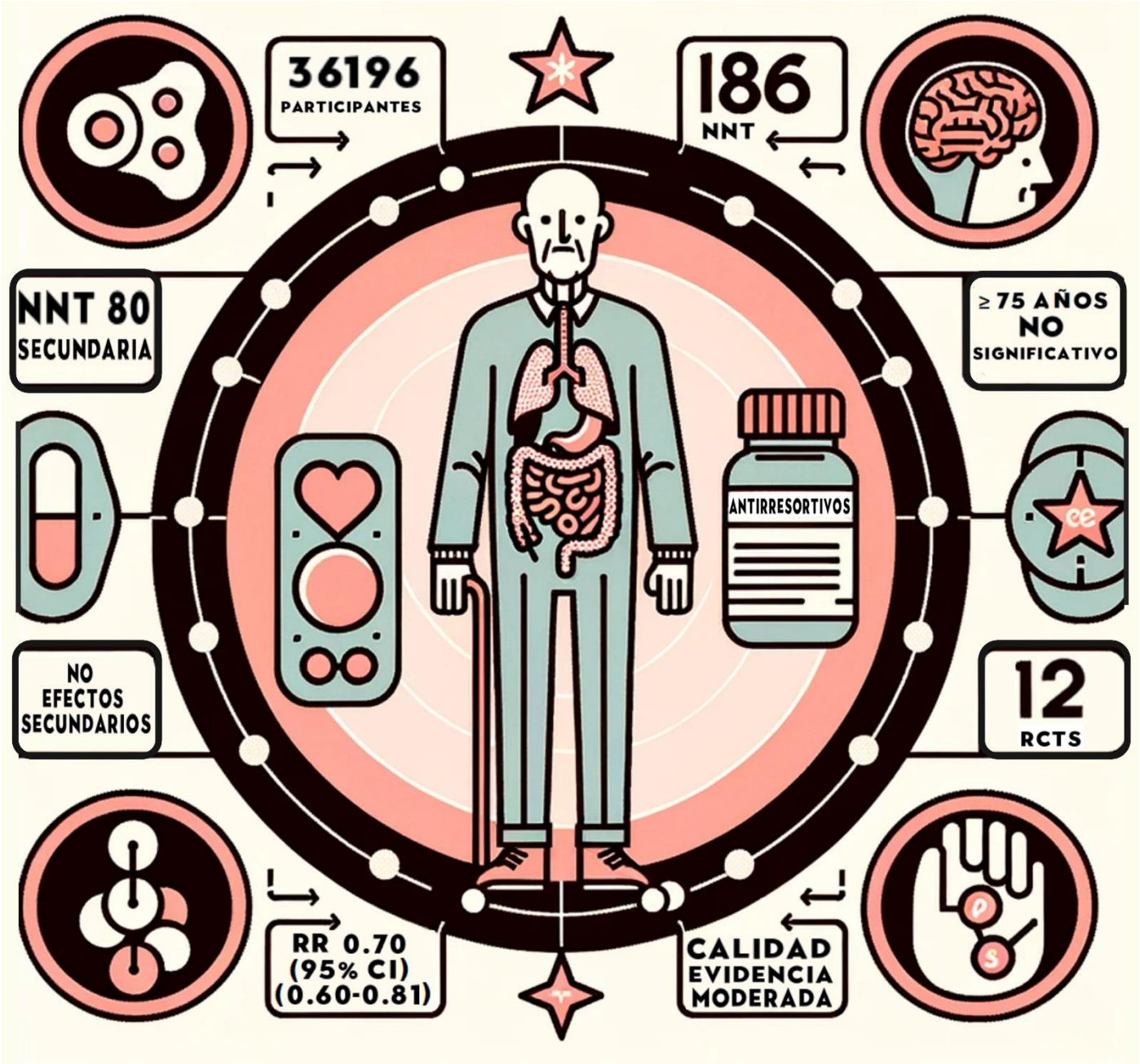
Research aim: To explore the relationships between serum inflammatory biomarkers and frailty in older adults with hip fractures and adverse outcomes.

Hypothesis: immunological biomarkers may serve as useful predictors of clinical outcomes in frailty patients with hip fractures.

Chapter 6: **Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail; exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial.**

Research aim: To assess the feasibility of implementing a multi-domain intervention based on telerehabilitation to improve the functional capacity of older patients after a hip fracture.

Hypothesis: multi-domain intervention may have a beneficial effect in functional status compared to usual care.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Chapter 1

Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials.

1. INTRODUCTION

Osteoporosis is an age-related syndrome that has been associated with poor outcomes such as disability, an increased risk of falls and fractures, loss of independence, high cost to healthcare systems and an increased risk of premature death[1].

Concerns surrounding antiresorptive treatment relate to the fact that studies have focused on surrogate variables, such as bone mineral density (BMD), bone turnover markers (BTM) and non-vertebral fractures[2], and the translation of these variable into clinical relevance, such as fracture prevention, is controversial[3]. These drugs have been shown to increase BMD (especially denosumab[4]), change BTM and reduce fragility fractures in osteoporotic patients[5,6]; however, hip fractures have a much greater clinical impact in term of ability, function, quality of life and accommodation[7], and cause more morbidity and mortality compared to other fractures[8].

Antiresorptive drugs are usually the first-line treatment in older adults (≥65 years)[5]; however, there is no clear evidence of their usefulness in this older population[3], and there are some concerns surrounding the benefits: firstly, a study based on screening for osteoporosis in older women did not reduce the incidence of osteoporosis-related fractures[9]; secondly, according to a recent meta-analysis, no significant association was found between all drug treatments for osteoporosis and the overall mortality rate[10]; finally, nonspecific exclusion criteria (comorbidities, severe illness, low life expectancy…) in many studies[11] mean that older adults were misrepresented in the studies, especially those over ≥75 years.

Thus, a systematic review was carried out to evaluate the efficacy of antiresorptive treatment in the prevention of osteoporotic hip fractures in older adults ≥65 years with osteoporosis. Other important outcomes in the management of osteoporosis[12] (BMD and BTM) were included, as well as clinically important outcomes for older adults[1] (any type of fractures, mortality, adverse events).

2. METHODS

2.1 Registering in PROSPERO

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[13]. The review protocol was registered in the PROSPERO database under registration number CRD42020165960.

2.2 Search strategy

The online databases MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, ISI Web of Science and Scopus were searched for studies from inception until 9th July 2021, using a combination of keywords and controlled vocabulary (sText1 in the Supplement), without restrictions on publication year. The review was restricted to studies published in English, Spanish, French, German and Portuguese.

2.3 Inclusion criteria

2.3.1 Type of studies:

Only randomised controlled trials (RCTs) were included. Trial reports had to present data for at least the primary outcome (hip fracture). When a published, updated study involving the same trial participants was identified, only the latest update was included in the analysis, unless both groups went on to receive drug treatment or there was some crossover between groups.

2.3.2 Type of participants:

Participants were older adults (65 years old and above) with osteoporosis, with or without a previous fragility fracture.

2.3.3 Type of intervention:

Trial participants were randomised to an antiresorptive treatment compared to placebo or non-osteoporotic treatment. Both arms could include calcium and/or vitamin D. The following drugs were considered as antiresorptives: alendronate, etidronate, ibandronate, risedronate, clodronate, minodronate, pamidronate, tiludronate, zoledronate and denosumab.

2.3.4 Exclusion criteria

Studies with secondary causes of osteoporosis (cancer-related and corticosteroid-induced osteoporosis) were not included. Participants younger than 65 years and studies that with no reported hip fracture were also not included.

2.4 Outcome measures

Primary outcome:

- Patients with hip fracture.

Secondary outcomes:

- Patients with fractures of any type.
- Total number of fractures.
- All-cause mortality.
- Vertebral and non-vertebral fractures.
- Change in BMD at the end of the study from baseline.
- Change in BTM at the end of the study from baseline.
- Total serious adverse events.
- Total cardiovascular events.
- Total gastrointestinal events.
- Withdrawal due to adverse event.

Clinical vertebral fracture events and new vertebral deformities identified by radiological morphometry were reported separately. Serious adverse events were defined according to the International Conference on Harmonisation Guidelines as any event that leads to death, which was life-threatening, required inpatient hospitalisation or the prolongation

of existing hospitalisation, resulted in persistent or significant disability, or was a congenital anomaly/birth defect (ICH 1995)[14]. All outcomes refer to the number of patients with events, unless otherwise indicated.

2.5 Subgroup analysis:

We carried out the following subgroup analysis:

- Participants aged ≥75 years (due to the increase of incidence of hip fractures[1], the worse outcomes[7] and the lack of representation of this population[11])
- Different drug types
- Participants with a previous osteoporotic fracture (secondary prevention)
-

2.6 Sensitivity analysis:

We restricted the analyses to the following:

- Trials including only participants of 65 years or older.
- Trials with a low or unclear risk of bias.

2.7 Data collection and analysis

2.7.1 Selection of studies:

We used Covidence to screen and classify the identified references. Two authors (BCV and MSL) independently screened the titles and abstracts of all the references to assess for eligibility, and the full text of every article considered for inclusion was obtained and screened for final selection. Discrepancies were resolved by a third author (ARG). A PRISMA flow diagram of the included and excluded articles is reported (shown in Fig. 1).

2.7.2 Data extraction and management:

Two review authors independently extracted data from the included trials using a previously prepared data extraction form. Any differences between review authors were resolved by discussion. Cochrane Review Manager 5 software was used for data synthesis and analysis. Quantitative analyses of outcomes were based on the intention-to-treat principle.

The data extraction form included details of study design, randomisation, blinding, assessment of risk of bias, duration of treatment, follow-up, baseline characteristics, number of participants lost to follow-up, interventions, outcomes, and statistical analysis.

In the case of studies that included both people younger and older than 65 years and where specific data for older adults were not included in the publication, data from individual participants related to our group of interest were requested and included in the review when available. If individual participant data for the subgroup of interest could not be obtained, we included studies if greater than or equal to 80% of the participants were older than 65 years; a sensitivity analysis was also carried out excluding these studies.

2.7.3 Assessment of risk of bias in included studies:

Two authors (BCV and MSL) independently assessed the methodological quality of the included studies, using the Cochrane risk of bias tool[15] to assess selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. These domains were judged as "unclear risk of bias", "low risk of bias" and "high risk of bias". Any disagreement was resolved by discussion with a third author. The quality of evidence for critical and important variables was assessed using Grading of Recommendations Assessment, Development, and Evaluation (GRADE), which includes an assessment of risk of bias, directness of the evidence, heterogeneity, precision of effect estimates and risk of publication bias.

2.7.4 Data synthesis and statistical analysis:

We performed the meta-analysis according to the Cochrane handbook version 6[15]. For binary outcomes, we calculated the risk ratio (RR), the absolute risk reduction (ARR) and the number needed to treat (NNT), with their 95% confidence intervals (CI). For continuous outcomes (change in BMD and change in BTM), we calculated the mean difference with standard deviation (SD). The Mantel–Haenszel method was used with a fixed-effect model. Heterogeneity between trials was tested with the Chi-square and I^2 test. A p-value of less than 0.1 and/or an I^2 value higher than 50% indicated significant heterogeneity. In this situation, we explored the possible causes of heterogeneity by performing sensitivity analyses. A random-effect model was used in the case of unexplained heterogeneity. A Begg's funnel plot was constructed to analyse the possibility of publication bias in the primary outcome. If a meta-analysis could not be performed, a narrative description of the results was provided.

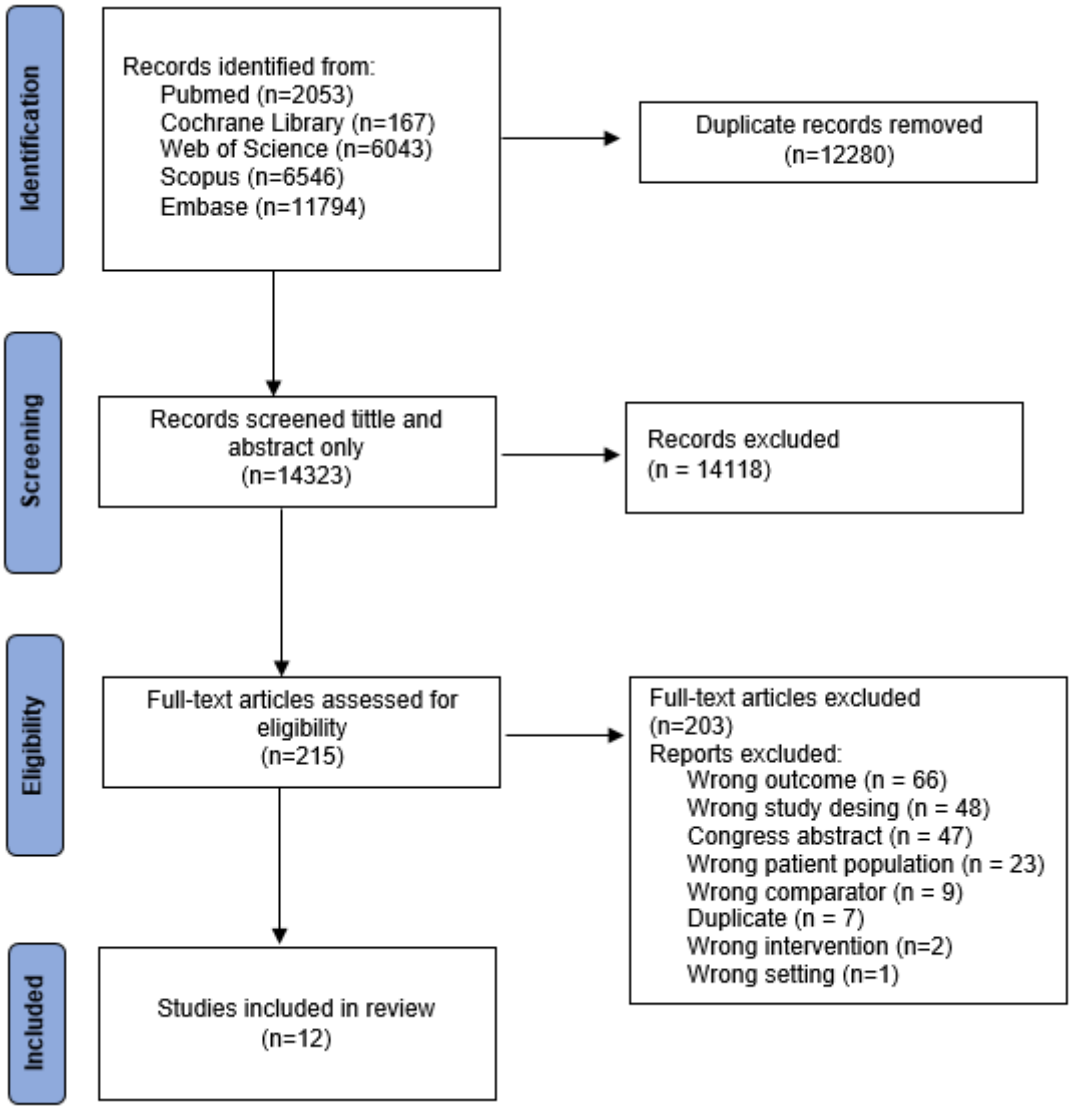
3. RESULTS

3.1 Search results and quality of the studies

We located 26,603 records, of which 14,323 were unique after removing duplicates. After screening the titles and abstracts, the full text eligibility of 215 studies was assessed. This resulted in the final inclusion of 12 RCTs (shown in Fig. 1)

Risk of bias was mostly unclear (shown in Fig. 2) due to allocation concealment, incomplete data and blinding of outcomes. Most of the studies had a low risk of bias from random sequences, blinding of participants and personnel, and selective reporting. The most important biases were the high number of studies with incomplete outcomes and the absence of funding reporting. The Begg's funnel plot was symmetrical, suggesting no publication bias (shown in Fig. S1 in the Supplementary data). The full bias assessment is in Table S1 in the Supplementary data. A summary of the findings and GRADE assessment of each outcome are shown in Table 1.

Figure 1: PRISMA flow chart of search results and included studies.



3.2 Study and participants characteristics

Alendronate [4 studies[16–19]], clodronate [1 study[20]], denosumab [1 study[21]], etidronate [1 study[22]], risedronate [1 study[23]] and zoledronate [4 studies[24–27]] were assessed as drug treatments against hip fracture. Sixteen studies were not included as there was no response to the request for information about participants aged 65 years or older[28–43]. One study was not included as no individual data were available[44]. Hip fracture as the main outcome was only observed in 3 studies[20,23,24].

Overall, the 12 studies included 36,196 participants (19,639 in treatment groups and 16,557 in control groups). The mean age across the studies was 75.2 years. The characteristics of the included studies and participants are listed in Table S2 in the Supplementary data.

Figure 2: Graph showing the risk of bias.

3.3 Antifracture effects in osteoporotic patients

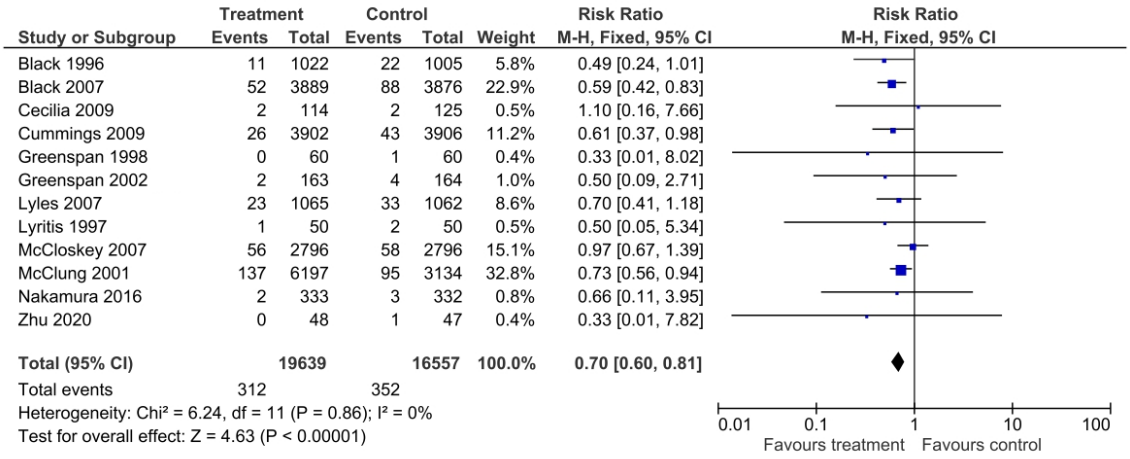
Antiresorptive treatment showed a lower risk of hip fracture than the control group: RR=0.70 (95%CI 0.60 to 0.81), 12 studies, I^2=0% (shown in Fig. 3). This means an ARR of 0.54% (95%CI 0.25 to 0.82%) and a NNT of 186 (95%CI 123 to 395).

When considering any type of fractures, treatment was associated with a lower risk: RR=0.67 (95%CI 0.62 to 0.71), 9 studies, I^2=39%; ARR=4.43% (95%CI 3.68 to 5.18%), NNT=23 (95%CI 19 to 27) (shown in Fig. S2 in the Supplementary data). Most of the studies reported this outcome as any clinical fracture[16,20,21,24–26].

Antiresorptive treatments decreased the risk of vertebral fractures [RR=0.39 (95%CI 0.30 to 0.50), 6 studies, I^2=64%]. Two studies[16,26] included only morphometric fractures in this outcome (shown in Fig. S2 in the Supplementary data). For clinical vertebral fractures, antiresorptive treatments also decreased the incidence [RR=0.38 (95%CI 0.28 to 0.53), 6 studies, I^2=55%, shown in Fig. S2 in the Supplementary data]. Furthermore,

antiresorptive treatments decreased the incidence of non-vertebral fractures [RR=0.79 (95%CI 0.74 to 0.86), 7 studies, I^2=0%, shown in Fig. S2 in the Supplementary data].

Figure 3: Forest plot for primary analysis of hip fracture in older adults



3.4 Bone mineral density (BMD) and bone turnover markers (BTM)

All studies showed a benefit in BMD and BTM when they were reported. It was not possible to carry out a meta-analysis, due to the lack of sufficient data. Treatment with antiresorptives increased the average bone mass at all sites compared to the control group. Differences between the groups in the change from basal measure to the last time point were as follows: lumbar spine 7.1% (SD 4.9), including 9 studies; hip 4.7% (SD 1.4), including 10 studies; and femoral neck 4.1% (SD 1.8), including 10 studies. The modification of BTM is hardly comparable among the studies when using different markers, although the difference between intervention and placebo was favourable to the drug in all cases.

3.5 Adverse events

No statistically significant association was found between active treatment and serious adverse events: RR=0.98 (95%CI 0.95 to 1.01), 8 studies, I^2=48%. Overall, there was no association between treatment and risk of mortality: RR=0.96 (95%CI 0.86 to 1.07), 7 studies, I^2=47% (shown in Fig. S3 in the Supplementary data).

Only 3 studies[21,24,25] reported cardiovascular events. Cummings *et al*.[21] reported the number of patients with a cardiovascular event and did not find any differences between groups (RR=1.04 (95%CI 0.85 to 1.27). Black *et al*. [24] and Lyles *et al*.[25] reported the total number of cardiovascular events and found no association with antiresorptive treatments: RR=1.08 (CI 0.94 to 1.24), I^2=0% (shown in Fig. S3 in the Supplementary data). No differences in gastrointestinal adverse events were observed either, but these were also underreported: RR=1.01 (95%CI 0.95 to 1.07), 6 studies, I^2=0% (shown in Fig. S3 in the Supplementary data). There may be a higher risk of withdrawal due to adverse events with antiresorptive treatments, although the significant heterogeneity did not allow firm conclusions to be drawn (8 studies, I^2=68%, shown in Fig. S3 in the Supplementary data). In Nakamura *et al*.[26], withdrawals were

differentiated according to adverse events, protocol violation, withdrew consent and others.

3.6 Subgroup analysis

Four studies provided data for patients aged 75 years or older: the study of McCloskey *et al*.[20], group 2 of the study by McClung *et al*.[23], Black *et al*.[45] (from The European Public Assessment Report) and a *post hoc* analysis published by Boonen *et al*.[46]. A statistically significant reduction in the risk of hip fracture was not observed in this age group: RR=0.81 (95%CI 0.66 to 1.00), I^2=40%; ARR 0.31% (-0.19% to 0.8%), NNT 324 (125 to -522), 4 studies. Treatment was associated with a reduced risk of fractures of any type and non-vertebral fractures (number of fractures). A higher risk of withdrawals due to adverse events was found, based on data from only one study. Results for this subgroup are shown in Table S3 and Fig. S4 in the Supplementary data.

Results according to drug type (bisphosphonates or denosumab) showed consistent results across the different subgroups (shown in Fig. S5 in the Supplementary data). Drug type did not seem to be a source of heterogeneity in those outcomes with significant heterogeneity (vertebral fractures, clinical vertebral fractures, and withdrawals due to adverse effects).

Six studies included only participants with previous osteoporotic fractures (secondary prevention)[16,17,19,22,25,27], while one reported separate results for those participants with vertebral fractures at baseline[23]. In this subgroup of patients, differences in the risk of hip fracture between treatment and control groups were also found RR=0.55 (95%CI 0.40 to 0.77), I^2=0%; ARR 1.3% (0.47% to 1.98%), NNT=80 (50 to 211), 7 studies. There was an association between treatment and a reduced risk of fractures of any type, mortality, serious adverse events, vertebral fractures (total and clinical) and non-vertebral fractures. Data are shown in Table S4 in the Supplementary data.

3.7 Sensitivity analyses

We performed a sensitivity analysis restricting to the seven studies including only participants aged 65 years or older[17,18,20,22–24,26]. Results were similar to the main analyses and are shown in Table S5 in the Supplementary data.

We also performed a sensitivity analysis restricting to trials with low or unclear risk of bias, with a total of seven studies[17,18,20,22–24,26] for the main outcome. Results were similar to the main analyses and are shown in Table S6 in the Supplementary data.

Table 1. Summary of findings table

Outcome	Studies (number)	Treatment	Control	RR (95% CI)	I^2	Quality of the evidence (GRADE)
Hip fracture	12 (36196)	312/19639	352/16557	0.70 (0.60-0.81)	0%	**MODERATE[a]**
Any fracture	9 (26502)	1172/13252	1759/13250	0.67 (0.62-0.71)	39%	**MODERATE[a]**
Total number of fractures	3 (6039)	283/3019	391/3020	0.72 (0.63-0.84)	21%	**MODERATE[b]**
Mortality	7 (26156)	604/13083	631/13073	0.96 (0.86-1.07)	47%	**MODERATE[c]**
Vertebral fractures	6 (17906)	291/8953	796/8953	0.39 (0.30-0.50)	64%	**LOW[a,d]**
Clinical vertebral fractures	6 (25955)	116/12979	314/12976	0.38 (0.28-0.53)	55%	**LOW[a,d]**
Non-vertebral fractures	7 (29823)	1342/16458	1329/13365	0.79 (0.74-0.86)	0%	**LOW[a,c]**
Serious adverse events	8 (35297)	6117/19198	5280/16099	0.98 (0.95-1.01)	48%	**MODERATE[a]**
Total cardiovascular events	2 (9825)	392/4916	363/4909	1.08 (0.94-1.24)	0%	**LOW[b,c]**
Total gastrointestinal events	6 (12709)	1883/7889	1198/4820	1.01 (0.95-1.07)	0%	**MODERATE[a]**
Withdrawal due to AE	8 (35302)	2074/19200	1427/16102	1.05 (0.91-1.22)	68%	**VERY LOW[a,c,d]**

GRADE Working Group grades of evidence

High quality: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a] Downgraded one level due risk of bias **[b] Downgraded one level due to publication bias**

[c] Downgraded one level due to imprecision **[d] Downgraded one level due to inconsistency**

CI: confidence interval; RR: risk ratio

4. DISCUSSION

To the best of our knowledge, this is the first systematic review on antiresorptive treatments to focus on **hip fracture** as an outcome for older adults. Despite the relatively large number of RCTs and individuals studied, results for hip fracture are only reported in 12 RCTs with older adults. Despite the fact that hip fracture is the most clinically relevant fracture, most reviews focus on the overall efficacy, and vertebral and non-vertebral fractures[47,48].

Antiresorptive drugs are widely used as a first-line drug therapy for the **prevention** of osteoporotic fracture, and their efficacy for hip fracture prevention has been confirmed in this review; however, the absolute magnitude of benefit is small **(NNT of 186)** (Fig. 3). This result is consistent with previous results in meta-analyses focusing on hip fractures in postmenopausal patients of all ages, with an NNT of 175[2]. Also, the GRADE analysis shows a moderate quality of evidence in the main outcome, with quality concerns in many of the outcomes. Most of the outcomes show a low to moderate quality of evidence.

When **secondary prevention** is analysed as a subgroup, the absolute magnitude of benefit increases **(NNT of 80)** (Table S4 in the Supplementary data), which is similar to previous evidence[6]. However, this benefit drops dramatically **(NNT 324) in those aged ≥75 years**, with a higher risk of withdrawals due to adverse events (Table S3 and Fig. S4 in the Supplementary data). This shows the need for multifactorial intervention in order to prevent fractures in much older adults[49].

Four studies could be included in the meta-analysis regarding much older adults. The reduction in the risk of hip fractures did not achieve statistical significance and the analysis shows higher heterogeneity in this population. The only study of the four studies that shows a reduction in the risk of hip fracture was the denosumab FREEDOM Trial, the results of which should be considered with caution, given the high risk of bias.

A *post hoc* analysis published by Boonen *et al*. presented the results of pooled data from the HORIZON Pivotal Fracture Trial[24] and the HORIZON Recurrent Fracture Trial[25], with women aged 75 years or older[50], and did not show a reduction in hip fractures. Another *post hoc* analysis combining three large randomised double-blind clinical trials with risedronate (HIP, VERT-NA and VERT-MN) in women over the age of 80 did not report hip fractures in this population[51]; however, the incidence of osteoporosis-related non-vertebral fractures was not significantly lower than the placebo group after 3 years. A *post hoc* analysis of the Fracture Intervention Trial (FIT)[52] including patients from 75 to 85 years, with and without previous fractures, showed an ARR of 53 women per 10,000 patient-years at risk (PYR) for hip fractures, but participants aged 75 years or older comprised only 25% of the FIT.

From these RCTs and *post hoc* analyses, it is clear that the evidence is not only sparse, but that it also suggests no (or marginal) treatment benefit in hip fractures in older adults aged over 75 years, and even use of in mortality reduction.

The other source of evidence are the observational studies. These studies found a relationship between the antiresorptive treatment and the reduction of fractures in older adults[53]. The complication of developing RCTs in older adults is always commented upon[54] and the use of observational studies is recommended for this

population. Nevertheless, the reduction of fractures was seen to be independent from the use of antiresorptives[55]; in fact, fractures decline despite from the reduction in antiresorptive regimen[56]. The inconsistency between these results and our findings highlights the need for more RCTs in older osteoporotic adults[57]. Moreover, studies of fall prevention strategies (the main cause of hip fractures in older adults), including bone active drugs, are needed in this high risk population[2,3].

Although not the main outcome in this study, other types of fracture were significantly reduced with antiresorptive treatment, with a more favourable NNT, but their definition and clinical relevance is controversial. Sensitivity analyses for trials with all participants ≥65 years are consistent with the overall results. For men, no RCTs with fracture as the primary endpoint exist, and this lack of information shows us the importance of trials in men with primary osteoporosis.

Surrogate outcomes in osteoporosis, like BMD and BTM, show a good response to antiresorptive treatment in our review. However, despite the importance that these outcomes have in osteoporosis studies and the literature[12,58], the clinical usefulness of osteoporosis approaches in older adults is doubtful[3].

Regarding mortality, our analysis does not show any association between osteoporosis treatments and a risk reduction. Despite previous favourable reviews of this fact[59], more recent analyses also show that treatment for osteoporosis does not reduce overall mortality[10]. Our findings are more aligned with the second hypothesis due to the lack of biological mechanisms that explain benefits other than fracture prevention. The low number of side effects in the studies contrasts with the later findings[6], where many are associated with gastrointestinal effects. Other potential adverse events, such as osteonecrosis of the jaw[60] and atypical femoral fractures[61], were not reported in these studies but are known side effects linked to these treatments. The median follow-up of the studies (36 months) may be the reason for this lack of events. New potential side effects, such as rebound-associated fractures after denosumab withdrawal[62], add more concern about the long-term safety of these drugs.

The benefits of antiresorptive treatment in older people with osteoporosis is unclear, considering the limited benefit in absolute terms in hip fractures or the lack of benefit with regard to mortality, the expected adverse effects, and concerns about conflicts of interest and risk of bias in the studies. In addition, the cost-opportunity (potential benefits that an individual misses out on when choosing one alternative over another) must be considered. While the focus is placed on pharmacological treatments, along with resources, not enough effort is put into measures that could be more effective, such as lifestyle modifications and physical exercise (progressive resistance exercises and balance training)[63]. It should not be forgotten that most fractures in older adults are caused by falls and not by osteoporosis, especially in frail patients. This shows the need for multifactorial intervention in order to prevent fractures in older adults[49], with antiresorptives as a treatment option, with doubtful efficacy, more likely to be used in secondary prevention (always after a comprehensive assessment).

The strengths of the study are the inclusion of all studies with hip fracture as an outcome in older adults and the focus on clinical outcomes (such as hip fracture) rather than surrogate outcomes. In addition, an extensive risk of bias and GRADE evaluation was

carried out. The main limitations are related to the quality of the studies, most of which showed an unclear/high risk of bias. Meta-analysis results should therefore be interpreted with caution. Furthermore, data in older participants are mostly based on subgroups from larger studies on postmenopausal studies in women, with no results in older men. The limited number of studies in some of the subgroup analyses is also a limitation, although cancer-related and corticosteroid-induced osteoporosis were not considered in our analysis. Some studies have nonspecific exclusion criteria, such as severe illness[20,23,32], low life expectancy[25] or number of prevalent fractures[21,50], making generalisation of the study data complicated. In addition, data on the baseline situation of the participants (very important in the older population) are missing.

5. CONCLUSION

Antiresorptive drugs have a statistically significant effect on preventing hip fracture, but with a moderate quality of evidence and low effect in absolute terms (NNT 186). Alendronate, denosumab, risedronate and zoledronate may have a significant role in preventing hip fracture, but the evidence is based on studies with a risk of bias and conflicts of interest. Evidence on very old adults (≥75 years) is not significant and comes from very few studies. The greatest advantage is found in secondary prevention (NNT 80). More RCTs in very old osteoporotic adults are therefore needed.

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SUPPLEMENTARY MATERIAL

Text S1. Search strategy that details the selection process

MEDLINE PUBMED 09/07/2021

1 "alendronate"[MeSH Terms] OR alendronate[Text Word] - 5692
2 "risedronic acid"[MeSH Terms] OR risedronate[Text Word] - 2021
3 "ibandronic acid"[MeSH Terms] OR ibandronate[Text Word] - 1183
4 "zoledronic acid"[MeSH Terms] OR zoledronic acid[Text Word] - 5154
5 "clodronic acid"[MeSH Terms] OR clodronate[Text Word] - 2793
6 "etidronic acid"[MeSH Terms] OR etidronate[Text Word] - 3230
7 "YM 529"[All Fields] OR minodronate[Text Word] - 172
8 "pamidronate"[MeSH Terms] OR pamidronate[Text Word] - 3172
9 "tiludronic acid"[All Fields] OR tiludronate[Text Word] - 171
10 "denosumab"[MeSH Terms] OR denosumab[Text Word] - 3531
11 "Osteoporosis"[Mesh] OR "Osteoporosis"[Text Word] - 91959
12 "Fractures, Bone"[Mesh] OR "Fractures, Bone"[Text Word] - 192558
13 Search 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 - 20934
14 Search 11 AND 12 AND 13 - 2053

COCHRANE LIBRARY 09/07/2021

#1 MeSH descriptor: [Alendronate] explode all trees 756
#2 MeSH descriptor: [Risedronic Acid] explode all trees 254
#3 MeSH descriptor: [Ibandronic Acid] explode all trees 206
#4 MeSH descriptor: [Zoledronic Acid] explode all trees 636
#5 MeSH descriptor: [Clodronic Acid] explode all trees189
#6 MeSH descriptor: [Etidronic Acid] explode all trees 476
#7 Minodronate 51
#8 MeSH descriptor: [Pamidronate] explode all trees 243
#9 Tiludronate 45
#10 MeSH descriptor: [Denosumab] explode all trees 349
#11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 2650
#12 MeSH descriptor: [Osteoporosis] explode all trees 4199
#13 MeSH descriptor: [Colles' Fracture] explode all trees 109
#14 MeSH descriptor: [Hip Fractures] explode all trees 1717
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#16 #13 OR #14 OR #15 2522
#17 #11 AND #12 AND #16 167

WOS 09/07/2021

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12 "Fractures, Bone"[Mesh] OR "Fractures, Bone"[Text Word] - 113081
13 Search 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 - 30091
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Scopus 13/02/2020
1 "alendronate"[MeSH Terms] OR alendronate[Text Word] - 6149
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6 "etidronic acid"[MeSH Terms] OR etidronate[Text Word] - 1612
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7 "minodronic acid” - 443
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9 "tiludronic acid" - 888
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13 Search 11 AND 12 AND 13 – 11794

Table S1. Bias assessment of each study

Author, year	Baseline imbalance	Allocation concealment	Blinding				Fracture assessment	Lost follow-up	Selective reporting	Funding	Overall Risk of Bias
			OA	DC	O	P					
Alendronate											
Black 1996[12]	No	No	NR	NR	NR	Yes	Medical records or radiologic reports	41 (4.01%) alendronate 40 (3.98%) placebo	No	Merck	Unclear
Greenspan 1998[13]	No	Unclear	NR	NR	NR	Yes	Radiologic reports	15 (25%) placebo 14 (23.3%) alendronate	Unclear	Merck	Unclear
Greenspan 2002[14]	Unclear	Probable yes	NR	NR	NR	Yes	Radiologic reports	NR	No	Merck	Unclear
Cecilia 2008[15]	No	Probable yes	No	No	No	No	Medical records or radiologic reports	23 (20.17%) alendronate 17 (13.6%) non alendronate	Yes	AIOE and Fundació Mutua Madrileña	High

Clodronate											
McCloskey 2007[16]	No	Yes	NR	NR	NR	Yes	Radiologic reports	13 (0.02%)	No	Schering Oy y Medical Research Council	**Low**
Denosumab											
Cummings 2009[17]	No	NR	NR	NR	NR	Yes	Medical records or radiologic reports	NR	Unclear	Amgen	High
Etidronate											
Lyritis 1997[18]	No	NR or unclear	NR	NR	NR	NR	Radiologic reports	15 (30%) placebo 11 (22%) etidronate	No	Not reported	High
Risedronate											
McClung 2001[19]	No	NR	NR	NR	NR	NR	Documented by medical personnel	1127 (35.9%) placebo 2197 (35.5%) risedronate	Nor	Procter & Gamble Pharmaceuticals and Aventis Pharma	High
Zoledronate											
Black 2007[20]	No	Probable Yes	NR	NR	NR	Yes	Documented by medical personnel	627 (16%) zoledronic	No	Novartis	Unclear

								592 (15.27%) placebo			
Lyles 2007[21]	No	Probable Yes	Yes	Yes	Yes	Yes	NR	35 (3.29%) zoledronic 28 (2.64%) placebo	No	Novartis	Unclear
Nakamura 2016[22]	No	Probably Yes	Yes	NR	NR	Yes	Medical records or radiologic reports	3 (0.9%) zoledronic 1 (0.3%) placebo	No	Asahi	Unclear
Zhu 2020[23]	No	Probable Yes	No	NR	NR	No	NR	0 zoledronic 1 (2.13%) non zoledronic	No	Shanghai Pujiang Young Rheumatologists Training Program	High

Abbreviations: OA (outcome assessors), DC (data collectors), O (others), P (patients), NR (not reported)

Fig. S1. Funnel plot for primary outcome

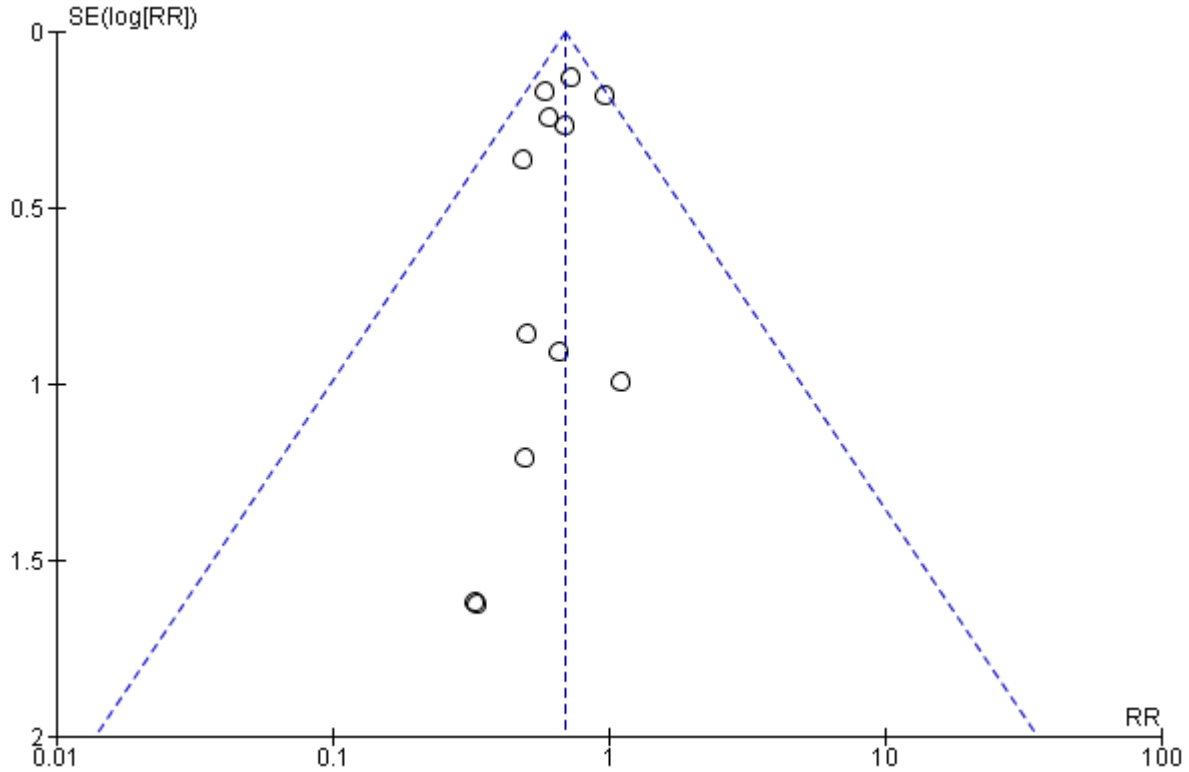


Table S2. Baseline characteristics of the studies

Study	Country	Intervention		Number of patients	Women (%)	Age (+/- SD)	≥65y(%)	Previous osteoporotic fracture (%)	Duration (moths)	Follow-up	Outcomes measured	BMD (lumbar; total hip; femoral neck) in g/cm2
Alendronate												
Black 1996[12]	USA	Placebo or Alendronate Both 500mg calcium and 250 UI D	Alendronate 5mg/day for 24 months. Then increased to 10 mg/day	1022	100	70.7+/-5.6	83.2	100	36	Clinic visit and radiographs at baseline 12-24-36	Clinical fractures Vertebral Fracture (decrease of 20% and at least 4 mm)	0.79 +/- 0.14 0.66 0.57 +/- 0.07
			Placebo	1005	100	71.0 +/- 5.6	84.2	100			Other fractures (b), BMD (secondary)	0.79 +/- 0.14 0.66 0.56 +/- 0.07
Greenspan 1998[13]	USA	Placebo or Alendronate 250 mg calcium and 125 IU of vitamin D if low calcium	Alendronate 5mg/day and increased to 10 mg/day from	60	100	69.7+/-4.4	100	100	30	baseline and every 6 months thereafter for a	BMD Non-vertebral Hip and Wrist; BTMs	0.844+/-0.165 0.741+/-0.13 0.629+/-0.102

		dietary intake (<1000)	November 1993							total of 30 months		
			Placebo	60	100	70.2+/-7.8	100	100				0.886+/-0.146 0.774+/-0.084 0.647+/-0.081
Greenspan 2002[14]	USA	Placebo or Alendronate Calcium and vitamin D 400 IU/day when low calcium dietary intake (<1500 mg/day)	alendronate 10 mg/day	163	100	78.5 years (range, 65 to 91 years)	100	55	24	baseline and every 6 months	BMD Non-vertebral Hip and Wrist; BTMs	NR
			Placebo	164	100		100					NR
Cecilia 2008[15]	ESP	Alendronate Calcium 500mg and	Alendronate 70mg/weekly	114	79.8	81 +/- 7	100	100	12	Baseline, 6 months and 12 months	BMD	0.798+/-0.166 0.619+/-0.132

		vitamin D 400 IU/day									BTMs, adverse outcomes	0.541+/- 0.102
			Non alendronate	125	78.4	81 +/- 7	100	100				0.798+/- 0.161 0.630+/- 0.161 0.550+/- 0.105
Clodronate												
McCloskey 2007[16]	UK	Placebo or clodronate. No calcium - vitamin D supplementation	Clodronate 800mg/day oral	2796	100	79.5+/-4.0 (≥75)	100	24.4	36	nurses at 6-month intervals	Hip Any clinical fracture Nonhip osteoporotic fractures	NR 0.754+/-0.14 NR
			Placebo	2796	100	79.6+/-4.0 (≥75)	100	21.1				NR 0.75+/-0.14 NR
Denosumab												

Cummings 2009[17]	INT	Placebo or denosumab at least 1000 mg calcium; vitamin D 800UI (serum level 12 to 20 ng) 400UI (above 20 ng)	denosumab 60 mg/6 months	3902	100	72.3 +/- 5.2	NR	23.8	36	Medical records or radiologic reports	Vertebral fracture (increase of at least 1 grade in a vertebral body that was at baseline) first nonvertebral fracture and the time to the first hip fracture	NR
			Placebo	3906	100	72.3 +/- 5.2	NR	23.4				NR
Etidronate												
Lyritis 1997[18]	GR	Placebo or etidronate cyclical 500mg calcium and 5 days of Calcitriol	90-day cyclical 400 mg etidronate	50	100	71.8+/-0.3 (aged 67-77)	100%	100	48	DEXA and radiographs at yearly	BMD lumbar BTMs, Vertebral fracture, other fractures	0.572+/-0.03 NA 0.419+/-0.02
			Placebo	50	100	72.2+/-0.4 (aged 67-77)	100%	100				0.565+/-0.02 NA 0.425+/-0.04
Risedronate												
McClung 2001[19]	INT	Risedronate 2.5 or 5	Risedronate overall	6197	100	NR	100	NR	36	Radriographs at	Hip fracture	NR

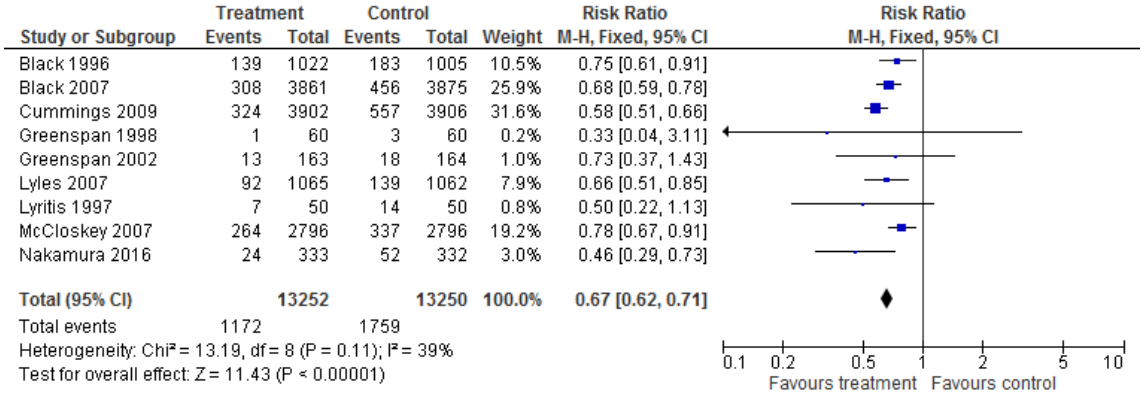
		mg/day vs. placebo.	Calcium 1000 mg/day. Vitamin D ≤500 IU/day when necessary							medical report	Non-vertebral fracture BMD AE, SAE Withdrawal due to AE	
			Placebo overall	3134	100	NR	100	NR				
			Risedronate Group 1	3624	100	74 +/- 3	100	38				
			Placebo Group 1	1821	100	74 +/- 3	100	39				
			Risedronate Group 2	2573	100	83+/- 3	100	44				
			Placebo Group 2	1313	100	83+/-3	100	45				
Zoledronate												
Black 2007[20]	INT	Placebo or zoledronic acid Both calcium (1000-1500 mg/day)	Zoledronic acid 5mg ev yearly	3889	100	73.1±5.34	100	73.1±5.34	36	Clinic visit 6-12-24-36 months	New vertebral fractures and hip fracture Non-vertebral fractures	Lumbar spine: 0.79±0.124 Total hip: 0.65±0.090 Femoral neck: 0.53±0.062

		and vitamin D (400-1200UI/day)	Placebo	3876	100	73.0±5.40	100	73.0±5.40			Clinical fracture Clinical vertebral fracture BMD, BTMs (secondary)	Lumbar spine: 0.79±0.140 Total hip: 0.65±0.091 Femoral neck: 0.53±0.064
Lyles 2007[21]	INT	Placebo or zoledronic acid Both calcium (1000-1500 mg/day) and vitamin D (800-1200UI/day)	zoledronic acid 5mg ev yearly	1065	76.7	74.4±9.48	83.8	100	36	Clinic visit 12-24-36-~~48-60~~	New clinical fracture (excluding facial, digital and abnormal fractures) BMD, new vertebral non-vertebral, hip fractures (secondary)	Femoral neck: 0.65±0.127 Total hip: 0.70±0.153
			Placebo	1062	75.5	74.6±9.86	81.9	100				Femoral neck: 0.65±0.122 Total hip: 0.70±0.152
Nakamura 2016[22]	JAP	Placebo or zoledronic acid	zoledronic acid 5mg ev yearly	333	93.6	74.0 +/- 5.4	100	92.8	24	radiographs at baseline and at 6, 12, 18,	new morphometric vertebral fractures	Lumbar spine: 0.66 ± 0.09 Total hip: 0.65 ± 0.10

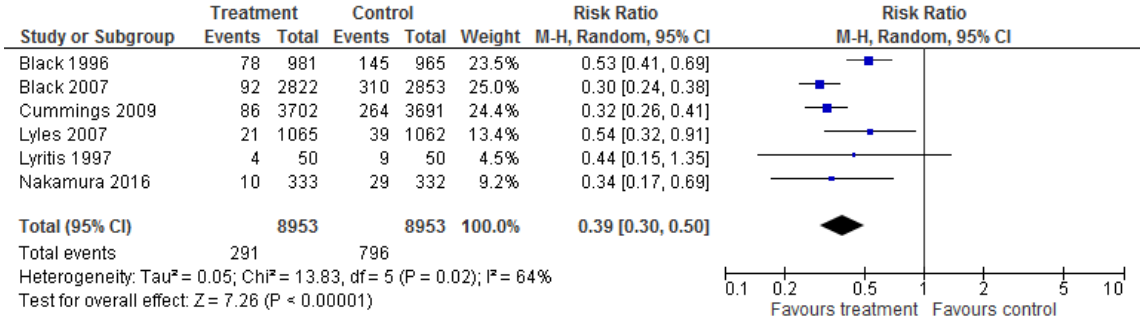
		Both of 610 mg/day calcium, 400 IU/day vitamin D, and 30 mg/day magnesium								and 24 months	Clinical fractures; BMD, BTMs (secondary), osteoporotic fractures	Femoral neck: 0.53 ± 0.08
			Placebo	332	94.3	74.3 +/-5.4	100	94.0				Lumbar spine: 0.66 ± 0.09 Total hip: 0.66 ± 0.09 Femoral neck: 0.53 ± 0.08
Zhu 2020[23]	CHN	Zoledronic acid or control Both of 0.5 µg/day calcitriol and 1000 mg/day	zoledronic acid 5mg ev once	48	77.08	74.58 +/- 8.45	100	100	12	BMD baseline, 6 and 12 months	BMD BTM Fractures (secondary)	NR
			Non-zoledronic	47	74.47	73.13 +/- 10.47	NR	100				NR

Fig. S2. Forest plot for other fractures

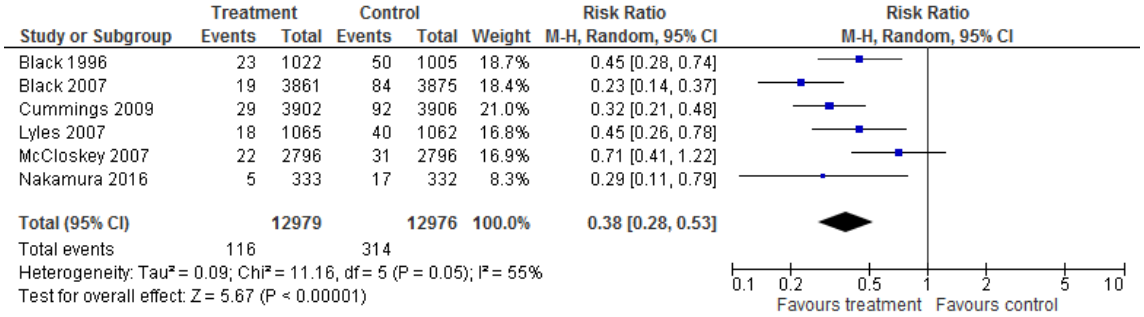
1. Any fracture



2. Vertebral fractures



3. Clinical vertebral fractures



4. Non-vertebral fractures

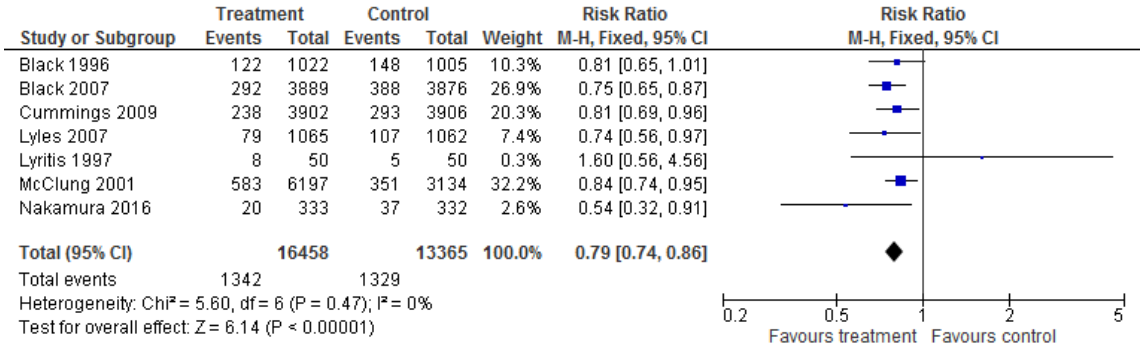
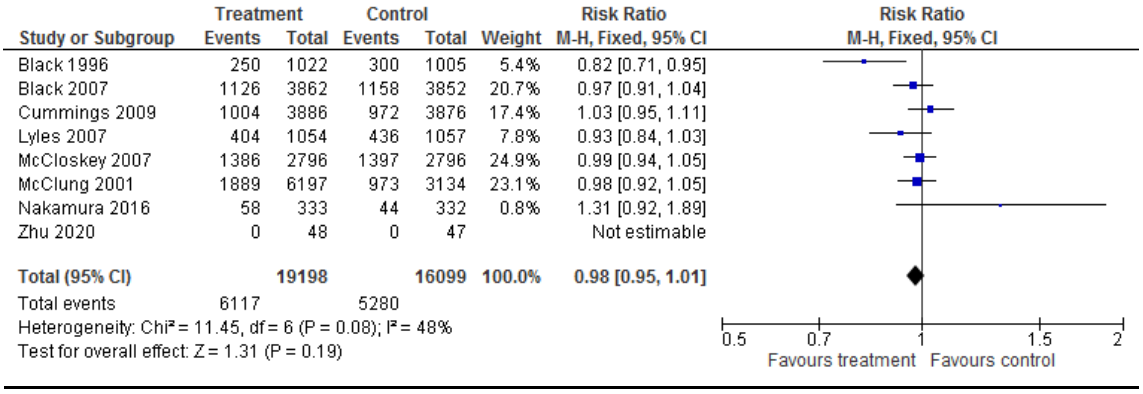


Fig. S3. Forest plot for other events

1. Serious adverse events



Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Black 1996	250	1022	300	1005	5.4%	0.82 [0.71, 0.95]
Black 2007	1126	3862	1158	3852	20.7%	0.97 [0.91, 1.04]
Cummings 2009	1004	3886	972	3876	17.4%	1.03 [0.95, 1.11]
Lyles 2007	404	1054	436	1057	7.8%	0.93 [0.84, 1.03]
McCloskey 2007	1386	2796	1397	2796	24.9%	0.99 [0.94, 1.05]
McClung 2001	1889	6197	973	3134	23.1%	0.98 [0.92, 1.05]
Nakamura 2016	58	333	44	332	0.8%	1.31 [0.92, 1.89]
Zhu 2020	0	48	0	47		Not estimable
Total (95% CI)		**19198**		**16099**	**100.0%**	**0.98 [0.95, 1.01]**
Total events	6117		5280			

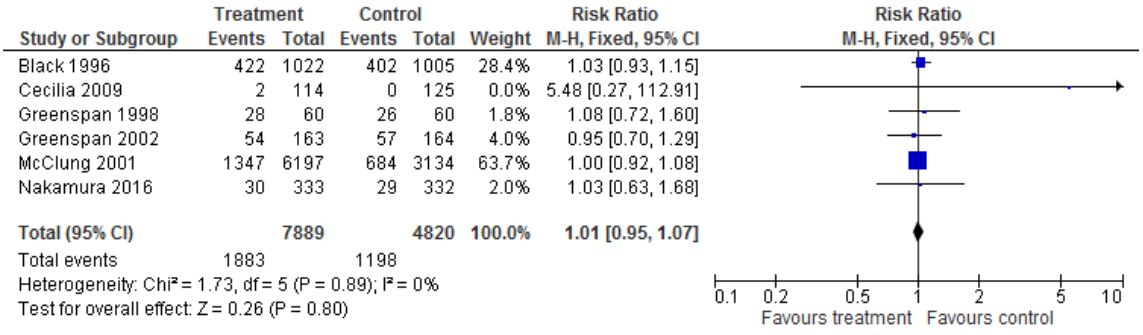
Heterogeneity: Chi² = 11.45, df = 6 (P = 0.08); I² = 48%
Test for overall effect: Z = 1.31 (P = 0.19)

2. Total cardiovascular events

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Black 2007	258	3862	239	3852	65.9%	1.08 [0.91, 1.28]
Lyles 2007	134	1054	124	1057	34.1%	1.08 [0.86, 1.36]
Total (95% CI)		**4916**		**4909**	**100.0%**	**1.08 [0.94, 1.24]**
Total events	392		363			

Heterogeneity: Chi² = 0.00, df = 1 (P = 0.96); I² = 0%
Test for overall effect: Z = 1.09 (P = 0.27)

3. Gastrointestinal events



Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Black 1996	422	1022	402	1005	28.4%	1.03 [0.93, 1.15]
Cecilia 2009	2	114	0	125	0.0%	5.48 [0.27, 112.91]
Greenspan 1998	28	60	26	60	1.8%	1.08 [0.72, 1.60]
Greenspan 2002	54	163	57	164	4.0%	0.95 [0.70, 1.29]
McClung 2001	1347	6197	684	3134	63.7%	1.00 [0.92, 1.08]
Nakamura 2016	30	333	29	332	2.0%	1.03 [0.63, 1.68]
Total (95% CI)		**7889**		**4820**	**100.0%**	**1.01 [0.95, 1.07]**
Total events	1883		1198			

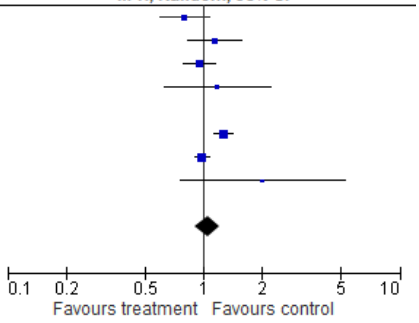
Heterogeneity: Chi² = 1.73, df = 5 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.26 (P = 0.80)

4. Withdrawals due to adverse events

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Black 1996	78	1022	96	1005	13.5%	0.80 [0.60, 1.06]
Black 2007	80	3862	70	3852	12.0%	1.14 [0.83, 1.57]
Cummings 2009	192	3886	202	3876	18.7%	0.95 [0.78, 1.15]
Lyles 2007	21	1054	18	1057	4.6%	1.17 [0.63, 2.18]
Lyritis 1997	0	50	0	50		Not estimable
McCloskey 2007	593	2796	471	2796	24.0%	1.26 [1.13, 1.40]
McClung 2001	1098	6197	564	3134	25.0%	0.98 [0.90, 1.08]
Nakamura 2016	12	333	6	332	2.1%	1.99 [0.76, 5.25]
Total (95% CI)		**19200**		**16102**	**100.0%**	**1.05 [0.91, 1.22]**
Total events	2074		1427			

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 19.02$, df = 6 (P = 0.004); $I^2 = 68\%$

Test for overall effect: Z = 0.65 (P = 0.51)



5. Mortality

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Black 1996	24	1022	21	1005	3.4%	1.12 [0.63, 2.01]
Black 2007	130	3862	112	3852	17.8%	1.16 [0.90, 1.48]
Cecilia 2009	13	114	15	125	2.3%	0.95 [0.47, 1.91]
Cummings 2009	70	3902	90	3906	14.3%	0.78 [0.57, 1.06]
Lyles 2007	101	1054	141	1057	22.3%	0.72 [0.56, 0.91]
McCloskey 2007	264	2796	249	2796	39.5%	1.06 [0.90, 1.25]
Nakamura 2016	2	333	3	332	0.5%	0.66 [0.11, 3.95]
Total (95% CI)		**13083**		**13073**	**100.0%**	**0.96 [0.86, 1.07]**
Total events	604		631			

Heterogeneity: $Chi^2 = 11.35$, df = 6 (P = 0.08); $I^2 = 47\%$

Test for overall effect: Z = 0.77 (P = 0.44)

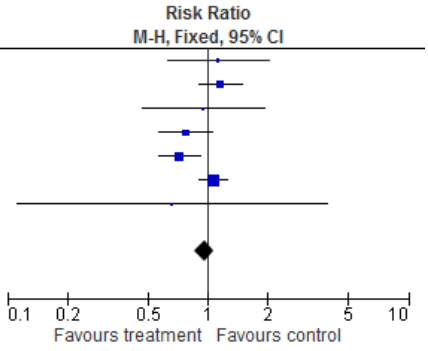


Table S3. Subgroup analyses for participants ≥75

Table E1 Results for participants ≥75					
Outcome	**Studies**	**Treatment**	**Control**	**RR (IC 95%)**	**I^2**
Hip fracture	Black 2007[20] Cummings 2009[17], McCloskey 2007[16], McClung 2001 (group 2) [19]	180/8101	172/6797	0.81 (0.66-1.00)	40%
Any fracture	McCloskey 2007[16]	264/2796	337/2796	0.78(0.67-0.91)	-
Total number of fractures	McCloskey 2007[16]	269/2796	360/2796	0.75 (0.64-0.87)	-
Mortality	McCloskey 2007[16]	264/2796	249/2796	1.06 (0.90-1.25)	-
SAE	Cummings 2009[17], McCloskey 2007[16]	1754/4021	1768/4025	0.99 (0.95-1.04)	0%
Clinical vertebral fractures	McCloskey 2007[16]	22/2796	31/2796	0.71 (0.41-1.22)	-
Non-vertebral fractures (participants)	McClung 2001 (group 2) [19]	278/2573	156/1313	0.91 (0.76-1.09)	-
Non-vertebral fractures (total number)	McCloskey 2007[16]	247/2796	329/2796	0.75 (0.64-0.88)	-
Withdrawal due to AE	McCloskey 2007[16]	593/2796	471/2796	1.26 (1.13-1.40)	-

Fig. S4. Forest plot of for hip fracture in participants ≥75

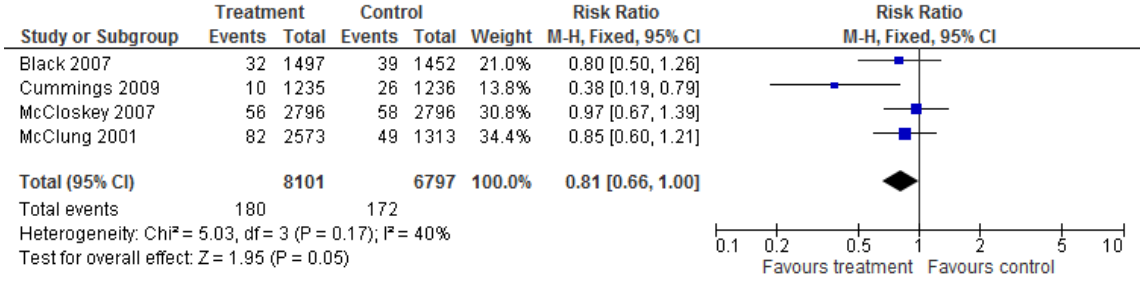
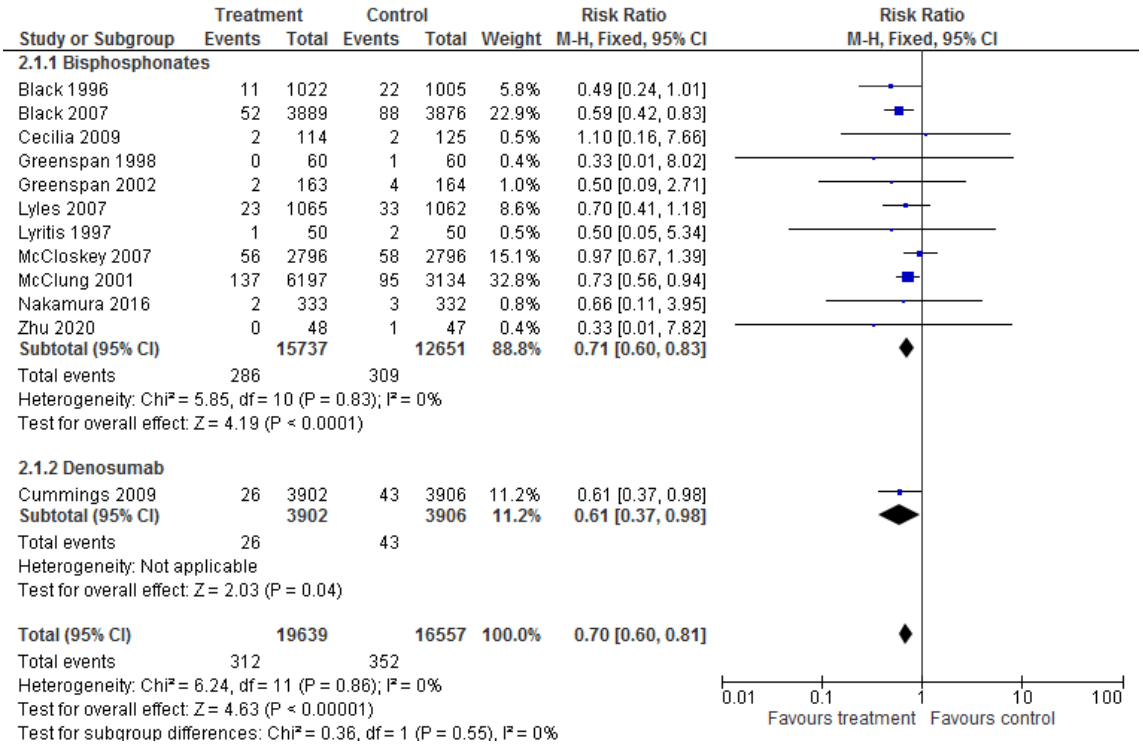
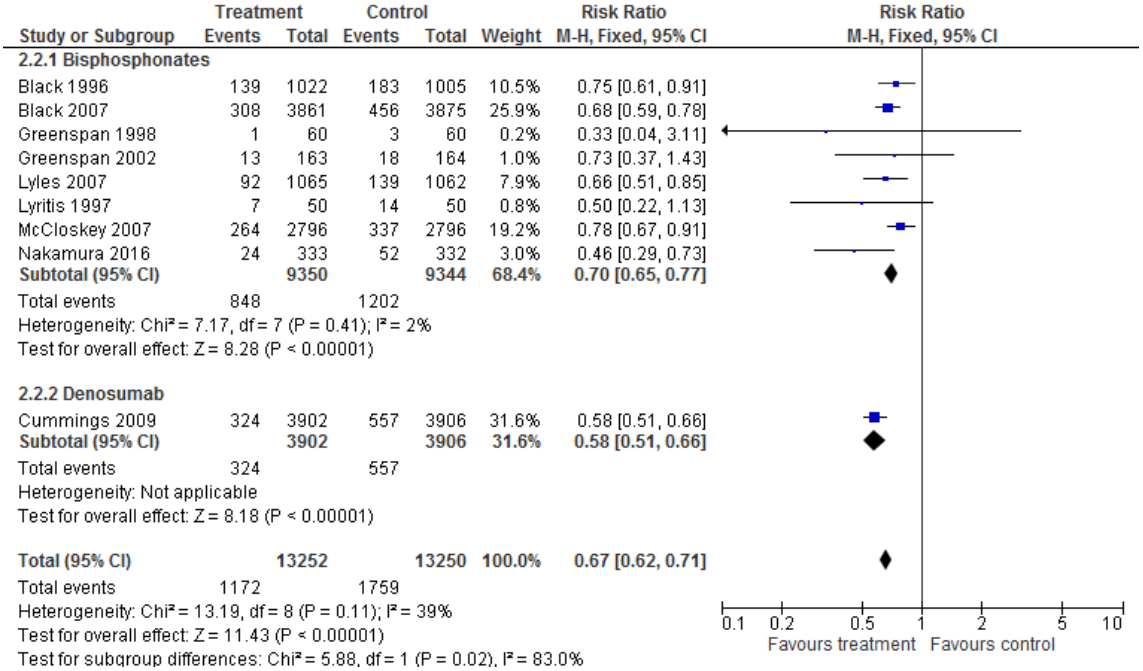


Fig. S5. Subgroup analyses

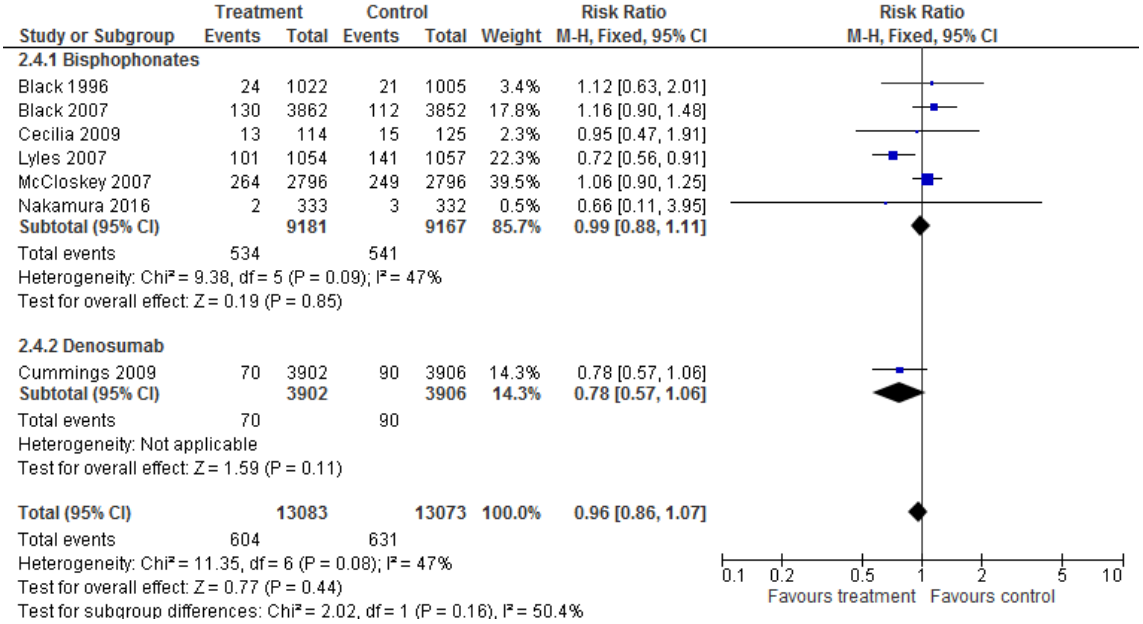
1: Hip fracture



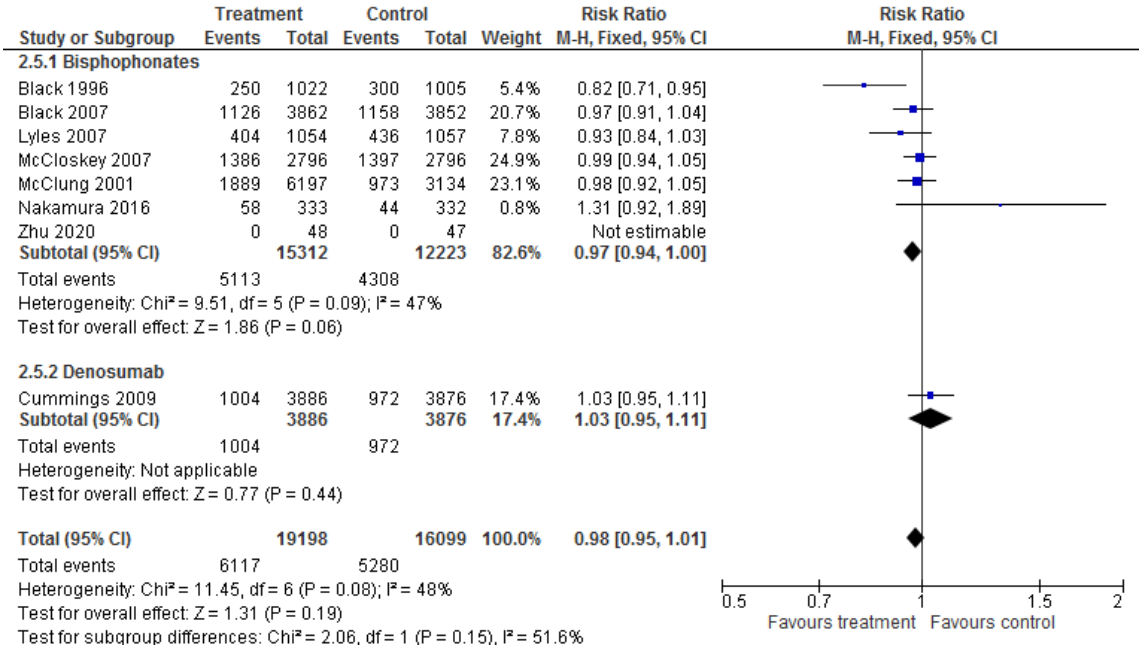
2: Any fracture



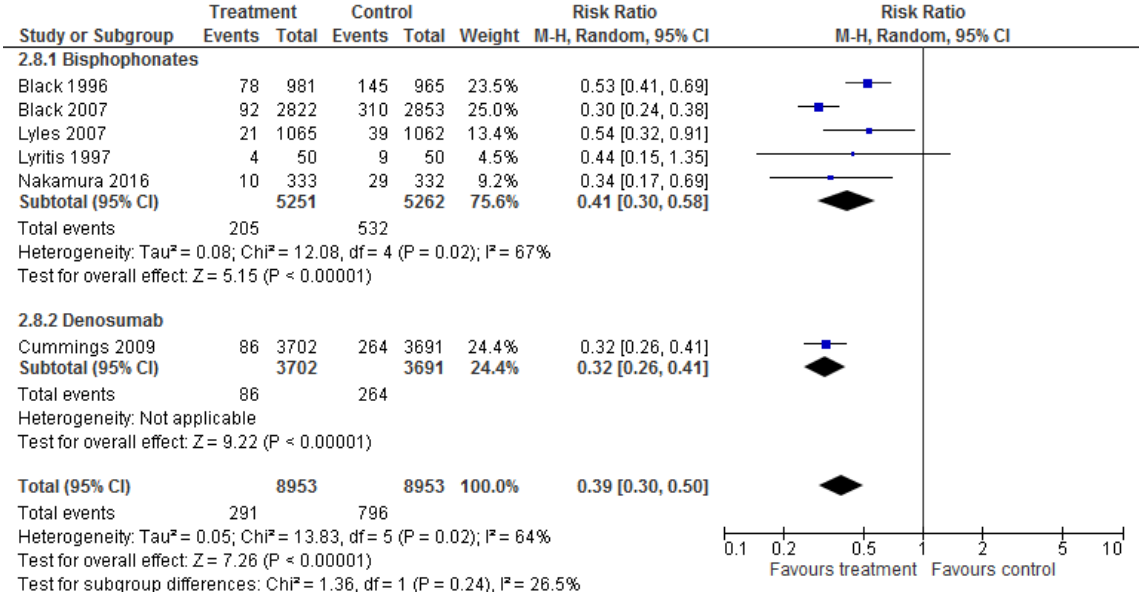
3: Mortality



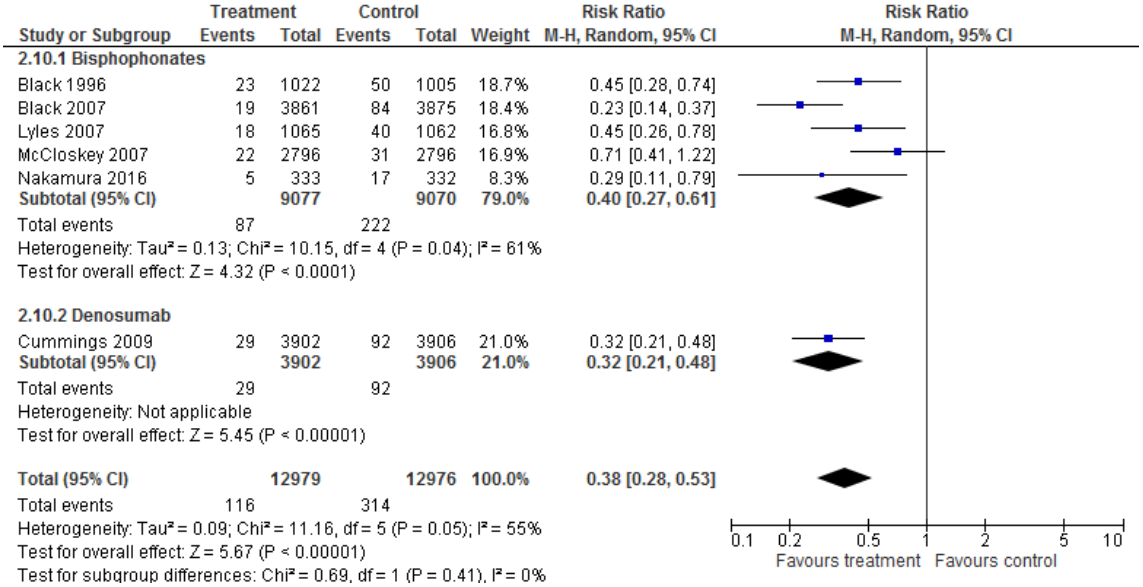
4: Serious adverse events



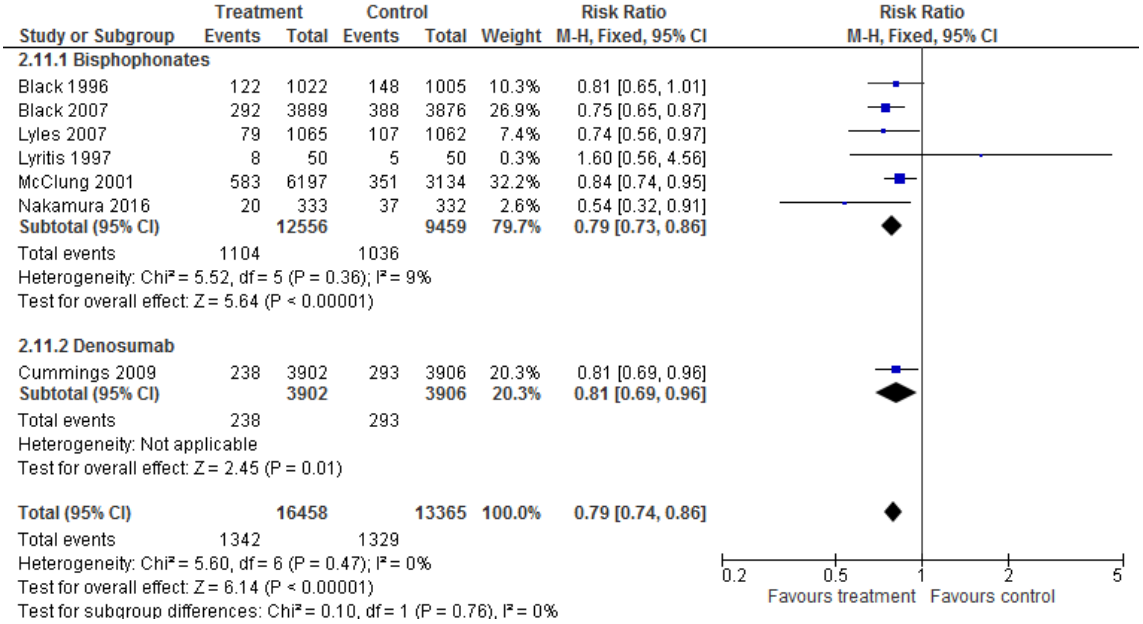
5: Vertebral fractures



6: Clinical Vertebral fractures



7: Non-vertebral fractures



8: Withdrawal due to adverse events

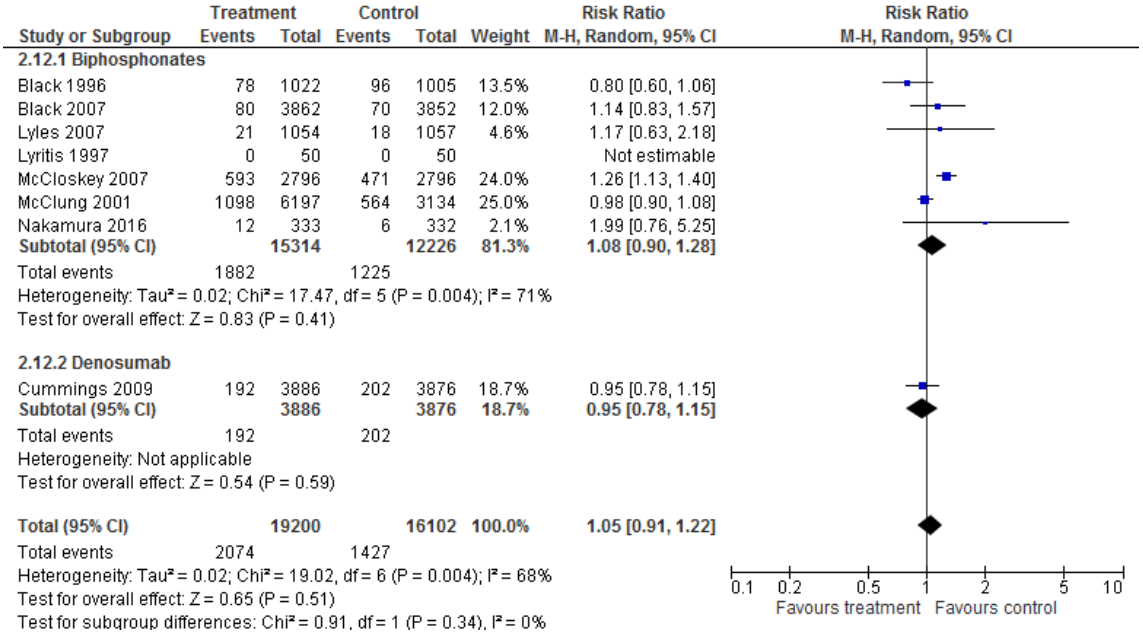


Table S4. Results for secondary prevention

Table 1. Results for secondary prevention only					
Outcome	**Number of studies**	**Treatment**	**Control**	**RR (IC 95%)**	**I^2**
Hip fracture	7	6 59/3487	86/2924	0.55 (0.40-0.77)	0%
Any fracture	4	239/2197	339/2177	0.70 (0.60-0.81)	0%
Total number of fractures	1 (Greenspan 1998[13])	1/60	3/60	0.33 (0.04-3.11)	-
Mortality	3	138/2190	177/2187	0.79 (0.64-0.97)	12%
SAE	3	654/2124	736/2109	0.88 (0.81-0.96)	48%
CV events	1 (Lyles 2007[21])	134/1054	124/1057	1.08 (0.86-1.36)	-
GI events	3	452/1196	428/1190	1.04 (0.94-1.15)	0%
Vertebral fractures	3	103/2096	193/2077	0.53 (0.42-0.66)	0%
Clinical vertebral fractures	2	41/2087	90/2067	0.45 (0.31-0.65)	0%
Non-vertebral fractures (participants)	3	209/2137	260/2117	0.80 (0.67-0.94)	2%
Withdrawal due to AE	3	99/2126	114/2112	0.86 (0.66-1.11)	16%

Table S5. Results of sensitive analyses for trials with all the participants ≥65

Table 1 Results for trials with all the participants ≥65					
Outcome	**Number of studies**	**Treatment**	**Control**	**RR (IC 95%)**	**I^2**
Hip fracture	7	250/13488	251/10412	0.73 (0.61-0.87)	0%
Any fracture	6	617/7263	880/7277	0.70 (0.64-0.77)	24%
Total number of fractures	3	283/3019	391/3020	0.72 (0.63-0.84)	21%
Mortality	3	396/6991	364/6980	1.09 (0.95-1.25)	0%
SAE	4	4459/13188	3572/10114	0.99 (0.95-1.02)	0%
CV events	1	258/3862	239/3852	1.08 (0.91-1.28)	-
GI events	4	1459/6753	796/3690	1.00 (0.92-1.08)	0%
Vertebral fractures	3	106/3205	348/3235	0.31 (0.25-0.38)	0%
Clinical vertebral fractures	3	46/6990	132/7003	0.37 (0.16-0.82)	79%
Non-vertebral fractures	4	903/10469	781/7392	0.79 (0.72-0.87)	42%
Withdrawal due to AE	5	1783/13238	1111/10164	1.14 (0.94-1.38)	77%

Table S6. Results of sensitive analyses excluding trials with high risk of bias

Table 1. Results of Sensitive analyses excluding trials with high risk of bias					
Outcome	**Number of studies**	**Treatment**	**Control**	**RR (95% CI)**	**I^2**
Hip fracture	7	146/9328	209/9295	0.70 (0.57-0.86)	0%
Any fracture	7	841/9300	1188/9294	0.71 (0.65-0.77)	7%
Total number of fractures	3	283/3019	391/3020	0.72 (0.63-0.84)	21%
Mortality	5	521/9067	526/9042	0.97 (0.78-1.21)	57%
SAE	5	3224/9067	3335/9042	0.95 (0.89-1.02)	57%
Total CV events	2	392/4916	363/4909	1.08 (0.94-1.24)	0%
Gastrointestinal AE	4	534/1578	514/1561	1.03 (0.93-1.13)	0%
Vertebral fractures	4	201/5201	523/5212	0.41 (0.29-0.60)	75%
Clinical vertebral fractures	5	87/9077	222/9070	0.39 (0.31-0.50)	61%
Non-vertebral fractures	4	513/6309	680/6275	0.75 (0.67-0.84)	0%
Withdrawal due to AE	5	784/9067	661/9042	1.12 (0.89-1.40)	59%


GEROCIENCIA
BIOMARCADORES
OSTEOPOROSIS

Chapter 2

Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult.

EDITORIAL

La gerociencia, una intersección entre la biogerontología y la medicina, es una disciplina prometedora que busca desentrañar los procesos moleculares y celulares del envejecimiento[1] y dentro de esta ciencia, el área de la geriatría tiene diferentes focos, entre los que destaca la osteoporosis. Debido a su naturaleza crónica y a su alta prevalencia en una población cada vez más envejecida, tiene importantes consecuencias humanas y socioeconómicas, que incluyen entre otras, morbilidad, discapacidad y mortalidad, además de conllevar un importante aumento del gasto sanitario derivado de las fracturas[2]. De hecho, en España se estima que ocurrieron 285000 fracturas osteoporóticas en 2019, con un coste de 4.3billones de € y un probable incremento del 29.6% en el periodo 2019-2034. Este impacto a nivel de morbilidad y gasto sanitario es más acusado en la población femenina, siendo el 79.2% del total[3]. En este contexto sociosanitario es evidente que debemos incrementar nuestros esfuerzos en detectar población con riesgo de fractura por el alto beneficio potencial que tendría una detección precoz de dicha situación clínica.

Históricamente, la predicción del riesgo de fractura relacionada con la osteoporosis ha sido subóptimo. Dentro de la evaluación de este riesgo, los factores más comúnmente estudiados han sido la densidad mineral ósea (DMO), los marcadores de recambio óseo (MRO) y la calculadora de riesgo Fracture Risk Assessment Tool FRAX®. El cálculo del riesgo mediante estas herramientas es un tema en debate, al considerarse que presentan una serie de limitaciones, especialmente en adultos mayores[4]. La DMO es un factor de riesgo clásico para las fracturas y se ha estudiado ampliamente, pero su baja sensibilidad es una de las razones por las cuales no se recomienda su uso exclusivo para evaluar el riesgo de fracturas en el cribado poblacional [5], siendo destacable la baja correlación observada entre la pérdida de DMO y su valor predictivo en el riesgo de fractura[6]. Del mismo modo, los MRO tampoco mejoran la predicción del riesgo de fractura o pérdida ósea del paciente, viéndose limitada su utilidad a la monitorización de la terapia con bisfosfonatos orales u otros fármacos antirresortivos[7]. A diferencia de los factores para la evaluación del riesgo de fractura antes mencionados, el FRAX es la calculadora de riesgo de fractura de referencia, la cual incluye múltiples parámetros, pero su aplicabilidad es limitada por tratarse, por diseño, de un método de cálculo simple para ser utilizado en atención primaria, y utiliza variables categóricas sin considerar los efectos dosis- dependientes de factores de riesgo claves[8]. De esta manera se está subestimando el riesgo de fracturas[9] y no es una calculadora adecuada para adultos mayores de 90 años ya que dicha tipología de pacientes fue excluida en su diseño[10]. Aunque es cierto que esta calculadora mejora la predicción de fracturas en comparación con la medición de la DMO aislada, su capacidad de predicción del riesgo de fractura varía en diferentes poblaciones de estudio[11] al ofrecer una perspectiva superficial y poco personalizada hasta el punto de haberse considerado de dudosa eficacia en la población española[4] y también a nivel mundial[12]. Es en este contexto en el que los biomarcadores podrían jugar un papel crucial en la identificación, seguimiento, evaluación y pronóstico de la osteoporosis en el adulto mayor, facilitando e integrando el concepto de medicina de precisión.

Es crucial desarrollar una nueva estrategia para comprender, predecir y abordar la osteoporosis desde el punto de vista de la medicina de precisión ya que el tratamiento de un paciente estratificado como de alto riesgo con los medios tradicionales resultaría

insuficiente para abordar el deterioro sistémico en la microestructura ósea [13]. Tanto es así, que desde sociedades como la European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO)[14], se han propuesto una serie de biomarcadores relevantes para el estudio de la salud musculo-esquelética. Con la ayuda analítica, estas tecnologías han estado proporcionando una imagen cada vez más detallada de las alteraciones moleculares y celulares que subyacen a la osteoporosis y la variabilidad entre pacientes a nivel molecular y celular[15].

Por otro lado, mediante el uso de técnicas multi-ómicas, se han identificado varias proteínas asociadas a la densidad mineral ósea y fractura durante el perfilado de proteomas humanos en diferentes poblaciones. En el estudio "Fracturas Osteoporóticas en Hombres", Nielson CM et al.[16] se encontró una asociación entre cinco proteínas y la aparición de fractura de cadera. También un reciente estudio de Al-Ansari et al[17] demostró una serie de biomarcadores diferenciados entre control, osteopenia y osteoporosis. Actualmente nuestro grupo ha obtenido resultado preliminares relacionados a una serie de biomarcadores diferenciados entre grupo de pacientes fracturados y no fracturados que se han relacionado con el riesgo de fractura según la escala FRAX[18], yendo pues un paso más allá al no solo relacionar biomarcadores con osteoporosis sino con el riesgo de fractura. Sin embargo, la mayoría de estos hallazgos son en un grupo pequeño, estudios transversales o solo en hombres, requiriendo su validación en estudios longitudinales con mayor presencia de mujeres al ser el grupo más afectado. Así mismo, aunque la identificación de las proteínas y las vías metabólicas involucradas en la regulación del metabolismo óseo en diferentes poblaciones ha aumentado, el conocimiento preciso de los mecanismos biológicos subyacentes a la baja densidad mineral ósea es incompleto.

Otras áreas de estudio dentro de la gerociencia de la osteoporosis serían senescencia celular, un estado de aumento de las células senescentes en el microentorno óseo característico del envejecimiento. Se ha demostrado que este acumulo contribuye a la patogénesis de la osteoporosis y por lo tanto, los biomarcadores de senescencia como la beta-galactosidasa ácida senescente y p16INK4a, tendrían el potencial de identificar pacientes con un alto grado de senescencia ósea y, por consiguiente, un mayor riesgo de osteoporosis. Así mismo se ha demostrado que enfoques que eliminan las células senescentes o afectan la producción del secretoma proinflamatorio previenen la pérdida ósea relacionada con la edad en ratones[19].

Los avances recientes en la ciencia del microbioma también han proporcionado nuevas perspectivas sobre la osteoporosis. Existe una correlación entre la composición del microbioma intestinal y la salud ósea, lo que sugiere que los biomarcadores basados en el microbioma podrían ser de utilidad futura para el diagnóstico y tratamiento de la osteoporosis[20]. Además, la investigación genómica, como la secuenciación de nueva generación (NGS), también está permitiendo el descubrimiento de biomarcadores genéticos. Por ejemplo, polimorfismos en genes que codifican proteínas implicadas en el metabolismo óseo, como RANK, RANKL, y OPG, podrían ser predictivos de la osteoporosis. Además, la metilación del ADN y la expresión de microARNs también están siendo investigados como posibles biomarcadores[21].

Al incrementar nuestra comprensión de la biología del envejecimiento, estamos en una posición cada vez más fortalecida para desarrollar e implementar terapias dirigidas para

la osteoporosis. La medicina de precisión[15], que busca personalizar los tratamientos basándose en el perfil biológico único de cada paciente, tiene un potencial inmenso en este dominio. La identificación y desarrollo de nuevos biomarcadores para la osteoporosis, particularmente aquellos que reflejan los mecanismos de envejecimiento subyacentes, pueden revolucionar nuestro abordaje terapéutico de esta enfermedad

En resumen, la gerociencia se encuentra en una etapa excitante de avance, y los biomarcadores de la osteoporosis continuarán siendo herramientas fundamentales para la medicina geriátrica. Estos indicadores no solo facilitarán el diagnóstico y monitoreo de la enfermedad, sino que también proporcionarán una ventana hacia los procesos biológicos subyacentes que conducen a la pérdida ósea. En esta era de medicina de precisión, la utilización estratégica de los biomarcadores será clave para la prevención y tratamiento óptimo de la osteoporosis, mejorando así la salud y la calidad de vida del adulto mayor

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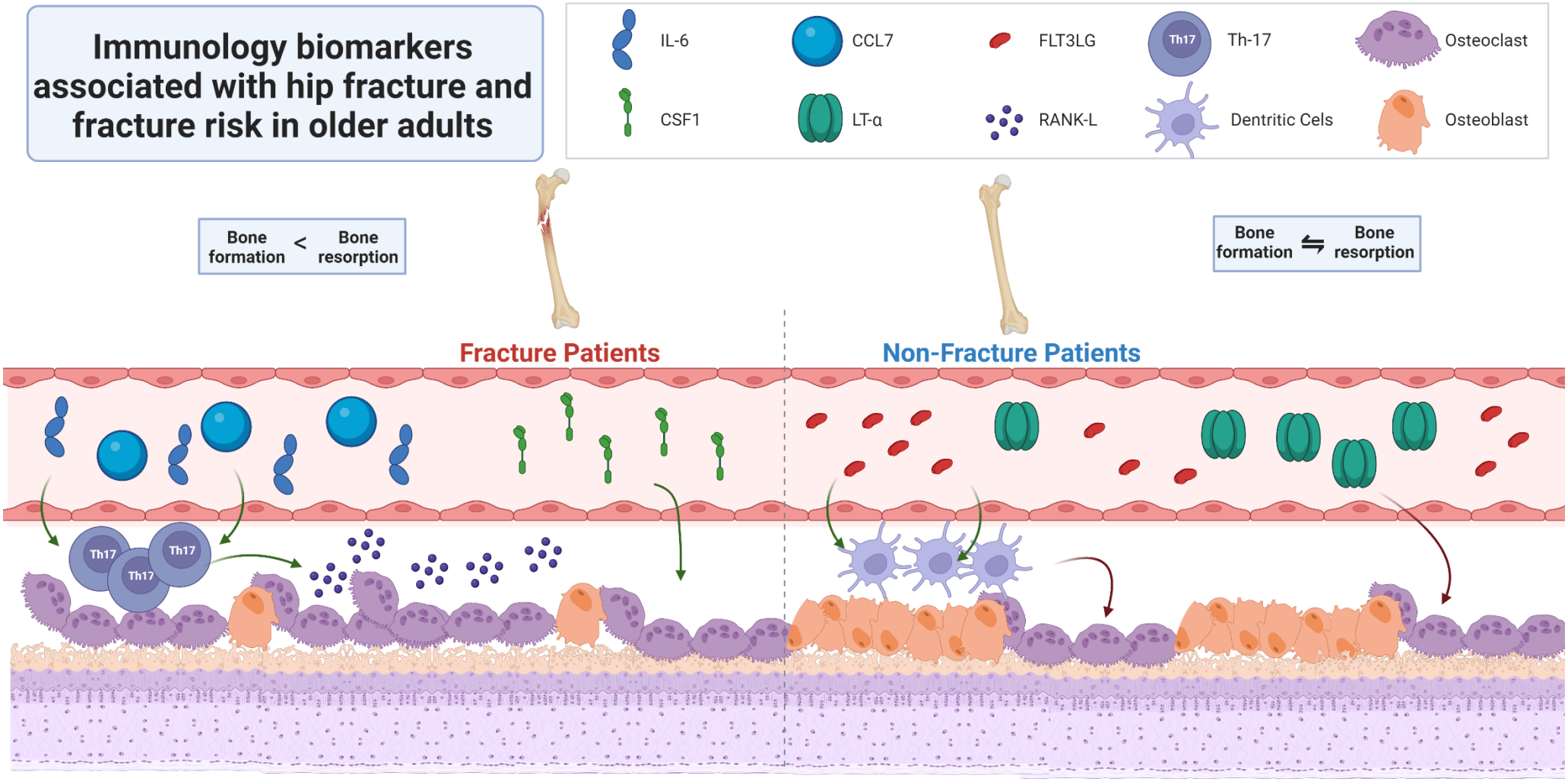
Immunology biomarkers associated with hip fracture and fracture risk in older adults



Chapter 3

Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults.

1. INTRODUCTION

Osteoporosis (OP) is a delineated systemic skeletal disorder associated with a reduced quantity of bone mineral mass and the microarchitectural degradation of the bone's tissue structure, which increases the risk of fragility fracture[1]. Due to its chronic nature and prevalence in an ageing population, OP has significant human and socioeconomic consequences, including morbi-mortality and disability[2]. Therefore, identifying high-risk populations and exploring potential biomarkers associated related to bone changes is crucial for effective health promotion [3].

Clinical guidelines serve as a foundation for assessing fracture risk[1] and promoting early interventions. Nonetheless, the most frequently examined parameters, such as bone mineral density (BMD), bone turnover markers (BMT) and FRAX®[4], exhibit limited efficacy, particularly in older population. BMD has been extensively researched and is recognized as a conventional risk determinant for fractures, but its low sensitivity is one of the reasons why population-based screening for BMD is not recommended for risk fracture assessment[1]. Another contributing factor is the relatively weak correlation between the loss of BMD and the capability to accurately forecast the risk of fractures[5]. BTM does not enhance fracture risk or bone loss prediction within an individual and is primarily useful in monitoring oral bisphosphonate therapy[6] or other osteoporosis treatments. FRAX, despite its widespread usage as a simple and primary care-applicable tool for estimating fracture risk and first-choice tool in most clinical guidelines[1], possesses a limitation in that it does not accommodate dose-response considerations for diverse risk factors[7, 8], potentially underestimating fracture risk[9], and is unsuitable for adults aged over 90[4]. While FRAX advances fracture prognostication beyond the capabilities of Bone Mineral Density (BMD) measurements alone, the accuracy of its fracture risk prediction displays variation across distinct study populations[10]. Consequently, there is a compelling need to investigate innovative approaches for estimating fracture risk. Presently, a revised version of FRAX is under development, with the intention of addressing the aforementioned limitations[11].

Bone loss in the ageing population is commonly attributed to its endocrine origin. However, comorbidities, genetics, and the immune system of the patient can also contribute to bone loss. A conventional approach to treatment is insufficient to address the systemic impairment in bone microstructure, making it crucial to develop a new strategy for understanding osteoporosis[12]. Analysing proteomes can provide insight into patients' pathophysiological status[13], which is particularly relevant given the observed link between pro-inflammatory states and fractures that are associated with an accelerated decrease in bone mineral density BMD[14, 15].

Chaput et al. [16] found three significant differences between osteoporosis and osteoarthritis (OA) in middle-aged women. In The Osteoporotic Fractures in Men Study, Nielson CM et al.[15] found an association between five proteins and incident hip fracture. When performing proteomic analyses on the osteoporotic population, the comparison population is usually patients with OA[17] due to the ease of obtaining bone tissue. Additionally, there are similarities and even overlaps between risk factors[18, 19] and an inverse relationship between hip fractures and hip OA[20]. In this overlap context, immunology biomarkers that enable differentiation between inflammation in bone (OP) and joint (OA) represent an encouraging possibility for the diagnosis and prognosis of osteoarticular diseases[21]. Even more, the role of immune system in the pathophysiology of osteoporosis[22] suggest that immune dysregulation can trigger

inflammatory conditions that negatively affect bone integrity[23]. Even in the acute phase, both hip fracture and hip replacement show a similar elevation of acute phase factors[24, 25]. Therefore, proteomic analyses can aid in understanding the pathophysiology of osteoporosis, the different with other chronic autoimmune rheumatic diseases and lead to the development of more effective treatment strategies.

Insufficient understanding of the pathophysiological and molecular mechanisms of OP and other chronic bone conditions has led to the lack of mechanism-based diagnoses [13]. However, proteomic approaches that examine changes in biomarkers show promise in developing **minimally invasive diagnostic biomarkers** for OP. Unfortunately, data from older adults are scarce, emphasizing the need to identify valid biomarkers for both diagnosing and evaluating treatments and interventions.

More studies are required to address the **knowledge gap** concerning the activated molecular mechanisms in OP and to identify potential biomarkers, including aspects of the clinical presentation. In this cross-sectional study, we used a targeted proteomic approach to examine the relationship between immunology biomarker profiles, fracture status, and fracture risk. Our primary aim was to compare immunology biomarker profiles between two patient groups: those with hip OA who were candidates for hip arthroplasty and those with hip fracture who were also candidates for hip arthroplasty. Subsequently, we investigated the association between these profiles and fracture risk, as determined using the FRAX reference tool (as the most extensively risk assessment tool).

2. MATERIAL AND METHODS

2.1 Patients and study design

This observational, cross-sectional study scrutinized patients who were referred to the Orthopedic Clinics and Traumatology Services at the University Hospital of Navarre (Pamplona, Spain) between March and October 2021. The criteria for participant inclusion were age ≥70 years, a diagnosis of osteoarthritis of the hip being a candidate for hip arthroplasty, a diagnosis of subcapital hip fracture being a candidate for hip arthroplasty, and spinal anaesthesia as the elective technique. The diagnosis of hip OA was based on the criteria of the American College of Rheumatology[26]. Exclusion criteria were diseases that cause secondary OP (e.g., glucocorticoid-induced osteoporosis, rheumatoid arthritis, and autoimmune diseases), terminal illness (advance stages pathologies and cancer) or refusal to participle in the study. We screened 256 older adults, with 83 meeting the inclusion criteria. In our selection process, 112 individuals were excluded due to secondary osteoporosis, 48 due to terminal illnesses, and 13 owing to their refusal to provide informed consent. Consequently, a final cohort of 40 participants was selected for the study, while an additional 43 were excluded. The main reason for exclusion at this point was the change of the day of surgery, which did not allow for the collection and processing of samples. The study flowchart is shown in **Appendix A.3**. The participants were classified into two groups: hip OA candidates for hip arthroplasty (n = 20) and hip fracture candidates for hip arthroplasty (n = 20). The study received approval from the Institutional Review Board of the University Hospital of Navarre (Pamplona, Spain), under the approval reference PI_2020/125. Every participant involved in the study furnished written informed consent prior to their inclusion in the research.

2.2 Clinical and functional parameters

A comprehensive medical assessment was performed including comorbidities (Cumulative Illness Rating Scale for Geriatrics, CIRS-G)[27], osteoporotic treatments and polypharmacy (defined as regular use of at least five medications). Functional status was assessed by the Barthel index[28], pre-intervention mobility by the FAC (Functional Ambulation Classification)[29] scale, and frailty status by the FRAIL scale[30]. We used pre-fracture values as baseline points. Handgrip strength was measured as part of the Groningen Fitness Test for the Elderly[31] using a Jamar Hydraulic Hand Dynamometer on the day of the surgery. The best of three attempts (with 30 seconds rest between each attempt) was recorded[32]. Nutritional assessment was performed by body mass index (BMI) calculation (weight/height^2), and by completing the Mini-nutritional Assessment (MNA) tool[33]. Cognitive status was assessed by Pfeiffer's Short Portable Mental State Questionnaire (SPMSQ)[34] and depression symptoms were assessed using the Geriatric Depression Scale (GDS-15)[35].

FRAX was determined by factors such as age, BMI, and a set of binary risk elements. These elements included prior fragility fracture, whether a parent has had a hip fracture, current smoking habits, long-term oral glucocorticoid usage, presence of rheumatoid arthritis, other underlying conditions leading to osteoporosis, and alcohol intake. Femoral neck BMD was inputted when it was possible[4].

2.3 Bone Mineral Density and Body Composition by Dual-Energy X-ray Absorptiometry (DXA)

BMD and body composition were assessed using dual X-ray absorptiometry (Lunar iDXA, GE Healthcare) one month after surgery. BMD was measured in the total hip, femur neck, posterior-anterior spine, and forearm[36]. Lean mass was measured as Appendicular Skeletal Muscle Mass (ASM) adjusted for height squared (Appendicular Skeletal Muscle Mass Index or ASMI), or body mass index (ASM/BMI)[37].

2.4 Blood Extraction and Analysis

On the morning of the intervention, fasting peripheral venous blood (PVB) samples were procured from the antecubital vein of the participants. Blood was inverted five times and allowed to sit for 30 min for clotting. Samples were then centrifuged at 2,000 × g for 10 min at 4 °C to obtain plasma and acellular supernatant. Serum aliquots were stored at − 80 °C until use. In order to investigate the viability of utilizing this technology for biomarker analysis, we conducted an assessment of the technical performance of Olink Proteomics' high-throughput, multiplex proximity extension assays (PEA), specifically the Target 48 Cytokine Panel, for protein screening purposes[38]. The panels had a positive correlation with other established technologies[39]. This emerging technology, developed by Olink Proteomics (Uppsala, Sweden), integrates quantitative real-time Polymerase Chain Reaction (qPCR) with multiplex immunoassays. Essentially, PEA is predicated on dual recognition of a targeted biomarker via a pair of antibodies, each labelled with unique DNA oligonucleotides. These biomarker-specific DNA 'barcodes' are quantified using microfluidic qPCR, which allows for high-throughput relative quantification of as many as 1161 human plasma proteins with a minimal volume of biofluids (1 µL suffices for the quantification of 92 biomarkers). The requirement for highly specific antibodies and the employment of target-designed primers augment the specificity and sensitivity of the assays in biological samples. These characteristics, coupled with the utilization of multiple internal controls that monitor each step of the reactions, help to avert unspecific events and minimize background noise[38].

Comprehensive details about PEA technology, its performance, and validation data can be obtained from the manufacturer's website (www.olink.com) and the biomarkers are listed in **Appendices A.1 and B**

The collected data were presented in standard units (pg/mL). For quality, a four-parameter logistic (4PL) curve was generated for the standard curve during product development. Within the limits of quantification (LOQ), the 4PL fitting described the standard curve well with high precision and accuracy, and the concentration could be correctly estimated. Beyond LOQ, the precision and accuracy of the 4PL fitting exhibited a decrease. Cytokine values that fell within the lower and upper limits of quantification (LLOQ and ULOQ, respectively) for each assay – parameters defined during the panel's development – were not incorporated into the analysis. In total, seven cytokines for which more than 35% of the values were below the limits of detection (LOD) were excluded from all analyses (grey-shaded biomarkers in **Appendix A.1**).

2.5 Statistical analysis

Background data were tested for normality using the Shapiro-Wilk method. Consequently, the non-parametric (Mann–Whitney U) or parametric (independent t-test) test was used to compare between groups (hip fracture cases versus controls) regarding the baseline characteristics in continuous variables. For dichotomous or nominal variables, Fisher's exact or Pearson X^2 were used. Data are presented as mean and standard deviation (SD) if not stated otherwise. The statistical package used to calculate group differences was SPSS version 26 (International Business Machines Corporation [IBM], Armonk, New York, USA). A two-tailed P-value of <0.05 was considered significant.

We used Tukey's fences method to detect observations out of the normal range by using interquartile ranges[40], which are often used for detecting outliers in various fields[41]. 55 outliers were excluded from the analysis out of the 1800 values analyzed using the Olink platform. Before performing Tukey's fences, the normality of the data was checked before fitting the curve. Features with >70% missing values in the real samples or >10% outlier values in the serum samples were deleted first, and 36 biomarkers passed quality control (**Appendix B**). Serum biomarkers in pg/mL values were analyzed using two unpaired t-tests, Benjamini–Hochberg method for p-value correction with a 5% false discovery rate, and a distribution boxplot. P values <0.05 were considered statistically significant after correction with the Benjamini–Hochberg method. Principal component analysis and Volcano plot (**Figure 1**) assessed the distribution groups, using singular value decomposition with imputation (pre-normalized data, no transformation), and visualized using ClustVis[42]. R-squared and goodness-of-fit measure for linear regression models was calculated including the clinical variables and significant biomarkers related to fracture risk (FRAX hip and major fracture). After these analyses, a one-way analysis of covariance (ANCOVA) was performed adjusted for age, sex, body mass index, and FRAX (hip and major) score with effect size of fracture vs. non-fracture. These analyses were performed using GraphPad Prism 9 program for Windows. Protein–protein association network analysis was created using the online database tool STRING version 11[43]. Protein accession numbers (UniProt) from significant proteins were entered in the search engine (multiple proteins) with the following parameters: Organism Homo sapiens, the maximum number of interactions was query proteins only, interaction score was set to medium confidence (0.400), and an FDR of ≤ 0.01 was used when classifying the Biological Process (GO) of each protein.

3. RESULTS

3.1. Baseline characteristics

We provided an overview of the demographic, clinical, and functional features of the patients included in the analysis (**Table 1**). The study included 40 older adults (72.5% female) with a mean age (SD) of 81.23 (8.23) years. As clinically expected, the scores for BMI, functional status, FRAX scores, bone mineral density and body composition parameters were all significantly lower in the fracture group than in the non-fracture group ($p<0.05$).

Table 1. Demographic, clinical, and functional characteristics of the patients included for analysis (values expressed as mean and standard deviation unless otherwise specified).

	Full sample (n=40)	**Fracture group (n=20)**	**Non-fracture group (n=20)**	**P value***
Demographic				
Age, years	81.23 (8.23)	87.25 (6.73)	75.20 (4.15)	**0.026**
Sex (men/female), n (%)	11 (27.5)/29 (72.5)	4 (20)/16 (80)	7 (35)/13 (65)	0.480
BMI (kg/m^2)[a]	27.39 (4.72)	24.91 (2.74)	29.87 (5.02)	**0.003**
Clinical status				
CIRS-G score	11.45 (4.21)	12.7 (4.81)	10.2 (3.17)	0.060
Polypharmacy score	6.28 (3.16)	7.25 (3.09)	5.3 (3)	0.534
Osteoporosis (n, %)	10 (25%)	4 (20%)	6 (30%)	0.716
Functional status				
Barthel Index (ADL), score[c]	81.63 (26.13)	67.5 (30.41)	95.75 (7.48)	**<0.001**
FAC (n, %)				
FAC 0 to 1	3 (7.5%)	3 (15%)	0 (0)	**0.032**
FAC 4 to 5	36 (92.5%)	17 (85%)	20 (100%)	
Frailty score[d]	2.18 (1.69)	3.05 (1.47)	1.3 (1.42)	**<0.001**
Hand grip strength (Kg)	17.63 (9.8)	11.3 (6.24)	23.95 (8.6)	**<0.001**
MNA score[e]	23.43 (6.51)	18.83 (6.08)	28.03 (2.33)	**<0.001**
Pfeiffer's SPMSQ[f]	2.55 (3.80)	5.05 (4.05)	0.5 (0.224)	**<0.001**
Depression score (n, %)[g]	8 (20%)	6 (42.9%)	2 (10%)	**0.026**
FRAX mayor score[h]	9.76 (7,15)	13.4 (6.99)	6.12 (5.29)	**<0.001**
FRAX hip score[i]	4.43 (3.85)	6.29 (3.79)	2.58 (2.94)	**<0.001**
Bone mineral density and body composition				
BMD[j] - total hip	0.873 (0.186)	0.735 (0.079)	0.976 (0.177)	**0.001**
BMD – femoral neck	0.869 (0.211)	0.739 (0.119)	0.966 (0.217)	**0.011**
BMD – lumbar spine	1.153 (0.256)	0.981 (0.18)	1.239 (0.247)	**0.007**
BMD – foreman	0.768 (0.314)	0.679 (0.127)	0.812 (0.37)	0.281
ASMI[k]	6.24 (1.63)	5.06 (1.27)	7.43 (0.95)	**<0.001**
ASM/BMI[l]	0.607 (0.188)	0.526 (0.155)	0.687 (0.187)	**0.005**

[a]BMI (body mass index).
[b]The Cumulative Illness Rating Scale for Geriatrics (CIRS-G) scale evaluates individual body systems, ranging from 0 (best) to 56 (worst).
[c]The Barthel Index ranges from 0 (severe functional dependence) to 100 (functional independence)
[d]Frail Scale ranges from 0 to 5 and indicates frailty with ≥ 3.
[e]Mini-Nutritional Assessment (MNA).

[f]Pfeiffer's Short Portable Mental State Questionnaire (SPMSQ) ranges errors from 0 (best) to 10 (worst).
[g]The Geriatric Depression Scale (GDS-15) ranges from 0 to 15 and indicates symptomatic depression with ≥ 5.
[h]FRAX 10-year fracture probability of mayor osteoporotic fracture (%). Mean and SD
[i]FRAX 10-year fracture probability of hip fracture (%).
[j]BMD (bone mineral density, g/cm^2).
[k]ASMI (Appendicular Skeletal Muscle Index, kg).
[l]ASM/BMI (Appendicular lean mass adjusted for BMI).
* p-value for different groups in percentage (Pearson X^2, expect no normal distribution; Fisher's exact test) or means (t-student, expect no normal distribution; U de Mann-Whitney). The bold values are statistically significant.

3.2. Principal component analysis, Volcano Plot and Protein association network analysis

A score plot was generated to show the separation between the fracture and non-fracture groups. The principal component analysis did not reveal any abnormal deviations between the two groups (**Figure 1, Panel A**) with a very similar pattern within the same group and differences between them. The outcome obtained using this selection criterion is presented in the volcano plot displayed in **Figure 1, Panel B**. It was possible to isolate five biomarkers that showed high differentiation between the study groups.

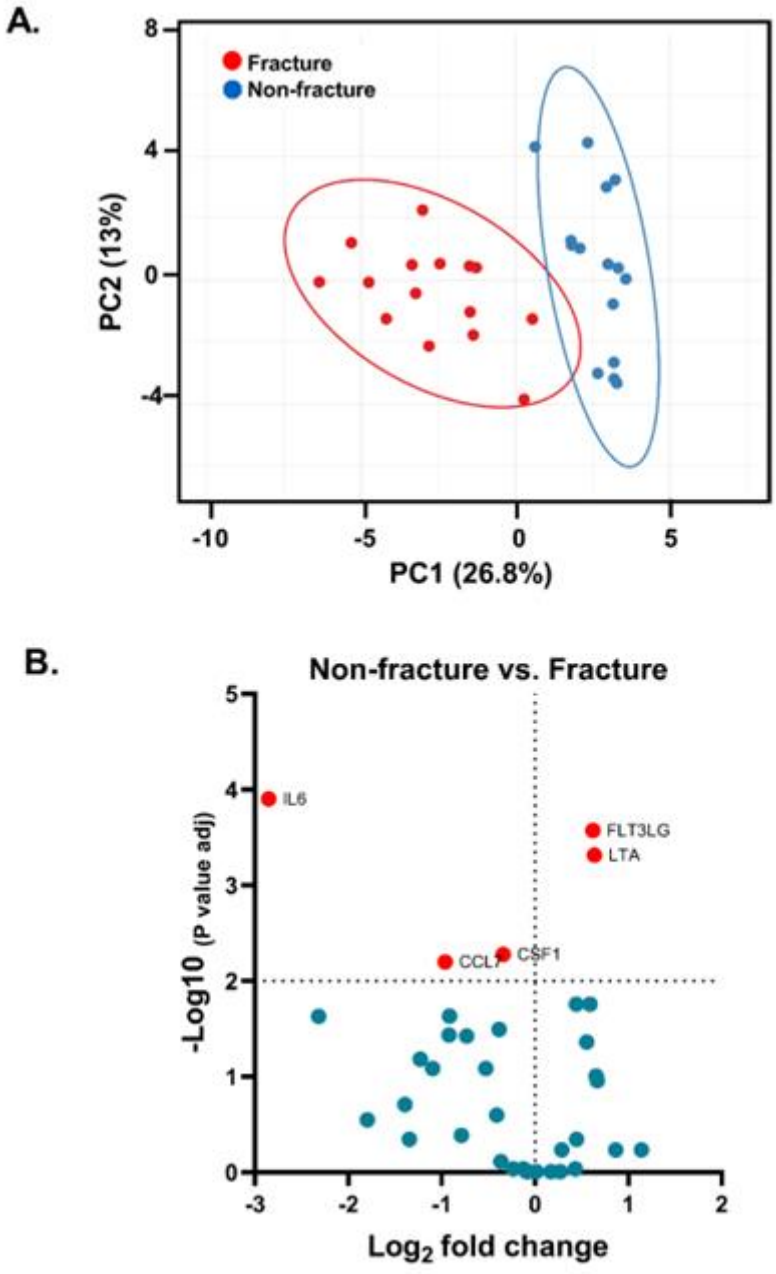


Figure 1. Principal component (PCA) and volcano plot analysis. Panel A, Principal component analysis (PCA) between the study groups. The ellipses show a probability of 95% that a new data point from the same group is located inside the ellipse. The red points correspond to fracture subjects, and the blue points correspond to non-fracture subjects. Panel B, Volcano plot of the paired t-test between non-fracture vs. fracture. Statistically significant differences in protein expression levels were found after correction with Benjamini–Hochberg, which is represented by all the proteins being presented as red dots, that is, the corrected p-values did reach <0.05. The dotted line represents the corrected significance threshold of 0.05. On the y-axis are $\log_{10}$ of p-values and on the x-axis is the $\log_2$ fold change between the two groups where a positive fold change indicates a lower protein level in the non-fracture than in the fracture.

Changes were observed in the five proteins included: Interleukin 6 (IL-6), Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7). Enrichment analysis with multiple testing corrections was used to assign related gene categories to their associated pathways using gene ontology (summarized in **Figure 2)**.

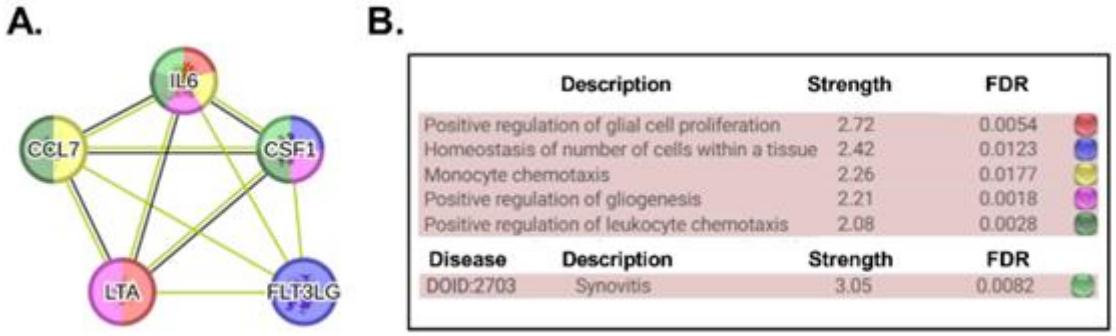


Figure 2. Pathway analysis of immunology proteins associated with the metabolic process in bone. Functional protein network analysis of significant proteins associated with metabolic process. The STRING version 11 was used to create the network analysis (https://string-db.org/). In the network, each protein is represented by a coloured node, and protein–protein interaction and association are represented by an edge visualized as a coloured lined (type of interaction). Known interactions used were from curated databases (turquoise) and experimentally determined (pink). Predicted interactions were gene neighbourhood (green), gene fusion (red) and gene-co-occurrence (dark blue), and other interactions were text mining (yellow), coexpression (black), and protein homology (purple). Interleukin 6 (IL-6), Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7).

3.3. Biomarkers difference and correlation with fracture risk

After conducting two unpaired t-tests with the Benjamini-Hochberg method for p-value correction, it was found that these five cytokines were significantly different between fracture and non-fracture patients ($p<0.05$). The mean plots in **Figure 3** (Panel A, D, G, J, and M) display the levels of these five proteins. LT-α and FLT3LG were found to be higher in non-fracture patients, whereas IL-6, CSF1, and CCL7 were found to be higher in fracture patients. **(Appendix A.2)** shows the immunology biomarkers that were not found to be significantly associated with fracture status.

Furthermore, linear regression models showed moderate ($R^2=0.409$) but significant ($p=0.001$) positive correlations between IL-6 levels and the risk of major fracture, as shown in **Figure 3**, Panel I. The levels of CSF1 ($R^2=0.267$; $p=0.005$) and CCL7 ($R^2=0.301$; $p=0.002$) had a weak correlation with the risk of fracture. On the other hand, LTA ($R^2=-0.157$; $p<0.001$) and FLT3LG ($R^2=-0.139$; $p<0.001$) exhibited a negative relation with the risk of fracture.

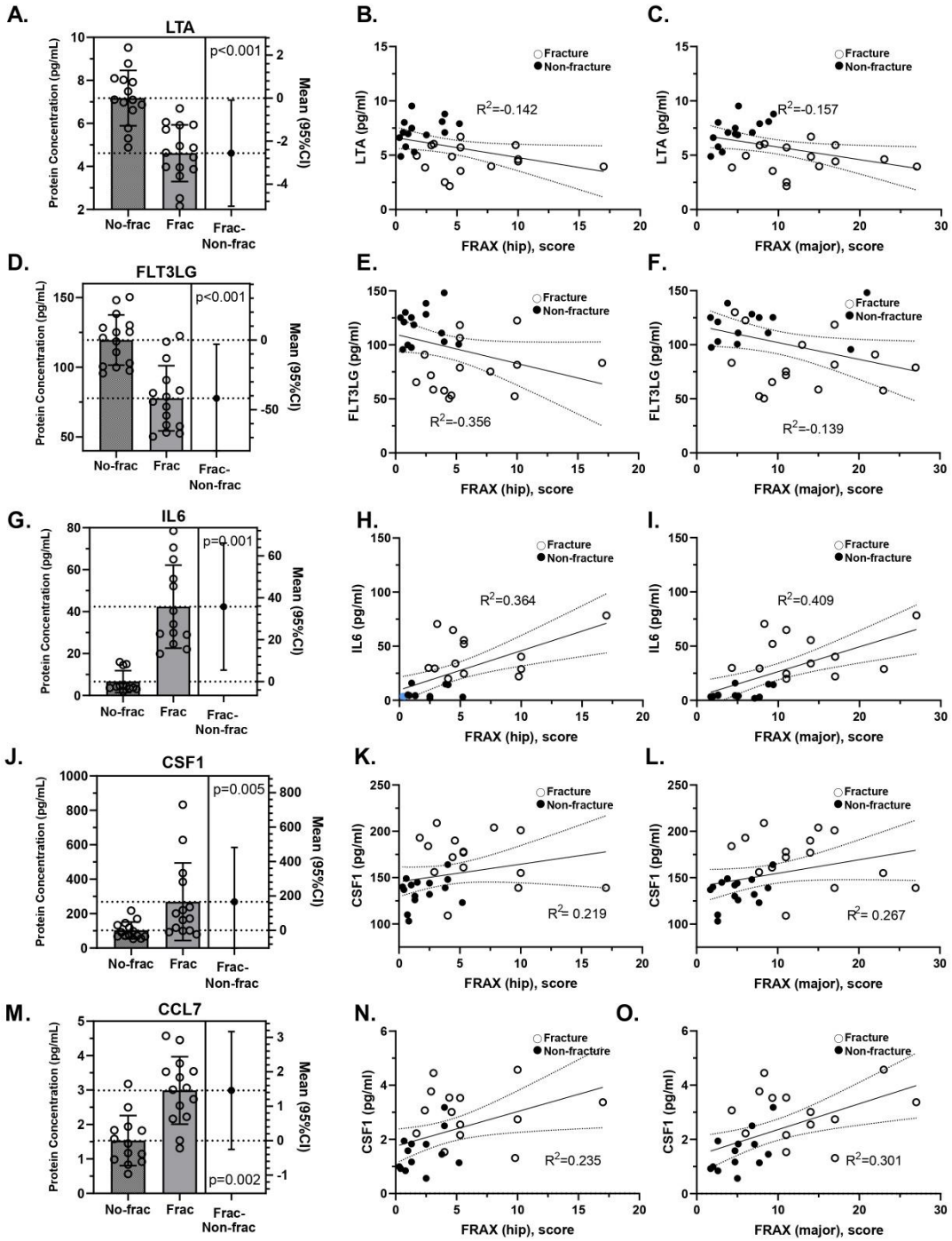


Figure 3. Group difference (fracture vs. non-fracture) and their association with FRAX (hip and major) score with significant plasma biomarkers. Panel A, D, G, J and M show mean plots of the five proteins with the most significant changes in protein expression levels following t-tests between fracture vs. non-fracture groups. Panel B, C, E, F, H, I, K, L, N, and O figures, show the lineal regression between fracture vs. non-fracture groups with FRAX (hip and major) scores with significant plasma biomarkers. Solid lines: estimation; dashed curved lines: 95% confidence interval limits. Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Interleukin 6 (IL-6), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7).

After the ANCOVA was performed adjusted for age, sex, body mass index, and FRAX (hip and major) score and with effect size of fracture vs. non-fracture, all immunology biomarkers maintained significant ($p<0.05$) expect for CSF1 (**Appendix A.4)**

4. DISCUSSION

This cross-sectional study utilized a targeted proteomic approach to identify potential biomarkers of hip fracture in older adults. The study identified five potential biomarkers, namely serum IL-6, CSF1, LT-α, FLT3LG, and CCL7, which may have significant implications for fracture risk. Out of these biomarkers, three (IL-6, CSF1, and CCL7) exhibited a positive relationship with fracture risk based on the FRAX reference tool, while two (LT-α and FLT3LG) had a negative relationship with fracture risk. While previous evidence has suggested an association between biomarkers and osteoporosis [23, 44], this study is the **first to examine the relationship between FRAX and serum cytokines**. These findings have the potential to pave the way for developing effective biomarker-based diagnostic tools and interventions for osteoporosis, which could significantly improve clinical outcomes for older adults at risk of hip fracture.

In this study, we utilized PEA to characterize serum cytokines related to signaling and inflammatory processes in older adults with hip fractures compared to other adults undergoing elective orthopedic surgery. Given the multitude of immunology biomarkers that are altered in rheumatic diseases[45], the choice of OA as the control group in this study allows us to confirm the association of these five biomarkers with OP[21], ruling out their association with OA as other most prevalent rheumatic disease in the older population. There are some similarities between osteoporosis (OP) and osteoarthritis (OA)[18–21], the characteristics of these groups are quite different due to factors such as age[46] and the presence of risk factors. As observed in our study and supported by existing literature, patients with OP and hip fractures are notably older[25, 46, 47] and often in a poorer nutritional state[48]. This age and nutritional disparity can inherently influence the outcomes of studies involving these populations. For instance, underweight is a risk factor for OP[49, 50] and while obesity stimulates the development of OA[19, 50] and maybe acts as OP protector factor[51]. Additionally, functional capacity is an independent factor for hip fracture[52], whereas hip arthroplasty is a common treatment for OA patients[53].

In this exploratory study, these clinical differences may have contributed to differences in cytokine profiles, which highlights the need for closer case-control clinical matching in further studies. Our interpretation of the functional mechanisms of the five identified proteins is that they are involved in **immune and inflammatory** processes. While these proteins have traditionally been associated with synovial membrane inflammation (synovitis), recent findings in **osteoimmunology** suggest that immune dysregulation can trigger inflammatory conditions that negatively affect bone integrity[23]. These findings may have important implications for understanding the complex interplay between inflammation and bone health in older adults.

Studying the molecules reported in this study is important because **low-grade inflammation** is a key factor in the pathogenesis of various widespread diseases, particularly osteoporosis[54]. Although it is not yet understood how circulating peptides reflect activity in musculoskeletal tissues, inflammatory mediators such as reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines directly or indirectly affect bone cells and contribute to the development of osteoporosis[15, 44]. Prior

endeavors have concentrated on the identification of prospective biomarkers capable of prognosticating the likelihood of osteoporosis, either as standalone predictors or in conjunction with clinical risk factors and BMD.

The biomarkers identified in this study have been previously investigated concerning osteoporosis. For example, increased levels of IL-6 induce osteoclastogenesis, the accumulation of T-cells (Th17), and the production of RANKL, which promotes bone resorption[23]. **IL-6** also upregulates bone destruction by releasing protease enzymes from inflammatory cells[44]. Even though the expression of RANKL in an array of cell types, including osteoblasts, research suggests that osteocytes predominantly contribute to the pool of RANKL essential for osteoclast genesis[55].

Despite the positive associations found between IL-6 and fracture risk (R^2 = 0.409 for major fracture risk, and R^2 = 0.364 for hip fracture risk), it is currently unclear whether blood IL-6 concentration can accurately predict fracture risk.

LT-α, also known as tumor necrosis factor-beta (TNF-β), is a cytokine belonging to the tumor necrosis factor superfamily that mediates a range of inflammatory, immunostimulatory, and antiviral responses[56]. Although involved in the genesis and treatment of osteoarthritis[57], it induces osteoclastogenesis alongside RANKL[58]. However, when TNF- α is present in abundance, studies suggest that its role is secondary to that of TNF- α [59]. The significant but weak (R^2 = −0.157 in the best case) correlation with the control group may be due to its relationship with both processes and its secondary role.

FLT3LG is a hematopoietic cytokine related to growth factors that increase the number of immune cells by activating hematopoietic progenitors. FLT3LG studies in the biomedical literature are more related to leukaemia than musculoskeletal diseases[60]. The role of this cytokine in bone joints is debated and has mainly been described in rheumatoid arthritis, where it is considered to be a negative regulator of osteoclastogenesis and a bone-protective factor[61]. This may explain the weak association with fracture risk seen in our study (R^2 = −0.356).

CSF1, also known as macrophage colony-stimulating factor (M-CSF), is a secreted cytokine that causes hematopoietic stem cells to differentiate into macrophages or other related cell types. CSF1 is involved in multiple functions throughout the body, including bone health. In bone, stromal cells secrete CSF1, which affects T-cell differentiation in osteoclastogenesis[23]. CSF1 is crucial for the proliferation, differentiation, and motility of osteoclasts[62], making it a key therapeutic target for osteoporosis[63]. In our study, we found that CSF1 levels were different between the fracture and control groups (p=0.005), but with a weak correlation to fracture risk. Despite its biological plausibility, CSF1 did not retain its significance after adjusting for multiple confounders, likely due to the sample size. While it was adequate for initial observations, it might not have been sufficiently large to detect subtle effects of CSF1 once other variables were taken into account.

CCL7 belongs to the CC chemokine family and its role in osteoporosis is currently under study[64]. RANKL induces the expression of many chemokines including CCL7, to enhance osteoclast formation. Currently, CCL7 is being studied as a potential target for postmenopausal osteoporosis[65]. Our findings support the relationship with OP (p=0.002), with a weak correlation with fracture risk.

Despite the importance of cytokines in bone regulation, other cytokines related to bone loss, such as IL-1B, IFNG, and TNF, did not show significance in our study[23, 44].

Considering the widely acknowledged limitations of utilizing BM in the evaluation of fracture risk within the bone health research community, there is an ongoing pursuit to discover and validate novel biomarkers for clinical application. This endeavor stems from the growing understanding of bone regulation, which contributes to an expanding pool of knowledge in the field. Our findings suggest that the weak association of IL-6, CSF1, and CCL7 with fracture risk may be related to the implications of these cytokines in inflammaging and other age-related diseases[66] in older adults with high comorbidity burden (especially OA[67]) and polypharmacy[68, 69]. The lack of differences in these cytokines may be due to similar inflammaging-related characteristics between the study groups. Hence, based on the current body of evidence, the utilization of these three prospective biomarkers as predictors of treatment responses to novel anti-osteoporotic medications is not supported[70].

The main strength of this exploratory analysis is its potential to provide a new tool for estimating an individual's risk of experiencing a hip fracture or a major osteoporotic fracture based on serum analysis, which could guide clinical decision-making and assist healthcare professionals in identifying individuals who may benefit from interventions to reduce their risk of fractures. The development of serum biomarkers for fracture risk in older adults is of interest in clinical practice due to the association of fractures with disability, premature mortality, and increased utilization of medical resources[3]. Moreover, Olink Proteomics' high-throughput allows for reliable analysis of these very low values of immunology biomarkers, such LTA and CCL7 (with levels <10pg/ml) but these results should be taken with caution.

However, it is essential to recognize and consider the limitations of our study. First, the analysis was cross-sectional, meaning causative relationships cannot be considered. Longitudinal studies will be necessary to determine the temporal relationship between changes in cytokine profiles and the development of a hip fracture. Second, the small study population comprised only Caucasians, so our findings cannot be generalized to other ethnic groups and limited the statistical strength (specially for CSF1). Additionally, although the cohort was extensively characterized, it was relatively small, and analyses involved a large set of variables. The two comparison groups were not closely matched in terms of demographic or clinical characteristics, which may have confounded our results, but after adjusted for age, sex, body mass index, and FRAX score; most of them were still significant different.

5. CONCLUSION

To summarize, our cross-sectional study identified five immunology biomarkers (IL-6, CSF1, LT-α, FLT3LG and CCL7) that were associated with hip fracture and have potential correlation with fracture risk. This study provides a potential contribution by highlighting immunology biomarkers that could be further studied to estimate fracture risk and potentially delay the onset of osteoporosis and fragility fractures in older adults. However, to increase the clinical relevance of these biomarkers and small sample, validation and replication in longitudinal cohorts with diverse populations are needed.

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SUPPLEMENTARY MATERIAL

Appendix A.1 List of biomarkers measured with Olink 48 Inflammatory Target

Biomarker name (Abbreviation)	Values < LOD
C-C motif chemokine 2 (CCL2)	0%
C-C motif chemokine 3 (CCL3)	3%
C-C motif chemokine 4 (CCL4)	3%
C-C motif chemokine 7 (CCL7)	0%
C-C motif chemokine 8 (CCL8)	0%
C-C motif chemokine 13 (CCL13)	0%
C-C motif chemokine 19 (CCL19)	2%
C-X-C motif chemokine 9 (CXCL9)	2%
C-X-C motif chemokine 10 (CXCL10)	0%
C-X-C motif chemokine 11 (CXCL11)	0%
Eotaxin (CCL11)	0%
Fms-related tyrosine kinase 3 ligand (FLT3LG)	0%
Granulocyte colony-stimulating factor (CSF3)	0%
Granulocyte-macrophage colony-stimulating factor (CSF2)	88%
Hepatocyte growth factor (HGF)	0%
Interferon gamma (IFNG)	0%
Interleukin-1 beta (IL-1β)	78%
Interleukin-2 (IL-2)	95%
Interleukin-4 (IL-4)	95%
Interleukin-6 (IL-6)	0%
Interleukin-7 (IL-7)	0%
Interleukin-8 (CXCL8)	3%
Interleukin-10 (IL-10)	0%
Interleukin-13 (IL-13)	90%
Interleukin-15 (IL-15)	0%
Interleukin-17A (IL-17α)	20%
Interleukin-17C (IL-17C)	0%
Interleukin-17F (IL-17F)	68%
Interleukin-18 (IL-18)	0%
Interleukin-27 (IL-27)	0%
Interleukin-33 (IL-33)	32%
Interstitial collagenase (MMP1)	18%
Lymphotoxin-alpha (LT-α)	0%
Macrophage colony-stimulating factor 1 (CSF1)	0%
Macrophage metalloelastase (MMP12)	0%
Oncostatin-M (OSM)	0%
Oxidized low-density lipoprotein receptor 1 (OLR1)	0%
Pro-epidermal growth factor (EGF)	2%
Protransforming growth factor alpha (TGF-α)	0%
Stromal cell-derived factor 1 (CXCL12)	0%
Tumor necrosis factor (TNF)	0%
Tumor necrosis factor ligand superfamily member 10 (TNFSF10)	0%

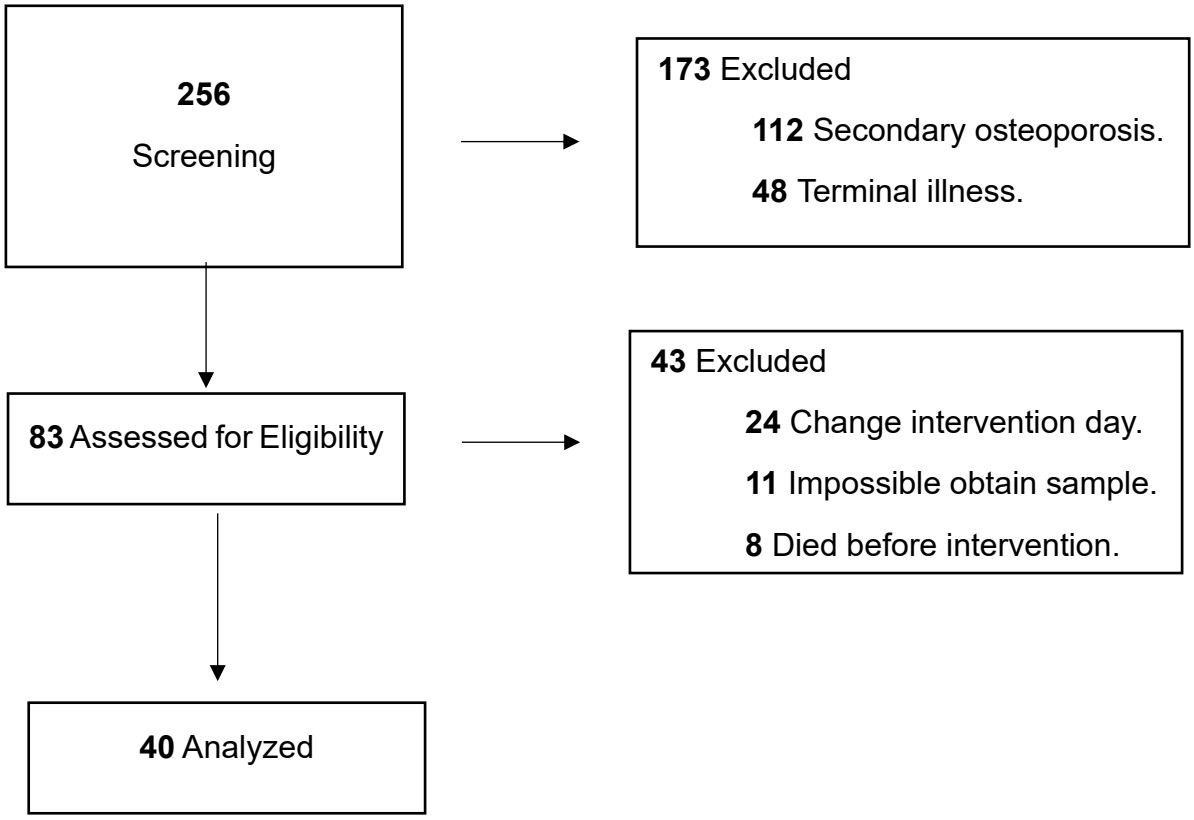
Thymic stromal lymphopoietin (TSLP)	97%
Tumor necrosis factor ligand superfamily member 12 (TNFSF12)	0%
Vascular endothelial growth factor A (VEGFA)	0%

Note: Grey shade indicates biomarkers with 35% or more of the values below the lower limit of detection (LOD).

Appendix A.2*:* Non-significantly associated Olink serum biomarker levels by groups.

Biomarker name (Abbreviation)	Non-fracture group (n=20)	Fracture group (n=20)	Difference	SE of difference	t-ratio	df	Adjusted-P value	Adjusted-P value (-log10)
CCL8	53.73	39.41	14.32	6.72	2.13	28	0.4510	0.3458
IL-33	0.51	0.38	0.13	0.12	1.09	26	0.9180	0.0372
CXCL12	185.50	202.00	-16.57	14.62	1.13	24	0.9180	0.0372
OLR1	404.80	400.70	4.17	83.60	0.05	28	0.9847	0.0067
IL-27	7.10	35.42	-28.32	7.55	3.75	27	0.0236	1.6280
CXCL9	98.40	170.00	-71.59	32.12	2.23	26	0.4111	0.3860
TGF-α	26.34	49.88	-23.54	6.66	3.53	28	0.0369	1.4330
TNFSF12	729.20	536.50	192.70	49.49	3.89	27	0.0175	1.7560
CCL11	180.40	119.80	60.64	15.61	3.89	28	0.0175	1.7560
IL-7	3.07	5.10	-2.03	0.58	3.51	28	0.0376	1.4240
IL-18	334.30	274.00	60.26	32.11	1.88	27	0.5819	0.2351
CCL13	238.90	150.30	88.65	29.90	2.97	28	0.1101	0.9581
TNFSF10	548.80	373.50	175.30	51.00	3.44	28	0.0435	1.3620
CXCL10	106.50	200.90	-94.44	24.94	3.79	26	0.0233	1.6320
IFNG	0.23	0.24	-0.01	0.05	0.22	25	0.9847	0.0067
IL-10	7.52	26.11	-18.60	7.56	2.46	27	0.2831	0.5480
CCL19	84.40	108.70	-24.26	16.35	1.48	24	0.7708	0.1131
TNF	83.09	45.60	37.49	19.88	1.89	27	0.5819	0.2351
IL-15	13.41	17.52	-4.11	1.14	3.60	28	0.0321	1.4940
CCL3	73.43	33.33	40.10	21.84	1.84	24	0.5819	0.2351
CXCL8	68.92	57.09	11.83	20.35	0.58	24	0.9847	0.0067
MMP12	363.50	523.80	-160.40	49.99	3.21	23	0.0825	1.0840
CSF3	102.50	269.10	-166.60	61.71	2.70	26	0.1959	0.7081
VEGFA	799.30	939.80	-140.40	129.20	1.09	28	0.9180	0.0372
IL-17C	28.05	65.78	-37.73	11.51	3.28	26	0.0660	1.1810
EGF	567.60	360.40	207.10	68.11	3.04	27	0.0988	1.0050
CCL2	592.10	630.30	-38.26	79.39	0.48	26	0.9847	0.0067
IL-17α	0.70	1.78	-1.08	0.51	2.13	27	0.4510	0.3458
OSM	10.03	21.43	-11.40	3.62	3.15	26	0.0825	1.0840
CCL4	350.70	311.30	39.36	94.14	0.42	25	0.9847	0.0067
CXCL11	52.76	70.11	-17.34	6.80	2.55	26	0.2525	0.5977

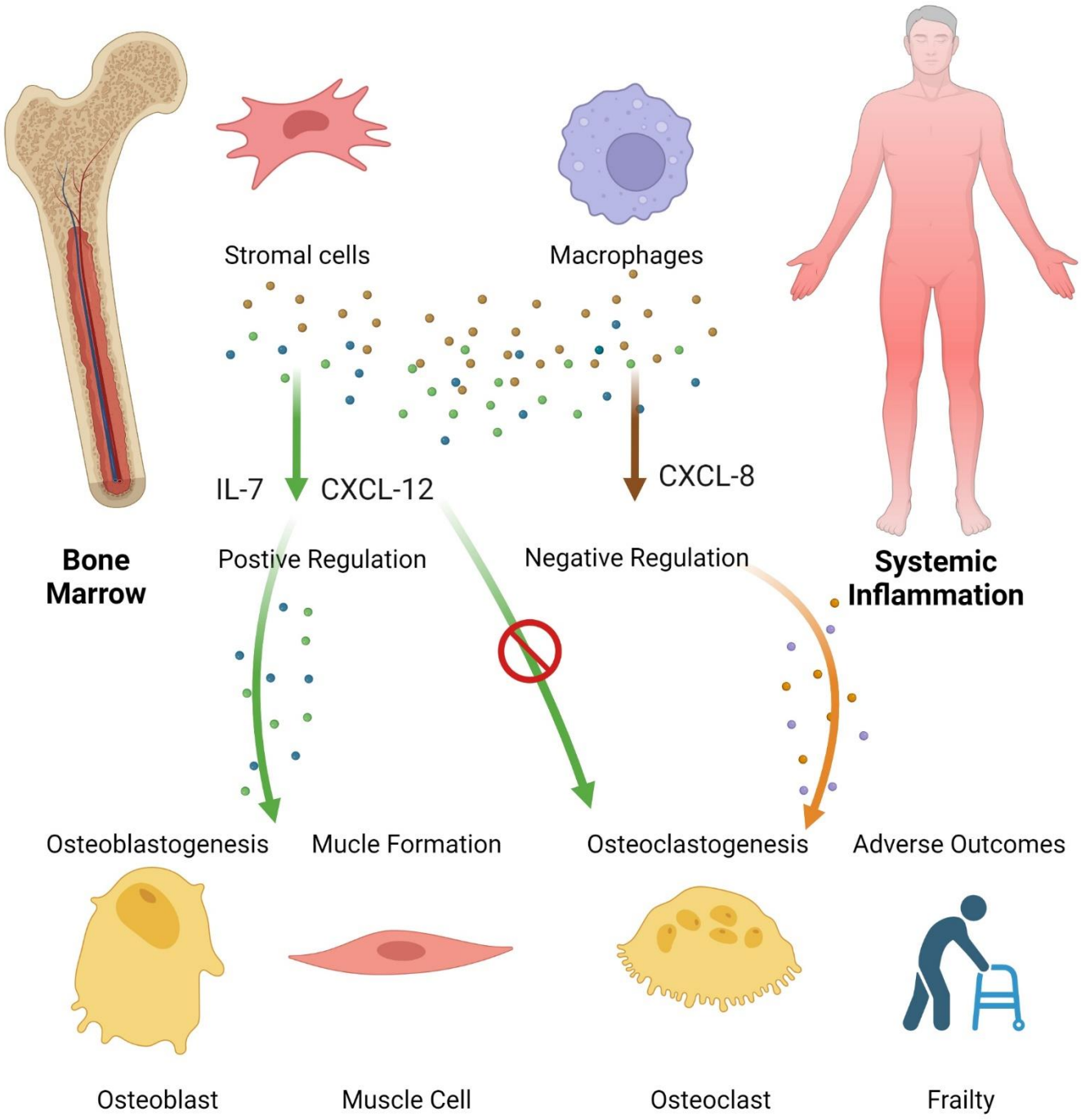
Appendix A.3*:* Flowchart of patients included in the study.



Appendix A.4*:* Group difference (fracture vs. non-fracture) with candidate metabolite markers

	Fracture Group (n=20)		**Non-fracture group (n=20)**		**Mean difference**		**η^2**	**P value***
	Mean	SE	Mean	SE	Mean	SE		
Lymphotoxin-alpha (LT-α)	1.68	0.31	2.85	0.29	1.16	0.52	0.191	**0.037**
Fms-related tyrosine kinase 3 ligand (FLT3LG)	121.01	7.34	76.30	7.34	44.70	12.89	0.334	**0.002**
Interleukin 6 (IL-6)	726.36	47.08	539.14	44.67	187.21	80.22	0.191	**0.029**
Colony stimulating factor 1 (CSF1)	336.15	82.22	319.67	97.43	16.47	156.81	0.001	0.917
Chemokine (C-C motif) ligand 7 (CCL7)	4879.64	562.72	2361.91	500.17	2517.72	929.60	0.268	**0.014**

* One-way analysis of covariance (ANCOVA) was performed to compare patients with fracture vs. non-fracture. All analysis were adjusted for age, sex, body mass index, and FRAX (hip and major) score. We calculated the partial eta squared (η^2) to estimate the effect size of fracture vs. non-fracture, considering the effect as small (0.0-0.13), substantial (0.13-0.26) and large (>0.26).



Chapter 4

Serum biomarkers related to frailty predicts negative outcomes in older adults with hip fracture.

1. INTRODUCTION

Hip fracture poses a rising public health concern, carrying substantial implications for older adults[1, 2], including elevated morbidity and mortality rates, along with significant social and economic burdens associated with hip fractures, make identifying populations at risk and developing predictive markers particularly important[3–6].

While traditional predictors of poor outcomes, such as age, co-morbidities and surgical factors [7, 8], have been identified, their performance as prognostic factors in older adults has proved to be limited. Frailty is recognized as a potential predictor of negative outcomes in patients with hip fractures[9]. Furthermore, delirium[10] and vitamin D[11] has also been highlighted as a significant predictor. The capacity of these newer predictors in forecasting outcomes is still under debate[12].

The complex pathophysiology of osteoporosis, frailty, and hip fractures hampers the identification of biomarkers, especially proinflammatory cytokines [13, 14], for predicting outcomes in frail older adults with hip fractures[15–17]. In this scenario, **proteomics** may serve as a powerful analytical approach for the definition of minimally invasive biomarkers for adverse outcomes in patients with hip fractures[18].

In this prospective cohort study, we aimed to explore the role of a targeted proteomic approach in better-characterizing frailty in older adults with hip fractures. The objective of this exploratory study is to use new analytical platforms, such as Olink, for the detection of **biomarkers associated with frailty and health outcomes after hip fracture**. Moreover, we sought to identify molecular features that could be useful in improving the prognosis of this group of patients. We hypothesized that levels of inflammatory biomarkers could be associated with frailty measures with the Clinical Frailty Scale (CFS) and health outcomes at one and three months after discharge independently of frailty status.

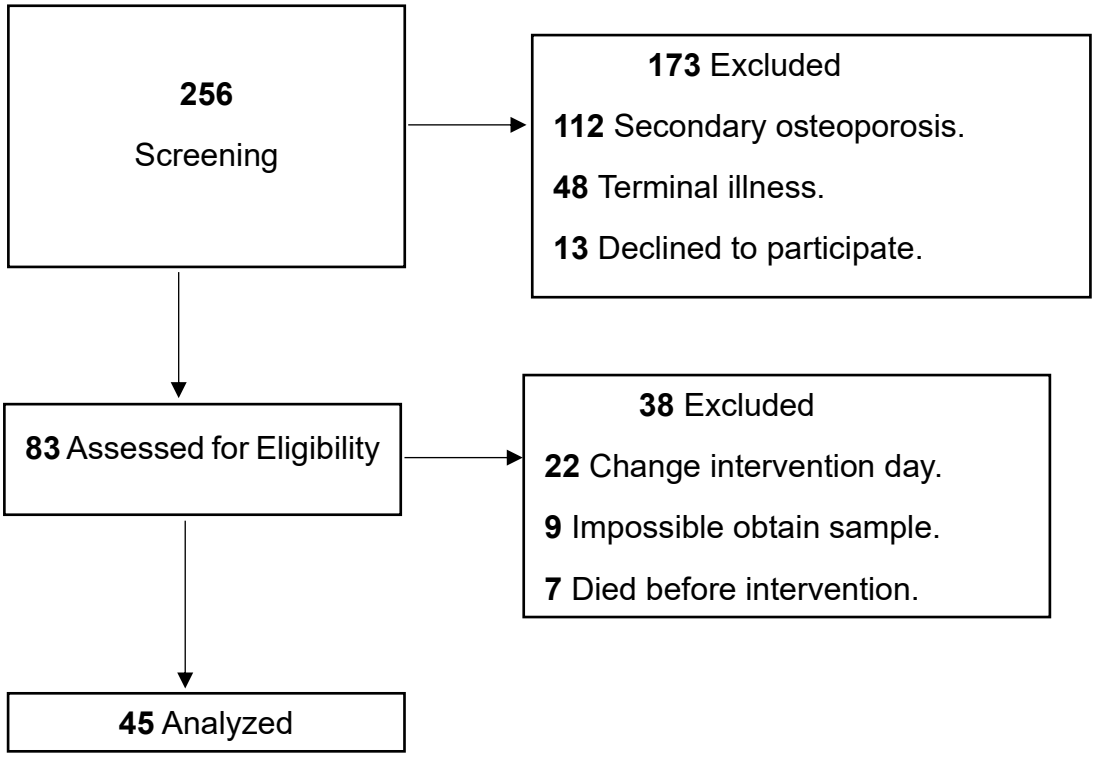
2. MATERIAL AND METHODS

2.1 Patients and study design

In this prospective cohort study, patients admitted to a tertiary hospital's Orthopedic ward were evaluated (Hospital Universitario de Navarra, Pamplona, Spain) between March and October 2021. Candidates for inclusion were patients aged ≥75 years undergoing surgery for hip fracture. The main exclusion criteria were the presence of diseases that cause secondary osteoporosis (glucocorticoid-induced osteoporosis, rheumatoid arthritis, etc.), terminal illness (defined as a progressive disease that is expected to result in death within six months wich included a CFS of 8 or 9) or unwillingness to provide informed consent. We screened 256 older adults, of whom 83 met the inclusion criteria. Exclusions at this point were 112 due to secondary osteoporosis, 48 due to terminal illness, and 13 due to unwillingness to provide informed consent. Finally, 45 participants were selected for the study with 38 excluded. The main reason for exclusion at this point was the change of the day of surgery, which did not allow for the collection and processing of samples. The study Flowchart is reported in Figure 1.

The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the local Research Ethics Committee (PI_2020/125). Before enrolling in the study, participants provided informed consent, which was approved by the ethics committee.

Figure 1: Flowchart of patients included in the study.



2.2 Clinical and functional parameters

A comprehensive medical assessment was performed during the hospital admission, which included the assessment of comorbidities (Charlson comorbidity index)[19], osteoporotic treatments, and polypharmacy (defined as regular use of at least five medications)[20].

Functional status was assessed by the Barthel[21] and Lawton index[22], and mobility using the FAC (Functional Ambulation Classification)[23] scale. We used the pre-fracture value as the baseline point.

The assessment of frailty status was conducted using the Clinical Frailty Scale (CFS)[24] by study investigators/geriatricians at hospital admission. Study participants were given a score from 1 (very fit) to 7 (living with severe frailty) based on clinical and functional information collected at hospital admission in the screening evaluation. Participants with a CFS above 5 were considered frail [25].

Handgrip strength was measured using a Jamar Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) following the Groningen Elderly Test protocol [26] on the day of the surgery, before the intervention. The best of three attempts (with 30 seconds rest between each attempt) was recorded[27].

Nutritional assessment was performed by body mass index (BMI) calculation (weight/height^2), and by completing the Mini-nutritional Assessment (MNA) tool[28] collected during medical assessment.

Cognitive status was assessed using the Global Deterioration Scale (GDS)[29], delirium by the Confusion Assessment Method (CAM)[30] and depression was assessed using the Geriatric Depression Scale (GDS-15)[31] collected during medical assessment.

The follow-up variables (mortality, hospital admission, Barthel and Lawton index, FAC and polypharmacy) were collected from a local database and by a phone call at one- and three months after discharge.

2.3 Blood Extraction and Analysis

Fasting peripheral venous blood (PVB) samples were collected on the morning of the intervention from the antecubital vein. Blood was inverted five times and left at room temperature for 30 min for clotting. Samples were then centrifuged at 2,000 × g for 10 min at 4 °C to obtain serum and acellular supernatant. Serum aliquots were stored at − 80 °C until use.

Cytokines analysis was performed using Olink® Target 48 Cytokine Panel. This analytical approach is based on the proximity extension assay (PEA) and showed high reproducibility and measurement correlation with other multimarker technologies, such as mass spectrometry[32]. The emerging PEA technology, developed by Olink Proteomics (Uppsala, Sweden), combines multiplex immunoassays with quantitative real-time polymerase chain reaction (PCR). In PEA, a targeted biomarker is recognized through a pair of antibodies labeled with distinct DNA oligonucleotides. These biomarker-specific DNA "barcodes" are then quantified using microfluidic qPCR, enabling high-throughput relative quantification of a wide range of human plasma proteins. The analysis requires only a few microliters of biofluids, with a minimal volume of 1 µL sufficient for quantifying 92 biomarkers. The use of highly specific antibodies and target-designed primers improves the specificity and sensitivity of the assays in biological samples. These features, along with the use of multiple internal controls that monitor each step of the reactions, circumvent unspecific events and reduce background noise[33]. Comprehensive details about PEA technology, its performance, and validation data can be obtained from the manufacturer's website (www.olink.com) and the biomarkers are listed in Table S1.

Data were reported in standard units (pg/mL). For quality, a four-parameter logistic (4PL) curve was generated for the standard curve during product development. Within the limits of quantification (LOQ), the 4PL fitting described the standard curve well with high precision and accuracy, and the concentration could be correctly estimated. Outside LOQ the precision and accuracy of the 4PL fitting decreased. When cytokines were within the lower and upper limits of quantification (LLOQ and ULOQ) for each assay (defined during the development of the panel), the values were not included in the analysis. In total, seven cytokines for which more than 35% of the values were below the limits of detection (LOD) were excluded from all analyses (grey-shaded biomarkers in Table S1).

2.4 Statistical Methods and outcome measure

Study participants were divided into 2 groups: frail and non-frail according to CFS (non-frail from 1 to 4; frail from 5 to 9). To characterize these groups, a descriptive analysis was performed for categorical variables using absolute and relative frequencies; and for quantitative variables using the mean and standard deviation or median and interquartile ranges, according to the normality of the data. Student's t-tests (for normally distributed data variables), Wilcoxon test (for non-normally distributed data variables) and chi-square tests (for categorical variables) were used to compare baseline characteristics between frail and non-frail patients.

The outliers were detected by Tukey's method and removed for analysis (103 outliers were excluded from the analysis out of the 2025 values analyzed using the Olink platform). Spearman correlations between all proteins and CFS were completed to investigate which cytokines were related to frailty in hip fracture patients.

Logistic regression was used to estimate the relationship between frailty and candidate biomarkers and binary outcome variables at one- and three-months follow-up: mortality, hospital admission, dependency according to Barthel index (≤60 points), dependency according to Lawton index (≤3 points), polypharmacy (≥5 prescriptions), and dependency in gait according to FAC scale (FAC ≤ 3). These results were presented as odds ratios (OR) with 95% confidence intervals (CI).

The discriminatory ability of the biomarkers was assessed by the receiver operating characteristics (ROC) curve with receiver operating characteristic (AUROC) calculation, and this was compared with the CFS. The ROC curve was calculated for outcomes with significant results in the logistic regression.

The principal components and heatmap were calculated from the proteomics dataset using singular value decomposition with imputation (pre-normalized data, no transformation) for missing data, and visualized using ClustVis[34].

All statistical calculations were completed using SPSS software ver. 28.0 (IBM, Armonk, NY, USA). Analyses were two-sided, and values of $p < 0.05$ or a 95% confidence interval (CI) non-containing the null value were considered statistically significant; except for Spearman correlation, which for exploratory reasons of the study, was considered $p<0.1$.

3. RESULTS

Our study included 45 older adults, of which 84.4% were female. The mean age was 85.67 years (SD 6.4). Among the participants, 28 were categorized as frail according to the CFS scale. Table 1 presents the reported clinical and functional characteristics of the study group. The scores for BMI, functional status, and body composition were all significantly lower in the frail group compared to non-frail participants. Unsupervised systems analysis was conducted to identify coregulated network responses (Figure 2) using Principal component analysis (A) and HeatMap (B) and revealed substantial overlap between frail and non-frail patients. These findings were related to the prevalence of hip fracture in this cohort.

Spearman correlation revealed a negative association between IL-7 and frailty status ($\rho = -0.302$, $p=0.046$) and between CXCL-12 and frailty status ($\rho = -0.284$, $p=0.068$). Both FLT3LG ($\rho = 0.264$, $p=0.079$) and CXCL-8 ($\rho = 0.274$, $p=0.083$) approached statistical significance. As an exploratory study, we used these cytokines for the follow-up analyses. The rest of the analysis in proteomics markers of patients were not significant and were available in Table S2.

Logistic regression analysis, as detailed in Table 2, revealed a significant association between CSF and dependency as measured by the Barthel index, as well as gait dependency at both one-month and three-month follow-ups. Independent of CFS, increased levels of CXCL-12 were associated with a reduction in dependency according to the Barthel index at three months (OR = 0.97, 95%CI 0.95-0.99, p=0.011). IL-7 levels were inversely associated with gait dependency (OR = 0.66, 95%CI 0.46-0.94, p=0.022). However, the association of IL-7 levels with dependency based on the Barthel index was not statistically significant (p=0.070). Elevated CXCL-8 levels were associated with an increased risk of hospital readmission at three months (OR = 1.07, 95%CI 1.01-1.14, p=0.019), although its association with dependency according to the Barthel index was not significant (OR = 1.05, 95%CI 1.00-1.10, p=0.058). No associations with mortality or polypharmacy were observed for any of the assessed candidate biomarkers.

AUROC analyses, depicted in Figure 3 and detailed in Table S3, showed that CXCL-12's ability to predict dependency based on the Barthel index at three months was comparable to that of CFS (AUROC = 0.845). IL-7 had an AUROC of 0.703 in predicting gait dependency at the three-month mark. Similarly, CXCL-8 had an AUROC of 0.815 related to hospital admissions at three months.

Figure 2: Unsupervised systems analysis to identify coregulated network responses. A: Principal component analysis (PCA). B: Heat MAP

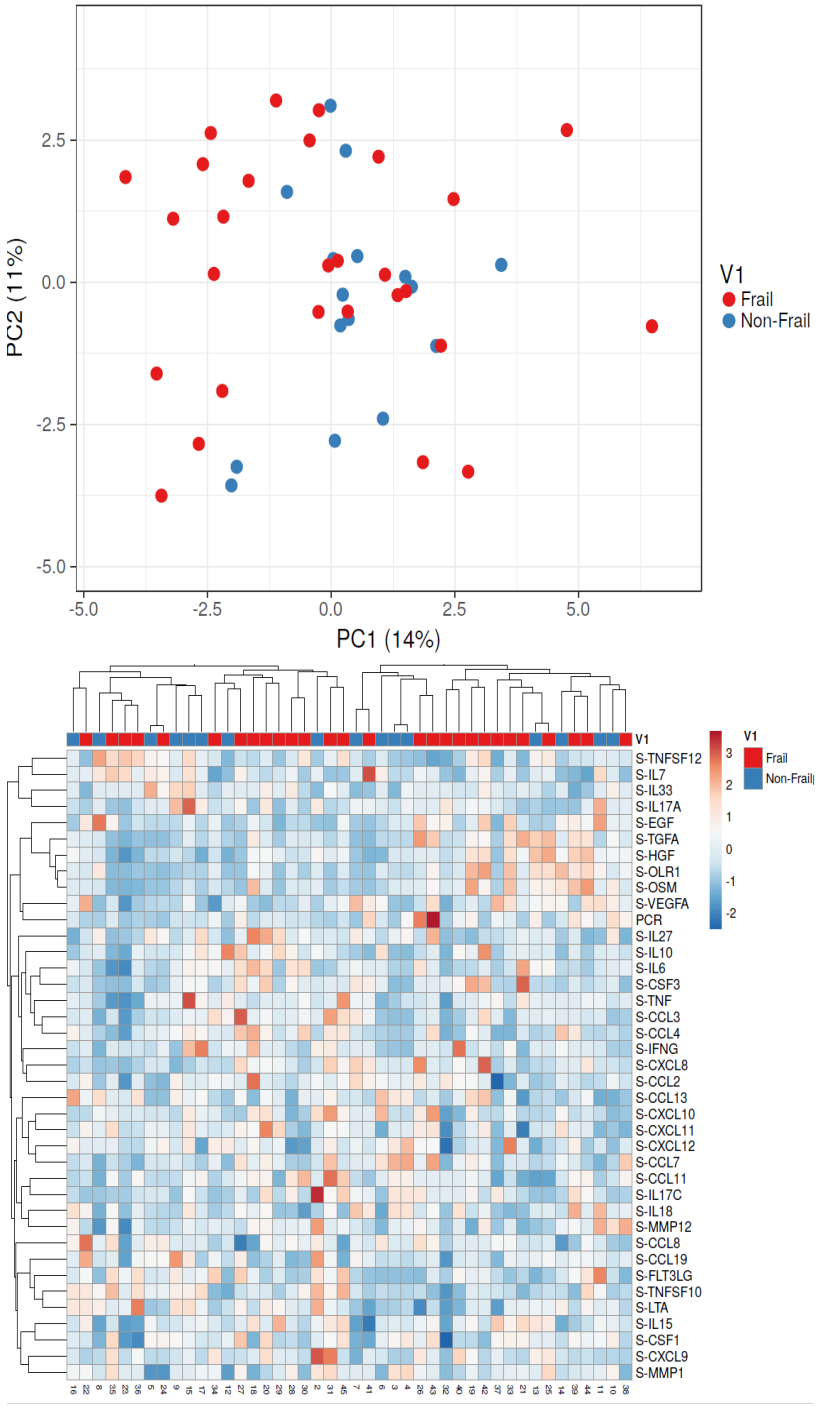


Table 1 Demographic and baseline characteristics of the patients included for analysis**.**

	Total (n=35)	Non-frail (n=17)	Frail (n=28)	P value*
Age	85.67 (6.4)	82.59 (5.43)	87.54 (6.25)	0.01
Sex, n (%)				
Men	7 (15.6%)	4 (57.1%)	3 (42.9%)	0.399
Female	38 (84.4%)	13 (34.2%)	25 (65.8%)	
Charlson score [a]	6.09 (2.31)	4.53 (1.38)	6.07 (2.72)	0.036
Polypharmacy	7.3 (3.8)	5.41 (2.85)	8.32 (3.95)	0.011
Functional Status				
Barthel Index[b]	84.60 (18.6)	97.65 (3.59)	77.14 (19.65)	<0.0001
Lawton Index[c]	4.8 (2.8)	6.65 (2.03)	3.79 (2.73)	0.001
Functional Ambulation Category	1.2 (1.3)	0.18 (0.39)	1.82 (1.22)	<0.0001
Hand Grip Strength (Kg)	12.88 (6.38)	18.71 (4.89)	9.89 (5.49)	<0.0001
MNA[d]	23.77 (5.22)	27.47 (2.21)	21.14 (4.87)	<0.0001
Depression (n, %)[e]	13 (28.9%)	2 (11.8%)	11 (39.3%)	0.03
Delirium (n, %)[f]	21 (46.7%)	4 (23.5%)	17 (60.7%)	0.03
Dementia (n, %)[g]	2.39 (1.59)	1.65 (1)	3 (1.56)	0.003
Body Composition				
BMI (kg/m2)[h]	25.4 (4.5)	24.9 (2.43)	25.68 (5.4)	0.623
ASMI[i]	4.98 (1.47)	5.19 (1.2)	4.49 (1.65)	0.134
ASM/BMI[j]	0.191 (0.051)	0.201 (0.042)	0.174 (0.053)	0.034

[a]The Charlson Comorbidity Index ranges from 0 (low comorbidity) to 37 (high comorbidity).
[b]The Barthel Index ranges from 0 (severe functional dependence) to 100 (functional independence).
[c]The Lawton Index ranges from 0 (dependence) to 8 (independence).
[d]Mini-Nutritional Assessment (MNA).
[e]The Geriatric Depression Scale (GDS-15) ranges from 0 to 15 and indicates symptomatic depression with ≥ 5.
[f]Confusion Assessment Method (CAM) is a standardized tool to identify and recognize delirium. The diagnosis of delirium by CAM requires the presence of features 1 and 2 and either 3 or 4.
[g]The Global Deterioration Scale (GDS) ranges from 0 to 7. Dementia stages are from 4 to 7.
[h]BMI (body mass index).
[i]ASMI (Appendicular Skeletal Muscle Index, kg).
[j]ASM/BMI (Appendicular lean mass adjusted for BMI).

* p-value for different groups in percentage (Pearson X2, expect no normal distribution; Fisher's exact test) or means (T-student, expect no normal distribution; U de Mann-Whitney). The bold values are statistically significant .

Table 2 Logistic Regression at one- and three-months follow-up between frailty scales and biomarkers with A: Mortality, B: Hospital admission, C: Dependency according Barthel index, D: Dependency according Lawton index, E: Polypharmacy and F: Dependency in gait according FAC scale. Clinical Frailty Scale (CFS). Functional Ambulation Classification (FAC)

A: Mortality

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS	2.19 (0.55, 8.78)	0.269	1.45 (0.44, 4.76)	0.538
FLT3LG	0.87 (0.61, 1.25)	0.452	1.00 (0.92, 1.09)	0.929
IL-7	-		0.84 (0.29, 2.47)	0.755
CXCL-12	0.99 (0.94, 1.04)	0.686	1.01 (0.96, 1.06)	0.741
CXCL-8	1.06 (0.95, 1.17)	0.300	1.03 (0.92, 1.14)	0.626

B: Hospital admission

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS	1.49 (0.72, 3.07)	0.280	1.18 (0.72, 1.95)	0.518
FLT3LG	0.99 (0.94, 1.05)	0.778	0.99 (9.95, 1.03)	0.577
IL-7	0.89 (0.48, 1.65)	0.706	1.28 (0.83, 1.98)	0.257
CXCL-12	0.98 (0.94, 1.01)	0.145	1.00 (0.98, 1.03)	0.764
CXCL-8	0.93 (0.76, 1.13)	0.460	1.07 (1.01, 1.14)	**0.019**

C: Dependency according Barthel index

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS	2.25 (1.32, 3.85)	0.003	3.64 (1.59, 8.32)	**0.002**
FLT3LG	1.01 (0.99, 1.04)	0.294	1.01 (0.98, 1.04)	0.441
IL-7	0.80 (0.58, 1.10)	0.161	0.71 (0.49, 1.03)	0.070
CXCL-12	0.98 (0.96, 1.00)	0.060	0.97 (0.95, 0.99)	**0.011**
CXCL-8	1.03 (0.99, 1.08)	0.169	1.05 (1.00, 1.10)	0.058

D: Dependency according Lawton index

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS	2.94 (1.52, 5.68)	0.001	2.01 (1.23, 3.29)	**0.005**
FLT3LG	1.02 (0.99, 1.05)	0.193	1.03 (1.00, 1.06)	0.084
IL-7	0.80 (0.58, 1,12)	0.192	0.85 (0.62, 1.16)	0.302
CXCL-12	0.99 (0.98, 1.01)	0.437	0.99 (0.97, 1.00)	0.123
CXCL-8	1.03 (0.98, 1.08)	0.298	1.03 (0.98, 1.08)	0.216

E: Polypharmacy

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS			0.71 (0.29, 1.71)	0.445
FLT3LG			0.98 (0.90, 1.05)	0.534
IL-7			0.95 (0.46, 1.96)	0.890
CXCL-12			1.01 (0.98, 1.06)	0.352
CXCL-8			0.98 (0.88, 1.10)	0.774

F: Dependency in gait

	One-month		Three-months	
	OR (IC95%)	p-value	OR (IC95%)	p-value
CFS	2.25 (1.32, 3.83)	0.003	2.21 (1.30, 3.75)	**0.003**
FLT3LG	1.00 (0.97, 1.02)	0.870	1.00 (0.98, 1.03)	0.787
IL-7	0.74 (0.53, 1.04)	0.079	0.66 (0.46, 0.94)	**0.022**
CXCL-12	0.99 (0.97, 1.01)	0.149	0.99 (0.97, 1.00)	0.098
CXCL-8	1.02 (0.97, 1.07)	0.394	1.01 (0.97, 1.06)	0.513

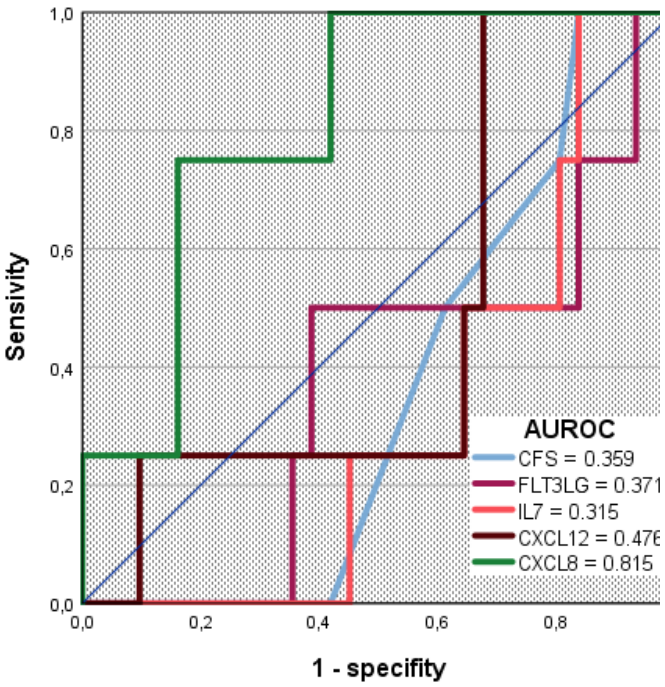
4. DISCUSSION

In our prospective cohort study, we identified three biomarkers (CXCL-12, CXCL-8, and IL-7) that may have significant implications for predicting adverse outcomes in older adults with hip fractures. While CXCL-12 and IL-7 levels were positively associated with improvements in activities of daily living and gait independence at three months respectively, we did not find associations with other outcomes such as mortality, rehospitalization, or dependency based on the Lawton index. On the other hand, CXCL-8 levels were linked to hospital readmissions, it was not significantly associated with other adverse outcomes. These lacks associations are associated with the prevalence of hip fracture. As we have mentioned, hip fracture is an event associated with numerous adverse outcomes[1, 2], and although these biomarkers may influence the outcomes, they may not carry sufficient weight to define differences among them, especially when frailty is in consideration[3].

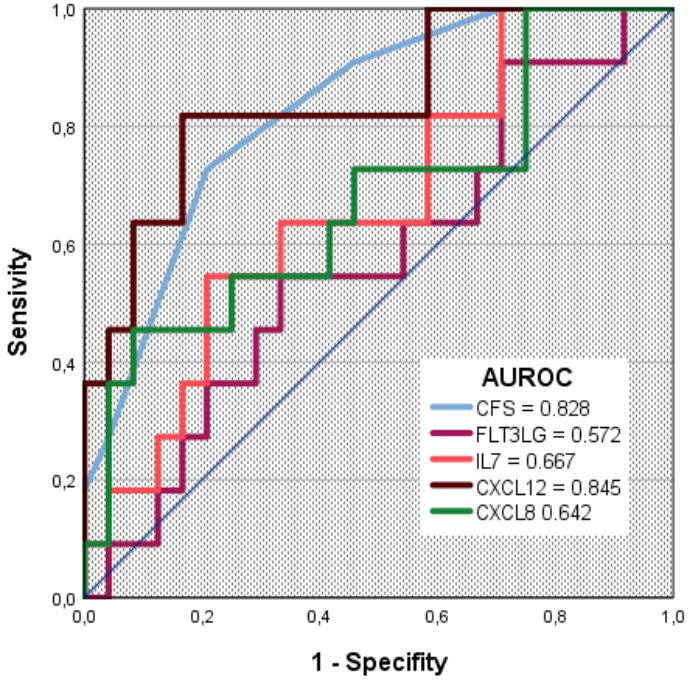
This exploratory study supports the previously established association between frailty and worse health outcomes after hip fractures, as shown in Figure 3. However, the study also found that CXCL-8 and CXCL-12 had a greater ability in predicting hospital readmission and decline in activities of daily living, respectively, compared to CFS. Additionally, the study found that neither CFS nor biomarkers were able to accurately predict polypharmacy. The relationship between inflammatory biomarkers and polypharmacy is a controversial topic, particularly for older adults with multimorbidity [35].

Figure 3: Performance of different biomarkers in prediction of A: Hospital admission, B: Dependency according Barthel index, and C: Dependency in gait according FAC scale. Receiver operating characteristic (AUROC), Clinical Frailty Scale (CFS)

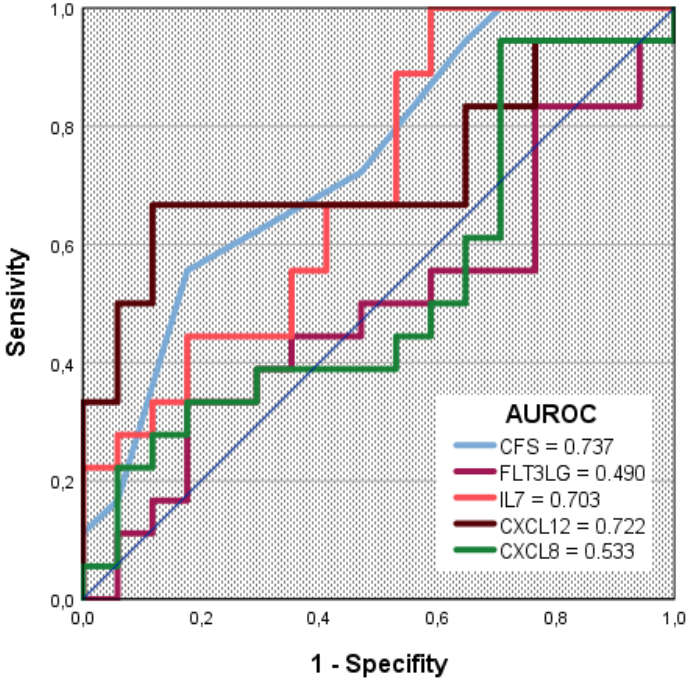
A: Hospital admission



B: Dependency according Barthel index



C: Dependency in gait according FAC



Low-grade inflammation plays a key role in the development of various highly prevalent age-related conditions, including frailty [18], and is associated with a higher risk of adverse events. The exact mechanism by which these circulating peptides exerts their detrimental actions on musculoskeletal tissues is not fully understood. However, it is well acknowledged that inflammatory mediators such as reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines can directly or indirectly affect body cells and contribute to worse outcomes in older adults [36]. It is important to understand the pathophysiological mechanism by which the inflammatory markers in this study were able to produce these results:

IL-7, as a growth factor synthesized by a diverse range of cell types, functions as a myokine and has an important role in the regulation of muscle cell development and bone metabolism. It is suggested that osteoblast-derived IL-7 might inhibit bone formation while simultaneously upregulating the expression of RANKL[37] but also increases osteoblasts[38]. In muscle tissue, IL-7 expression has been associated with improvements in both muscle strength and mass[39] and elevated levels have been observed in active older adults[40]. Our study findings revealed that patients exhibiting higher levels of IL-7 demonstrated a reduced risk of experiencing a decline in walking independence at the three-month mark post-discharge (OR = 0.66, 95%CI 0.46-0.94, p=0.022). Based on these findings, increasing IL-7's level through strength exercises[39] may enhance patient function. However, in other studies, elevated levels of IL-7 increased the likelihood of falls [41] so further work is needed to clarify the mechanisms that link IL-7 to adverse outcomes.

CXCL-12 also has a role in musculoskeletal system. It is expressed in the area of inflammatory bone destruction, where it mediates their suppressive effect on osteoclastogenesis and stimulates osteogenic differentiation [42]. In muscle tissue, the presence of CXCL-12 has been observed to significantly enhance the regenerative properties of these cells, promoting muscle repair and recovery[43]. Our study revealed a relationship between elevated levels of CXCL-12 and a decreased risk of functional impairment at the three-month follow-up after discharge (OR = 0.97, 95%CI 0.95-0.99, p=0.011). These findings may suggest the beneficial impact of CXCL-12 on muscle tropism and overall functional recovery throw muscle regeneration after hip fracture [44].

CXCL-8, also known as Interleukin 8 (IL-8), has many roles. One of them is as an osteoclastogenic cytokine, inducing RANK-mediated NFATc1 activation [45]. Several studies have shown that pro-inflammatory cytokines such as CXCL-8 and IL-6 are associated with frailty and adverse outcomes [46–48]. Moreover, in a study conducted by Edvardsson et al., it was observed that heightened levels of CXCL-8 and C-reactive protein correlated with decreased survival rates among older nursing home adults during a one-year follow-up period[49]. In line with these studies, our own investigation found that elevated levels of CXCL-8 were linked to an augmented risk of hospital readmission at the three-month mark following discharge (OR = 1.07, 95%CI 1.01-1.14, p=0.019).

In our study, we did not observe significant associations between negative outcomes and other cytokines commonly associated with frailty, such as IL-1β, IL-6, IFN-γ, and TNF-α[18]. These mediators play a relevant role in inflammaging and other age-related conditions [50], including multimorbidity, osteoporosis[51] and polypharmacy[52]. The absence of differences in IL-1, IL-6, IFN and TNF observed in the present investigation

may be due to the comparable characteristics of frail and non-frail participants regarding parameters associated with inflammation. Other clinical factors related to the frailty group, such as reductions in BMI, functional status, and body composition scores, align with the established pathophysiology of frailty[53].

This study has several strengths and limitations. The main strengths are the high validity and reproducibility of the analytical approach adopted. Olink technology allowed the measurement of a large panel of cytokines which proved to track changes not related to inflammaging with higher sensitivity. Moreover, more than 90% of proteins included in the Olink panels were detected above the limit of detection in all samples, indicating excellent detectability of the assays in human blood plasma from the general population[54]. Given the complexity of hip fracture patients, exploratory approaches will be needed to allow the identification of specific signatures relevant to distinguishing the risk of adverse outcomes in this group of patients[55]. The development of immunology biomarkers for hip fracture patients would be a field of interest in clinical practice due to its association with disability, premature mortality, and increased medical resources[3]. Our study has identified three potential biomarkers that hold promise in predicting adverse outcomes associated with hip fracture risk in older adults. These findings offer a potential clinical tool for managing complex patients and present a new avenue for further investigation. Future longitudinal studies with larger sample sizes are necessary to explore the potential of these biomarkers in accurately identifying patient groups with poorer outcomes and optimizing resource allocation. The present study has also some limitations that should be mentioned. First, the study population consisted of a small cohort of only Caucasians, preventing the generalization of our findings to other ethnic groups. However, as the objective of this study is exploratory to detect potential biomarkers, these findings are useful to open up new lines of research in larger cohorts. Secondly, measuring inflammation markers on the day of the surgery could result in disproportionately high results due to the acute inflammation associated with fracture. However, this potential increase would be similar in both groups (frail and non-frail patients). Additionally, due to the targeted approach used, we cannot rule out the presence of other circulatory proteins with a potential impact on the risk prediction of frailty and hip fracture. Furthermore, although the cohort was extensively characterized, it was relatively small, and analyses involved a large set of variables. Even considering these limitations, the study included 46 cytokines and Olink-enhanced PEA was used for the analyzes, which has been established as a straightforward, sensitive and highly reliable method for biomarker analysis[33].

5. CONCLUSION

In summary, CXCL-12, IL-7, and CXCL-8 levels have potential roles as prognostic biomarkers for adverse outcomes related to hip fractures at a three-month follow-up: CXCL-12 is associated with improvements in activities of daily living, IL-7 with gait independence, and CXCL-8 with hospital readmission. These findings were independent of the patients' frailty status.

Our PEA-based high-throughput proteomic approach produced a differential serum prototype, paving the way towards the development and implementation of new screening tools. However, as an exploratory study, further analysis is needed. These

approaches, together with functional analyses, could help clarify the underlying mechanisms involved in the development of frailty among hip fracture patients.

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SUPPLEMENTARY MATERIAL

Table S1: List of biomarkers measured with Olink 48 Inflammatory Target

Biomarker name (Abbreviation)	Values < LOD
C-C motif chemokine 2 (CCL2)	0%
C-C motif chemokine 3 (CCL3)	3%
C-C motif chemokine 4 (CCL4)	3%
C-C motif chemokine 7 (CCL7)	0%
C-C motif chemokine 8 (CCL8)	0%
C-C motif chemokine 13 (CCL13)	0%
C-C motif chemokine 19 (CCL19)	2%
C-X-C motif chemokine 9 (CXCL9)	2%
C-X-C motif chemokine 10 (CXCL10)	0%
C-X-C motif chemokine 11 (CXCL11)	0%
Eotaxin (CCL11)	0%
Fms-related tyrosine kinase 3 ligand (FLT3LG)	0%
Granulocyte colony-stimulating factor (CSF3)	0%
Granulocyte-macrophage colony-stimulating factor (CSF2)	88%
Hepatocyte growth factor (HGF)	0%
Interferon gamma (IFNG)	0%
Interleukin-1 beta (IL1B)	78%
Interleukin-2 (IL2)	95%
Interleukin-4 (IL4)	95%
Interleukin-6 (IL6)	0%
Interleukin-7 (IL7)	0%
Interleukin-8 (CXCL8)	3%
Interleukin-10 (IL10)	0%
Interleukin-13 (IL13)	90%
Interleukin-15 (IL15)	0%
Interleukin-17A (IL17A)	20%
Interleukin-17C (IL17C)	0%
Interleukin-17F (IL17F)	68%
Interleukin-18 (IL18)	0%
Interleukin-27 (IL27)	33%
Interleukin-33 (IL33)	32%
Interstitial collagenase (MMP1)	18%
Lymphotoxin-alpha (LTA)	0%
Macrophage colony-stimulating factor 1 (CSF1)	0%
Macrophage metalloelastase (MMP12)	0%
Oncostatin-M (OSM)	0%
Oxidized low-density lipoprotein receptor 1 (OLR1)	0%
Pro-epidermal growth factor (EGF)	2%
Protransforming growth factor alpha (TGFA)	0%
Stromal cell-derived factor 1 (CXCL12)	0%
Tumor necrosis factor (TNF)	0%
Tumor necrosis factor ligand superfamily member 10 (TNFSF10)	0%
Thymic stromal lymphopoietin (TSLP)	97%
Tumor necrosis factor ligand superfamily member 12 (TNFSF12)	0%
Vascular endothelial growth factor A (VEGFA)	0%

Note: Grey shade indicates biomarkers with 35% or more of the values below the limit of detection (LOD)

Table S2: Differences in proteomics markers of patients.

Biomarker	Rho (p-valor)	Biomarker	Rho (p-valor)
CCL8	-0.172 (0.289)	CCL19	-0.184 (0.270)
IL33	-0.099 (0.623)	TNF	0.168 (0.320)
CXCL12	-0.284 (0.068)	IL15	0.072 (0.644)
OLR1	0.096 (0.530)	CCL3	0.260 (0.105)
IL27	-0.142 (0.357)	CXCL8	0.274 (0.083)
CXCL9	0.238 (0.128)	MMP12	0.176 (0.278)
TGFA	0.196 (0.197)	CSF3	0.144 (0.369)
IL6	-0.015 (0.924)	VEGFA	0.205 (0.177)
TNFSF12	-0.067 (0.660)	IL17C	0.177 (0.262)
CCL11	0.227 (0.148)	EGF	0.115 (0.457)
HGF	0.127 (0.415)	CCL2	-0.031 (0.858)
FLT3LG	0.264 (0.079)	IL17A	-0.222 (0.192)
IL7	-0.302 (0.046)	OSM	0.043 (0.786)
IL18	-0.026 (0.867)	CSF1	-0.058 (0.704)
CCL13	-0.084 (0.588)	CCL4	0.101 (0.523)
TNFSF10	-0.117 (0.449)	CXCL11	0.119 (0.458)
CXCL10	-0.042 (0.792)	LTA	-0.128 (0.407)
IFNG	0.205 (0.212)	CCL7	0.052 (0.739)
IL10	0.023 (0.889)	MMP1	-0.187 (0.283)

Note: Frailty according to Clinical Frailty Scale (CFS) with Spearman Correlation

Table S3: Performance of ROC-derived cut-off values for three-months follow-up with A: Hospital admission, B: Dependency according Barthel index, and C: Dependency in gait according FAC scale

A: Hospital admission,

Parameter	CFS	FLT3LG	IL-7	CXCL-12	CXCL-8
AUC (SE)	0.359 (0.101)	0.371 (0.144)	0.315 (0.103)	0.476 (0.142)	0.815 (0.092)
95% CI	0.161-0.557	0.089-0.653	0.112-0.517	0.198-0.754	0.635-0.994

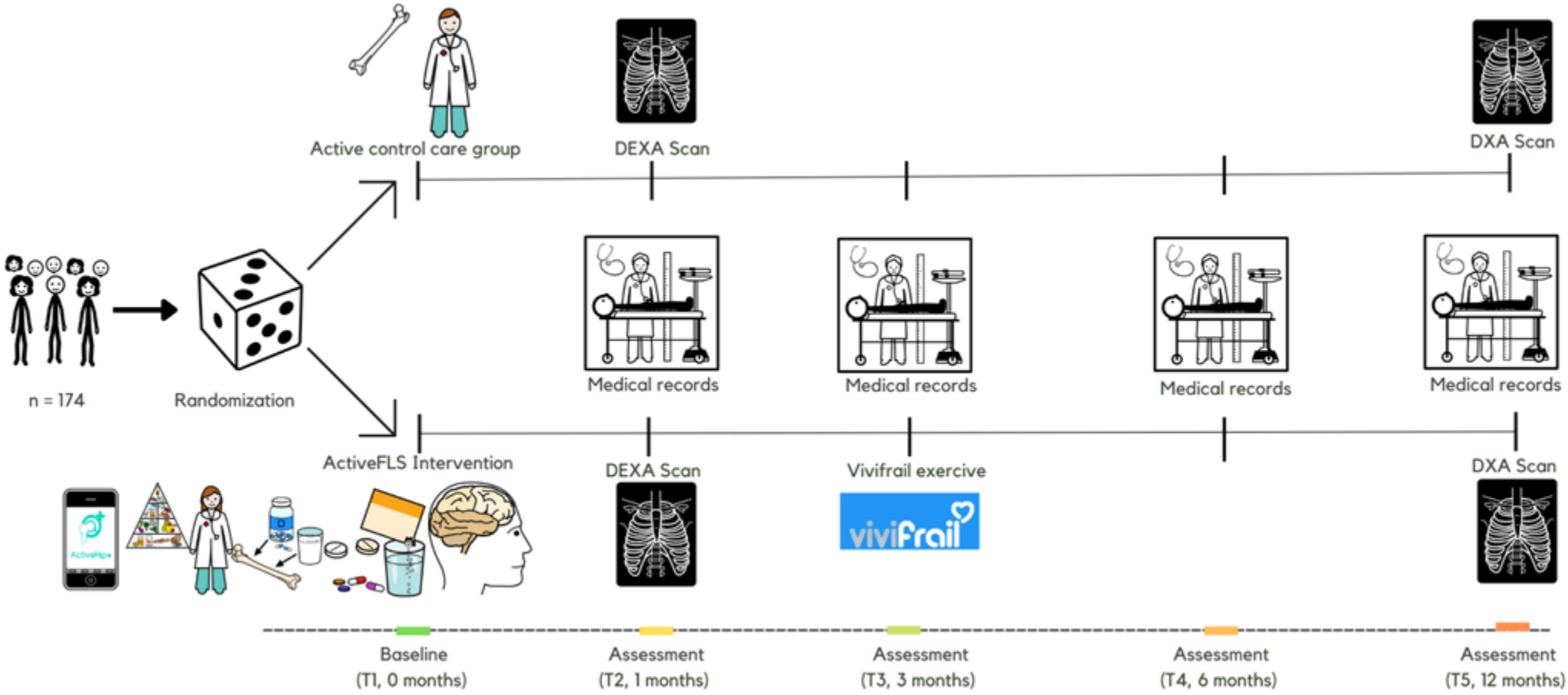
B: Dependency according Barthel index

Parameter	CFS	FLT3LG	IL-7	CXCL-12	CXCL-8
AUC (SE)	0.828 (0.072)	0.572 (0.105)	0.667 (0.097)	0.845 (0.074)	0.642 (0.043)
95% CI	0.687-0.968	0.366-0.777	0.476-0.858	0.7-0.990	0.471-0.877

C: Dependency in gait according FAC scale

Parameter	CFS	FLT3LG	IL-7	CXCL-12	CXCL-8
AUC (SE)	0.737 (0.084)	0.490 (0.1)	0.703 (0.089)	0.722 (0.091)	0.533 (0.101)
95% CI	0.572-0.902	0.294-0.687	0.528-0.877	0.545-0.9	0.334-0.731

CFS, Clinical Frailty Scale; AUC, area under the curve; SE, standard error; CI, confidence interval.


Active control care group
DEXA Scan
DXA Scan
n = 174
Randomization
Medical records
Medical records
Medical records
Medical records
ActiveFLS Intervention
DEXA Scan
Vivifrail exercive
DXA Scan
vivifrail
Baseline
(T1, 0 months)
Assessment
(T2, 1 months)
Assessment
(T3, 3 months)
Assessment
(T4, 6 months)
Assessment
(T5, 12 months)

Chapter 5

Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial.

1. BACKGROUND AND RATIONALE {6a}

Osteoporosis is a prevalent disease globally and fragility fractures, especially hip fractures in older adults, impose a significant burden on health and economics [1,2]. Despite efforts to curb the increasing incidence of hip fractures, it remains a "silent epidemic" [1] affecting populations worldwide. The projected rise in the number of fragility fractures is alarming and many fracture liaison services (FLS) primarily focus on bone metabolism treatments, therapeutic adherence and mortality [3], ignoring other critical factors that affect older adults. Among these factors, we find functional decline, cognitive impairment, malnutrition, frailty, sarcopenia, pain, falls and comorbidities [4].

FLS have not yet studied the special approach required for frail and vulnerable individuals at risk of experiencing fragility fractures [5–7]. Although there is a consensus on the importance of nutrition, calcium, vitamin D and certain osteoporosis medications [8], the effectiveness and suitability of exercise guidelines for older adults remain controversial [9].

Tele-rehabilitation is a new way of providing rehabilitation remotely through information and communication technologies [10]. The @ctivehip [11] application is an example of a program that has shown promising results in enhancing functional recovery, physical independence, quality of life, fear of falling and emotional status, as well as reducing the emotional state and perceived burden of informal caregivers [12]. However, the long-term effectiveness of such programs among older hip fracture patients, including exercise interventions like Vivifrail and ActiveHip, and their combination with comprehensive geriatric assessment (CGA) remain uncertain, as most studies have focused on evaluating their short-term effects over a three-month intervention period.

This study aims to contribute to the development of clinical integrated practice guidelines for the implementation of functional recovery after hip fracture with tele-rehabilitation (physical exercise based on the @ctivehip and Vivifrail programs [13]), nutrition, secondary prevention of osteoporosis, polypharmacy adjustment and other major comorbidities. Pathways for clinical management for older adults who are at risk of chronic illnesses moreover than osteoporosis are essential to approach the complexity of these patients.

2. OBJECTIVES {7}

2.1 Hypothesis

We hypothesize that a multicomponent intervention with tele-rehabilitation and the Vivifrail exercise program will improve hip fracture recovery at the 12-month follow-up.

3. METHODS AND ANALYSIS

3.1 Trial design {8}

This study will follow the recommendations of the International Conference on Frailty and Sarcopenia Research ICFSR Task Force 2020 [14]. This is a prospective, randomized controlled trial (RCT), two-group repeated measures experimental design. Patients will be assigned in a 1:1 allocation ratio.

3.2 Study setting {9}

The study will take place in the Department of Orthopaedics Clinics and Traumatology of Navarre University Hospital (Pamplona, Spain). Hospitalized patients who meet the inclusion criteria during the screening will be informed about the study. After signing the consent form, the subjects will be randomly assigned to either the intervention or active control care group.

3.3 Eligibility criteria {10} and recruitment {15}

The study participants will be older inpatient adults ≥ 75 years in the Trauma Ward of Navarre University Hospital (Pamplona, Spain) after a hip fracture. This study was approved by the Navarre University Hospital Research Ethics Committee (PI_2022/7) on 25 April 2022. It is estimated that the study dates will be from 1 June 2022 to 31 December 2025.

Patients will be eligible to participate if the following apply: (i) age ≥ 75 years with a diagnosis of hip fracture fragility; (ii) Barthel index score for activities of daily living (ADL) of ≥ 60 (scale: 0, severe functional dependence; 100, functional independence) 2 weeks before fracture [15]; (iii) mobility independence on the Functional Ambulation Classification (FAC) scale of ≥ 3 (scale: 0, non-functional ambulatory; 5, independent ambulator) 2 weeks before fracture [16]; (iv) ability/support to use the ActiveHIP app (defined as the presence of a patient or caregiver willing to use the platform and ability to operate it after installing it on the cell phone in the presence of the recruiter and understand Spanish); and (v) informed consent by patients (if possible), relatives or legal representatives.
Patients will be excluded if the following apply: (i) moderate–severe cognitive impairment with a Global Deterioration Scale (GDS) score of ≥ 5; (ii) secondary osteoporosis [17]; (iii) institutionalized in a permanent nursing home; (iv) refusal to give informed consent by patient/primary caregiver/legal guardian or inability to obtain it; (v) terminal illness (life expectancy less than 3 months); and (vi) any factor that precludes the performance of physical exercise, including acute myocardial infarction in the past 3 months, unstable angina, severe heart valve insufficiency, arrhythmia/uncontrolled arterial hypertension or pulmonary embolism in the past 3 months and haemodynamic instability. Only the conditions specifically mentioned will be taken into consideration.

3.4 Who will take informed consent? {26a}

Study recruitment will be done through posters and other tools. We will provide explanations using the consent explanatory document and consent forms, and written consent will be obtained from all participants and their guardians. These consent forms will be under the scrutiny of the Ethics Committee to ensure all ethical standards will be met.

3.5 Additional consent provisions for collection and use of participant data and biological specimens {26b}

The study consent process includes permission for additional analyses of collected data. Blood samples will be collected.

3.6 Explanation of the choice of comparators {6b}

- **Interventions**

In the active control group (control), participants will receive outpatient care in line with standard clinical practice. This sets it apart from traditional control groups in other studies that have no

planned interventions. The intervention group (ActiveFLS), on the other hand, will receive an individualized multicomponent physical exercise program based on the ActiveHip+ for 3 months, in addition to standard care. In subsequent revisions, after finishing the ActiveHip+ program, the Vivifrail program will be given according to the patient's functional capacity. A CGA will be performed, evaluating nutrition status, polypharmacy, cognitive impairment, and mood disorders. Nutritional intervention, adjustment of polypharmacy according to the Screening Tool of Older Person's Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) criteria, management of anxiety, depression, cognitive impairment, and fear of falling will be done, as well as protocolized secondary fracture prevention treatment. Throughout the study, all participants will be permitted to maintain their regular physical activity levels. The interventions and follow-up are time-matched (Figure 1), ensuring both groups' experiences are synchronized over the same time period.

- **Intervention description {11a}**

ActiveFLS intervention: We will propose a comprehensive geriatric assessment program that includes a multicomponent physical exercise program guideline based on ActiveHip+. ActiveHip+ is a mobile app that is loaded onto the patient's smartphone. Given the limitations of older adults with smartphone apps, the caregiver will play a crucial role in ensuring the ongoing monitoring of the patient's rehabilitation program.

The ActiveHip+ program will include a health education program with five modules designed for patients and caregivers, as well as two additional modules specifically for caregivers. These modules will provide information on hip fracture recovery and strategies to prevent a second fracture. A detailed description of the program can be found [18]. The home-based tele-rehabilitation program, developed by a multidisciplinary team of health professionals and engineers, will include physical exercise and occupational therapy, with three smartphone-based sessions per week. The exercise program will comprise two physical exercise sessions and one occupational therapy session, ideally scheduled on alternate days, each lasting 30-60 minutes.

We will provide exercise guidelines based on the Vivifrail program. The focus of this program is to provide personalized exercise plans consisting of multiple components, tailored to the functional abilities of older individuals and to be performed at home. The program includes exercises for resistance/power, balance, flexibility, and cardiovascular endurance. A detailed description of the Vivifrail program can be found at http://vivifrail.com/resources/ [13].

After T3 (3-month assessment, Figure 1), patients in the intervention group will be enrolled in one of the four individualized Vivifrail training programs, based on their physical functional status: Disability (0–3 points in the SPPB score), Frailty (4–6 points), Prefrailty (7–9 points) and Robust (10–12 points). A copy of the patient's specific exercise protocol will be provided to each patient.

The exercise intervention will consist of a 5-day-a-week routine of multicomponent exercises for 12 consecutive weeks. This routine will include resistance, balance and flexibility exercises 3 days per week and walking 5 days per week. At the 6-month assessment, a new exercise program will be given to patients and caregivers based on the patients' functional status at that time. This program will remain the same until the final assessment.

A protocolized nutritional intervention will be carried out [19] based on the Global Leadership Initiative on Malnutrition (GLIM) criteria [20], with a focus on recommendations for protein intake, calcium and vitamin D [21]. Oral nutritional supplementation, if needed, will consist of supplements enriched in β-hydroxy-β-methylbutyrate (HMB) [22]. Vitamin D and anti-osteoporosis treatments will be prescribed following national guidelines [23], with zoledronic acid as the preferred choice

due to better tolerance and adherence [24]. The patient's treatment will be reviewed and adapted based on the STOPP/START criteria [25]. Additionally, the patient's mood, cognitive impairment and fear of falling will be evaluated and addressed. The evaluation of depression will follow established clinical practices, utilizing a comprehensive approach that includes both pharmacological strategies, such as the use of prescribed medications, and non-pharmacological strategies, encompassing treatments like psychotherapy, cognitive-behavioural therapy, and lifestyle changes [26]. The training protocol is shown in **Figure 1**.

Active control care group (control): Participants allocated to the usual care group will receive standard outpatient care. This consists of multidisciplinary and multicomponent follow-up during hospital admission by Traumatology, Rehabilitation and Internal Medicine/Geriatrics. At discharge, a continuity of care report is made for follow-up by the Primary Care team and a 1-month review by Traumatology with a control X-ray to check consolidation of the surgical fracture.

3.7 Participant timeline {13}

The Barthel index, FAC scale, GDS and institutionalization status will be conducted as a screening test to assess the general functional capacity of the patient's previous hip fracture. The study will have four major data collection points (baseline during acute hospitalization and at 3, 6 and 12 months) and one minor point (at 1 month). The times of measurement of the different outcomes are shown in **Table 1**. **Figure 2** displays the study flow diagram.

Table 1. Schedule for the different primary and secondary variables for the participants of the study

Measure	**Screening**	**T1 Baseline**	**T2 1 month**	**T3 3 months**	**T4 6 months**	**T5 12 months**
Primary outcome						
Short Physical Performance Battery (SPPB).		x	x	x	x	x
Secondary outcomes						
Barthel index.	x		x	x	x	x
Functional Ambulation Classification (FAC).	x		x	x	x	x
Lawton's Instrumental Activities of Daily Living (IADL).		x	x	x	x	x
Global Deterioration Scale (GDS).	x		x	x	x	x
Mini-Mental State Examination (MMSE).		x	x	x	x	x

Abbreviated Mental Test 4 (4AT).		x	x	x	x	x
Yesavage Geriatric Depression Scale (YE-GDS).		x	x	x	x	x
Falls Efficacy Scale International (FES-I).		x		x		x
Frailty.		x	x	x	x	x
Handgrip.		x	x	x	x	x
Quality of Life (EuroQol-5D).		x	x	x	x	x
Sarcopenia and Quality of Life (SarQoL).		x		x		x
FRAX, QFracture.		x				x
Geriatric syndromes.		x	x	x	x	x
Polypharmacy.		x	x	x	x	x
Rate and risk of falls.		x	x	x	x	x
Visual Analogue Scale (VAS).		x	x	x	x	x
Cumulative Illness Rating Scale for Geriatrics (CIRS-G).		x				
Mini-Nutritional Assessment (MNA).		x	x	x	x	x
Adverse effects.			x	x	x	x
Mortality.			x	x	x	x
Admission and readmission to the hospital.			x	x	x	x
Institutionalization.	x		x	x	x	x
Blood test.		x	x	x	x	x
Bone turnover markers (BTMs).			x			x
Dual-energy X-ray absorptiometry (DXA).			x			x

Figure 1: Intervention timeline through the "ActiveFLS randomized control trial". Participants will be randomly assigned to intervention group (ActiveFLS Intervention, n=87) or control group (Active control care, n=87). T: time-point; DXA: Dual energy x-ray absorptiometry

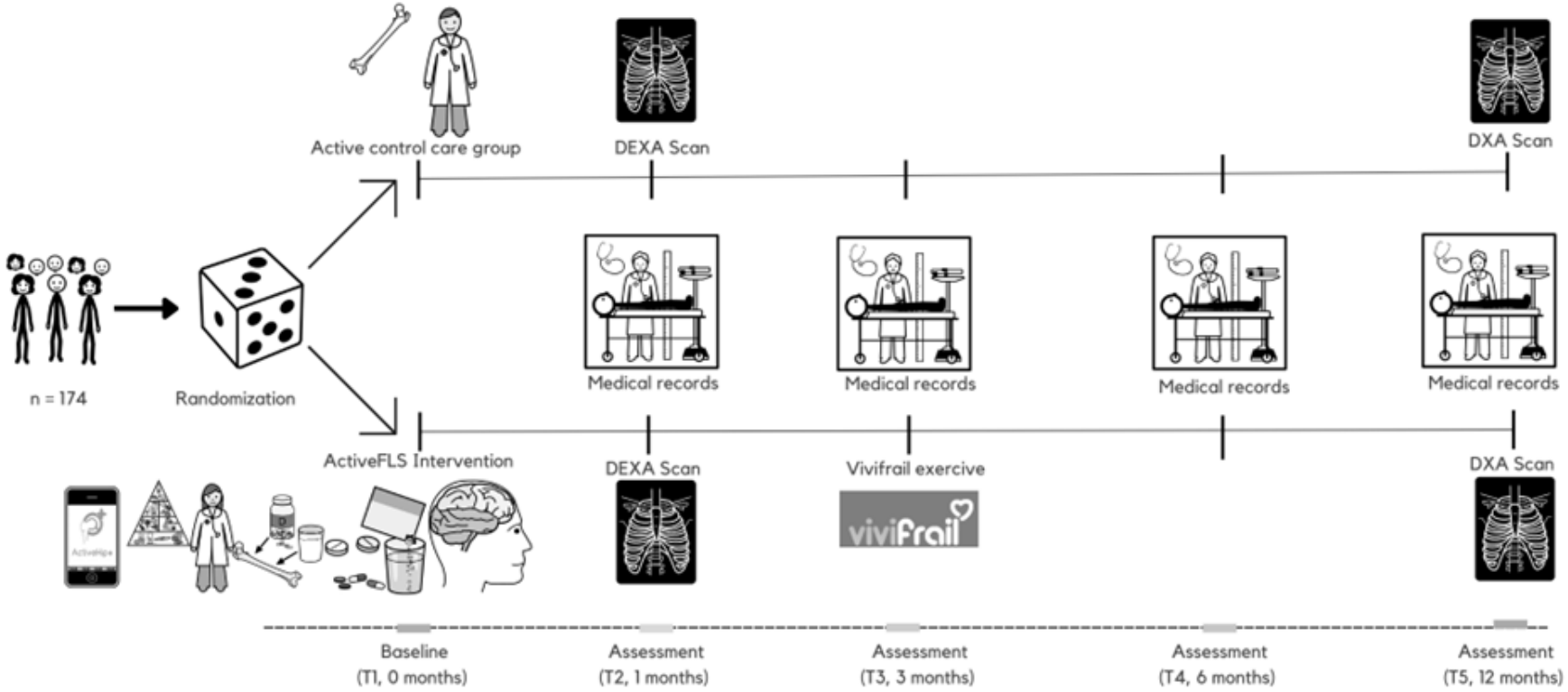
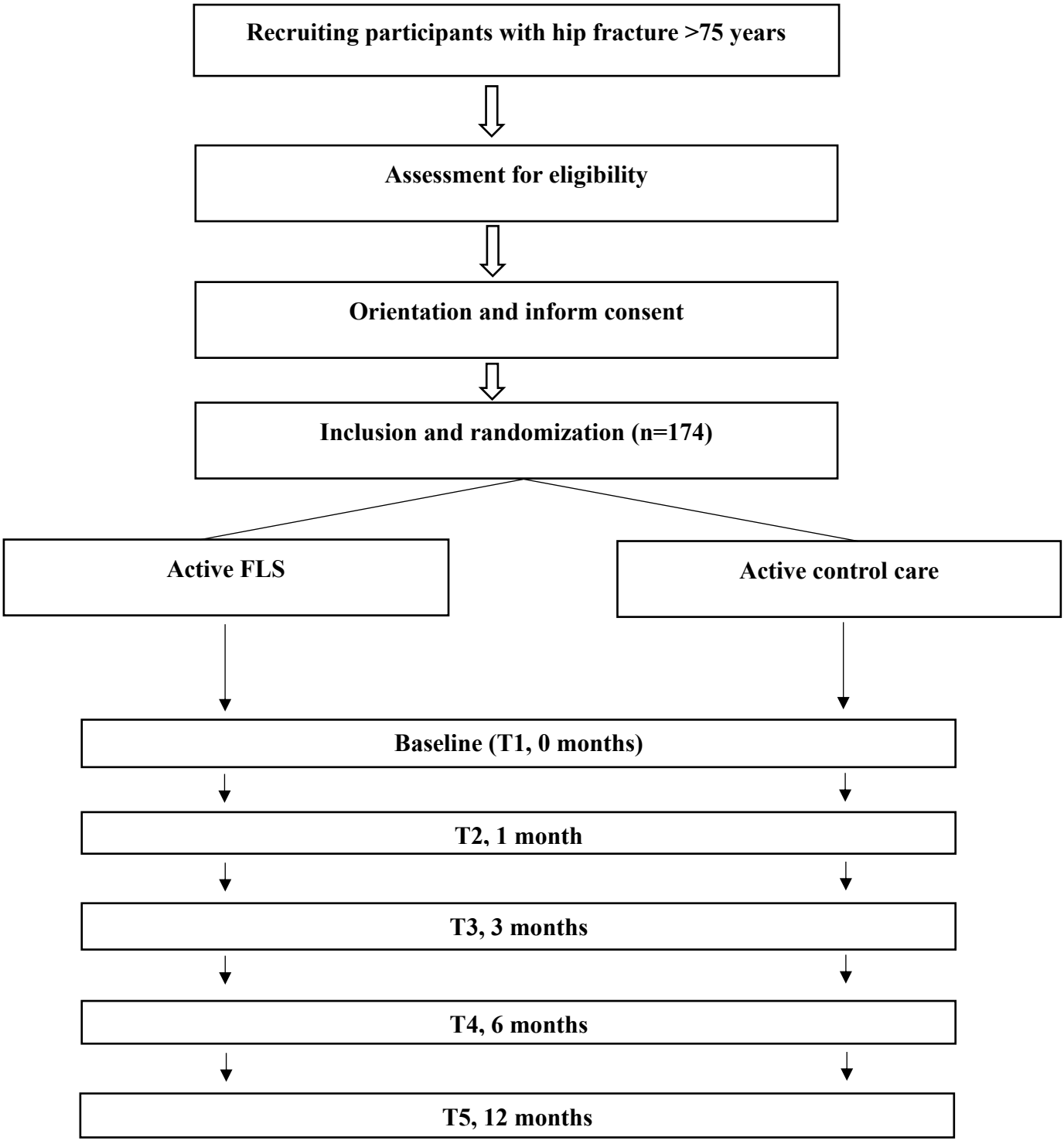


Figure 2. Flow diagram of the study protocol.



3.8 Criteria for discontinuing or modifying allocated interventions {11b}

Participants randomly assigned to the intervention group will be encouraged to use the Vivifrail program and/or usual care completely and sequentially as prescribed. As this practice-level intervention poses a low risk, there are no predefined rules for early termination.

Strategies to improve adherence to interventions {11c}

This study will aim to promote adherence to the intervention by designing a multifactorial intervention rehabilitation program after hip fracture based on a comprehensive geriatric assessment, secondary prevention of fracture and home-based rehabilitation with ActiveHip and Vivifrail intervention based on high-quality evidence of FLS follow-up and international guidelines. The adherence to the Vivifrail programme will be based on the patient's daily record, which will be collected at each follow-up visit throughout the study.

3.9 Relevant concomitant care permitted or prohibited during the trial {11d}

During the trial, participants will not take part in other research projects that involve physical exercise interventions. However, participants are allowed to continue with any other non-conflicting interventions or therapies prescribed by their healthcare providers during the training period.

3.10 Provisions for post-trial care {30}

Not applicable. The intervention in this study will be implemented as part of the usual clinical practice for 12 months. Participants will have access to post-trial care and may choose to incorporate other strategies to improve their medical practice through consultation with a physician.

4. OUTCOMES {12}

4.1 Primary outcome

The primary outcome measure will be the change in functional status over the study period. Functional capacity will be assessed using the Short Physical Performance Battery (SPPB) [27], a single tool that evaluates balance, gait ability and leg strength. The SPPB test has been demonstrated to be a valid instrument for evaluating functional capacity and quality of life following a hip fracture [28]. The total score ranges from 0 (indicating worst functional capacity) to 12 points (indicating best functional capacity). A 1-point change in the score has been demonstrated to be clinical relevance [29].

4.2 Secondary outcomes

The secondary measures will assess constructs related to hip fracture, such as physical and cognitive decline, sarcopenia, nutrition, quality of life and healthcare system utilization. Furthermore, osteoporosis-related parameters will be measured using instrumented examinations, blood tests and dual-energy x-ray absorptiometry (DXA) (see Table 1).

- Functional status: The Barthel index of independence during ADL (0, worst; 100, best) [15], Lawton's Instrumental Activities of Daily Living (IADL) scale (0, worst; 8, best) [30]

and the FAC scale (0, non-functional ambulatory; 5, independent ambulator) [16] will be used.

- Cognitive status [31]**:** The GDS, which describes seven clinically distinguishable global stages from normality to severe dementia of the Alzheimer type, and the 16-question Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), where each question is scored from 1 (much improved) to 5 (much worse) and a cut-off point (average score) of 3.31/3.38 achieves a balance of sensitivity and specificity of cognitive impairment [32], will be used. Delirium assessment during hospitalization will be carried out with the Abbreviated Mental Test 4 (4AT) [33].

- Mood status: Depression will be screened using the 15-item Yesavage Geriatric Depression Scale (YE-GDS; scale: 0, best; 15, worst), which is independently associated with hip fracture [34], and fear of falling will assessed with the Falls Efficacy Scale International (FES-I), where the validated cut-off points are low concern (16–19 points), moderate concern (20–27 points) and high concern (28–64 points) [35].

- Frailty and sarcopenia: Frailty will be screened by the FRAIL questionnaire and verified by modified Fried's frailty criteria [36]. Sarcopenia will be determined by: (i) handgrip strength < 16 kg for women or <27 kg for men; and (ii) appendicular skeletal muscle mass (ASMM)/ height^2 < 7.0 kg/m^2 for men or < 5.5 kg/m^2 for women [37]. Handgrip strength will be measured following the Groningen Elderly Test using a Smedley hand dynamometer [38]. The best of three attempts (with 30 seconds of rest between each attempt) will be recorded. The severity will be defined as gait speed ≤ 0.8 m/s or SPPB ≤ 8 points.

- Quality of life: The EuroQol-5D and the Sarcopenia and Quality of Life (SarQoL) scales will be used to measure the quality of life: the former measures five dimensions of health status (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and is a valid instrument for hip fracture patients [39]; and the latter is a novel validated instrument for measuring the quality of life in sarcopenia patients [40].

- Other clinical assessment: A comprehensive geriatric assessment will be conducted to evaluate geriatric syndromes [41], including falls (defined as unexpected and involuntary loss of balance, causing the person an undesired contact with the ground), polypharmacy (defined as five or more medications) [42] and pain (visual analogue scale: 0, best; 10, worst). Height will be measured with a digital stadiometer. Nutritional assessment will be performed by body mass index (BMI) calculation (weight/height^2) and by completing the Mini-Nutritional Assessment (MNA) tool [43]. Comorbidities will be evaluated with the Cumulative Illness Rating Scale for Geriatrics (CIRS-G) [44], ranging from 0 (best) to 56 (worst). Osteoporosis risk assessment is evaluated using the FRAX and QFracture tools [45] and pain using the Visual Analogue Scale (VAS).

- Adverse events: As per the International Conference on Harmonization Guidelines, serious adverse events will be defined as any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability, or is a congenital anomaly/birth defect [46].

- Use of health sources: this will include hospital admissions, nursing home admissions, visits to primary care physicians, and visits to the emergency department.

- Biochemical analyses: Blood samples will be collected in Vacutainer tubes and centrifuged at 3300 rpm for 10 min at room temperature using a fixed-angle rotor. After centrifugation, the serum in the upper layer will be carefully extracted from the plasma in the bottom layer, divided into 100-µl aliquots and immediately stored at −80°C. Plasma and buffy coat will be also extracted and stored in polypropylene plastic tubes at −80°C until analysis. Bone turnover markers (BTMs) will be measured at the Clinical Neuroproteomics Unit (Navarrabiomed), whereas other measurements will be performed at the Central Laboratory Unit of Navarra (LUNA). All biological samples will be obtained after overnight fasting, between 8 and 10 am. Alkaline phosphatase, 25-hydroxyvitamin D_3 (vitamin D), parathyroid hormone (PTH), calcium, phosphorus, thyroid-stimulating hormone (TSH), creatinine and albumin will be run clinically, immediately after bringing the samples to the laboratory. Due to the high prevalence of hypoalbuminaemia in older adults, the serum concentrations of albumin and calcium will be used to correct the calcium value (calcium-corrected value = Ca + 0.8 [40-albumin]). The calcium-corrected value will be used in the subsequent analysis. C-terminal crosslinked telopeptide of type I collagen (CTX), sclerostin (SCL), bone-specific alkaline phosphatase (B-ALP), procollagen type 1 N propeptide (P1NP) and osteocalcin (OC) will be measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions from frozen samples [47].

- Dual-energy x-ray absorptiometry (DXA): Bone mineral density (BMD) and body composition (fat and lean mass) will be assessed using a Hologic DPX-IQ Discovery dual-energy x-ray absorptiometry (DXA) machine (GE Healthcare, Pollards Wood, UK). To minimize variability, all measurements will be performed by the same operator. The densitometer will be calibrated daily. BMD will be measured in grams per square centimetre at the non-predominant wrist, lumbar spine and proximal femur (neck, trochanter, intertrochanter area and Ward triangle) [48]. The L1 to L4 area will be included by aligning the patient with the axis of the examining table. To measure BMD in the proximal femur, the patient's position will be adjusted by rotating the legs 15–30° to discreetly visualize the smaller femur trochanter. The Z-score and T-score will be calculated in both locations. The coefficient of variation will be 1.14%. Osteopenia and osteoporosis are defined using the World Health Organization standard criteria of a BMD T-score between −1.0SD and −2.49SD less than the young adult mean and less than −2.5SD, respectively [49]. Lean mass will be measured as appendicular skeletal muscle mass (ASM) adjusted for height squared (appendicular skeletal muscle mass index, ASMI) or body mass index (ASM/BMI) [37].

4.3 Sample size {14}

Assuming an alpha error of $\alpha = 5\%$, the simple sample size will be required to achieve a power of 90%, a $\rho = 0.5$, a standard deviation for the SPPB of $\sigma = 2.5$ and detect a 10% difference in the frequency of patients obtaining a functional improvement of more than 1 point in the SPPB between each group will be 138 (69 per group), with an expected proportion of success in the usual clinical practice arm set at 30%. Given the characteristics of the study and the complexity of the patients (older adults after hip fracture), and assuming a loss of 20% of patients in the follow-up, we calculated a sample size of 174 subjects (87 patients in each arm). These calculations are based on a

two-sided test. The 10% difference between both the intervention and the control group, representing a functional improvement greater than 1 point in the SPPB at 12 months between each group, will be considered clinically relevant based on the most relevant clinical variables involved in the functional decline after hip fracture.[50,51].

4.4 Assignment of interventions: Allocation

Sequence generation {16a}

Eligible practices will be allocated to either the intervention or control group using a randomized block approach, with blocks of four (www.randomizer.org).

Concealment mechanism {16b}

The assessment staff at the clinic will be kept blinded to the participant's randomized assignment as well as the main study design and the expected changes in study outcomes for each group. However, it will not be possible to conceal the group assignment from staff who are involved in training the intervention group. Patients and their families will be informed of their random inclusion in one group, but not of which specific group they belong to. If patients or their families inquire about the specific group to which they belong, they will be informed.

Implementation {16c}

When a participant will be deemed eligible and ready to be randomized, one of the research staff will determine which block-group they belong to and opens the next randomization block. The principal investigator will be notified of the site's randomization status and then will send an email to the practice and will inform the study staff.

4.5 Assignment of interventions: Blinding

Who will be blinded {17a}

Once a study participant will be randomized, their assigned study arm won't be kept blinded. However, the principal investigator, assessors and data analysis staff will be kept blinded to the identities of the intervention participants within their group.

Procedure for unblinding if needed {17b}

Not applicable. This study will be an unblinded intervention conducted at the practice level.

4.6 Data collection methods (plans for assessment {18a} and plans to complete follow-up {18b}) and data outcome management {19}

At each visit, data collection and procedures will be carried out. The study data will be stored on an encrypted hard disk partition that can only be accessed by the research team. Only authorized researchers will have access to this password. Participants will be identified using numbers or symbols, and any information that could easily identify them (such as name or address) will not be stored in the dataset. If a participant is prematurely discontinued from the study, they will be considered off-study and will follow the same schedule of events as those who continue in the study.

4.7 Confidentiality {27}

The study will adhere to the Spanish regulations including Law 3/2018 (5 December 2018) for the protection of personal data and to guarantee digital rights; Regulation 2016/679 of the European Union Parliament (27 April 2016) on data protection (RGPD); and Law 41/2002 (14 November 2002), which is a basic regulatory law on patient autonomy.

5. STATISTICAL METHODS

5.1 Statistical methods for primary and secondary outcomes {20a–20c}

We will use the intention-to-treat approach, incorporating all participants as originally allocated post-randomization. Missing data due to drop-outs or deaths will be addressed using multiple imputations. For qualitative variables, we will calculate frequencies and confidence intervals in an initial descriptive analysis. For continuous variables, we will report statistics of central tendency and dispersion, such as means, standard error and confidence intervals, or the median and interquartile range. We will check the normality of continuous variables graphically and through K-M and Shapiro-Wilk tests, comparing their differences between groups using either parametric tests (*t*-tests, mixed-effects models) or non-parametric tests (Mann–Whitney U, Kruskal-Wallis). We will employ a Bonferroni post-hoc test to evaluate statistically significant ($p < 0.05$) group and time differences. Sperarman's (rho) rank correlation coefficients and level of significance (p) will be used to assess the relationship between clinical/functional parameters and biochemical parameters, adjusted for age and sex. The values of *r* will be used to indicate small ($r = 0.10$), medium ($r = 0.30$) and large ($r = 0.50$) size correlations (i.e. effect size). Finally, we will assess the relationship between categorical and dichotomous variables through $\chi 2$ and Fisher exact tests. The level of statistical significance will be set at 0.05. We will analyse the data using SPSS package 23.0.

5.2 Interim analyses {21b}

Not applicable. The study will not include interim analyses or stopping guidelines since the medical practice-level intervention is considered low-risk.

5.3 Methods for additional analyses (e.g. subgroup analyses) {20b}

A secondary analysis of the primary endpoint will account for pre-randomization variables that could potentially predict positive outcomes. These groups will include frailty, sarcopenia, osteosarcopenia and the degree of cognitive impairment. A Bonferroni post-hoc test will be used to evaluate statistically significant ($p < 0.05$) group and time differences.

5.4 Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

Data Monitoring Committee: Mikel Izquierdo (Chair), Fabrizo Zambom-Ferrasi and Lucia Lozano-Vicario.

Trial Steering Committee: Nicolás Martinez-Velilla (Chair), Robinson Ramírez-Vélez and María Gonzalo Lázaro.

Composition, role and reporting structure of the data monitoring committee {21a}

The ActiveFLS study will have an independent data and safety monitoring committee that advises the investigators. The committee members will provide their expertise and recommendations in an individual capacity and report directly to the principal investigator.

Adverse event reporting and harms {22}

To ensure safety, the occurrence of falls and severe fall-related injuries will be monitored. Data on falls are based on medical records during follow-up. Other adverse events relative to the intervention protocol (nutrition, vitamin D, osteoporosis treatment, etc.) will also be monitored. The study team will conduct data monitoring to keep track of any minor or major events that may be associated with the intervention or usual care groups during the study. The chief investigators will review any adverse events or unintended effects detected.

Frequency and plans for auditing trial conduct {23}

There will not plans for auditing trial conduct.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Any changes made to the study protocol will be electronically communicated to all members of the research team and will be reviewed following the policies of the Institutional Review Board.

Dissemination plans {31a}

Dissemination is a recurring item on the agenda for the Department of Orthopaedics Clinics and Geriatrics of Navarre University Hospital (Pamplona, Spain) and the International Conference on Frailty and Sarcopenia Research ICFSR Task Force 2020 [14]. Patient advisors will be involved in reviewing all study materials to ensure that the findings are presented in an understandable and usable way for a broad audience. The study results will be disseminated in various formats, including peer-reviewed publications, conference presentations, blog posts, and policy briefs.

6. DISCUSSION

For this study, we will be developed a multifactorial intervention rehabilitation program after hip fracture. The program will be based on a comprehensive geriatric assessment, secondary prevention of fractures and home-based rehabilitation with ActiveHip and Vivifrail. We will aim to examine whether this intervention could improve functional status after hip fracture. Our ActiveFLS intervention will be developed based on high-quality evidence of FLS follow-up[52,53] and international guidelines [19,23] on hip fracture management, and it is feasible for most types of patients with little support. The use of integrated models of care based on comprehensive geriatric assessment can help align clinical practice with the individual needs of patients and enhance their quality of life [54]. Due to the crucial role of supervision during exercise programs on fracture reduction [55], this protocol will try to adapt current exercise programs to produce consistent supervision and monitoring results.

This study will have several strengths. First, it will be a combination of multiple interventions that were studied separately. This will also generate a problem in which

the hypothetical expected benefit cannot be attributed to a specific intervention. However, given the complexity of managing older adults after a hip fracture, an approach in this direction will be possible to provide greater benefits. Secondly, very old adults will be included with a few exclusion criteria, making this study of broad impact on this heterogeneous population. Thirdly, it will be easily applicable to various regions as it is based on home-based rehabilitation and will not require any specific infrastructure for implementation. The study will also have several limitations. Firstly, it will not include patients with advanced dementia defined as GDS ≥ 5 (a group with a high incidence of hip fracture) because the exercise interventions will not be adapted to this type of population [56]. Secondly, secondary osteoporosis will be also an exclusion criterion due to the variability of management in this population [17]. Thirdly, nursing-home patients will be excluded from the study due to the difficulty of follow-up and adherence to the intervention protocol (especially tele-rehabilitation). It should be noted that the usual care group, although involved in the study, will receive certain components of the ActiveFLS intervention. This is because this arm will include an assessment by Internal Medicine/Geriatrics and a follow-up by Primary Care.

To our knowledge, many studies have been developed for hip fracture management but they usually address issues from the fracture separately (exercise [55], nutrition [57], osteoporosis management [58]) or have low-quality evidence. If our hypothesis will be confirmed and demonstrates that our multifactorial and multicomponent program will improve functional status, it will lead to the development of a new targeted therapeutic pathway for use after hip fracture discharge.

Contribution to the field

Hip fracture is a frequent complication of osteoporosis that is linked to increased morbidity, mortality, and poorer functional recovery. Despite the numerous studies carried out in recent years, the best management in complex cases is still lacking. We hypothesize that multicomponent intervention with tele-rehabilitation could have a role in the evolution of hip fracture, given its multiple levels. This is the first study to assess the effect of a multifactorial intervention that includes tele-rehabilitation based on physical exercise on the recovery of hip fracture patients. If our findings align with our expectations, a possible new pathway and therapeutic protocol after hip fracture could be developed and implemented.

Trial status

The trial commenced recruitment on 1 June 2022 and is currently open for recruitment. Recruitment will cease when 174 participants have been randomized. It is anticipated that this target will be reached by December 2025.

7. ETHICS AND DISSEMINATION

Ethics statement {24}

This study was approved by the Navarre University Hospital Research Ethics Committee (PI_2022/7) on 25 April 2022. **Trial registration:** ClinicalTrials.gov. Identifier: NCT05435534 (Date of registration 25.05.2022). At the point of screening and enrollment, we will acquire written consent from participants or their legal representatives using two

distinct documents. To guarantee participant understanding, we will employ meticulous and comprehensive explanations while securing consent during both the screening and enrollment processes.

Modification of the protocol {25}

Any adjustments made to the protocol that could influence the implementation of the study, potential benefits to the patient, or jeopardize patient safety - including alterations to the study objectives, design, patient population, sample sizes, study procedures, or significant administrative aspects - will necessitate a formal amendment to the protocol. This amendment will be agreed upon by the research team and approved by the Ethics Committee before implementation.

Availability of data and materials {29}

Within 12 months of the conclusion of the study, we will publicly release the de-identified participant-level data used for analyzing research questions through an online data repository.

Conflict of interest {28}

Dr Cedeno-Veloz reports receiving lecture fees from Amgen, Grünenthal, Italfarmaco, Nutricia, Angelini and Abbott, and grant support from Amgen and Abbott.

The authors declare that the research will be conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

BC-V, IC-M, ARG and LL-V will develop the protocol in consultation with FZ-F, AMHO, RR-V, MI and NM-V; BC-V, IC-M, ARG and MGL will be involved in the recruitment and evaluation of the patients, and all authors listed will make an intellectual contribution to the work and approved it for publication.

Funding {5c}

This research will not receive any specific grant from funding agencies in the public or not-for-profit sectors. No sponsors have a role in the design of this study and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

C-VB has received a grant from Amgen and Abbott. MV-N received funding from “la Caixa” Foundation (ID 100010434) under agreement LCF/PR/PR15/51100006.

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DISCUSIÓN GENERAL

Chapter 6

General Discussion.

General discussion

The present doctoral thesis aims to explore a **translational approach to secondary hip fracture prevention and functional improvement in older adults**. This approach is based on the current evidence on the efficacy of pharmacological treatment in preventing hip fractures (chapter 1) and in a real-world database (chapter 2), analysis of biomarkers related to fracture risk (chapter 3), frailty and adverse events (chapter 4) with an editorial resume of their importance of biomarkers in osteoporosis (chapter 5) and, finally, a proposal of a multi-domain intervention system based on telerehabilitation to improve the functional capacity of older patients after a hip fracture (chapter 6).

Efficacy of pharmacological treatment in preventing hip fractures (chapter 1)

Given the gap that exists regarding the treatment of osteoporosis, many authors associated with the world of bone metabolism attribute these circumstances to a mix of factors[1]: discrepancy between the severity of the condition and the associated perceptions by professionals, patients, and healthcare managers[2], the "erroneous" perception of the low efficacy of treatments[3], the "excessive importance" given to side effects (which would be largely overshadowed by the benefits of the medication)[4]. We have commented on the first chapter about the impact on people's lives after a hip fracture and about the absence of adverse events among patients who take and do not take bisphosphonate treatment. However, regarding the efficacy of the treatment, we have reinforced these doubts about its effectiveness.

In Chapter 1, we observed how, despite being statistically significant, the absolute magnitude of benefit is small (NNT of 186) for hip fracture prevention in older adults. This absolute magnitude of benefit increases (NNT of 80) in a secondary prevention scenario but drops dramatically (NNT 324) in those aged ≥75 years, with a higher risk of withdrawals due to adverse events. The benefits of antiresorptive treatment in older people with osteoporosis remain unclear, considering the limited benefit in absolute terms in hip fractures or the lack of benefit concerning mortality, the expected adverse effects, and concerns about conflicts of interest and risk of bias in the studies. In addition, the cost-opportunity (potential benefits that an individual misses out on when choosing one alternative over another) must be considered. While the focus is placed on pharmacological treatments, along with resources, not enough effort is put into measures that could be more effective, such as lifestyle modifications and physical exercise (progressive resistance exercises and balance training)[5]. It should not be forgotten that most fractures in older adults are caused by falls and not by osteoporosis, especially in frail patients. This shows the need for multifactorial intervention to prevent fractures in older adults[6], with antiresorptive as a treatment option, with doubtful efficacy, more likely to be used in secondary prevention (always after a comprehensive assessment).

In summary, this disruptive approach to the situation of the pharmacological treatment of osteoporosis, along with the found evidence, should prompt consideration as to whether healthcare professionals are making a correct approach to osteoporosis through antiresorptives or if new therapeutic targets or different approaches (inflammatory biomarkers for example) should be sought.

Biomarkers related to fracture risk, frailty and adverse events (chapter 2, 3 and 4)

Geroscience is becoming a major hope for preventing age-related diseases and loss of function by targeting biological mechanisms of aging[7]. Historically, the prediction of fracture risk related to osteoporosis has been suboptimal. Within the evaluation of this risk, the most commonly studied factors have been bone mineral density (BMD), bone turnover markers (BTMs), and the Fracture Risk Assessment Tool (FRAX®). The risk calculation through these tools is a topic of debate, as they are considered to have several limitations, especially in older adults[8–10]. It is in this context that biomarkers could play a crucial role in the identification, monitoring, evaluation, and prognosis of osteoporosis in older adults, facilitating and integrating the concept of precision medicine.

In our studies, we utilized PEA to characterize serum cytokines related to signalling and inflammatory processes in older adults with hip fractures. Studying the molecules reported in this study is important because low-grade inflammation is a key factor in the pathogenesis of various widespread diseases, particularly osteoporosis[11]. Although it is not yet understood how circulating peptides reflect activity in musculoskeletal tissues, inflammatory mediators such as reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines directly or indirectly affect bone cells and contribute to the development of osteoporosis[12,13] and plays a key role in the development of various highly prevalent age-related conditions, including frailty [14], and is associated with a higher risk of adverse events[15]. Prior endeavours have concentrated on the identification of prospective biomarkers capable of prognosticating the likelihood of osteoporosis, either as standalone predictors or in conjunction with clinical risk factors and BMD. It is important to understand the pathophysiological mechanism by which the inflammatory markers in this study were able to produce these results.

In our studies, we did not observe significant associations between negative outcomes and other cytokines commonly associated with frailty and bone loss, such as IL-1β, IL-6, IFN-γ, and TNF-α[14]. These mediators play a relevant role in inflammaging and other age-related conditions [16], including multimorbidity, osteoporosis[12,17] and polypharmacy[18]. The absence of differences in IL-1, IL-6, IFN and TNF observed in the present investigation may be due to the comparable characteristics of frail and non-frail participants regarding parameters associated with inflammation and other clinical factors related, such as reductions in BMI, functional status, and body composition scores, align with the established pathophysiology of frailty[19]. The lack of differences in these cytokines may be due to similar inflammaging-related characteristics between the study groups. Hence, based on the current body of evidence, the utilization of these

three prospective biomarkers as predictors of treatment responses to novel anti-osteoporotic medications is not supported[20].

The main strength of these exploratory analyses is their potential to provide a new tool for estimating an individual's risk of experiencing a hip fracture or a major osteoporotic fracture based on serum analysis and identification of specific signatures relevant to distinguishing the risk of adverse outcomes in this group of patients[21]. This could guide clinical decision-making and assist healthcare professionals in identifying individuals who may benefit from interventions to reduce their risk of fractures and adverse outcomes. The development of serum biomarkers for fracture risk in older adults is of interest in clinical practice due to the association of fractures with disability, premature mortality, and increased utilization of medical resources[22].

Effect of Multi-domain intervention system based on telerehabilitation to improve the functional capacity of older patients after a hip fracture (chapter 5)

Clinical guidelines for post-hip-fracture surgery rehabilitation have been introduced in various countries, and several reports summarizing various rehabilitation methods have been published. However, because of the diversity of research methods and differences in the results among published studies, there is insufficient data to conclusively substantiate this potential benefit[23].

For this study, we will develop a multifactorial intervention rehabilitation program after hip fracture. The program will be based on a comprehensive geriatric assessment, secondary prevention of fractures and home-based rehabilitation with ActiveHip and Vivifrail. We will aim to examine whether this intervention could improve functional status after hip fracture. Our ActiveFLS intervention will be developed based on high-quality evidence of FLS follow-up[24,25] and international guidelines [5,26] on hip fracture management, and it is feasible for most types of patients with little support. The use of integrated models of care based on comprehensive geriatric assessment can help align clinical practice with the individual needs of patients and enhance their quality of life [27]. Due to the crucial role of supervision during exercise programs on fracture reduction [28], this protocol will try to adapt current exercise programs to produce consistent supervision and monitoring results. Digital technologies offer tremendous potential for shifting from traditional medical routines to remote medicine and transforming our ability to manage health and independence in aging populations[29].

To our knowledge, many studies have been developed for hip fracture management but they usually address issues from the fracture separately (exercise [28], nutrition [30], osteoporosis management [31]) or have low-quality evidence. If our hypothesis will be confirmed and demonstrates that our multifactorial and multicomponent program will improve functional status, it will lead to the development of a new targeted therapeutic pathway for use after hip fracture discharge.

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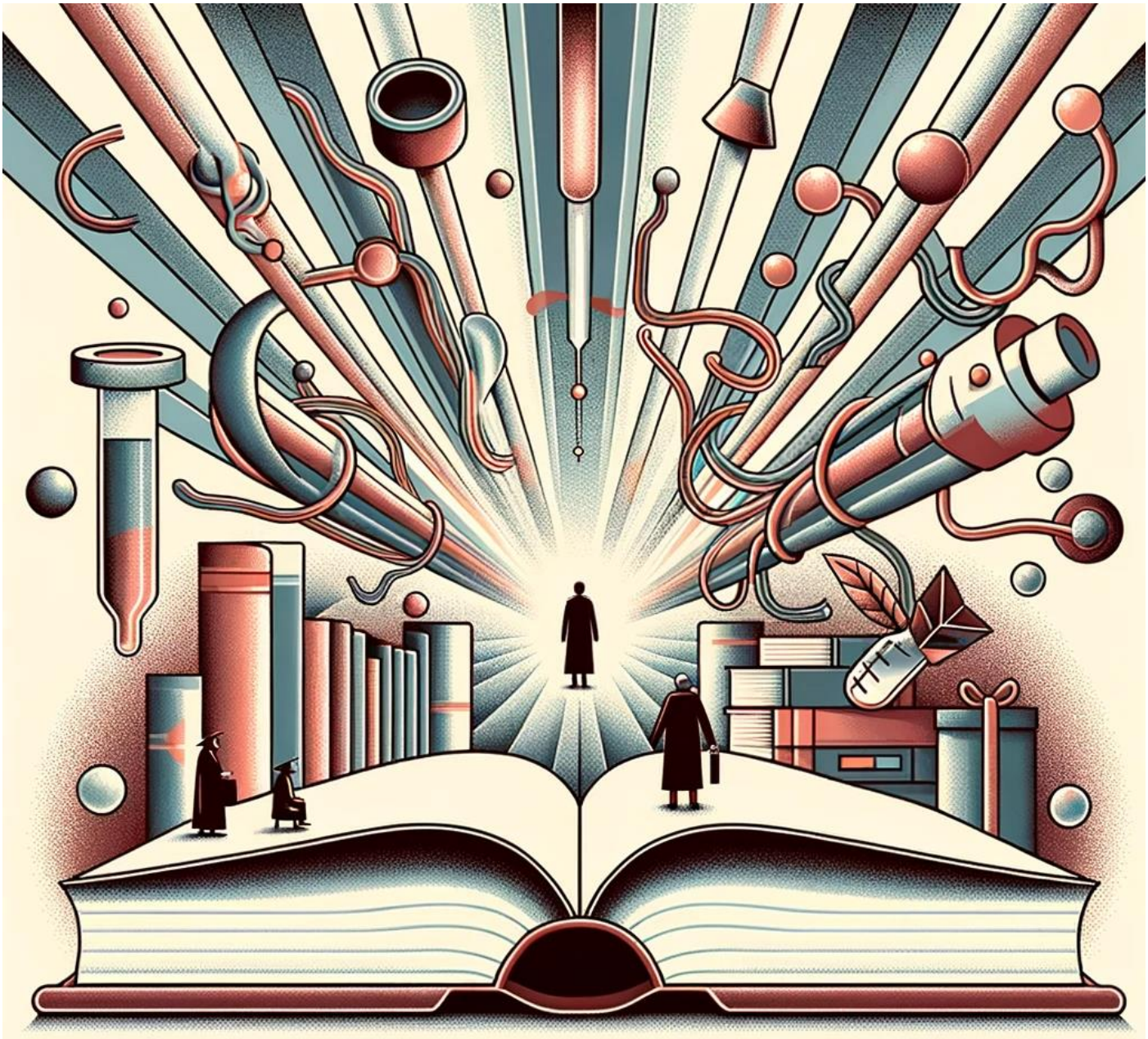
TESIS DOCTORAL
CONCLUSIONES, APLICACIONES PRACTICAS Y PERSPECTIVAS DE FUTURO

Chapter 7

Conclusions, practical applications, and future perspective.

Conclusiones, aplicaciones prácticas y perspectiva de futuro.

Conclusions

Chapter 1: **Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials.**

Conclusion 1: The benefits of antiresorptive treatment in older people with osteoporosis remain unclear, considering the limited benefit in absolute terms (NNT of 186 for hip fracture prevention in older adults; NNT of 80 in a secondary prevention scenario and NNT 324 in those aged ≥75 years), the lack of benefit concerning mortality, and concerns about conflicts of interest and risk of bias in the studies.

Practical Application 1: These doubts about its efficacy underscore the need to undertake a multifactorial approach in the prevention of hip fractures that goes beyond antiresorptive treatment.

Future Perspective 1: This disruptive approach to the pharmacological treatment of osteoporosis should encourage investigators to explore new therapeutic targets or different approaches (such as inflammatory biomarkers, for example) for osteoporosis and hip fracture prevention.

Chapter 2: **Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult.**

Conclusion 2: Biomarkers for osteoporosis will continue to be fundamental tools in geriatric medicine, not only for diagnosis and disease monitoring but also for assessing the underlying biological processes leading to bone loss

Practical application 2: The identification and development of new biomarkers for osteoporosis, especially those that reflect underlying aging mechanisms, have the potential to revolutionize our therapeutic approach to this disease.

Future perspective 2: The strategic use of biomarkers will be key to the prevention and optimal treatment of osteoporosis.

Chapter 3: **Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults.**

Conclusion 3: Three biomarkers (IL-6, CSF1, and CCL7) exhibited a positive relationship with fracture risk based on the FRAX reference tool, while two (LT-α and FLT3LG) had a negative relationship with fracture risk.

Practical Application 3: The potential of this exploratory analysis lies in its ability to provide a new tool for estimating an individual's risk of experiencing a hip fracture. This could guide clinical decision-making and assist healthcare professionals in identifying

individuals who may benefit from interventions to reduce their risk of fractures due to it association with disability, mortality, and increased utilization of medical resources.

Future Perspective 3: Immunology biomarkers could be further studied to estimate fracture risk and potentially delay the onset of osteoporosis and fragility fractures in older adults. However, to increase the clinical relevance of these findings, validation, and replication in longitudinal cohorts with diverse populations are needed.

Chapter 4: **Serum biomarkers related to frailty predicts negative outcomes in older adults with hip fracture.**

Conclusion 4: some biomarkers have potential roles as prognostic biomarkers for adverse outcomes related to hip fractures at a three-month follow-up. CXCL-12 and IL-7 levels were positively associated with improvements in activities of daily living and gait independence, while CXCL-8 levels were linked to hospital readmissions.

Practical Application 4: These findings offer a potential clinical tool for managing complex patients and present a new avenue for further investigation in predicting adverse outcomes associated with hip fracture risk in older adults.

Future Perspective 4: A high-throughput proteomic approach produced a differential serum prototype, paving the way towards the development and implementation of new screening tools. However, to increase the clinical relevance of these findings, validation, and replication in longitudinal cohorts with diverse populations are needed.

Chapter 5: **Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail; exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial.**

Conclusion 5: As most health systems are fragmented and cannot ensure the adequate management of frail and complex individuals after experiencing hip fractures, a multicomponent intervention with tele-rehabilitation could play a role in the evolution of hip fracture care, given its multifaceted levels.

Practical Application 5: Development of integrated clinical practice guidelines for the implementation of functional recovery after hip fracture with tele-rehabilitation (physical exercise based on the @ctivehip and Vivifrail programs), nutrition, secondary prevention of osteoporosis, polypharmacy adjustment, and management of other major comorbidities is advocated.

Future Perspective 5: If our hypothesis is confirmed and demonstrates that our multifactorial and multicomponent program improves functional status, it will lead to the development of a new targeted therapeutic pathway for use after hip fracture discharge.

Capítulo 1: **Eficacia del Tratamiento Antirresortivos en Adultos Mayores con Osteoporosis: Una Revisión Sistemática y Meta-análisis de Ensayos Clínicos Aleatorizados.**

Conclusión 1: Los beneficios del tratamiento antirresortivo en personas mayores con osteoporosis permanecen inciertos, considerando el beneficio limitado en términos absolutos (NNT de 186 para la prevención de fracturas de cadera en adultos mayores; NNT de 80 en un escenario de prevención secundaria y NNT 324 en aquellos de edad ≥75 años), la falta de beneficio en lo que respecta a la mortalidad y las preocupaciones sobre conflictos de interés y riesgo de sesgo en los estudios.

Aplicación Práctica 1: Estas dudas sobre su eficacia resaltan la necesidad de emprender un enfoque multifactorial en la prevención de fracturas de cadera que vaya más allá del tratamiento antirresortivo.

Perspectiva Futura 1: Este enfoque disruptivo para el tratamiento farmacológico de la osteoporosis debería alentar a los investigadores a explorar nuevas dianas terapéuticas o diferentes enfoques (como los biomarcadores inflamatorios, por ejemplo) para la prevención de la osteoporosis y las fracturas de cadera.

Capítulo 2: **Importancia de Biomarcadores en la Osteoporosis: Avances en la Gerociencia del Adulto Mayor**

Conclusión 2: los biomarcadores de la osteoporosis continuarán siendo herramientas fundamentales para la medicina geriátrica no solo en el diagnóstico y monitorización de la enfermedad, así como valoración de los procesos biológicos subyacentes que conducen a la pérdida ósea

Aplicación práctica 2: la identificación y desarrollo de nuevos biomarcadores para la osteoporosis, particularmente aquellos que reflejan los mecanismos de envejecimiento subyacentes, pueden revolucionar nuestro abordaje terapéutico de esta enfermedad

Perspectiva de futuro 2: utilización estratégica de los biomarcadores será clave para la prevención y tratamiento óptimo de la osteoporosis

Capítulo 3: **Efecto de los biomarcadores inmunológicos asociados con la fractura de cadera y el riesgo de fractura en adultos mayores.**

Conclusión 3: Tres biomarcadores (IL-6, CSF1 y CCL7) mostraron una relación positiva con el riesgo de fractura basada en la herramienta de referencia FRAX, mientras que dos (LT-α y FLT3LG) tuvieron una relación negativa con el riesgo de fractura.

Aplicación Práctica 3: El potencial de este análisis exploratorio radica en su capacidad para proporcionar una nueva herramienta para estimar el riesgo de un individuo de experimentar una fractura de cadera. Esto podría guiar la toma de decisiones clínicas y ayudar a los profesionales de la salud a identificar a los individuos que podrían beneficiarse de intervenciones para reducir su riesgo de fracturas ya que las fracturas se asocian con la discapacidad, mortalidad y el aumento de la utilización de recursos médicos.

Perspectiva Futura 3: Los biomarcadores inmunológicos podrían estudiarse más a fondo para estimar el riesgo de fractura y potencialmente retrasar la aparición de osteoporosis y fracturas por fragilidad en adultos mayores. Sin embargo, para aumentar la relevancia clínica de estos hallazgos, se necesita validación y replicación en cohortes longitudinales con poblaciones diversas.

Capítulo 4: **Marcadores séricos relacionados con la fragilidad predicen resultados negativos en adultos mayores con fractura de cadera.**

Conclusión 4: diversos biomarcadores tienen utilidad pronóstica para resultados adversos relacionados con fracturas de cadera a los tres meses. . Los niveles de CXCL-12 y IL-7 estuvieron positivamente asociados con mejoras en las actividades de la vida diaria y la independencia de la marcha, mientras que los niveles de CXCL-8 estuvieron vinculados con reingresos hospitalarios.

Aplicación Práctica 4: Estos hallazgos ofrecen una herramienta clínica potencial para manejar pacientes complejos y presentan una nueva vía para futuras investigaciones de cara a predicción de resultados adversos asociados con el riesgo de fractura de cadera en adultos mayores.

Perspectiva Futura 4: Un enfoque proteómico de alto rendimiento produjo un prototipo de suero diferencial, allanando el camino hacia el desarrollo e implementación de nuevas herramientas de detección. Sin embargo, para aumentar la relevancia clínica de estos hallazgos, se necesitan validación y replicación en cohortes longitudinales con poblaciones diversas.

Capítulo 5: **Efecto de una intervención multicomponente con tele-rehabilitación y el programa de ejercicios Vivifrail sobre la capacidad funcional tras una fractura de cadera: Protocolo de estudio para el ensayo controlado aleatorizado ActiveFLS.**

Conclusión 5: Dado que la mayoría de los sistemas de salud están fragmentados y no pueden garantizar la gestión adecuada de individuos frágiles y complejos después de sufrir fracturas de cadera, una intervención multicomponente con tele-rehabilitación podría desempeñar un papel en la evolución del cuidado de las fracturas de cadera, dada su multifacética aplicación.

Aplicación Práctica 5: Se aboga por el desarrollo de guías de práctica clínica integradas para la implementación de la recuperación funcional después de una fractura de cadera que incluyan tele-rehabilitación (ejercicio físico basado en los programas @ctivehip y Vivifrail), nutrición, prevención secundaria de la osteoporosis, ajuste de la polifarmacia y gestión de otras comorbilidades importantes.

Perspectiva Futura 5: Si nuestra hipótesis se confirma y demuestra que nuestro programa multifactorial y multicomponente mejora el estado funcional, esto conducirá al desarrollo de una nueva vía terapéutica dirigida para su uso después del alta tras una fractura de cadera.


RELEVANT
PAPER

Chapter 8

Relevant Papers.

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Review

Efficacy of Antiresorptive Treatment in Osteoporotic Older Adults: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

B.A. Cedeno-Veloz[1,2], J. Erviti Lopez[3], M. Gutiérrez-Valencia[2,3], L. Leache Alegría[3], L.C. Saiz[3], A.M. Rodríguez García[1], M. Sánchez Latorre[1], R. Ramírez Vélez[2,4,5], M. Izquierdo[2,4,5], N. Martínez-Velilla[1,2,4,5]

1. Geriatric Department, Hospital Universitario de Navarra (HUN), Pamplona, Navarra, Spain; 2. Navarrabiomed, IdiSNA, Navarra Institute for Health Research, Pamplona, Navarra, Spain; 3. Unit of Innovation and Organization, Navarre Health Service, Pamplona, Navarra, Spain; 4. Department of Health Sciences, Public University of Navarra, Pamplona, Navarra, Spain; 5. CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain

Corresponding Author: Bernardo Abel Cedeño Veloz, MD, Geriatric Department (Hospital Universitario de Navarra), Irunlarrea Street 4, 31008 Pamplona, Navarra, Spain, E-mail: ba.cedeno.veloz@navarra.es

Abstract

OBJECTIVES: To investigate concerns surrounding the benefits of antiresorptive drugs in older adults, a systematic review was carried out to evaluate the efficacy of these treatments in the prevention of osteoporotic hip fractures in older adults.

DESIGN: a systematic review and meta-analysis of randomized clinical trials.

SETTING AND PARTICIPANTS: older adults ≥65 years with osteoporosis, with or without a previous fragility fracture. Studies with cancer-related and corticosteroid-induced osteoporosis, participants <65 years and no reported hip fracture were not included.

METHODS: MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, ISI Web of Science and Scopus databases were searched. The primary outcome was hip fracture, and subgroup analysis (≥75 years, with different drug types and secondary prevention) and sensitivity analysis was carried out using a GRADE evaluation. Secondary outcomes were any type of fractures, vertebral fracture, bone markers and adverse events. The risk of bias was assessment with the Cochrane risk of bias tool.

RESULTS: A total of 12 randomised controlled trials (RCTs) qualified for this meta-analysis, with 36,196 participants. Antiresorptive drugs have a statistically significant effect on the prevention of hip fracture (RR=0.70; 95%CI 0.60 to 0.81), but with a moderate GRADE quality of evidence and a high number needed to treat (NNT) of 186. For other outcomes, there is a statistically significant effect, but with a low to moderate quality of evidence. Antiresorptives showed no reduction in the risk of hip fracture in people ≥75 years. The results for different drug types, secondary prevention and sensitivity analysis are similar to the main analyses and have the same concerns.

CONCLUSIONS: Antiresorptive drugs have a statistically significant effect on preventing hip fracture but with a moderate quality (unclear/high risk of bias) and high NNT (186). This small benefit disappears in those ≥75 years, but increases in secondary prevention. More RCTs in very old osteoporotic adults are needed.

Key words: Age-related changes, bone, drug-related, hip fracture, osteoporosis.

Introduction

Osteoporosis is an age-related syndrome that has been associated with poor outcomes such as disability, an increased risk of falls and fractures, loss of independence, high cost to healthcare systems and an increased risk of premature death (1).

Concerns surrounding antiresorptive treatment relate to the fact that studies have focused on surrogate variables, such as bone mineral density (BMD), bone turnover markers (BTM) and non-vertebral fractures (2), and the translation of these variable into clinical relevance, such as fracture prevention, is controversial (3). These drugs have been shown to increase BMD (especially denosumab (4)), change BTM and reduce fragility fractures in osteoporotic patients (5, 6); however, hip fractures have a much greater clinical impact in term of ability, function, quality of life and accommodation (7), and cause more morbidity and mortality compared to other fractures (8).

Antiresorptive drugs are usually the first-line treatment in older adults (≥65 years) (5); however, there is no clear evidence of their usefulness in this older population (3), and there are some concerns surrounding the benefits: firstly, a study based on screening for osteoporosis in older women did not reduce the incidence of osteoporosis-related fractures (9); secondly, according to a recent meta-analysis, no significant association was found between all drug treatments for osteoporosis and the overall mortality rate (10); finally, nonspecific exclusion criteria (comorbities, severe illness, low life expectancy…) in many studies (11) mean that older adults were misrepresented in the studies, especially those over ≥75 years.

Thus, a systematic review was carried out to evaluate the efficacy of antiresorptive treatment in the prevention of osteoporotic hip fractures in older adults ≥65 years with osteoporosis. Other important outcomes in the management of osteoporosis (12) (BMD and BTM) were included, as well as clinically important outcomes for older adults(1) (any type of fractures, mortality, adverse events).

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Methods

Registering in PROSPERO

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (13). The review protocol was registered in the PROSPERO database under registration number CRD42020165960.

Search strategy

The online databases MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, ISI Web of Science and Scopus were searched for studies from inception until 9th July 2021, using a combination of keywords and controlled vocabulary (sText1 in the Supplement), without restrictions on publication year. The review was restricted to studies published in English, Spanish, French, German and Portuguese.

Inclusion criteria

Type of studies

Only randomised controlled trials (RCTs) were included. Trial reports had to present data for at least the primary outcome (hip fracture). When a published, updated study involving the same trial participants was identified, only the latest update was included in the analysis, unless both groups went on to receive drug treatment or there was some crossover between groups.

Type of participants

Participants were older adults (65 years old and above) with osteoporosis, with or without a previous fragility fracture.

Type of intervention

Trial participants were randomised to an antiresorptive treatment compared to placebo or non-osteoporotic treatment. Both arms could include calcium and/or vitamin D. The following drugs were considered as antiresorptives: alendronate, etidronate, ibandronate, risedronate, clodronate, minodronate, pamidronate, tiludronate, zoledronate and denosumab.

Exclusion criteria

Studies with secondary causes of osteoporosis (cancer-related and corticosteroid-induced osteoporosis) were not included. Participants younger than 65 years and studies that with no reported hip fracture were also not included.

Outcome measures

Primary outcome

- Patients with hip fracture.

Secondary outcomes

- Patients with fractures of any type.
- Total number of fractures.
- All-cause mortality.
- Vertebral and non-vertebral fractures.
- Change in BMD at the end of the study from baseline.
- Change in BTM at the end of the study from baseline.
- Total serious adverse events.
- Total cardiovascular events.
- Total gastrointestinal events.
- Withdrawal due to adverse event.

Clinical vertebral fracture events and new vertebral deformities identified by radiological morphometry were reported separately. Serious adverse events were defined according to the International Conference on Harmonisation Guidelines as any event that leads to death, which was life-threatening, required inpatient hospitalisation or the prolongation of existing hospitalisation, resulted in persistent or significant disability, or was a congenital anomaly/birth defect (ICH 1995) (14). All outcomes refer to the number of patients with events, unless otherwise indicated.

Subgroup analysis

We carried out the following subgroup analysis:

- Participants aged ≥75 years (due to the increase of incidence of hip fractures(1), the worse outcomes(7) and the lack of representation of this population(11))
- Different drug types
- Participants with a previous osteoporotic fracture (secondary prevention)

Sensitivity analysis

We restricted the analyses to the following:

- Trials including only participants of 65 years or older.
- Trials with a low or unclear risk of bias.

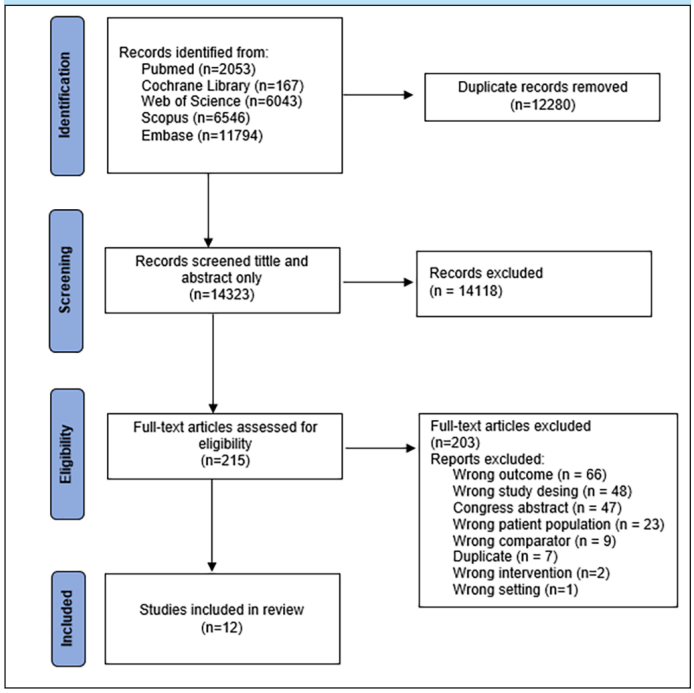
Data collection and analysis

Selection of studies

We used Covidence to screen and classify the identified references. Two authors (BCV and MSL) independently screened the titles and abstracts of all the references to assess for eligibility, and the full text of every article considered for inclusion was obtained and screened for final selection.

Discrepancies were resolved by a third author (ARG). A PRISMA flow diagram of the included and excluded articles is reported (shown in Fig. 1).

Figure 1. PRISMA flow chart of search results and included studies



Data extraction and management

Two review authors independently extracted data from the included trials using a previously prepared data extraction form. Any differences between review authors were resolved by discussion. Cochrane Review Manager 5 software was used for data synthesis and analysis. Quantitative analyses of outcomes were based on the intention-to-treat principle.

The data extraction form included details of study design, randomisation, blinding, assessment of risk of bias, duration of treatment, follow-up, baseline characteristics, number of participants lost to follow-up, interventions, outcomes and statistical analysis.

In the case of studies that included both people younger and older than 65 years and where specific data for older adults were not included in the publication, data from individual participants related to our group of interest were requested and included in the review when available. If individual participant data for the subgroup of interest could not be obtained, we included studies if greater than or equal to 80% of the participants were older than 65 years; a sensitivity analysis was also carried out excluding these studies.

Assessment of risk of bias in included studies

Two authors (BCV and MSL) independently assessed the methodological quality of the included studies, using the Cochrane risk of bias tool(15) to assess selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. These domains were judged as "unclear risk of bias", "low risk of bias" and "high risk of bias". Any disagreement was resolved by discussion with a third author. The quality of evidence for critical and important variables was assessed using Grading of Recommendations Assessment, Development, and Evaluation (GRADE), which includes an assessment of risk of bias, directness of the evidence, heterogeneity, precision of effect estimate and risk of publication bias.

Data synthesis and statistical analysis

We performed the meta-analysis according to the Cochrane handbook version 6 (15). For binary outcomes, we calculated the risk ratio (RR), the absolute risk reduction (ARR) and the number needed to treat (NNT), with their 95% confidence intervals (CI). For continuous outcomes (change in BMD and change in BTM), we calculated the mean difference with standard deviation (SD). The Mantel–Haenszel method was used with a fixed-effect model. Heterogeneity between trials was tested with the Chi-square and I2 test. A p-value of less than 0.1 and/or an I2 value higher than 50% indicated significant heterogeneity. In this situation, we explored the possible causes of heterogeneity by performing sensitivity analyses. A random-effect model was used in the case of unexplained heterogeneity. A Begg's funnel plot was constructed to analyse the possibility of publication bias in the primary outcome. If a meta-analysis could not be performed, a narrative description of the results was provided.

Results

Search results and quality of the studies

We located 26,603 records, of which 14,323 were unique after removing duplicates. After screening the titles and abstracts, the full text eligibility of 215 studies was assessed. This resulted in the final inclusion of 12 RCTs (shown in Fig. 1)

Risk of bias was mostly unclear (shown in Fig. 2) due to allocation concealment, incomplete data and blinding of outcomes. Most of the studies had a low risk of bias from random sequences, blinding of participants and personnel, and selective reporting. The most important biases were the high number of studies with incomplete outcomes and the absence of funding reporting. The Begg's funnel plot was symmetrical, suggesting no publication bias (shown in Fig. S1 in the Supplementary data). The full bias assessment is in Table S1 in the Supplementary data. A summary of the findings and GRADE assessment of each outcome are shown in Table 1.

Study and participants characteristics

Alendronate [4 studies (16–19)], clodronate [1 study (20)], denosumab [1 study (21)], etidronate [1 study (22)], risedronate [1 study (23)] and zoledronate [4 studies (24–27)] were

Table 1. Summary of findings table

Outcome	Studies (number)	Treatment	Control	RR (95% CI)	I^2	Quality of the evidence (GRADE)
Hip fracture	12 (36196)	312/19639	352/16557	0.70 (0.60-0.81)	0%	MODERATE[a]
Any fracture	9 (26502)	1172/13252	1759/13250	0.67 (0.62-0.71)	39%	MODERATE[a]
Total number of fractures	3 (6039)	283/3019	391/3020	0.72 (0.63-0.84)	21%	MODERATE[b]
Mortality	7 (26156)	604/13083	631/13073	0.96 (0.86-1.07)	47%	MODERATE[c]
Vertebral fractures	6 (17906)	291/8953	796/8953	0.39 (0.30-0.50)	64%	LOW[a,d]
Clinical vertebral fractures	6 (25955)	116/12979	314/12976	0.38 (0.28-0.53)	55%	LOW[a,d]
Non-vertebral fractures	7 (29823)	1342/16458	1329/13365	0.79 (0.74-0.86)	0%	LOW[a,c]
Serious adverse events	8 (35297)	6117/19198	5280/16099	0.98 (0.95-1.01)	48%	MODERATE[a]
Total cardiovascular events	2 (9825)	392/4916	363/4909	1.08 (0.94-1.24)	0%	LOW[b,c]
Total gastrointestinal events	6 (12709)	1883/7889	1198/4820	1.01 (0.95-1.07)	0%	MODERATE[a]
Withdrawal due to AE	8 (35302)	2074/19200	1427/16102	1.05 (0.91-1.22)	68%	VERY LOW[a,c,d]

GRADE Working Group grades of evidence; High quality: we are very confident that the true effect lies close to that of the estimate of the effect; Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect; Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect; a .Downgraded one level due risk of bias; b . Downgraded one level due to publication bias; c .Downgraded one level due to imprecision d Downgraded one level due to inconsistency; CI: confidence interval; RR: risk ratio

assessed as drug treatments against hip fracture. Sixteen studies were not included as there was no response to the request for information about participants aged 65 years or older (28–43). One study was not included as no individual data were available (44). Hip fracture as the main outcome was only observed in 3 studies (20, 23, 24).

Overall, the 12 studies included 36,196 participants (19,639 in treatment groups and 16,557 in control groups). The mean age across the studies was 75.2 years. The characteristics of the included studies and participants are listed in Table S2 in the Supplementary data.

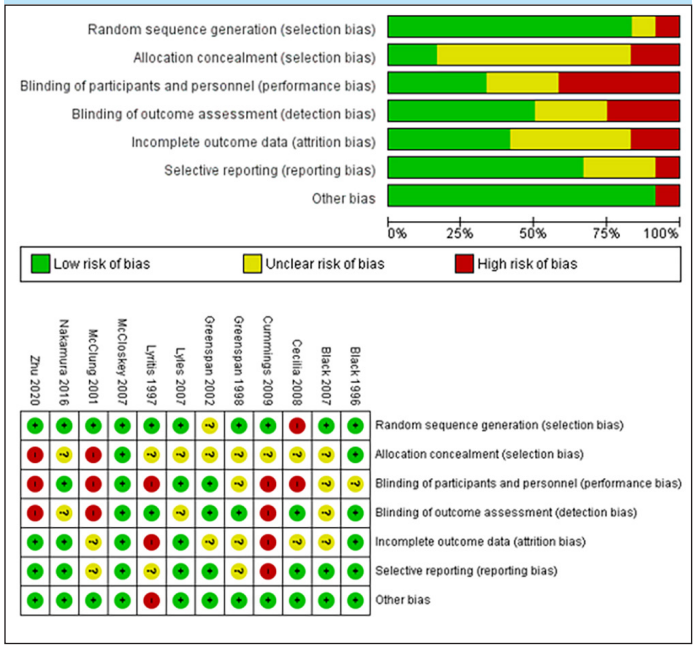
Antifracture effects in osteoporotic patients

Antiresorptive treatment showed a lower risk of hip fracture than the control group: RR=0.70 (95%CI 0.60 to 0.81), 12 studies, I2=0% (shown in Fig. 3). This means an ARR of 0.54% (95%CI 0.25 to 0.82%) and a NNT of 186 (95%CI 123 to 395).

When considering any type of fractures, treatment was associated with a lower risk: RR=0.67 (95%CI 0.62 to 0.71), 9 studies, I2=39%; ARR=4.43% (95%CI 3.68 to 5.18%), NNT=23 (95%CI 19 to 27) (shown in Fig. S2 in the Supplementary data). Most of the studies reported this outcome as any clinical fracture (16, 20, 21, 24–26).

Antiresorptive treatments decreased the risk of vertebral fractures [RR=0.39 (95%CI 0.30 to 0.50), 6 studies, I2=64%]. Two studies (16, 26) included only morphometric fractures in this outcome (shown in Fig. S2 in the Supplementary data). For clinical vertebral fractures, antiresorptive treatments also decreased the incidence [RR=0.38 (95%CI 0.28 to 0.53), 6 studies, I2=55%, shown in Fig. S2 in the Supplementary data]. Furthermore, antiresorptive treatments decreased the incidence of non-vertebral fractures [RR=0.79 (95%CI 0.74 to 0.86), 7 studies, I2=0%, shown in Fig. S2 in the Supplementary data].

Figure 2. Graph showing the risk of bias

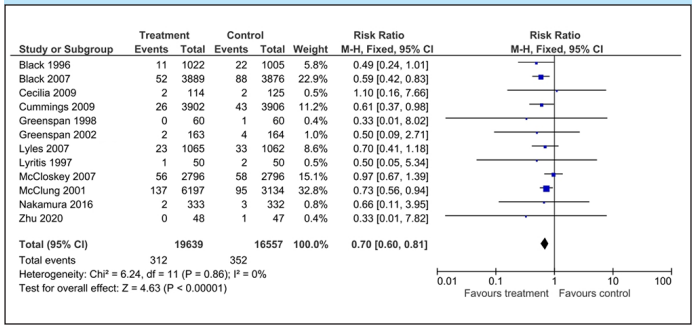


Bone mineral density (BMD) and bone turnover markers (BTM)

All studies showed a benefit in BMD and BTM when they were reported. It was not possible to carry out a meta-analysis, due to the lack of sufficient data. Treatment with antiresorptives increased the average bone mass at all sites compared to the control group. Differences between the groups in the change from basal measure to the last time point were as follows: lumbar spine 7.1% (SD 4.9), including 9 studies; hip 4.7% (SD 1.4), including 10 studies; and femoral neck 4.1% (SD 1.8), including 10 studies. The modification of BTM is hardly

comparable among the studies when using different markers, although the difference between intervention and placebo was favourable to the drug in all cases.

Figure 3. Forest plot for primary analysis of hip fracture in older adults



Adverse events

No statistically significant association was found between active treatment and serious adverse events: RR=0.98 (95%CI 0.95 to 1.01), 8 studies, I2=48%. Overall, there was no association between treatment and risk of mortality: RR=0.96 (95%CI 0.86 to 1.07), 7 studies, I2=47% (shown in Fig. S3 in the Supplementary data).

Only 3 studies (21,24,25) reported cardiovascular events. Cummings et al. (21) reported the number of patients with a cardiovascular event and did not find any differences between groups (RR=1.04 (95%CI 0.85 to 1.27). Black et al. (24) and Lyles et al. (25) reported the total number of cardiovascular events and found no association with antiresorptive treatments: RR=1.08 (CI 0.94 to 1.24), I2=0% (shown in Fig. S3 in the Supplementary data). No differences in gastrointestinal adverse events were observed either, but these were also underreported: RR=1.01 (95%CI 0.95 to 1.07), 6 studies, I2=0% (shown in Fig. S3 in the Supplementary data). There may be a higher risk of withdrawal due to adverse events with antiresorptive treatments, although the significant heterogeneity did not allow firm conclusions to be drawn (8 studies, I2=68%, shown in Fig. S3 in the Supplementary data). In Nakamura et al.(26), withdrawals were differentiated according to adverse events, protocol violation, withdrew consent and others.

Subgroup analysis

Four studies provided data for patients aged 75 years or older: the study of McCloskey et al. (20), group 2 of the study by McClung et al. (23), Black et al. (45) (from The European Public Assessment Report) and a post hoc analysis published by Boonen et al.(46). A statistically significant reduction in the risk of hip fracture was not observed in this age group: RR=0.81 (95%CI 0.66 to 1.00), I2=40%; ARR 0.31% (-0.19% to 0.8%), NNT 324 (125 to -522), 4 studies. Treatment was associated with a reduced risk of fractures of any type and non-vertebral fractures (number of fractures). A higher risk of withdrawals due to adverse events was found, based on data from only one study. Results for this subgroup are shown in Table S3 and Fig. S4 in the Supplementary data.

Results according to drug type (bisphosphonates or denosumab) showed consistent results across the different subgroups (shown in Fig. S5 in the Supplementary data). Drug type did not seem to be a source of heterogeneity in those outcomes with significant heterogeneity (vertebral fractures, clinical vertebral fractures and withdrawals due to adverse effects).

Six studies included only participants with previous osteoporotic fractures (secondary prevention) (16, 17, 19, 22, 25, 27), while one reported separate results for those participants with vertebral fractures at baseline (23). In this subgroup of patients, differences in the risk of hip fracture between treatment and control groups were also found: RR=0.55 (95%CI 0.40 to 0.77), I2=0%; ARR 1.3% (0.47% to 1.98%), NNT=80 (50 to 211), 7 studies. There was an association between treatment and a reduced risk of fractures of any type, mortality, serious adverse events, vertebral fractures (total and clinical) and non-vertebral fractures. Data are shown in Table S4 in the Supplementary data.

Sensitivity analyses

We performed a sensitivity analysis restricting to the seven studies including only participants aged 65 years or older (17, 18, 20, 22–24, 26). Results were similar to the main analyses and are shown in Table S5 in the Supplementary data.

We also performed a sensitivity analysis restricting to trials with low or unclear risk of bias, with a total of seven studies (17, 18, 20, 22–24, 26) for the main outcome. Results were similar to the main analyses and are shown in Table S6 in the Supplementary data.

Discussion

To the best of our knowledge, this is the first systematic review on antiresorptive treatments to focus on hip fracture as an outcome for older adults. Despite the relatively large number of RCTs and individuals studied, results for hip fracture are only reported in 12 RCTs with older adults. Despite the fact that hip fracture is the most clinically relevant fracture, most reviews focus on the overall efficacy, and vertebral and non-vertebral fractures (47, 48).

Antiresorptive drugs are widely used as a first-line drug therapy for the prevention of osteoporotic fracture, and their efficacy for hip fracture prevention has been confirmed in this review; however, the absolute magnitude of benefit is small (NNT of 186) (Fig. 3). This result is consistent with previous results in meta-analyses focusing on hip fractures in postmenopausal patients of all ages, with an NNT of 175 (2). Also, the GRADE analysis shows a moderate quality of evidence in the main outcome, with quality concerns in many of the outcomes. Most of the outcomes show a low to moderate quality of evidence.

When secondary prevention is analysed as a subgroup, the absolute magnitude of benefit increases (NNT of 80) (Table S4 in the Supplementary data), which is similar to

previous evidence (6). However, this benefit drops dramatically (NNT 324) in those aged ≥75 years, with a higher risk of withdrawals due to adverse events (Table S3 and Fig. S4 in the Supplementary data). This shows the need for multifactorial intervention in order to prevent fractures in much older adults (49).

Four studies could be included in the meta-analysis regarding much older adults. The reduction in the risk of hip fractures did not achieve statistical significance and the analysis shows higher heterogeneity in this population. The only study of the four studies that shows a reduction in the risk of hip fracture was the denosumab FREEDOM Trial, the results of which should be considered with caution, given the high risk of bias.

A post hoc analysis published by Boonen et al. presented the results of pooled data from the HORIZON Pivotal Fracture Trial (24) and the HORIZON Recurrent Fracture Trial (25), with women aged 75 years or older (50), and did not show a reduction in hip fractures. Another post hoc analysis combining three large randomised double-blind clinical trials with risedronate (HIP, VERT-NA and VERT-MN) in women over the age of 80 did not report hip fractures in this population (51); however, the incidence of osteoporosis-related non-vertebral fractures was not significantly lower than the placebo group after 3 years. A post hoc analysis of the Fracture Intervention Trial (FIT) (52) including patients from 75 to 85 years, with and without previous fractures, showed an ARR of 53 women per 10,000 patient-years at risk (PYR) for hip fractures, but participants aged 75 years or older comprised only 25% of the FIT.

From these RCTs and post hoc analyses, it is clear that the evidence is not only sparse, but that it also suggests no (or marginal) treatment benefit in hip fractures in older adults aged over 75 years, and even use of in mortality reduction.

The other source of evidence are the observational studies. These studies found a relationship between the antiresorptive treatment and the reduction of fractures in older adults (53). The complication of developing RCTs in older adults is always commented upon (54) and the use of observational studies is recommended for this population. Nevertheless, the reduction of fractures was seen to be independent from the use of antiresorptives (55); in fact, fractures decline despite from the reduction in antiresorptive regimen (56). The inconsistency between these results and our findings highlights the need for more RCTs in older osteoporotic adults (57). Moreover, studies of fall prevention strategies (the main cause of hip fractures in older adults), including bone active drugs, are needed in this high risk population (2, 3).

Although not the main outcome in this study, other types of fracture were significantly reduced with antiresorptive treatment, with a more favourable NNT, but their definition and clinical relevance is controversial. Sensitivity analyses for trials with all participants ≥65 years are consistent with the overall results. For men, no RCTs with fracture as the primary endpoint exist, and this lack of information shows us the importance of trials in men with primary osteoporosis.

Surrogate outcomes in osteoporosis, like BMD and BTM, show a good response to antiresorptive treatment in our review. However, despite the importance that these outcomes have in osteoporosis studies and the literature (12, 58), the clinical usefulness of osteoporosis approaches in older adults is doubtful (3).

Regarding mortality, our analysis does not show any association between osteoporosis treatments and a risk reduction. Despite previous favourable reviews of this fact (59), more recent analyses also show that treatment for osteoporosis does not reduce overall mortality (10). Our findings are more aligned with the second hypothesis due to the lack of biological mechanisms that explain benefits other than fracture prevention. The low number of side effects in the studies contrasts with the later findings (6), where many are associated with gastrointestinal effects. Other potential adverse events, such as osteonecrosis of the jaw (60) and atypical femoral fractures (61), were not reported in these studies but are known side effects linked to these treatments. The median follow-up of the studies (36 months) may be the reason for this lack of events. New potential side effects, such as rebound-associated fractures after denosumab withdrawal (62), add more concern about the long-term safety of these drugs.

The benefits of antiresorptive treatment in older people with osteoporosis is unclear, considering the limited benefit in absolute terms in hip fractures or the lack of benefit with regard to mortality, the expected adverse effects, and concerns about conflicts of interest and risk of bias in the studies. In addition, the cost-opportunity (potential benefits that an individual misses out on when choosing one alternative over another) must be considered. While the focus is placed on pharmacological treatments, along with resources, not enough effort is put into measures that could be more effective, such as lifestyle modifications and physical exercise (progressive resistance exercises and balance training) (63). It should not be forgotten that most fractures in older adults are caused by falls and not by osteoporosis, especially in frail patients. This shows the need for multifactorial intervention in order to prevent fractures in older adults (49), with antiresorptives as a treatment option, with doubtful efficacy, more likely to be used in secondary prevention (always after a comprehensive assessment).

The strengths of the study are the inclusion of all studies with hip fracture as an outcome in older adults and the focus on clinical outcomes (such as hip fracture) rather than surrogate outcomes. In addition, an extensive risk of bias and GRADE evaluation was carried out. The main limitations are related to the quality of the studies, most of which showed an unclear/high risk of bias. Meta-analysis results should therefore be interpreted with caution. Furthermore, data in older participants are mostly based on subgroups from larger studies on postmenopausal studies in women, with no results in older men. The limited number of studies in some of the subgroup analyses is also a limitation, although cancer-related and corticosteroid-induced osteoporosis were not considered in our analysis. Some studies have nonspecific exclusion criteria, such as severe illness (20, 23, 32), low life expectancy (25) or number of prevalent fractures (21, 50), making generalisation of the study data complicated. In addition, data on the baseline situation of the participants (very important in the older population) are missing.

Conclusion

Antiresorptive drugs have a statistically significant effect on preventing hip fracture, but with a moderate quality of evidence and low effect in absolute terms (NNT 186). Alendronate, denosumab, risedronate and zoledronate may have a significant role in preventing hip fracture, but the evidence is based on studies with a risk of bias and conflicts of interest. Evidence on very old adults (≥75 years) is not significant and comes from very few studies. The greatest advantage is found in secondary prevention (NNT 80). More RCTs in very old osteoporotic adults are therefore needed.

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Data Availability: All data generated or analysed during this study (template data collection forms; data extracted from included studies; data used for all analyses; analytic code) are included in this article or its supplementary material files. The review protocol can be accessed on the PROSPERO database (CRD42020165960). Further enquiries can be directed to the corresponding author.

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EDITORIAL

Importancia de Biomarcadores en la Osteoporosis: Avances en la Gerociencia del Adulto Mayor

Importance of Biomarkers in Osteoporosis: Advances in the Geroscience of the Older Adult

Bernardo Cedeno-Veloz[a,b,*] y Nicolás Martínez-Velilla[a,c]

[a] Geriatric Department, Hospital Universitario de Navarra (HUN), Pamplona 31008, Navarra, Spain
[b] Navarrabiomed, IdiSNA, Navarra Institute for Health Research, Pamplona 31008, Navarra, Spain
[c] CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain

La gerociencia, una intersección entre la biogerontología y la medicina, es una disciplina prometedora que busca desentrañar los procesos moleculares y celulares del envejecimiento[1] y dentro de esta ciencia, el área de la geriatría tiene diferentes focos, entre los que destaca la osteoporosis. Debido a su naturaleza crónica y a su alta prevalencia en una población cada vez más envejecida, tiene importantes consecuencias humanas y socioeconómicas, que incluyen entre otras, morbilidad, discapacidad y mortalidad, además de conllevar un importante aumento del gasto sanitario derivado de las fracturas[2]. De hecho, en España se estima que ocurrieron 285000 fracturas osteoporóticas en 2019, con un coste de 4.3 billones de € y un probable incremento del 29.6% en el periodo 2019-2034. Este impacto a nivel de morbilidad y gasto sanitario es más acusado en la población femenina, siendo el 79.2% del total[3]. En este contexto sociosanitario es evidente que debemos incrementar nuestros esfuerzos en detectar población con riesgo de fractura por el alto beneficio potencial que tendría una detección precoz de dicha situación clínica.

Históricamente, la predicción del riesgo de fractura relacionada con la osteoporosis ha sido subóptimo. Dentro de la evaluación de este riesgo, los factores más comúnmente estudiados han sido la densidad mineral ósea (DMO), los marcadores de recambio óseo (MRO) y la calculadora de riesgo Fracture Risk Assessment Tool FRAX®. El cálculo del riesgo mediante estas herramientas es un tema en debate, al considerarse que presentan una serie de limitaciones, especialmente en adultos mayores[4]. La DMO es un factor de riesgo clásico para las fracturas y se ha estudiado ampliamente, pero su baja sensibilidad es una de las razones por las cuales no se recomienda su uso exclusivo para evaluar el riesgo de fracturas en el cribado poblacional [5], siendo destacable la baja correlación observada entre la pérdida de DMO y su valor predictivo en el riesgo de fractura[6]. Del mismo modo, los MRO tampoco mejoran la predicción del riesgo de fractura o pérdida ósea del paciente, viéndose limitada su utilidad a la monitorización de la terapia con bisfosfonatos orales u otros fármacos antirresortivos[7]. A diferencia de los factores para la evaluación del riesgo de fractura antes mencionados, el FRAX es la calculadora de riesgo de fractura de referencia, la cual incluye múltiples parámetros, pero su aplicabilidad es limitada por tratarse, por diseño, de un método de cálculo simple para ser utilizado en atención primaria, y utiliza variables categóricas sin considerar los efectos dosisdependientes de factores de riesgo claves[8]. De esta manera se estásubestimando el riesgo de fracturas[9] y no es una calculadora adecuada para adultos mayores de 90 años ya que dicha tipología de pacientes fueexcluida en su diseño[10]. Aunque es cierto que esta calculadora mejora la predicción de fracturas en comparación con la medición de la DMO aislada, su capacidad de predicción del riesgo de fractura varía en diferentes poblaciones de estudio[11] al ofrecer una perspectiva superficial y poco personalizada hasta el punto de haberse considerado de dudosa eficacia en la población española[4] y también a nivel mundial[12]. Es en este contexto en el que los biomarcadores podrían jugarun papel crucial en la identificación, seguimiento, evaluación y pronóstico de la osteoporosis en el adulto mayor, facilitando e integrando el concepto de medicina de precisión.

Es crucial desarrollar una nueva estrategia para comprender, predecir y abordar la osteoporosis desde el punto de vista de la medicina de precisión ya que el tratamiento de un paciente estratificado como de alto riesgo con los medios tradicionales resultaría insuficiente para abordar el deterioro sistémico en la microestructura ósea [13]. Tanto es así, que desde sociedades como la European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO)[14], se han propuesto una serie de biomarcadores relevantes para el estudio de la salud musculo-esquelética. Con la ayuda analítica, estas tecnologías han estado proporcionando una imagen cada vez más detallada de las alteraciones moleculares y celulares que subyacen a la osteoporosis y la variabilidad entre pacientes a nivel molecular y celular[15].

Por otro lado, mediante el uso de técnicas multi-ómicas, se han identificado varias proteínas asociadas a la densidad mineral ósea y fractura durante el perfilado de proteomas humanos en diferentes poblaciones. En el estudio “Fracturas Osteoporóticas en Hombres”,

* Autor para correspondencia.
Correo electrónico: abel.medifmsa@gmail.com (B. Cedeno-Veloz).

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Nielson CM et al.[16] encontró una asociación entre cinco proteínas y la aparición de fractura de cadera. También un reciente estudio de Al-Ansari et al[17] demostró una serie de biomarcadores diferenciados entre control, osteopenia y osteoporosis. Actualmente nuestro grupo ha obtenido resultado preliminares relacionados a una serie de biomarcadores diferenciados entre grupo de pacientes fracturados y no fracturados que se han relacionado con el riesgo de fractura según la escala FRAX[18], yendo pues un paso más allá al no solo relacionar biomarcadores con osteoporosis sino con el riesgo de fractura. Sin embargo, la mayoría de estos hallazgos son en un grupo pequeño, estudios transversales o solo en hombres, requiriendo su validación en estudios longitudinales con mayor presencia de mujeres al ser el grupo más afectado. Así mismo, aunque la identificación de las proteínas y las vías metabólicas involucradas en la regulación del metabolismo óseo en diferentes poblaciones ha aumentado, el conocimiento preciso de los mecanismos biológicos subyacentes a la baja densidad mineral ósea es incompleto.

Otra área de estudio dentro de la gerociencia de la osteoporosis es la senescencia celular, un estado de aumento de las células senescentes en el microentorno óseo característico del envejecimiento. Se ha demostrado que este acumulo contribuye a la patogénesis de la osteoporosis y por lo tanto, los biomarcadores de senescencia como la beta-galactosidasa ácida senescente y p16INK4a, tendrían el potencial de identificar pacientes con un alto grado de senescencia ósea y, por consiguiente, un mayor riesgo de osteoporosis. Así mismo se ha demostrado que enfoques que eliminan las células senescentes o afectan la producción del secretoma proinflamatorio previenen la pérdida ósea relacionada con la edad en ratones[19].

Los avances recientes en la ciencia del microbioma también han proporcionado nuevas perspectivas sobre la osteoporosis. Existe una correlación entre la composición del microbioma intestinal y la salud ósea, lo que sugiere que los biomarcadores basados en el microbioma podrían ser de utilidad futura para el diagnóstico y tratamiento de la osteoporosis[20]. Además, la investigación genómica, como la secuenciación de nueva generación (NGS), también está permitiendo el descubrimiento de biomarcadores genéticos. Por ejemplo, polimorfismos en genes que codifican proteínas implicadas en el metabolismo óseo, como RANK, RANKL, y OPG, podrían ser predictivos de la osteoporosis. Además, la metilación del ADN y la expresión de microARNs también están siendo investigados como posibles biomarcadores[21].

Al incrementar nuestra comprensión de la biología del envejecimiento, estamos en una posición cada vez más fortalecida para desarrollar e implementar terapias dirigidas para la osteoporosis. La medicina de precisión[15], que busca personalizar los tratamientos basándose en el perfil biológico único de cada paciente, tiene un potencial inmenso en este dominio. La identificación y desarrollo de nuevos biomarcadores para la osteoporosis, particularmente aquellos que reflejan los mecanismos de envejecimiento subyacentes, pueden revolucionar nuestro abordaje terapéutico de esta enfermedad

En resumen, la gerociencia se encuentra en una etapa excitante de avance, y los biomarcadores de la osteoporosis continuarán siendo herramientas fundamentales para la medicina geriátrica. Estos indicadores no solo facilitarán el diagnóstico y monitoreo de la enfermedad, sino que también proporcionarán una ventana hacia los procesos biológicos subyacentes que conducen a la pérdida ósea. En esta era de medicina de precisión, la utilización estratégica de los biomarcadores será clave para la prevención y tratamiento óptimo de la osteoporosis, mejorando así la salud y la calidad de vida del adulto mayor

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Immunity & Ageing

BRIEF REPORT Open Access

Effect of immunology biomarkers associated with hip fracture and fracture risk in older adults

Bernardo Abel Cedeno-Veloz[1,2,3*], Lucía Lozano-Vicario[1,2], Fabricio Zambom-Ferraresi[2,3,4], Joaquín Fernández-Irigoyen[2,5], Enrique Santamaría[2,5], Alba Rodríguez-García[1], Roman Romero-Ortuno[6], Jaime Mondragon-Rubio[7], Javier Ruiz-Ruiz[7], Robinson Ramírez-Vélez[2,3,4], Mikel Izquierdo[2,3,4] and Nicolás Martínez-Velilla[1,2,3]

Abstract

Osteoporosis is a skeletal disease that can increase the risk of fractures, leading to adverse health and socioeconomic consequences. However, current clinical methods have limitations in accurately estimating fracture risk, particularly in older adults. Thus, new technologies are necessary to improve the accuracy of fracture risk estimation. In this observational study, we aimed to explore the association between serum cytokines and hip fracture status in older adults, and their associations with fracture risk using the FRAX reference tool. We investigated the use of a proximity extension assay (PEA) with Olink. We compared the characteristics of the population, functional status and detailed body composition (determined using densitometry) between groups. We enrolled 40 participants, including 20 with hip fracture and 20 without fracture, and studied 46 cytokines in their serum. After conducting a score plot and two unpaired t-tests using the *Benjamini-Hochberg* method, we found that Interleukin 6 (IL-6), Lymphotoxin-alpha (LT-α), Fms-related tyrosine kinase 3 ligand (FLT3LG), Colony stimulating factor 1 (CSF1), and Chemokine (C-C motif) ligand 7 (CCL7) were significantly different between fracture and non-fracture patients ($p < 0.05$). IL-6 had a moderate correlation with FRAX ($R^2 = 0.409$, $p < 0.001$), while CSF1 and CCL7 had weak correlations with FRAX. LT-α and FLT3LG exhibited a negative correlation with the risk of fracture. Our results suggest that targeted proteomic tools have the capability to identify differentially regulated proteins and may serve as potential markers for estimating fracture risk. However, longitudinal studies will be necessary to validate these results and determine the temporal patterns of changes in cytokine profiles.

Keywords Cytokines, Hip fractures, Biomarkers, Prognosis, FRAX

[†]Mikel Izquierdo and Nicolás Martínez-Velilla jointly supervised this work.

*Correspondence:
Bernardo Abel Cedeno-Veloz
ba.cedeno.veloz@navarra.es; cedeno.136621@e.unavarra.es
[1]Geriatric Department, Hospital Universitario de Navarra (HUN), 2 Navarrabiomed, Pamplona, Navarra, IdiSNA 31008, Spain
[2]Navarrabiomed, Navarra Medical Research Institute, Pamplona, Navarra 31008, Spain
[3]Department of Health Sciences, Public University of Navarra, Pamplona, Navarra 31008, Spain
[4]CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Madrid 28029, Spain
[5]Clinical Neuroproteomics Unit, Navarrabiomed, Pamplona 31008, Spain
[6]Discipline of Medical Gerontology, School of Medicine, Trinity College Dublin, Dublin, Ireland
[7]Department of Orthopaedics Clinics and Traumatology, University Hospital of Navarre (HUN), Pamplona, Navarra 31008, Spain

Introduction

Osteoporosis (OP) is delineated systemic skeletal disorder associated with a reduced quantity of bone mineral mass and the microarchitectural degradation of the bone's tissue structure, which increases the risk of fragility fracture [1]. Due to its chronic nature and prevalence in an ageing population, OP has significant human and socioeconomic consequences, including morbi-mortality and disability [2]. Therefore, identifying high risk populations and exploring potential biomarkers associated related to bone changes is crucial for effective health promotion [3].

Clinical guidelines serve as a foundation for assessing fracture risk [1] and promoting early interventions. Nonetheless, the most frequently examined parameters, such as bone mineral density (BMD), bone turnover markers (BMT) and FRAX® [4], exhibit limited efficacy, particularly in older population. BMD has been extensively researched and is recognized as a conventional risk determinant for fractures, but its low sensitivity is one of the reasons why population-based screening for BMD is not recommended for risk fracture assessment [1]. Another contributing factor is the relatively weak correlation between the loss of BMD and the capability to accurately forecast the risk of fractures [5]. BTM does not enhance fracture risk or bone loss prediction within an individual and is primarily useful in monitoring oral bisphosphonate therapy [6] or other osteoporosis treatments. FRAX, despite its widespread usage as a simple and primary care-applicable tool for estimating fracture risk and first-choice tool in most of clinical guidelines [1], possesses a limitation in that it does not accommodate dose-response considerations for diverse risk factors [7, 8], potentially underestimating fracture risk [9], and is unsuitable for adults aged over 90 [4]. While FRAX advances fracture prognostication beyond the capabilities of Bone Mineral Density (BMD) measurements alone, the accuracy of its fracture risk prediction displays variation across distinct study populations [10]. Consequently, there is a compelling need to investigate innovative approaches for estimating fracture risk. Presently, a revised version of FRAX is under development, with the intention of addressing the aforementioned limitations [11].

Bone loss in the ageing population is commonly attributed to its endocrine origin. However, comorbidities, genetics, and the immune system of the patient can also contribute to bone loss. A conventional approach to treatment is insufficient to address the systemic impairment in bone microstructure, making it crucial to develop a new strategy for understanding osteoporosis [12]. Analysing proteomes can provide insight into patients' pathophysiological status [13], which is particularly relevant given the observed link between pro-inflammatory states and fractures that are associated with an accelerated decrease in bone mineral density BMD [14, 15].

Chaput et al. [16] found three significant differences between osteoporosis and osteoarthritis (OA) in middle-aged women. In The Osteoporotic Fractures in Men Study, Nielson CM et al. [15] found an association between five proteins and incident hip fracture. When performing proteomic analyses on the osteoporotic population, the comparison population is usually patients with OA [17] due to the ease of obtaining bone tissue. Additionally, there are similarities and even overlaps between risk factors [18, 19] and an inverse relationship between hip fractures and hip OA [20]. In this overlap context, immunology biomarkers that enable differentiation between inflammation in bone (OP) and joint (OA) represent an encouraging possibility for the diagnosis and prognosis of osteoarticular diseases [21]. Even more, the role of immune system in the pathophysiology of osteoporosis [22] suggest that immune dysregulation can trigger inflammatory conditions that negatively affect bone integrity [23]. Even in the acute phase, both hip fracture and hip replacement show a similar elevation of acute phase factors [24, 25]. Therefore, proteomic analyses can aid in understanding the pathophysiology of osteoporosis, the different with other chronic autoimmune rheumatic diseases and lead to the development of more effective treatment strategies.

Insufficient understanding of the pathophysiological and molecular mechanisms of OP and other chronic bone conditions has led to the lack of mechanism-based diagnoses [13]. However, proteomic approaches that examine changes in biomarkers show promise in developing minimally invasive diagnostic biomarkers for OP. Unfortunately, data from older adults are scarce, emphasizing the need to identify valid biomarkers for both diagnosing and evaluating treatments and interventions.

More studies are required to address the knowledge gap concerning the activated molecular mechanisms in OP and to identify potential biomarkers, including aspects of the clinical presentation. In this cross-sectional study, we used a targeted proteomic approach to examine the relationship between immunology biomarker profiles, fracture status, and fracture risk. Our primary aim was to compare immunology biomarker profiles between two patient groups: those with hip OA who were candidates for hip arthroplasty and those with hip fracture who were also candidates for hip arthroplasty. Subsequently, we investigated the association between these profiles and fracture risk, as determined using the FRAX reference tool (as the most extensively risk assessment tool).

Materials and methods

Patients and study design

This observational, cross-sectional study scrutinized patients who were referred to the Orthopedic Clinics and Traumatology Services at the University Hospital of Navarre (Pamplona, Spain) between March and October 2021. The criteria for participant inclusion were age≥70 years, a diagnosis of osteoarthritis of the hip being a candidate for hip arthroplasty, a diagnosis of subcapital hip fracture being a candidate for hip arthroplasty, and spinal anaesthesia as the elective technique. The diagnosis of hip OA was based on the criteria of the American College of Rheumatology [26]. Exclusion criteria were diseases that cause secondary OP (e.g., glucocorticoid-induced osteoporosis, rheumatoid arthritis, and autoimmune diseases), terminal illness (advance stages pathologies and cancer) or refusal to participle in the study. We screened 256 older adults, with 83 meeting the inclusion criteria. In our selection process, 112 individuals were excluded due to secondary osteoporosis, 48 due to terminal illnesses, and 13 owing to their refusal to provide informed consent. Consequently, a final cohort of 40 participants was selected for the study, while an additional 43 were excluded. The main reason for exclusion at this point was the change of the day of surgery, which did not allow for the collection and processing of samples. The study flowchart is shown in Appendix A.3. The participants were classified into two groups: hip OA candidates for hip arthroplasty (n=20) and hip fracture candidates for hip arthroplasty (n=20). The study received approval from the Institutional Review Board of the University Hospital of Navarre (Pamplona, Spain), under the approval reference PI_2020/125. Every participant involved in the study furnished written informed consent prior to their inclusion in the research.

Clinical and functional parameters

A comprehensive medical assessment was performed including comorbidities (Cumulative Illness Rating Scale for Geriatrics, CIRS-G) [27], osteoporotic treatments and polypharmacy (defined as regular use of at least five medications). Functional status was assessed by the Barthel index [28], pre-intervention mobility by the FAC (Functional Ambulation Classification) [29] scale, and frailty status by the FRAIL scale [30]. We used pre-fracture values as baseline points. Handgrip strength was measured as part of the Groningen Fitness Test for the Elderly [31] using a Jamar Hydraulic Hand Dynamometer on the day of the surgery. The best of three attempts (with 30 s rest between each attempt) was recorded [32]. Nutritional assessment was performed by body mass index (BMI) calculation (weight/height^2), and by completing the Mini-nutritional Assessment (MNA) tool [33]. Cognitive status was assessed by Pfeiffer's Short Portable Mental State Questionnaire (SPMSQ) [34] and depression symptoms were assessed using the Geriatric Depression Scale (GDS-15) [35].

FRAX was determined by factors such as age, BMI, and a set of binary risk elements. These elements included prior fragility fracture, whether a parent has had a hip fracture, current smoking habits, long-term oral glucocorticoid usage, presence of rheumatoid arthritis, other underlying conditions leading to osteoporosis, and alcohol intake. Femoral neck BMD was inputted when it was possible [4].

Bone mineral density and body composition by dual-energy X-ray absorptiometry (DXA)

BMD and body composition were assessed using dual X-ray absorptiometry (Lunar iDXA, GE Healthcare) one month after surgery. BMD was measured in the total hip, femur neck, posterior-anterior spine, and forearm [36]. Lean mass was measured as Appendicular Skeletal Muscle Mass (ASM) adjusted for height squared (Appendicular Skeletal Muscle Mass Index or ASMI), or body mass index (ASM/BMI) [37].

Blood extraction and analysis

On the morning of the intervention, fasting peripheral venous blood (PVB) samples were procured from the antecubital vein of the participants. Blood was inverted five times and allowed to sit for 30 min for clotting. Samples were then centrifuged at 2,000 × g for 10 min at 4 °C to obtain plasma and acellular supernatant. Serum aliquots were stored at −80 °C until use. In order to investigate the viability of utilizing this technology for biomarker analysis, we conducted an assessment of the technical performance of Olink Proteomics' high-throughput, multiplex proximity extension assays (PEA), specifically the Target 48 Cytokine Panel, for protein screening purposes [38]. The panels had a positive correlation with other established technologies [39]. This emerging technology, developed by Olink Proteomics (Uppsala, Sweden), integrates quantitative real-time Polymerase Chain Reaction (qPCR) with multiplex immunoassays. Essentially, PEA is predicated on dual recognition of a targeted biomarker via a pair of antibodies, each labelled with unique DNA oligonucleotides. These biomarker-specific DNA 'barcodes' are quantified using microfluidic qPCR, which allows for high-throughput relative quantification of as many as 1161 human plasma proteins with a minimal volume of biofluids (1 µL suffices for the quantification of 92 biomarkers). The requirement for highly specific antibodies and the employment of target-designed primers augment the specificity and sensitivity of the assays in biological samples. These characteristics, coupled with the utilization of multiple internal controls that monitor each step of the reactions, help to

avert unspecific events and minimize background noise [38]. Comprehensive details about PEA technology, its performance, and validation data can be obtained from the manufacturer's website (www.olink.com) and the biomarkers are listed in Appendices A.1 and B.

The collected data were presented in standard units (pg/mL). For quality, a four-parameter logistic (4PL) curve was generated for the standard curve during product development. Within the limits of quantification (LOQ), the 4PL fitting described the standard curve well with high precision and accuracy, and the concentration could be correctly estimated. Beyond LOQ, the precision and accuracy of the 4PL fitting exhibited a decrease. Cytokine values that fell within the lower and upper limits of quantification (LLOQ and ULOQ, respectively) for each assay – parameters defined during the panel's development – were not incorporated into the analysis. In total, seven cytokines for which more than 35% of the values were below the limits of detection (LOD) were excluded from all analyses (grey-shaded biomarkers in Appendix A.1).

Statistical analysis

Background data were tested for normality using the Shapiro-Wilk method. Consequently, the non-parametric (Mann–Whitney U) or parametric (independent t-test) test was used to compare between groups (hip fracture cases *versus* controls) regarding the baseline characteristics in continuous variables. For dichotomous or nominal variables, *Fisher's* exact or *Pearson* X^2 were used. Data are presented as mean and standard deviation (SD) if not stated otherwise. The statistical package used to calculate group differences was SPSS version 26 (International Business Machines Corporation [IBM], Armonk, New York, USA). A two-tailed P-value of <0.05 was considered significant.

We used *Tukey's* fences method to detect observations out of the normal range by using interquartile ranges [40], which are often used for detecting outliers in various fields [41]. 55 outliers were excluded from the analysis out of the 1800 values analyzed using the Olink platform. Before performing Tukey's fences, the normality of the data was checked before fitting the curve. Features with >70% missing values in the real samples or >10% outlier values in the serum samples were deleted first, and 36 biomarkers passed quality control (Appendix B). Serum biomarkers in pg/mL values were analyzed using two unpaired t-tests, *Benjamini–Hochberg* method for *p*-value correction with a 5% false discovery rate, and a distribution boxplot. *P* values <0.05 were considered statistically significant after correction with the *Benjamini–Hochberg* method. Principal component analysis and Volcano plot (Fig. 1) assessed the distribution groups, using singular value decomposition with imputation (pre-normalized data, no transformation), and visualized using ClustVis [42]. R-squared and goodness-of-fit measure for linear regression models was calculated including the clinical variables and significant biomarkers related to fracture risk (FRAX hip and major fracture). After these analyses, a one-way analysis of covariance (ANCOVA) was performed adjusted for age, sex, body mass index, and FRAX (hip and major) score with effect size of fracture vs. non-fracture. These analyses were performed using GraphPad Prism 9 program for Windows. Protein–protein association network analysis was created using the online database tool STRING version 11 [43]. Protein accession numbers (UniProt) from significant proteins were entered in the search engine (multiple proteins) with the following parameters: Organism *Homo sapiens*, the maximum number of interactions was query proteins only, interaction score was set to medium confidence (0.400), and an FDR of ≤ 0.01 was used when classifying the Biological Process (GO) of each protein.

Results

Baseline characteristics

We provided an overview of the demographic, clinical, and functional features of the patients included in the analysis (Table 1). The study included 40 older adults (72.5% female) with a mean age (SD) of 81.23 (8.23) years. As clinically expected, the scores for BMI, functional status, FRAX scores, bone mineral density and body composition parameters were all significantly lower in the fracture group than in the non-fracture group ($p<0.05$).

Principal component analysis, Volcano plot and protein association network analysis

A score plot was generated to show the separation between the fracture and non-fracture groups. The principal component analysis did not reveal any abnormal deviations between the two groups (Fig. 1A) with a very similar pattern within the same group and differences between them. The outcome obtained using this selection criterion is presented in the volcano plot displayed in Fig. 1B. It was possible to isolate five biomarkers that showed high differentiation between the study groups.

Changes were observed in the five proteins included: Interleukin 6 (IL-6), Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7). Enrichment analysis with multiple testing corrections was used to assign related gene categories to their associated pathways using gene ontology (summarized in Fig. 2).

Table 1 Demographic, clinical, and functional characteristics of the patients included for analysis (values expressed as mean and standard deviation unless otherwise specified)

	Full sample (n = 40)	Fracture group (n = 20)	Non-fracture group (n = 20)	P value*
Demographic				
Age, years	81.23 (8.23)	87.25 (6.73)	75.20 (4.15)	**0.026**
Sex (men/female), n (%)	11 (27.5)/29 (72.5)	4 (20)/16 (80)	7 (35)/13 (65)	0.480
BMI (kg/m^2)[a]	27.39 (4.72)	24.91 (2.74)	29.87 (5.02)	**0.003**
Clinical status				
CIRS-G score	11.45 (4.21)	12.7 (4.81)	10.2 (3.17)	0.060
Polypharmacy score	6.28 (3.16)	7.25 (3.09)	5.3 (3)	0.534
Osteoporosis (n, %)	10 (25%)	4 (20%)	6 (30%)	0.716
Functional status				
Barthel Index (ADL), score[c]	81.63 (26.13)	67.5 (30.41)	95.75 (7.48)	**< 0.001**
Functional Ambulation Category (n, %)				
FAC 0 to 1	3 (7.5%)	3 (15%)	0 (0)	**0.032**
FAC 4 to 5	36 (92.5%)	17 (85%)	20 (100%)	
Frailty score[d]	2.18 (1.69)	3.05 (1.47)	1.3 (1.42)	**< 0.001**
Hand grip strength (Kg)	17.63 (9.8)	11.3 (6.24)	23.95 (8.6)	**< 0.001**
MNA score[e]	23.43 (6.51)	18.83 (6.08)	28.03 (2.33)	**< 0.001**
Pfeiffer's SPMSQ[f]	2.55 (3.80)	5.05 (4.05)	0.5 (0.224)	**< 0.001**
Depression score (n, %)[g]	8 (20%)	6 (42.9%)	2 (10%)	**0.026**
FRAX mayor score[h]	9.76 (7,15)	13.4 (6.99)	6.12 (5.29)	**< 0.001**
FRAX hip score[i]	4.43 (3.85)	6.29 (3.79)	2.58 (2.94)	**< 0.001**
Bone mineral density and body composition				
BMD[j] - total hip	0.873 (0.186)	0.735 (0.079)	0.976 (0.177)	**0.001**
BMD – femoral neck	0.869 (0.211)	0.739 (0.119)	0.966 (0.217)	**0.011**
BMD – lumbar spine	1.153 (0.256)	0.981 (0.18)	1.239 (0.247)	**0.007**
BMD – foreman	0.768 (0.314)	0.679 (0.127)	0.812 (0.37)	0.281
ASMI[k]	6.24 (1.63)	5.06 (1.27)	7.43 (0.95)	**< 0.001**
ASM/BMI[l]	0.607 (0.188)	0.526 (0.155)	0.687 (0.187)	**0.005**

[a]BMI (body mass index)

[b]The Cumulative Illness Rating Scale for Geriatrics (CIRS-G) scale evaluates individual body systems, ranging from 0 (best) to 56 (worst)

[c]The Barthel Index ranges from 0 (severe functional dependence) to 100 (functional independence)

[d]Frail Scale ranges from 0 to 5 and indicates frailty with ≥ 3

[e]Mini-Nutritional Assessment (MNA).

[f]Pfeiffer's Short Portable Mental State Questionnaire (SPMSQ) ranges errors from 0 (best) to 10 (worst)

[g]The Geriatric Depression Scale (GDS-15) ranges from 0 to 15 and indicates symptomatic depression with ≥ 5

[h]FRAX 10-year fracture probability of mayor osteoporotic fracture (%). Mean and SD

[i]FRAX 10-year fracture probability of hip fracture (%)

[j]BMD (bone mineral density, g/cm^2)

[k]ASMI (Appendicular Skeletal Muscle Index, kg)

[l]ASM/BMI (Appendicular lean mass adjusted for BMI).

* p-value for different groups in percentage (Pearson X^2, expect no normal distribution; Fisher's exact test) or means (t-student, expect no normal distribution; U de Mann-Whitney). The bold values are statistically significant

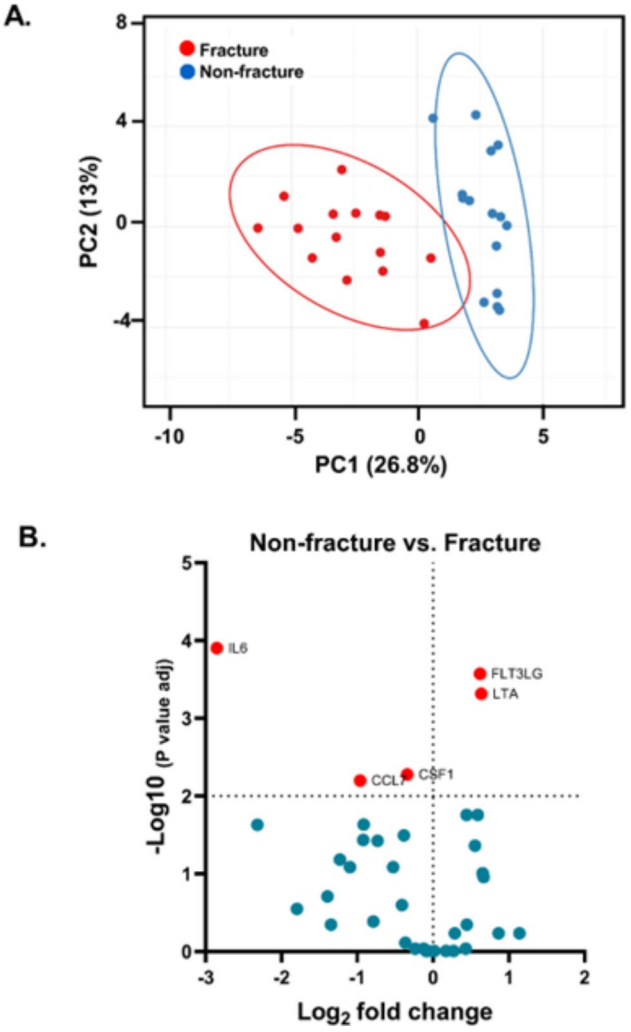

Fig. 1 Principal component (PCA) and volcano plot analysis. Panel **A**, Principal component analysis (PCA) between the study groups. The ellipses show a probability of 95% that a new data point from the same group is located inside the ellipse. The red points correspond to fracture subjects, and the blue points correspond to non-fracture subjects. Panel **B**, Volcano plot of the paired t-test between non-fracture vs. fracture. Statistically significant differences in protein expression levels were found after correction with Benjamini–Hochberg, which is represented by all the proteins being presented as red dots, that is, the corrected p-values did reach < 0.05. The dotted line represents the corrected significance threshold of 0.05. On the y-axis are $\log_{10}$ of p-values and on the x-axis is the $\log_2$ fold change between the two groups where a positive fold change indicates a lower protein level in the non-fracture than in the fracture

Biomarkers difference and correlation with fracture risk

After conducting two unpaired t-tests with the *Benjamini-Hochberg* method for p-value correction, it was found that these five cytokines were significantly different between fracture and non-fracture patients ($p<0.05$). The mean plots in Fig. 3A, D, G, J, and M display the levels of these five proteins. LT-α and FLT3LG were found to be higher in non-fracture patients, whereas IL-6, CSF1, and CCL7 were found to be higher in fracture patients. (Appendix A.2) shows the immunology biomarkers that were not found to be significantly associated with fracture status.

Furthermore, linear regression models showed moderate ($R^2=0.409$) but significant ($p=0.001$) positive correlations between IL-6 levels and the risk of major fracture, as shown in Fig. 3I. The levels of CSF1 ($R^2=0.267$; $p=0.005$) and CCL7 ($R^2=0.301$; $p=0.002$) had a weak correlation with the risk of fracture. On the other hand, LTA (R^2=-0.157; $p<0.001$) and FLT3LG (R^2=-0.139; $p<0.001$) exhibited a negative relation with the risk of fracture.

After the ANCOVA was performed adjusted for age, sex, body mass index, and FRAX (hip and major) score and with effect size of fracture vs. non-fracture, all immunology biomarkers maintained significant ($p<0.05$) expect for CSF1 (Appendix A.4).

Discussion

This cross-sectional study utilized a targeted proteomic approach to identify potential biomarkers of hip fracture in older adults. The study identified five potential biomarkers, namely serum IL-6, CSF1, LT-α, FLT3LG, and CCL7, which may have significant implications for fracture risk. Out of these biomarkers, three (IL-6, CSF1, and CCL7) exhibited a positive relationship with fracture risk based on the FRAX reference tool, while two (LT-α and FLT3LG) had a negative relationship with fracture risk. While previous evidence has suggested an association between biomarkers and osteoporosis [23, 44], this study is the first to examine the relationship between FRAX and serum cytokines. These findings have the potential to pave the way for developing effective biomarker-based diagnostic tools and interventions for osteoporosis, which could significantly improve clinical outcomes for older adults at risk of hip fracture.

In this study, we utilized PEA to characterize serum cytokines related to signaling and inflammatory processes in older adults with hip fractures compared to other adults undergoing elective orthopedic surgery. Given the multitude of immunology biomarkers that are altered in rheumatic diseases [45], the choice of OA as the control group in this study allows us to confirm the association of these five biomarkers with OP [21], ruling out their association with OA as other most prevalent rheumatic disease in the older population. There are some similarities between osteoporosis (OP) and osteoarthritis (OA) [18–21], the characteristics of these groups are quite different due to factors such as age [46] and the presence of risk factors. As observed in our study and supported by existing literature, patients with OP and hip fractures are notably older [25, 46, 47] and often in a poorer nutritional state [48]. This age and nutritional disparity can inherently influence the outcomes of studies involving these populations. For instance, underweight is

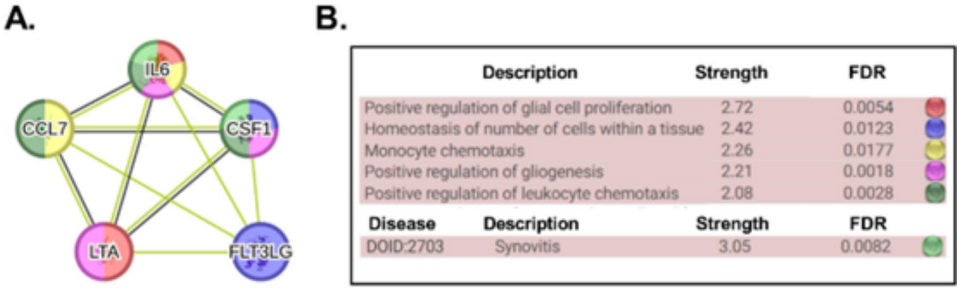

Fig. 2 Pathway analysis of immunology proteins associated with the metabolic process in bone. Functional protein network analysis of significant proteins associated with metabolic process. The STRING version 11 was used to create the network analysis (https://string-db.org/). In the network, each protein is represented by a coloured node, and protein–protein interaction and association are represented by an edge visualized as a coloured lined (type of interaction). Known interactions used were from curated databases (turquoise) and experimentally determined (pink). Predicted interactions were gene neighbourhood (green), gene fusion (red) and gene-co-occurrence (dark blue), and other interactions were text mining (yellow), coexpression (black), and protein homology (purple). Interleukin 6 (IL-6), Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7)

a risk factor for OP [49, 50] and while obesity stimulates the development of OA [19, 50] and maybe acts as OP protector factor [51]. Additionally, functional capacity is an independent factor for hip fracture [52], whereas hip arthroplasty is a common treatment for OA patients [53].

In this exploratory study, these clinical differences may have contributed to differences in cytokine profiles, which highlights the need for closer case-control clinical matching in further studies. Our interpretation of the functional mechanisms of the five identified proteins is that they are involved in immune and inflammatory processes. While these proteins have traditionally been associated with synovial membrane inflammation (synovitis), recent findings in osteoimmunology suggest that immune dysregulation can trigger inflammatory conditions that negatively affect bone integrity [23]. These findings may have important implications for understanding the complex interplay between inflammation and bone health in older adults.

Studying the molecules reported in this study is important because low-grade inflammation is a key factor in the pathogenesis of various widespread diseases, particularly osteoporosis [54]. Although it is not yet understood how circulating peptides reflect activity in musculoskeletal tissues, inflammatory mediators such as reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines directly or indirectly affect bone cells and contribute to the development of osteoporosis [15, 44]. Prior endeavors have concentrated on the identification of prospective biomarkers capable of prognosticating the likelihood of osteoporosis, either as standalone predictors or in conjunction with clinical risk factors and BMD.

The biomarkers identified in this study have been previously investigated concerning osteoporosis. For example, increased levels of IL-6 induce osteoclastogenesis, the accumulation of T-cells (Th17), and the production of RANKL, which promotes bone resorption [23]. IL-6 also upregulates bone destruction by releasing protease enzymes from inflammatory cells [44]. Even though the expression of RANKL in an array of cell types, including osteoblasts, research suggests that osteocytes predominantly contribute to the pool of RANKL essential for osteoclast genesis [55].

Despite the positive associations found between IL-6 and fracture risk (R^2=0.409 for major fracture risk, and R^2=0.364 for hip fracture risk), it is currently unclear whether blood IL-6 concentration can accurately predict fracture risk.

LT-α, also known as tumor necrosis factor-beta (TNF-β), is a cytokine belonging to the tumor necrosis factor superfamily that mediates a range of inflammatory, immunostimulatory, and antiviral responses [56]. Although involved in the genesis and treatment of osteoarthritis [57], it induces osteoclastogenesis alongside RANKL [58]. However, when TNF- α is present in abundance, studies suggest that its role is secondary to that of TNF- α [59]. The significant but weak (R^2=−0.157 in the best case) correlation with the control group may be due to its relationship with both processes and its secondary role.

FLT3LG is a hematopoietic cytokine related to growth factors that increase the number of immune cells by activating hematopoietic progenitors. FLT3LG studies in the biomedical literature are more related to leukaemia than musculoskeletal diseases [60]. The role of this cytokine in bone joints is debated and has mainly been described in rheumatoid arthritis, where it is considered to be a negative regulator of osteoclastogenesis and a bone-protective factor [61]. This may explain the weak association with fracture risk seen in our study (R^2=−0.356).

CSF1, also known as macrophage colony-stimulating factor (M-CSF), is a secreted cytokine that causes hematopoietic stem cells to differentiate into macrophages or other related cell types. CSF1 is involved in multiple functions throughout the body, including bone health. In bone, stromal cells secrete CSF1, which affects T-cell

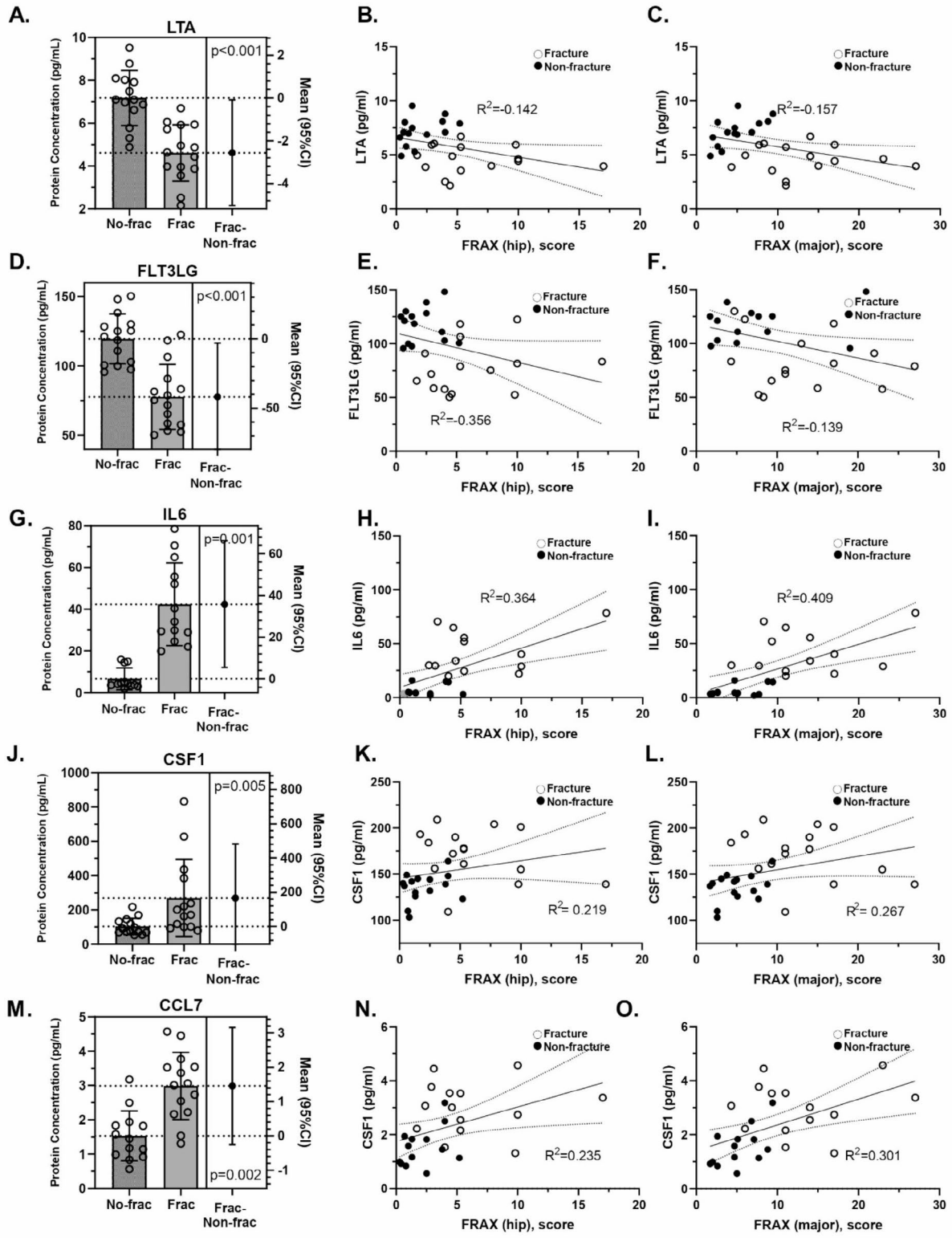

Fig. 3 Group difference (fracture vs. non-fracture) and their association with FRAX (hip and major) score with significant plasma biomarkers. Panel **A**, **D**, **G**, **J** and **M** show mean plots of the five proteins with the most significant changes in protein expression levels following t-tests between fracture vs. non-fracture groups. Panel **B**, **C**, **E**, **F**, **H**, **I**, **K**, **L**, **N**, and **O** figures, show the lineal regression between fracture vs. non-fracture groups with FRAX (hip and major) scores with significant plasma biomarkers. Solid lines: estimation; dashed curved lines: 95% confidence interval limits. Lymphotoxin-alpha (LT-α) or tumor necrosis factor-beta (TNF-β), Fms-related tyrosine kinase 3 ligand (FLT3LG), Interleukin 6 (IL-6), Colony stimulating factor 1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), and Chemokine (C-C motif) ligand 7 (CCL7)

differentiation in osteoclastogenesis [23]. CSF1 is crucial for the proliferation, differentiation, and motility of osteoclasts [62], making it a key therapeutic target for osteoporosis [63]. In our study, we found that CSF1 levels were different between the fracture and control groups ($p=0.005$), but with a weak correlation to fracture risk. Despite its biological plausibility, CSF1 did not retain its significance after adjusting for multiple confounders, likely due to the sample size. While it was adequate for initial observations, it might not have been sufficiently large to detect subtle effects of CSF1 once other variables were taken into account.

CCL7 belongs to the CC chemokine family and its role in osteoporosis is currently under study [64]. RANKL induces the expression of many chemokines including CCL7, to enhance osteoclast formation. Currently, CCL7 is being studied as a potential target for postmenopausal osteoporosis [65]. Our findings support the relationship with OP ($p=0.002$), with a weak correlation with fracture risk.

Despite the importance of cytokines in bone regulation, other cytokines related to bone loss, such as IL-1B, IFNG, and TNF, did not show significance in our study [23, 44]. Considering the widely acknowledged limitations of utilizing BM) in the evaluation of fracture risk within the bone health research community, there is an ongoing pursuit to discover and validate novel biomarkers for clinical application. This endeavor stems from the growing understanding of bone regulation, which contributes to an expanding pool of knowledge in the field. Our findings suggest that the weak association of IL-6, CSF1, and CCL7 with fracture risk may be related to the implications of these cytokines in inflammaging and other age-related diseases [66] in older adults with high comorbidity burden (especially OA [67]) and polypharmacy [68, 69]. The lack of differences in these cytokines may be due to similar inflammaging-related characteristics between the study groups. Hence, based on the current body of evidence, the utilization of these three prospective biomarkers as predictors of treatment responses to novel anti-osteoporotic medications is not supported [70].

The main strength of this exploratory analysis is its potential to provide a new tool for estimating an individual's risk of experiencing a hip fracture or a major osteoporotic fracture based on serum analysis, which could guide clinical decision-making and assist healthcare professionals in identifying individuals who may benefit from interventions to reduce their risk of fractures. The development of serum biomarkers for fracture risk in older adults is of interest in clinical practice due to the association of fractures with disability, premature mortality, and increased utilization of medical resources [3]. Moreover, Olink Proteomics' high-throughput allows for reliable analysis of these very low values of immunology biomarkers, such LTA and CCL7 (with levels < 10pg/ml) but these results should be taken with caution.

However, it is essential to recognize and consider the limitations of our study. First, the analysis was cross-sectional, meaning causative relationships cannot be considered. Longitudinal studies will be necessary to determine the temporal relationship between changes in cytokine profiles and the development of a hip fracture. Second, the small study population comprised only Caucasians, so our findings cannot be generalized to other ethnic groups and limited the statistical strength (specially for CSF1). Additionally, although the cohort was extensively characterized, it was relatively small, and analyses involved a large set of variables. The two comparison groups were not closely matched in terms of demographic or clinical characteristics, which may have confounded our results, but after adjusted for age, sex, body mass index, and FRAX score; most of them were still significant different.

Conclusion

To summarize, our cross-sectional study identified five immunology biomarkers (IL-6, CSF1, LT-α, FLT3LG and CCL7) that were associated with hip fracture and have potential correlation with fracture risk. This study provides a potential contribution by highlighting immunology biomarkers that could be further studied to estimate fracture risk and potentially delay the onset of osteoporosis and fragility fractures in older adults. However, to increase the clinical relevance of these biomarkers and small sample, validation and replication in longitudinal cohorts with diverse populations are needed.

Abbreviations

ASM	Appendicular Skeletal Muscle Mass
ASMI	Appendicular Skeletal Muscle Mass Index
BMD	Bone mineral density
BMI	Body mass index
BMT	Bone turnover markers
CCL7	Chemokine (C-C motif) ligand 7
CIRS-G	Cumulative Illness Rating Scale for Geriatrics
CSF1	Colony stimulating factor 1
DXA	Dual-Energy X-ray Absorptiometry
FLT3LG	Fms-related tyrosine kinase 3 ligand
IL-6	Interleukin 6
LT-α	Lymphotoxin-alpha
LOQ	Limits of Quantification
MNA	Mini-nutritional Assessment
M-CSF	Macrophage colony-stimulating factor
OA	Osteoarthritis
OP	Osteoporosis
PEA	Proximity extension assay
PVB	Fasting peripheral venous blood
ROS	Reactive oxygen species
SPMSQ	Pfeiffer's Short Portable Mental State Questionnaire
TNF-β	Tumor necrosis factor-beta

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-023-00379-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions
All authors participated in data acquisition. BC-V, AMHO, MI and NM-V contributed to the conception and design of the study. BC-V, FZF, JF-I, ES, MI, RRV and RR-O did the data analysis and interpretation. BC-V, AR-G, RR-O, MI and NM-V contributed to the drafting and revision of the manuscript. All authors read and approved the final manuscript.

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Data Availability
All data relevant to the study are included in the article or uploaded as supplementary information.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The study followed the principles of the Declaration of Helsinki and was approved by the Navarra Research Ethics Committee (PI_2020/125), Spain.

Consent for publication
Written informed consent was obtained from each patient for publication of this study.

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BRIEF REPORT

Serum biomarkers related to frailty predict negative outcomes in older adults with hip fracture

B. Cedeno-Veloz[1,2,3] · L. Lozano-Vicario[1,2,3] · A. Rodríguez-García[1] · F. Zambom-Ferraresi[1,2,3] · A. Galbete[3] · J. Fernández-Irigoyen[2] · E. Santamaría[2] · A. García-Hermoso[2] · R. Calvani[5] · R. Ramírez-Vélez[2,3,4] · M. Izquierdo[2,3,4] · N. Martínez-Velilla[1,2,3,4]

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Abstract

Purpose Hip fracture is a public health problem worldwide. Traditional prognostic models do not include blood biomarkers, such as those obtained by proteomics. This study aimed to investigate the relationships between serum inflammatory biomarkers and frailty in older adults with hip fracture as well as adverse outcomes at one and three months after discharge.

Methods A total of 45 patients aged 75 or older who were admitted for hip fracture were recruited. At admission, a Comprehensive Geriatric Assessment (CGA) was conducted, which included a frailty assessment using the Clinical Frailty Scale (CFS). Blood samples were collected before surgery. Participants were followed up at one and three months after discharge. The levels of 45 cytokines were analyzed using a high-throughput proteomic approach. Binary logistic regression was used to determine independent associations with outcomes, such as functional recovery, polypharmacy, hospital readmission, and mortality.

Results The results showed that IL-7 (OR 0.66 95% CI 0.46–0.94, $p=0.022$) and CXCL-12 (OR 0.97 95% CI 0.95–0.99, $p=0.011$) were associated with better functional recovery at three months after discharge, while CXCL-8 (OR 1.07 95% CI 1.01–1.14, $p=0.019$) was associated with an increased risk of readmission.

Conclusions These findings suggest that immunology biomarkers may represent useful predictors of clinical outcomes in hip fracture patients.

M. Izquierdo and N. Martínez-Velilla share senior authorship.

✉ B. Cedeno-Veloz
ba.cedeno.veloz@navarra.es

1 Geriatric Department, Navarre University Hospital (HUN), Irunlarrea 3, 31008 Pamplona, Navarra, Spain

2 Navarrabiomed-Institute for Health Research of Navarra (IDISNA), Irunlarrea 3, 31008 Pamplona, Navarra, Spain

3 Public University of Navarre, Av Cataluña s/n, 31006 Pamplona, Navarra, Spain

4 CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Av Monforte de Lemos, 3-5, Pabellón 11, Planta 0, 28029 Madrid, Spain

5 Department of Geriatrics and Orthopedics, Università Cattolica del Sacro Cuore and Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, 00168 Rome, Italy

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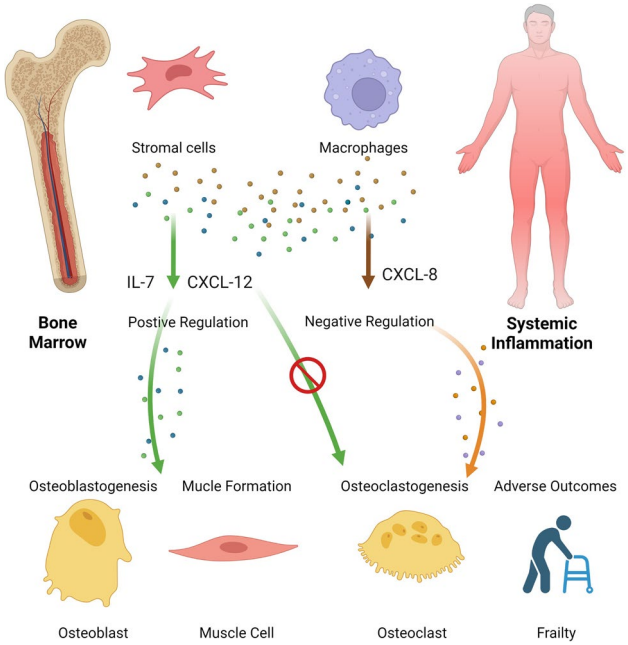
Graphical abstract



Keywords Aging · Fragility fracture · Osteoimmunology · Osteoporosis

Introduction

Hip fracture poses a rising public health concern, carrying substantial implications for older adults[1, 2], including elevated morbidity and mortality rates, along with significant social and economic burdens associated with hip fractures, makes identifying populations at risk and developing predictive markers particularly important [3–6].

While traditional predictors of poor outcomes, such as age, co-morbidities, and surgical factors [7, 8], have been identified, their performance as prognostic factors in older adults has proved to be limited. Frailty is recognized as a potential predictor of negative outcomes in patients with hip fractures [9]. Furthermore, delirium [10] and vitamin D [11] have also been highlighted as a significant predictor. The capacity of these newer predictors in forecasting outcomes is still under debate [12].

The complex pathophysiology of osteoporosis, frailty, and hip fractures hampers the identification of biomarkers, especially pro-inflammatory cytokines [13, 14], for predicting outcomes in frail older adults with hip fractures [15–17]. In this scenario, proteomics may serve as a powerful analytical approach for the definition of minimally invasive biomarkers for adverse outcomes in patients with hip fractures [18].

In this prospective cohort study, we aimed to explore the role of a targeted proteomic approach in better-characterizing frailty in older adults with hip fractures. The objective of this exploratory study is to use new analytical platforms, such as Olink, for the detection of biomarkers associated with frailty and health outcomes after hip fracture. Moreover, we sought to identify molecular features that could be useful in improving the prognosis of this group of patients. We hypothesized that levels of inflammatory biomarkers could be associated with frailty measures with the Clinical Frailty Scale (CFS) and health outcomes at one and three months after discharge independently of frailty status.

Materials and methods

Patients and study design

In this prospective cohort study, patients admitted to a tertiary hospital's Orthopedic ward were evaluated (Hospital Universitario de Navarra, Pamplona, Spain) between March and October 2021. Candidates for inclusion were patients aged ≥ 75 years undergoing surgery for hip fracture. The main exclusion criteria were the presence of diseases that cause secondary osteoporosis (glucocorticoid-induced osteoporosis, rheumatoid arthritis, etc.), terminal illness (defined as a progressive disease that is expected to result in death within six months which included a CFS of 8 or 9), and unwilling to provide informed consent. We screened 256

older adults, of whom 83 met the inclusion criteria. Exclusions at this point were 112 due to secondary osteoporosis, 48 due to terminal illness, and 13 due to unwillingness to provide informed consent. Finally, 45 participants were selected for the study with 38 excluded. The main reason for exclusion at this point was the change of the day of surgery, which did not allow for the collection and processing of samples. The study flowchart is reported in Fig. 1.

The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the local Research Ethics Committee (PI_2020/125). Before enrolling in the study, participants provided informed consent, which was approved by the ethics committee.

Clinical and functional parameters

A comprehensive medical assessment was performed during the hospital admission, which included the assessment of comorbidities (Charlson comorbidity index) [19], osteoporotic treatments, and polypharmacy (defined as regular use of at least five medications) [20].

Functional status was assessed by the Barthel [21] and Lawton index [22], and mobility using the FAC (Functional Ambulation Classification) [23] scale. We used the pre-fracture value as the baseline point.

The assessment of frailty status was conducted using the Clinical Frailty Scale (CFS) [24] by study investigators/geriatricians at hospital admission. Study participants were given a score from 1 (very fit) to 7 (living with severe frailty) based on clinical and functional information collected at hospital admission in the screening evaluation. Participants with a CFS above 5 were considered frail [25].

Handgrip strength was measured using a Jamar Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) following the Groningen Elderly Test protocol [26] on the day of the surgery, before the intervention. The best of three attempts (with 30 s rest between each attempt) was recorded [27].

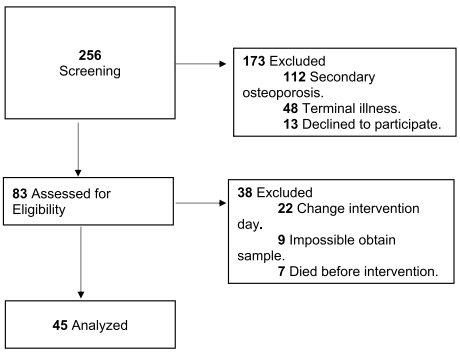


Fig. 1 Flowchart of patients included in the study

Nutritional assessment was performed by body mass index (BMI) calculation (weight/height^2), and by completing the Mini-nutritional Assessment (MNA) tool [28] collected during medical assessment.

Cognitive status was assessed using the Global Deterioration Scale (GDS) [29] and delirium by the Confusion Assessment Method (CAM) [30], and depression was assessed using the Geriatric Depression Scale (GDS-15) [31] collected during medical assessment.

The follow-up variables (mortality, hospital admission, Barthel and Lawton index, FAC, and polypharmacy) were collected from a local database and by a phone call at one and three months after discharge.

Blood extraction and analysis

Fasting peripheral venous blood (PVB) samples were collected in the morning through the intervention from the antecubital vein. Blood was inverted five times and left at room temperature for 30 min for clotting. Samples were then centrifuged at 2000 × g for 10 min at 4 °C to obtain serum and acellular supernatant. Serum aliquots were stored at − 80 °C until use.

Cytokines analysis was performed using Olink® Target 48 Cytokine Panel. This analytical approach is based on the proximity extension assay (PEA) and showed high reproducibility and measurement correlation with other multimarker technologies such as mass spectrometry [32]. The emerging PEA technology, developed by Olink Proteomics (Uppsala, Sweden), combines multiplex immunoassays with quantitative real-time polymerase chain reaction (PCR). In PEA, a targeted biomarker is recognized through a pair of antibodies labeled with distinct DNA oligonucleotides. These biomarker-specific DNA "barcodes" are then quantified using microfluidic qPCR, enabling high-throughput relative quantification of a wide range of human plasma proteins. The analysis requires only a few microliters of bio-fluids, with a minimal volume of 1 µL sufficient for quantifying 92 biomarkers. The use of highly specific antibodies and target-designed primers improves the specificity and the sensitivity of the assays in biological samples. These features, along with the use of multiple internal controls that monitor each step of the reactions, circumvent unspecific events and reduce background noise [33]. Comprehensive details about PEA technology, its performance, and validation data can be obtained from the manufacturer's website (www.olink.com), and the biomarkers are listed in Table S1.

Data were reported in standard units (pg/mL). For quality, a four-parameter logistic (4PL) curve was generated for the standard curve during product development. Within the limits of quantification (LOQ), the 4PL fitting described the standard curve well with high precision and accuracy, and the concentration could be correctly estimated. Outside

LOQ, precision and accuracy of the 4PL fitting decreased. When cytokines were within lower and upper limits of quantification (LLOQ and ULOQ) for each assay (defined during the development of the panel), the values were not included in the analysis. In total, seven cytokines for which more than 35% of the values were below the limits of detection (LOD) were excluded from all analyses (gray-shaded biomarkers in Table S1).

Statistical methods and outcome measure

Study participants were divided into 2 groups: frail and non-frail according to CFS (non-frail from 1 to 4; frail from 5 to 9). To characterize these groups, a descriptive analysis was performed for categorical variables using absolute and relative frequencies; and for quantitative variables using the mean and the standard deviation or median and interquartile ranges, according to the normality of the data. Student's *t* tests (for normally distributed data variables), Wilcoxon test (for non-normally distributed data variables), and chi-square tests (for categorical variables) were used to compare baseline characteristics between frail and non-frail patients.

The outliers were detected by Tukey's method and removed for analysis (103 outliers were excluded from the analysis out of the 2025 values analyzed using the Olink platform). Spearman correlations between all proteins and CFS were completed to investigate in which cytokines were related to frailty in hip fracture patients.

Logistic regression was used to estimate the relationship between frailty and candidate biomarkers and binary outcome variables at one- and three-month follow-up: mortality, hospital admission, dependency according to Barthel index (≤ 60 points), dependency according to Lawton index (≤ 3 points), poly-pharmacy (≥ 5 prescriptions), and dependency in gait according to FAC scale (FAC ≤ 3). These results were presented as odds ratios (OR) with 95% confidence intervals (CI).

The discriminatory ability of the biomarkers was assessed by the receiver operating characteristics (ROC) curve with receiver operating characteristic (AUROC) calculation, and this was compared with the CFS. The ROC curve was calculated for outcomes with significant results in the logistic regression.

The principal components and the heatmap were calculated from the proteomics dataset using singular value decomposition with imputation (pre-normalized data, no transformation) for missing data, and visualized using ClustVis [34].

All statistical calculations were completed using SPSS software ver. 28.0 (IBM, Armonk, NY, USA). Analyses were two-sided, and values of $p < 0.05$ or a 95% confidence interval (CI) non-containing the null value were considered statistically significant, except for Spearman correlation, for which exploratory reasons of the study, were considered $p < 0.1$.

Results

Our study included 45 older adults, of which 84.4% were female. The mean age was 85.67 years (SD 6.4). Among the participants, 28 were categorized as frail according to the CFS scale. Table 1 presents the reported clinical and functional characteristics of the study group. The scores for BMI, functional status, and body composition were all significantly lower in the frail group compared to non-frail participants. Unsupervised system analysis was conducted to identify co-regulated network responses (Fig. 2) using principal component analysis (A) and heatmap (B) and revealed substantial overlap between frail and non-frail patients. These findings were related to the prevalence of hip fracture in this cohort.

Spearman correlation revealed a negative association between IL-7 and frailty status ($\rho = -0.302$, $p = 0.046$) and between CXCL-12 and frailty status ($\rho = -0.284$, $p = 0.068$). Both FLT3LG ($\rho = 0.264$, $p = 0.079$) and CXCL-8 ($\rho = 0.274$, $p = 0.083$) approached statistical significance. As an exploratory study, we used these cytokines for the follow-up analyses. The rest of the analysis in proteomics markers of patients was not significant and available in Table S2.

Logistic regression analysis, as detailed in Table 2, revealed a significant association between CSF and dependency as measured by the Barthel index, as well as gait dependency at both one-month and three-month follow-ups. Independent of CFS, increased levels of CXCL-12 were associated with a reduction in dependency according to the Barthel index at three months (OR = 0.97, 95% CI 0.95–0.99, $p = 0.011$). IL-7 levels were inversely associated with gait dependency (OR = 0.66, 95% CI 0.46–0.94, $p = 0.022$). However, the association of IL-7 levels with dependency based on the Barthel index was not statistically significant ($p = 0.070$). Elevated CXCL-8 levels were associated with an increased risk of hospital readmission at three months (OR = 1.07, 95% CI 1.01–1.14, $p = 0.019$), although its association with dependency according to the Barthel index was not significant (OR = 1.05, 95% CI 1.00–1.10, $p = 0.058$). No associations with mortality or polypharmacy were observed for any of the assessed candidate biomarkers.

AUROC analyses, depicted in Fig. 3 and detailed in Table S3, showed that CXCL-12's ability to predict dependency based on the Barthel index at three months was comparable to that of CFS (AUROC = 0.845). IL-7 had an AUROC of 0.703 in predicting gait dependency at the three-month mark. Similarly, CXCL-8 had an AUROC of 0.815 related to hospital admissions at three months.

Table 1 Demographic and baseline characteristics of the patients included for analysis

	Total ($n=35$)	Non-frail ($n=17$)	Frail ($n=28$)	P value*
Age	85.67 (6.4)	82.59 (5.43)	87.54 (6.25)	0.01
Sex, n (%)				0.399
Men	7 (15.6%)	4 (57.1%)	3 (42.9%)	
Female	38 (84.4%)	13 (34.2%)	25 (65.8%)	
Charlson score[a]	6.09 (2.31)	4.53 (1.38)	6.07 (2.72)	0.036
Polypharmacy	7.3 (3.8)	5.41 (2.85)	8.32 (3.95)	0.011
Functional status				
Barthel index[b]	84.60 (18.6)	97.65 (3.59)	77.14 (19.65)	<0.0001
Lawton index[c]	4.8 (2.8)	6.65 (2.03)	3.79 (2.73)	0.001
Functional ambulation category	1.2 (1.3)	0.18 (0.39)	1.82 (1.22)	<0.0001
Hand-grip strength (Kg)	12.88 (6.38)	18.71 (4.89)	9.89 (5.49)	<0.0001
MNA[d]	23.77 (5.22)	27.47 (2.21)	21.14 (4.87)	<0.0001
Depression (n, %)[e]	13 (28.9%)	2 (11.8%)	11 (39.3%)	0.03
Delirium (n, %)[f]	21 (46.7%)	4 (23.5%)	17 (60.7%)	0.03
Dementia (n, %)[g]	2.39 (1.59)	1.65 (1)	3 (1.56)	0.003
Body composition				
BMI (kg/m^2)[h]	25.4 (4.5)	24.9 (2.43)	25.68 (5.4)	0.623
ASMI[i]	4.98 (1.47)	5.19 (1.2)	4.49 (1.65)	0.134
ASM/BMI[j]	0.191 (0.051)	0.201 (0.042)	0.174 (0.053)	0.034

[a]The Charlson Comorbidity Index ranges from 0 (low comorbidity) to 37 (high comorbidity)

[b]The Barthel Index ranges from 0 (severe functional dependence) to 100 (functional independence)

[c]The Lawton Index ranges from 0 (dependence) to 8 (independence)

[d]Mini-Nutritional Assessment (MNA)

[e]The Geriatric Depression Scale (GDS-15) ranges from 0 to 15 and indicates symptomatic depression with ≥ 5

[f]Confusion Assessment Method (CAM) is a standardized tool to identify and recognize delirium. The diagnosis of delirium by CAM requires the presence of features 1 and 2 and either 3 or 4

[g]The Global Deterioration Scale (GDS) ranges from 0 to 7. Dementia stages are from 4 to 7

[h]BMI (body mass index)

[i]ASMI (Appendicular Skeletal Muscle Index, kg)

[j]ASM/BMI (Appendicular lean mass adjusted for BMI)

*p value for different groups in percentage (Pearson X2, expect no normal distribution; Fisher's exact test) or means (T Student, expect no normal distribution; U de Mann–Whitney). The bold values are statistically significant

Discussion

In our prospective cohort study, we identified three biomarkers (CXCL-12, CXCL-8, and IL-7) that may have significant implications for predicting adverse outcomes in older adults with hip fractures. While CXCL-12 and IL-7 levels were positively associated with improvements in activities of daily living and gait independence at three months, respectively, we did not find associations with other outcomes, such as mortality, re-hospitalization, or dependency, based on the Lawton index. On the other hand, CXCL-8 levels were linked to hospital readmissions, it was not significantly associated with other adverse outcomes. These lack of associations are associated with the prevalence of hip fracture. As we have mentioned, hip fracture is an event associated with numerous adverse outcomes [1, 2], and although these biomarkers may influence the outcomes, they may not carry sufficient weight to define differences among them, especially when frailty is in consideration [3].

This exploratory study supports the previously established association between frailty and worse health outcomes after hip fractures, as shown in Fig. 3. However, the study also found that CXCL-8 and CXCL-12 had a greater ability in predicting hospital readmission and decline in activities of daily living, respectively, compared to CFS. Additionally, the study found that neither CFS nor biomarkers were able to accurately predict polypharmacy. The relationship between inflammatory biomarkers and polypharmacy is a controversial topic, particularly for older adults with multimorbidity [35].

Low-grade inflammation plays a key role in the development of various highly prevalent age-related conditions,

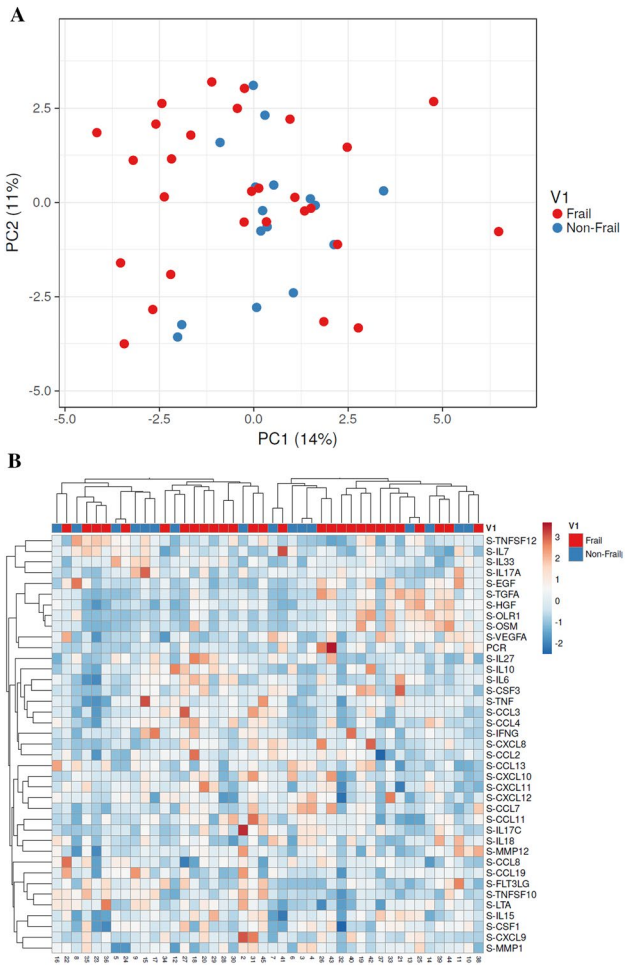


Fig. 2 Unsupervised systems analysis to identify co-regulated network responses. **A**: principal component analysis (PCA). **B**: heat MAP

including frailty [18], and is associated with a higher risk of adverse events. The exact mechanism by which these circulating peptides exerts their detrimental actions on musculoskeletal tissues is not fully understood. However, it is well acknowledged that inflammatory mediators, such as reactive oxygen species (ROS), pro-inflammatory cytokines, and chemokines, can directly or indirectly affect body cells and contribute to worse outcomes in older adults [36]. It is important to understand the pathophysiological mechanism by which the inflammatory markers in this study were able to produce these results:

IL-7, as a growth factor synthesized by a diverse range of cell types, functions as a myokine and has an important role in the regulation of muscle cell development and bone metabolism. It is suggested that osteoblast-derived IL-7 might inhibit bone formation while simultaneously upregulating the expression of RANKL [37] but also increases osteoblasts [38]. In muscle tissue, IL-7 expression has been associated with improvements in both muscle strength and

Table 2 Logistic Regression at one- and three-month follow-up between frailty scales and biomarkers with *A* mortality, *B* hospital admission, *C* dependency according Barthel index, *D* dependency according Lawton index, *E* polypharmacy and *F* dependency in gait according FAC scale

	One-month		Three-months	
	OR (IC95%)	*p* value	OR (IC95%)	*p* value
A: mortality				
CFS	2.19 (0.55, 8.78)	0.269	1.45 (0.44, 4.76)	0.538
FLT3LG	0.87 (0.61, 1.25)	0.452	1.00 (0.92, 1.09)	0.929
IL-7	–		0.84 (0.29, 2.47)	0.755
CXCL-12	0.99 (0.94, 1.04)	0.686	1.01 (0.96, 1.06)	0.741
CXCL-8	1.06 (0.95, 1.17)	0.300	1.03 (0.92, 1.14)	0.626
B: hospital admission				
CFS	1.49 (0.72, 3.07)	0.280	1.18 (0.72, 1.95)	0.518
FLT3LG	0.99 (0.94, 1.05)	0.778	0.99 (9.95, 1.03)	0.577
IL-7	0.89 (0.48, 1.65)	0.706	1.28 (0.83, 1.98)	0.257
CXCL-12	0.98 (0.94, 1.01)	0.145	1.00 (0.98, 1.03)	0.764
CXCL-8	0.93 (0.76, 1.13)	0.460	1.07 (1.01, 1.14)	0.019
C: dependency according Barthel index				
CFS	2.25 (1.32, 3.85)	0.003	3.64 (1.59, 8.32)	0.002
FLT3LG	1.01 (0.99, 1.04)	0.294	1.01 (0.98, 1.04)	0.441
IL-7	0.80 (0.58, 1.10)	0.161	0.71 (0.49, 1.03)	0.070
CXCL-12	0.98 (0.96, 1.00)	0.060	0.97 (0.95, 0.99)	0.011
CXCL-8	1.03 (0.99, 1.08)	0.169	1.05 (1.00, 1.10)	0.058
D: dependency according Lawton index				
CFS	2.94 (1.52, 5.68)	0.001	2.01 (1.23, 3.29)	0.005
FLT3LG	1.02 (0.99, 1.05)	0.193	1.03 (1.00, 1.06)	0.084
IL-7	0.80 (0.58, 1,12)	0.192	0.85 (0.62, 1.16)	0.302
CXCL-12	0.99 (0.98, 1.01)	0.437	0.99 (0.97, 1.00)	0.123
CXCL-8	1.03 (0.98, 1.08)	0.298	1.03 (0.98, 1.08)	0.216
E: polypharmacy				
CFS			0.71 (0.29, 1.71)	0.445
FLT3LG			0.98 (0.90, 1.05)	0.534
IL-7			0.95 (0.46, 1.96)	0.890
CXCL-12			1.01 (0.98, 1.06)	0.352

Table 2 (continued)

	One-month		Three-months	
	OR (IC95%)	*p* value	OR (IC95%)	*p* value
CXCL-8			0.98 (0.88, 1.10)	0.774
F: dependency in gait				
CFS	2.25 (1.32, 3.83)	0.003	2.21 (1.30, 3.75)	0.003
FLT3LG	1.00 (0.97, 1.02)	0.870	1.00 (0.98, 1.03)	0.787
IL-7	0.74 (0.53, 1.04)	0.079	0.66 (0.46, 0.94)	0.022
CXCL-12	0.99 (0.97, 1.01)	0.149	0.99 (0.97, 1.00)	0.098
CXCL-8	1.02 (0.97, 1.07)	0.394	1.01 (0.97, 1.06)	0.513

CFS clinical frailty scale, *FAC* functional ambulation classification

mass [39] and elevated levels have been observed in active older adults [40]. Our study findings revealed that patients exhibiting higher levels of IL-7 demonstrated a reduced risk of experiencing a decline in walking independence at the three-month mark post-discharge (OR = 0.66, 95% CI 0.46–0.94, $p = 0.022$). Based on these findings, increasing IL-7's level through strength exercises [39] may enhance patient function. However, in other studies, elevated levels of IL-7 increased the likelihood of falls [41] so further work is needed to clarify the mechanisms that link IL-7 to adverse outcomes.

CXCL-12 also has a role in musculoskeletal system. It is expressed in the area of inflammatory bone destruction, where it mediates their suppressive effect on osteoclastogenesis and stimulates osteogenic differentiation [42]. In muscle tissue, the presence of CXCL-12 has been observed to significantly enhance the regenerative properties of these cells, promoting muscle repair and recovery [43]. Our study revealed a relationship between elevated levels of CXCL-12 and a decreased risk of functional impairment at the three-month follow-up after discharge (OR = 0.97, 95% CI 0.95–0.99, $p = 0.011$). These findings may suggest the beneficial impact of CXCL-12 on muscle tropism and overall functional recovery throw muscle regeneration after hip fracture [44].

CXCL-8, also known as interleukin 8 (IL-8), has many roles. One of them is as an osteoclastogenic cytokine, inducing RANK-mediated NFATc1 activation [45]. Several studies have shown that pro-inflammatory cytokines, such as CXCL-8 and IL-6, are associated with frailty and adverse outcomes [46–48]. Moreover, in a study conducted by Edvardsson et al., it was observed that heightened levels of CXCL-8 and C-reactive protein correlated with decreased survival rates among older nursing home adults

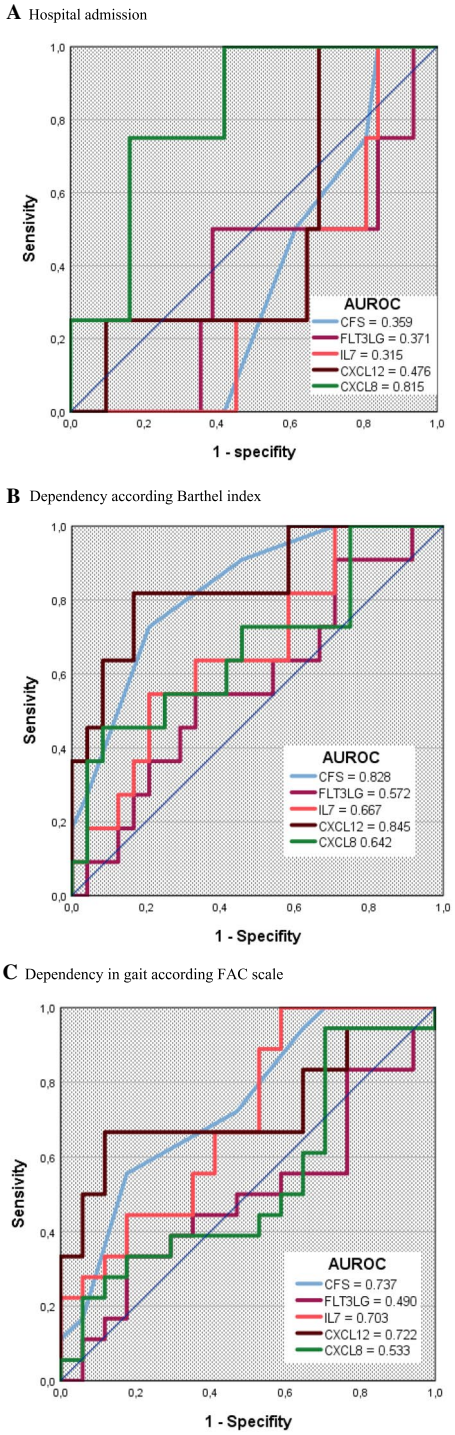


Fig. 3 Performance of different biomarkers in prediction of **A** hospital admission, **B** dependency according Barthel index, and **C** dependency in gait according FAC scale. Receiver operating characteristic (AUROC), clinical frailty scale (CFS). **A** hospital admission

during a one-year follow-up period [49]. In line with these studies, our own investigation found that elevated levels of CXCL-8 were linked to an augmented risk of hospital

readmission at the three-month mark following discharge (OR = 1.07, 95% CI 1.01–1.14, $p = 0.019$).

In our study, we did not observe significant associations between negative outcomes and other cytokines commonly associated with frailty, such as IL-1β, IL-6, IFN-γ, and TNF-α [18]. These mediators play a relevant role in inflammaging and other age-related conditions [50], including multi-morbidity, osteoporosis [51] and polypharmacy [52]. The absence of differences in IL-1, IL-6, IFN and TNF observed in the present investigation may be due to the comparable characteristics of frail and non-frail participants regarding parameters associated with inflammation. Other clinical factors related to the frailty group, such as reductions in BMI, functional status, and body composition scores, align with the established pathophysiology of frailty [53].

This study has several strengths and limitations. The main strengths are the high validity and reproducibility of the analytical approach adopted. Olink technology allowed the measurement of a large panel of cytokines which proved to track changes not related to inflammaging with higher sensitivity. Moreover, more than 90% of proteins included in the Olink panels were detected above the limit of detection in all samples, indicating excellent detectability of the assays in human blood plasma from the general population [54]. Given the complexity of hip fracture patients, exploratory approaches will be needed to allow the identification of specific signatures relevant to distinguishing the risk of adverse outcomes in this group of patients [55]. The development of immunology biomarkers for hip fracture patients would be a field of interest in clinical practice due to its association with disability, premature mortality, and increased medical resources [3]. Our study has identified three potential biomarkers that hold promise in predicting adverse outcomes associated with hip fracture risk in older adults. These findings offer a potential clinical tool for managing complex patients and present a new avenue for further investigation. Future longitudinal studies with larger sample sizes are necessary to explore the potential of these biomarkers in accurately identifying patient groups with poorer outcomes and optimizing resource allocation. The present study has also some limitations that should be mentioned. First, the study population consisted of a small cohort of only Caucasians, preventing the generalization of our findings to other ethnic groups. However, as the objective of this study is exploratory to detect potential biomarkers, these findings are useful to open up new lines of research in larger cohorts. Secondly, measuring inflammation markers on the day of the surgery could result in disproportionately high results due to the acute inflammation associated with fracture. However, this potential increase would be similar in both groups (frail and non-frail patients). Additionally, due to the targeted approach used, we cannot rule out the presence of other circulatory proteins with a potential impact on the risk prediction of frailty and hip fracture. Furthermore, although the cohort was extensively characterized, it was relatively small, and analyses involved a large set of variables. Even considering these limitations, the study included 46 cytokines and Olink-enhanced PEA was used for the analyses, which has been established as a straightforward, sensitive and highly reliable method for biomarker analysis [33].

Conclusion

In summary, CXCL-12, IL-7, and CXCL-8 levels have potential roles as prognostic biomarkers for adverse outcomes related to hip fractures at a three-month follow-up: CXCL-12 is associated with improvements in activities of daily living, IL-7 with gait independence, and CXCL-8 with hospital readmission. These findings were independent of the patients' frailty status.

Our PEA-based high-throughput proteomic approach produced a differential serum prototype, paving the way toward the development and implementation of new screening tools. However, as an exploratory study, further analysis is needed. These approaches, together with functional analyses, could help clarify the underlying mechanisms involved in the development of frailty among hip fracture patients.

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Author contributions Conceptualization, BC-V, LL-V, MI and NM-V; methodology, BC-V, LL-V, FZ-F, JF-I, ES and NM-V; formal analysis, AG.; investigation and data curation, BC-V, LL-V, AR-G and FZ-F; writing—original draft preparation, BC-V; writing—reviewing and editing, BC-V, LL-V, and NM-V; supervision and project administration, NM-V. All authors have read and agreed to the published version of the manuscript.

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Data availability All data relevant to the study are included in the article or uploaded as supplementary information. The data utilized and analyzed in the present study can be obtained from the corresponding author upon a reasonable request.

Declarations

Conflict of interest The authors declare no competing interests. The funders played no part in the study design, data collection, analysis, interpretation, manuscript preparation, or decision to publish the findings.

Research involving human participants and/or animals The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Navarra Research Ethics Committee (PI_2020/125), Spain. All authors read and approved the final manuscript.

Informed consent Written informed consent was obtained from each patient for publication of this study.

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Study Protocol

Effect of a multicomponent intervention with tele-rehabilitation and the Vivifrail© exercise programme on functional capacity after hip fracture: Study protocol for the ActiveFLS randomized controlled trial.

Bernardo Cedeno-Veloz[1-3], Irache Casadamon-Munarriz[1], Alba Rodríguez García[1], Lucia Lozano-Vicario[1-3], Fabricio Zambom-Ferraresi[1-3,5], María Gonzalo Lázaro[1], Ángel María Hidalgo Ovejero[4], Mikel Izquierdo[2-3,5] and **Nicolás Martínez-Velilla[1-3,5]**

[1] Navarre University Hospital (HUN), Irunlarrea 3, 31008 Pamplona, Navarra, Spain.
[2] Navarrabiomed, Institute for Health Research of Navarra (IDISNA), Irunlarrea 3, 31008 Pamplona, Navarra, Spain.
[3] Public University of Navarre, Av Cataluña s/n 31006 Pamplona, Navarra, Spain.
[4] Department of Orthopaedics Clinics and Traumatology, University Hospital of Navarre (HUN), Pamplona, Navarra, Spain.
[5] CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Av Monforte de Lemos, 3-5, Pabellón 11, Planta 0, 28029 Madrid, Spain.
* Correspondence: Bernardo Abel Cedeño Veloz, MD. Geriatric Department (Hospital Universitario de Navarra). Irunlarrea Street 4, 31008 Pamplona, Navarra, Spain. E-mail: ba.cedeno.veloz@navarra.es ORCID: https://orcid.org/0000-0002-0190-4402

Abstract: Introduction: Hip fractures are the most common reason for hospitalization and are associated with high costs, mortality rates and functional decline. Although several guidelines exist for preventing new fractures and promoting functional recovery, they tend to focus on osteoporosis treatment and do not take into account the complexity of frailty in older adults and geriatric syndromes, which are important factors in individuals at risk of suffering from frailty fractures. Moreover, most health systems are fragmented and are incapable of providing appropriate management for frail and vulnerable individuals who are at risk of experiencing fragility fractures. Multicomponent interventions and physical exercise using tele-rehabilitation could play a role in the management of hip fracture recovery. However, the effectiveness of exercise prescription and its combination with a comprehensive geriatric assessment (CGA) is still unclear. **Methods:** This randomized clinical trial will be conducted at the Hospital Universitario de Navarra (Pamplona, Spain). A total of 174 older adults who have suffered a hip fracture and meet the inclusion criteria will be randomly assigned to either the intervention or control group. The intervention group will receive a multicomponent intervention consisting of individualized home-based exercise using the @ctive hip app for three months, followed by nine months of exercise using Vivifrail. Additionally, the intervention group will receive nutrition intervention, osteoporosis treatment, polypharmacy adjustment and evaluation of patient mood, cognitive impairment, and fear of falling. The control group will receive standard outpatient care according to local guidelines. The primary objective of this study will be to assess the effectiveness of the intervention in modifying the primary outcomes, which include changes in functional status during the study period based on the Short Physical Performance Battery. **Discussion:** The findings of this study will offer valuable insights into the efficacy of a comprehensive approach that considers the complexity of frailty in older adults and geriatric syndromes, which are important factors in individuals at risk of suffering from frailty fractures. The outcomes of this study will have implications for the development of more effective interventions that address the needs of these vulnerable populations.

Keywords: hip fracture; tele-rehabilitation; FLS; multicomponent intervention; physical exercise

Trial registration: *ClinicalTrials.gov. Identifier: NCT05435534 (Date of registration 25.05.2022).*

Administrative information

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Author details {5a}	1 Navarre University Hospital (HUN), Irunlarrea 3, 31008 Pamplona, Navarra, Spain. 2 Navarrabiomed, Institute for Health Research of Navarra (IDISNA), Irunlarrea 3, 31008 Pamplona, Navarra, Spain. 3 Public University of Navarre, Av Cataluña s/n 31006 Pamplona, Navarra, Spain. 4 Department of Orthopaedics Clinics and Traumatology, University Hospital of Navarre (HUN), Pamplona, Navarra, Spain. 5 CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Av Monforte de Lemos, 3-5, Pabellón 11, Planta 0, 28029 Madrid, Spain. 6 Institute for Health Research of Navarre (IDISNA), Irunlarrea 3, 31008 Pamplona, Navarra, Spain.

1. BACKGROUND AND RATIONALE {6a}

Osteoporosis is a prevalent disease globally and fragility fractures, especially hip fractures in older adults, impose a significant burden on health and economics [1,2]. Despite efforts to curb the increasing incidence of hip fractures, it remains a "silent epidemic" [1] affecting populations worldwide. The projected rise in the number of fragility fractures is alarming and many fracture liaison services (FLS) primarily focus on bone metabolism treatments, therapeutic adherence and mortality [3], ignoring other critical factors that affect older adults. Among these factors, we find functional decline, cognitive impairment, malnutrition, frailty, sarcopenia, pain, falls and comorbidities [4].

FLS have not yet studied the special approach required for frail and vulnerable individuals at risk of experiencing fragility fractures [5–7]. Although there is a consensus on the importance of nutrition, calcium, vitamin D and certain osteoporosis medications [8], the effectiveness and suitability of exercise guidelines for older adults remain controversial [9].

Tele-rehabilitation is a new way of providing rehabilitation remotely through information and communication technologies [10]. The @ctivehip [11] application is an

example of a program that has shown promising results in enhancing functional recovery, physical independence, quality of life, fear of falling and emotional status, as well as reducing the emotional state and perceived burden of informal caregivers [12]. However, the long-term effectiveness of such programs among older hip fracture patients, including exercise interventions like Vivifrail and ActiveHip, and their combination with comprehensive geriatric assessment (CGA) remain uncertain, as most studies have focused on evaluating their short-term effects over a three-month intervention period.

This study aims to contribute to the development of clinical integrated practice guidelines for the implementation of functional recovery after hip fracture with tele-rehabilitation (physical exercise based on the @ctivehip and Vivifrail programs [13]), nutrition, secondary prevention of osteoporosis, polypharmacy adjustment and other major comorbidities. Pathways for clinical management for older adults who are at risk of chronic illnesses moreover than osteoporosis are essential to approach the complexity of these patients.

2. OBJECTIVES {7}

2.1. Hypothesis

We hypothesize that a multicomponent intervention with tele-rehabilitation and the Vivifrail exercise program will improve hip fracture recovery at the 12-month follow-up.

3. METHODS AND ANALYSIS

3.1. Trial design {8}

This study will follow the recommendations of the International Conference on Frailty and Sarcopenia Research ICFSR Task Force 2020 [14]. This is a prospective, randomized controlled trial (RCT), two-group repeated measures experimental design. Patients will be assigned in a 1:1 allocation ratio.

3.2. Study setting {9}

The study will take place in the Department of Orthopaedics Clinics and Traumatology of Navarre University Hospital (Pamplona, Spain). Hospitalized patients who meet the inclusion criteria during the screening will be informed about the study. After signing the consent form (Supplementary Material 3), the subjects will be randomly assigned to either the intervention or active control care group.

3.3. Eligibility criteria {10} and recruitment {15}

The study participants will be older inpatient adults ≥ 75 years in the Trauma Ward of Navarre University Hospital (Pamplona, Spain) after a hip fracture. This study was approved by the Navarre University Hospital Research Ethics Committee (PI_2022/7) on 25 April 2022. It is estimated that the study dates will be from 1 June 2022 to 31 December 2025.

Patients will be eligible to participate if the following apply: (i) age ≥ 75 years with a diagnosis of hip fracture fragility; (ii) Barthel index score for activities of daily living (ADL) of ≥ 60 (scale: 0, severe functional dependence; 100, functional independence) 2 weeks before fracture [15]; (iii) mobility independence on the Functional Ambulation Classification (FAC) scale of ≥ 3 (scale: 0, non-functional ambulatory; 5, independent ambulator) 2 weeks before fracture [16]; (iv) ability/support to use the ActiveHIP app (defined as the presence of a patient or caregiver willing to use the platform and ability to operate it after installing it on the cell phone in the presence of the recruiter and understand Spanish); and (v) informed consent by patients (if possible), relatives or legal representatives.

Patients will be excluded if the following apply: (i) moderate–severe cognitive impairment with a Global Deterioration Scale (GDS) score of ≥ 5; (ii) secondary osteoporosis [17]; (iii) institutionalized in a permanent nursing home; (iv) refusal to give informed

consent by patient/primary caregiver/legal guardian or inability to obtain it; (v) terminal illness (life expectancy less than 3 months); and (vi) any factor that precludes the performance of physical exercise, including acute myocardial infarction in the past 3 months, unstable angina, severe heart valve insufficiency, arrhythmia/uncontrolled arterial hypertension or pulmonary embolism in the past 3 months and haemodynamic instability. Only the conditions specifically mentioned will be taken into consideration.

3.4. Who will take informed consent? {26a}

Study recruitment will be done through posters and other tools. We will provide explanations using the consent explanatory document and consent forms, and written consent will be obtained from all participants and their guardians. These consent forms will be under the scrutiny of the Ethics Committee to ensure all ethical standards will be met.

3.5. Additional consent provisions for collection and use of participant data and biological specimens {26b}

The study consent process includes permission for additional analyses of collected data. Blood samples will be collected.

3.6. Explanation of the choice of comparators {6b}

3.6.1. Interventions

In the active control group (control), participants will receive outpatient care in line with standard clinical practice. This sets it apart from traditional control groups in other studies that have no planned interventions. The intervention group (ActiveFLS), on the other hand, will receive an individualized multicomponent physical exercise program based on the ActiveHip+ for 3 months, in addition to standard care. In subsequent revisions, after finishing the ActiveHip+ program, the Vivifrail program will be given according to the patient's functional capacity. A CGA will be performed, evaluating nutrition status, polypharmacy, cognitive impairment and mood disorders. Nutritional intervention, adjustment of polypharmacy according to the Screening Tool of Older Person's Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) criteria, management of anxiety, depression, cognitive impairment and fear of falling will be done, as well as protocolized secondary fracture prevention treatment. Throughout the study, all participants will be permitted to maintain their regular physical activity levels. The interventions and follow-up are time-matched (Figure 1), ensuring both groups' experiences are synchronized over the same time period.

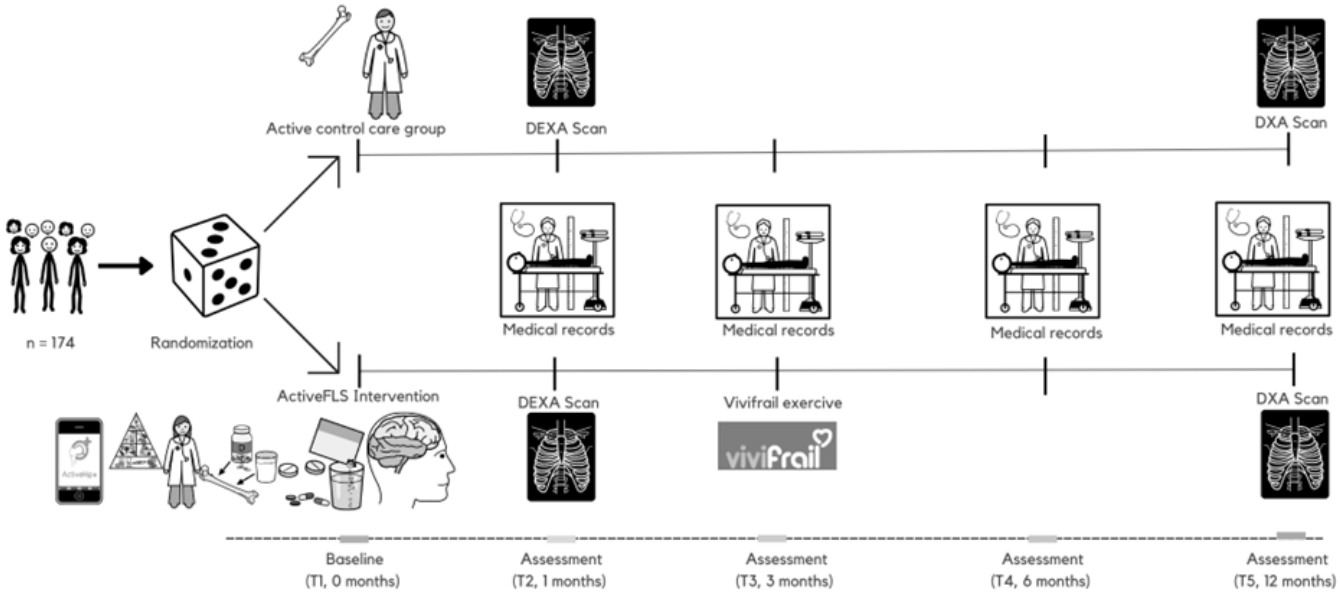

Figure 1. Intervention timeline through the "ActiveFLS randomized control trial". Participants will be randomly assigned to intervention group (ActiveFLS Intervention, n=87) or control group (Active control care, n=87). T: time-point; DXA: Dual energy x-ray absorptiometry.

3.6.2. Intervention description {11a}

ActiveFLS intervention: We will propose a comprehensive geriatric assessment program that includes a multicomponent physical exercise program guideline based on ActiveHip+. ActiveHip+ is a mobile app that is loaded onto the patient's smartphone. Given the limitations of older adults with smartphone apps, the caregiver will play a crucial role in ensuring the ongoing monitoring of the patient's rehabilitation program.

The ActiveHip+ program will include a health education program with five modules designed for patients and caregivers, as well as two additional modules specifically for caregivers. These modules will provide information on hip fracture recovery and strategies to prevent a second fracture. A detailed description of the program can be found [18]. The home-based tele-rehabilitation program, developed by a multidisciplinary team of health professionals and engineers, will include physical exercise and occupational therapy, with three smartphone-based sessions per week. The exercise program will comprise two physical exercise sessions and one occupational therapy session, ideally scheduled on alternate days, each lasting 30-60 minutes.

We will provide exercise guidelines based on the Vivifrail program. The focus of this program is to provide personalized exercise plans consisting of multiple components, tailored to the functional abilities of older individuals and to be performed at home. The program includes exercises for resistance/power, balance, flexibility, and cardiovascular endurance. A detailed description of the Vivifrail program can be found at http://vivifrail.com/resources/ [13].

After T3 (3-month assessment, Figure 1), patients in the intervention group will be enrolled in one of the four individualized Vivifrail training programs, based on their physical functional status: Disability (0–3 points in the SPPB score), Frailty (4–6 points), Prefrailty (7–9 points) and Robust (10–12 points). A copy of the patient's specific exercise protocol will be provided to each patient.

The exercise intervention will consist of a 5-day-a-week routine of multicomponent exercises for 12 consecutive weeks. This routine will include resistance, balance and flexibility exercises 3 days per week and walking 5 days per week. At the 6-month assessment, a new exercise program will be given to patients and caregivers based on the patients' functional status at that time. This program will remain the same until the final assessment.

A protocolized nutritional intervention will be carried out [19] based on the Global Leadership Initiative on Malnutrition (GLIM) criteria [20], with a focus on recommendations for protein intake, calcium and vitamin D [21]. Oral nutritional supplementation, if needed, will consist of supplements enriched in β-hydroxy-β-methylbutyrate (HMB) [22]. Vitamin D and anti-osteoporosis treatments will be prescribed following national guidelines [23], with zoledronic acid as the preferred choice due to better tolerance and adherence [24]. The patient's treatment will be reviewed and adapted based on the STOPP/START criteria [25]. Additionally, the patient's mood, cognitive impairment and fear of falling will be evaluated and addressed. The evaluation of depression will follow established clinical practices, utilizing a comprehensive approach that includes both pharmacological strategies, such as the use of prescribed medications, and non-pharmacological strategies, encompassing treatments like psychotherapy, cognitive-behavioural therapy, and lifestyle changes [26]. The training protocol is shown in **Figure 1**.

Active control care group (control): Participants allocated to the usual care group will receive standard outpatient care. This consists of multidisciplinary and multicomponent follow-up during hospital admission by Traumatology, Rehabilitation and Internal Medicine/Geriatrics. At discharge, a continuity of care report is made for follow-up by the

Primary Care team and a 1-month review by Traumatology with a control X-ray to check consolidation of the surgical fracture.

3.7. Participant timeline {13}

The Barthel index, FAC scale, GDS and institutionalization status will be conducted as a screening test to assess the general functional capacity of the patient's previous hip fracture. The study will have four major data collection points (baseline during acute hospitalization and at 3, 6 and 12 months) and one minor point (at 1 month). The times of measurement of the different outcomes are shown in **Table 1**. **Figure 2** displays the study flow diagram.

Table 1. Schedule for the different primary and secondary variables for the participants of the study.

Measure	Screening	T1 Baseline	T2 1 month	T3 3 months	T4 6 months	T5 12 months
Primary outcome						
Short Physical Performance Battery (SPPB).		x	x	x	x	x
Secondary outcomes						
Barthel index.	x		x	x	x	x
Functional Ambulation Classification (FAC).	x		x	x	x	x
Lawton's Instrumental Activities of Daily Living (IADL).		x	x	x	x	x
Global Deterioration Scale (GDS).	x		x	x	x	x
Mini-Mental State Examination (MMSE).		x	x	x	x	x
Abbreviated Mental Test 4 (4AT).		x	x	x	x	x
Yesavage Geriatric Depression Scale (YE-GDS).		x	x	x	x	x
Falls Efficacy Scale International (FES-I).		x		x		x
Frailty.		x	x	x	x	x
Handgrip.		x	x	x	x	x
Quality of Life (EuroQol-5D).		x	x	x	x	x
Sarcopenia and Quality of Life (SarQoL).		x		x		x
FRAX, QFracture.		x				x
Geriatric syndromes .		x	x	x	x	x
Polypharmacy.		x	x	x	x	x
Rate and risk of falls.		x	x	x	x	x
Visual Analogue Scale (VAS).		x	x	x	x	x

Cumulative Illness Rating Scale for Geriatrics (CIRS-G).		x				
Mini-Nutritional Assessment (MNA).		x	x	x	x	x
Adverse effects.			x	x	x	x
Mortality.			x	x	x	x
Admission and readmission to the hospital.			x	x	x	x
Institutionalization.	x		x	x	x	x
Blood test.		x	x	x	x	x
Bone turnover markers (BTMs).			x			x
Dual-energy X-ray absorptiometry (DXA).			x			x

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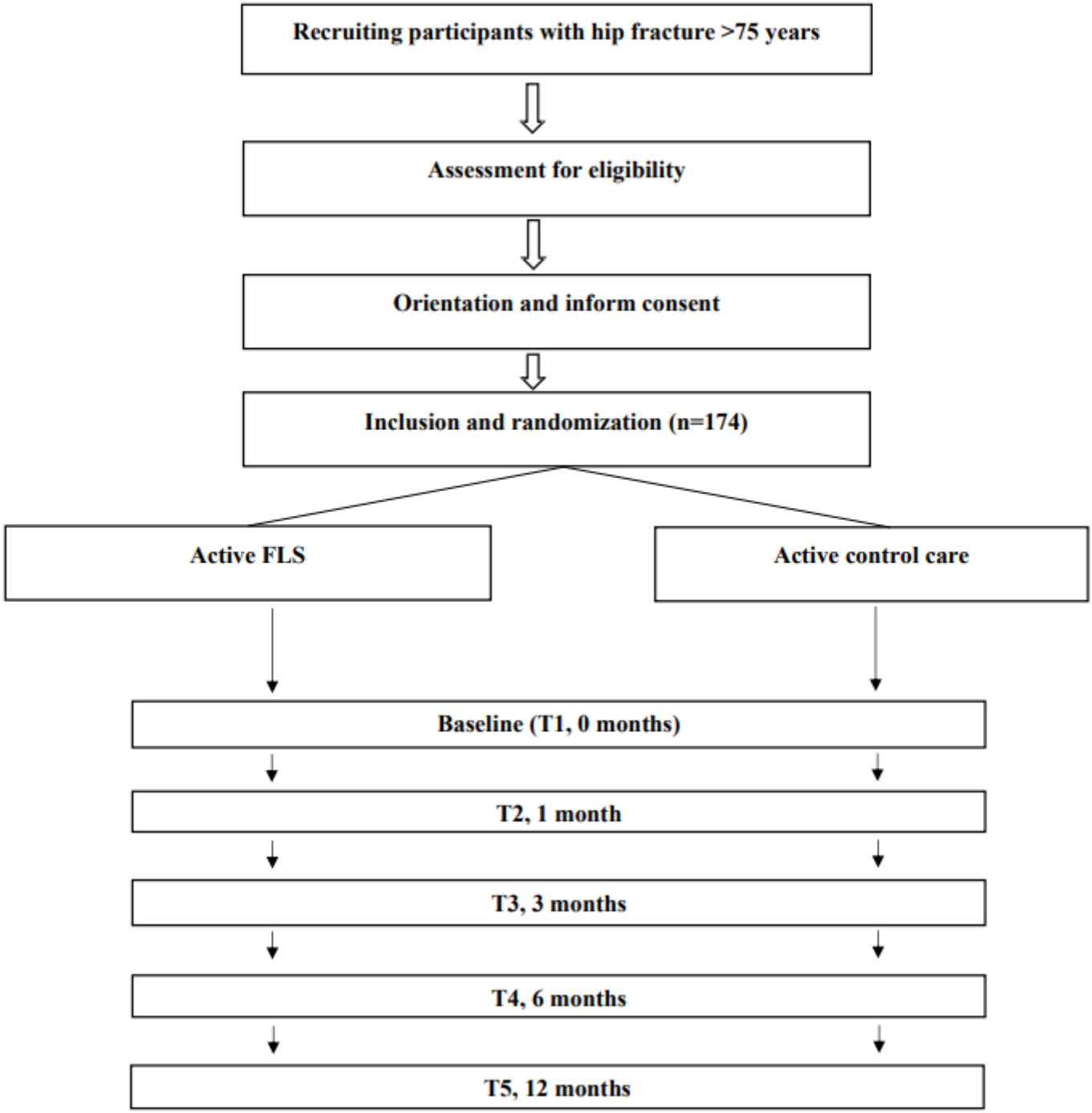

Figure 2. Flow diagram of the study protocol.

3.8. Criteria for discontinuing or modifying allocated interventions {11b}

Participants randomly assigned to the intervention group will be encouraged to use the Vivifrail program and/or usual care completely and sequentially as prescribed. As this practice-level intervention poses a low risk, there are no predefined rules for early termination.

Strategies to improve adherence to interventions {11c}

This study will aim to promote adherence to the intervention by designing a multifactorial intervention rehabilitation program after hip fracture based on a comprehensive geriatric assessment, secondary prevention of fracture and home-based rehabilitation

with ActiveHip and Vivifrail intervention based on high-quality evidence of FLS follow-up and international guidelines. The adherence to the Vivifrail programme will be based on the patient's daily record, which will be collected at each follow-up visit throughout the study.

3.9. Relevant concomitant care permitted or prohibited during the trial {11d}

During the trial, participants will not take part in other research projects that involve physical exercise interventions. However, participants are allowed to continue with any other non-conflicting interventions or therapies prescribed by their healthcare providers during the training period.

3.10. Provisions for post-trial care {30}

Not applicable. The intervention in this study will be implemented as part of the usual clinical practice for 12 months. Participants will have access to post-trial care and may choose to incorporate other strategies to improve their medical practice through consultation with a physician.

4. OUTCOMES {12}

4.1. Primary outcome

The primary outcome measure will be the change in functional status over the study period. Functional capacity will be assessed using the Short Physical Performance Battery (SPPB) [27], a single tool that evaluates balance, gait ability and leg strength. The SPPB test has been demonstrated to be a valid instrument for evaluating functional capacity and quality of life following a hip fracture [28]. The total score ranges from 0 (indicating worst functional capacity) to 12 points (indicating best functional capacity). A 1-point change in the score has been demonstrated to be clinical relevance [29].

4.2. Secondary outcomes

The secondary measures will assess constructs related to hip fracture, such as physical and cognitive decline, sarcopenia, nutrition, quality of life and healthcare system utilization. Furthermore, osteoporosis-related parameters will be measured using instrumented examinations, blood tests and dual-energy x-ray absorptiometry (DXA) (see Table 1).

- Functional status: The Barthel index of independence during ADL (0, worst; 100, best) [15], Lawton's Instrumental Activities of Daily Living (IADL) scale (0, worst; 8, best) [30] and the FAC scale (0, non-functional ambulatory; 5, independent ambulator) [16] will be used.

- Cognitive status [31]: The GDS, which describes seven clinically distinguishable global stages from normality to severe dementia of the Alzheimer type, and the 16-question Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), where each question is scored from 1 (much improved) to 5 (much worse) and a cut-off point (average score) of 3.31/3.38 achieves a balance of sensitivity and specificity of cognitive impairment [32], will be used. Delirium assessment during hospitalization will be carried out with the Abbreviated Mental Test 4 (4AT) [33].

- Mood status: Depression will be screened using the 15-item Yesavage Geriatric Depression Scale (YE-GDS; scale: 0, best; 15, worst), which is independently associated with hip fracture [34], and fear of falling will assessed with the Falls Efficacy Scale International (FES-I), where the validated cut-off points are low concern (16–19 points), moderate concern (20–27 points) and high concern (28–64 points) [35].

- Frailty and sarcopenia: Frailty will be screened by the FRAIL questionnaire and verified by modified Fried's frailty criteria [36]. Sarcopenia will be determined by: (i) handgrip strength < 16 kg for women or <27 kg for men; and (ii) appendicular skeletal muscle mass (ASMM)/ height^2 < 7.0 kg/m^2 for men or < 5.5 kg/m^2 for women [37].

Handgrip strength will be measured following the Groningen Elderly Test using a Smedley hand dynamometer [38]. The best of three attempts (with 30 seconds of rest between each attempt) will be recorded. The severity will be defined as gait speed ≤ 0.8 m/s or SPPB ≤ 8 points.

- Quality of life: The EuroQol-5D and the Sarcopenia and Quality of Life (SarQoL) scales will be used to measure the quality of life: the former measures five dimensions of health status (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and is a valid instrument for hip fracture patients [39]; and the latter is a novel validated instrument for measuring the quality of life in sarcopenia patients [40].

- Other clinical assessment: A comprehensive geriatric assessment will be conducted to evaluate geriatric syndromes [41], including falls (defined as unexpected and involuntary loss of balance, causing the person an undesired contact with the ground), polypharmacy (defined as five or more medications) [42] and pain (visual analogue scale: 0, best; 10, worst). Height will be measured with a digital stadiometer. Nutritional assessment will be performed by body mass index (BMI) calculation (weight/height^2) and by completing the Mini-Nutritional Assessment (MNA) tool [43]. Comorbidities will be evaluated with the Cumulative Illness Rating Scale for Geriatrics (CIRS-G) [44], ranging from 0 (best) to 56 (worst). Osteoporosis risk assessment is evaluated using the FRAX and QFracture tools [45] and pain using the Visual Analogue Scale (VAS).

- Adverse events: As per the International Conference on Harmonization Guidelines, serious adverse events will be defined as any event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability, or is a congenital anomaly/birth defect [46].

- Use of health sources: this will include hospital admissions, nursing home admissions, visits to primary care physicians, and visits to the emergency department.

- Biochemical analyses: Blood samples will be collected in Vacutainer tubes and centrifuged at 3300 rpm for 10 min at room temperature using a fixed-angle rotor. After centrifugation, the serum in the upper layer will be carefully extracted from the plasma in the bottom layer, divided into 100-µl aliquots and immediately stored at −80°C. Plasma and buffy coat will be also extracted and stored in polypropylene plastic tubes at −80°C until analysis. Bone turnover markers (BTMs) will be measured at the Clinical Neuroproteomics Unit (Navarrabiomed), whereas other measurements will be performed at the Central Laboratory Unit of Navarra (LUNA). All biological samples will be obtained after overnight fasting, between 8 and 10 am. Alkaline phosphatase, 25-hydroxyvitamin D_3 (vitamin D), parathyroid hormone (PTH), calcium, phosphorus, thyroid-stimulating hormone (TSH), creatinine and albumin will be run clinically, immediately after bringing the samples to the laboratory. Due to the high prevalence of hypoalbuminaemia in older adults, the serum concentrations of albumin and calcium will be used to correct the calcium value (calcium-corrected value = Ca + 0.8 [40-albumin]). The calcium-corrected value will be used in the subsequent analysis. C-terminal crosslinked telopeptide of type I collagen (CTX), sclerostin (SCL), bone-specific alkaline phosphatase (B-ALP), procollagen type 1 N propeptide (P1NP) and osteocalcin (OC) will be measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions from frozen samples [47].

- Dual-energy x-ray absorptiometry (DXA): Bone mineral density (BMD) and body composition (fat and lean mass) will be assessed using a Hologic DPX-IQ Discovery dual-energy x-ray absorptiometry (DXA) machine (GE Healthcare, Pollards Wood, UK). To minimize variability, all measurements will be performed by the same operator. The densitometer will be calibrated daily. BMD will be measured in grams per square centimetre at the non-predominant wrist, lumbar spine and proximal femur (neck, trochanter, intertrochanter area and Ward triangle) [48]. The L1 to L4 area will be included by aligning the patient with the axis of the examining table. To measure BMD in the proximal femur, the patient's position will be adjusted by rotating the legs 15–30° to discreetly visualize the smaller femur trochanter. The Z-score and T-score will be calculated in both locations. The

coefficient of variation will be 1.14%. Osteopenia and osteoporosis are defined using the World Health Organization standard criteria of a BMD T-score between −1.0SD and −2.49SD less than the young adult mean and less than −2.5SD, respectively [49]. Lean mass will be measured as appendicular skeletal muscle mass (ASM) adjusted for height squared (appendicular skeletal muscle mass index, ASMI) or body mass index (ASM/BMI) [37].

4.3. Sample size {14}

Assuming an alpha error of $\alpha = 5\%$, the simple sample size will be required to achieve a power of 90%, a $\varrho = 0.5$, a standard deviation for the SPPB of $\sigma = 2.5$ and detect a 10% difference in the frequency of patients obtaining a functional improvement of more than 1 point in the SPPB between each group will be 138 (69 per group), with an expected proportion of success in the usual clinical practice arm set at 30%. Given the characteristics of the study and the complexity of the patients (older adults after hip fracture), and assuming a loss of 20% of patients in the follow-up, we calculated a sample size of 174 subjects (87 patients in each arm). These calculations are based on a two-sided test. The 10% difference between both the intervention and the control group, representing a functional improvement greater than 1 point in the SPPB at 12 months between each group, will be considered clinically relevant based on the most relevant clinical variables involved in the functional decline after hip fracture.[50,51].

4.4. Assignment of interventions: Allocation

4.4.1. Sequence generation {16a}

Eligible practices will be allocated to either the intervention or control group using a randomized block approach, with blocks of four (www.randomizer.org).

4.4.2. Concealment mechanism {16b}

The assessment staff at the clinic will be kept blinded to the participant's randomized assignment as well as the main study design and the expected changes in study outcomes for each group. However, it will not be possible to conceal the group assignment from staff who are involved in training the intervention group. Patients and their families will be informed of their random inclusion in one group, but not of which specific group they belong to. If patients or their families inquire about the specific group to which they belong, they will be informed.

4.4.3. Implementation {16c}

When a participant will be deemed eligible and ready to be randomized, one of the research staff will determine which block-group they belong to and opens the next randomization block. The principal investigator will be notified of the site's randomization status and then will send an email to the practice and will inform the study staff.

4.5. Assignment of interventions: Blinding

4.5.1. Who will be blinded {17a}

Once a study participant will be randomized, their assigned study arm won't be kept blinded. However, the principal investigator, assessors and data analysis staff will be kept blinded to the identities of the intervention participants within their group.

4.5.2. Procedure for unblinding if needed {17b}

Not applicable. This study will be an unblinded intervention conducted at the practice level.

4.6. Data collection methods (plans for assessment {18a} and plans to complete follow-up {18b}) and data outcome management {19}

At each visit, data collection and procedures will be carried out. The study data will be stored on an encrypted hard disk partition that can only be accessed by the research team. Only authorized researchers will have access to this password. Participants will be identified using numbers or symbols, and any information that could easily identify them (such as name or address) will not be stored in the dataset. If a participant is prematurely discontinued from the study, they will be considered off-study and will follow the same schedule of events as those who continue in the study.

4.7. Confidentiality {27}

The study will adhere to the Spanish regulations including Law 3/2018 (5 December 2018) for the protection of personal data and to guarantee digital rights; Regulation 2016/679 of the European Union Parliament (27 April 2016) on data protection (RGPD); and Law 41/2002 (14 November 2002), which is a basic regulatory law on patient autonomy.

5. STATISTICAL METHODS

5.1. Statistical methods for primary and secondary outcomes {20a–20c}

We will use the intention-to-treat approach, incorporating all participants as originally allocated post-randomization. Missing data due to drop-outs or deaths will be addressed using multiple imputations. For qualitative variables, we will calculate frequencies and confidence intervals in an initial descriptive analysis. For continuous variables, we will report statistics of central tendency and dispersion, such as means, standard error and confidence intervals, or the median and interquartile range. We will check the normality of continuous variables graphically and through K-M and Shapiro-Wilk tests, comparing their differences between groups using either parametric tests (*t*-tests, mixed-effects models) or non-parametric tests (Mann–Whitney U, Kruskal-Wallis). We will employ a Bonferroni post-hoc test to evaluate statistically significant ($p < 0.05$) group and time differences. Sperarman's (rho) rank correlation coefficients and level of significance (p) will be used to assess the relationship between clinical/functional parameters and biochemical parameters, adjusted for age and sex. The values of r will be used to indicate small ($r = 0.10$), medium ($r = 0.30$) and large ($r = 0.50$) size correlations (i.e. effect size). Finally, we will assess the relationship between categorical and dichotomous variables through $\chi 2$ and Fisher exact tests. The level of statistical significance will be set at 0.05. We will analyse the data using SPSS package 23.0.

5.2. Interim analyses {21b}

Not applicable. The study will not include interim analyses or stopping guidelines since the medical practice-level intervention is considered low-risk.

5.3. Methods for additional analyses (e.g. subgroup analyses) {20b}

A secondary analysis of the primary endpoint will account for pre-randomization variables that could potentially predict positive outcomes. These groups will include frailty, sarcopenia, osteosarcopenia and the degree of cognitive impairment. A Bonferroni post-hoc test will be used to evaluate statistically significant ($p < 0.05$) group and time differences.

5.4. Oversight and monitoring

5.4.1. Composition of the coordinating centre and trial steering committee {5d}

Data Monitoring Committee: Mikel Izquierdo (Chair), Fabrizo Zambom-Ferrasi and Lucia Lozano-Vicario.

Trial Steering Committee: Nicolás Martinez-Velilla (Chair), Robinson Ramírez-Vélez and María Gonzalo Lázaro.

5.4.2. Composition, role and reporting structure of the data monitoring committee {21a}

The ActiveFLS study will have an independent data and safety monitoring committee that advises the investigators. The committee members will provide their expertise and recommendations in an individual capacity and report directly to the principal investigator.

5.4.3. Adverse event reporting and harms {22}

To ensure safety, the occurrence of falls and severe fall-related injuries will be monitored. Data on falls are based on medical records during follow-up. Other adverse events relative to the intervention protocol (nutrition, vitamin D, osteoporosis treatment, etc.) will also be monitored. The study team will conduct data monitoring to keep track of any minor or major events that may be associated with the intervention or usual care groups during the study. The chief investigators will review any adverse events or unintended effects detected.

5.4.4. Frequency and plans for auditing trial conduct {23}

There will not plans for auditing trial conduct.

5.4.5. Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Any changes made to the study protocol will be electronically communicated to all members of the research team and will be reviewed following the policies of the Institutional Review Board.

5.4.6. Dissemination plans {31a}

Dissemination is a recurring item on the agenda for the Department of Orthopaedics Clinics and Geriatrics of Navarre University Hospital (Pamplona, Spain) and the International Conference on Frailty and Sarcopenia Research ICFSR Task Force 2020 [14]. Patient advisors will be involved in reviewing all study materials to ensure that the findings are presented in an understandable and usable way for a broad audience. The study results will be disseminated in various formats, including peer-reviewed publications, conference presentations, blog posts, and policy briefs.

6. DISCUSSION

For this study, we will be developed a multifactorial intervention rehabilitation program after hip fracture. The program will be based on a comprehensive geriatric assessment, secondary prevention of fractures and home-based rehabilitation with ActiveHip and Vivifrail. We will aim to examine whether this intervention could improve functional status after hip fracture. Our ActiveFLS intervention will be developed based on high-quality evidence of FLS follow-up[52,53] and international guidelines [19,23] on hip fracture management, and it is feasible for most types of patients with little support. The use of integrated models of care based on comprehensive geriatric assessment can help align clinical practice with the individual needs of patients and enhance their quality of life [54]. Due to the crucial role of supervision during exercise programs on fracture reduction [55], this protocol will try to adapt current exercise programs to produce consistent supervision and monitoring results.

This study will have several strengths. First, it will be a combination of multiple interventions that were studied separately. This will also generate a problem in which the hypothetical expected benefit cannot be attributed to a specific intervention. However, given the complexity of managing older adults after a hip fracture, an approach in this direction will be possible to provide greater benefits. Secondly, very old adults will be included with a few exclusion criteria, making this study of broad impact on this heterogeneous population. Thirdly, it will be easily applicable to various regions as it is based

on home-based rehabilitation and will not require any specific infrastructure for implementation. The study will also have several limitations. Firstly, it will not include patients with advanced dementia defined as GDS ≥ 5 (a group with a high incidence of hip fracture) because the exercise interventions will not be adapted to this type of population [56]. Secondly, secondary osteoporosis will be also an exclusion criterion due to the variability of management in this population [17]. Thirdly, nursing-home patients will be excluded from the study due to the difficulty of follow-up and adherence to the intervention protocol (especially tele-rehabilitation). It should be noted that the usual care group, although involved in the study, will receive certain components of the ActiveFLS intervention. This is because this arm will include an assessment by Internal Medicine/Geriatrics and a follow-up by Primary Care.

To our knowledge, many studies have been developed for hip fracture management but they usually address issues from the fracture separately (exercise [55], nutrition [57], osteoporosis management [58]) or have low-quality evidence. If our hypothesis will be confirmed and demonstrates that our multifactorial and multicomponent program will improve functional status, it will lead to the development of a new targeted therapeutic pathway for use after hip fracture discharge.

6.1. Contribution to the field

Hip fracture is a frequent complication of osteoporosis that is linked to increased morbidity, mortality, and poorer functional recovery. Despite the numerous studies carried out in recent years, the best management in complex cases is still lacking. We hypothesize that multicomponent intervention with tele-rehabilitation could have a role in the evolution of hip fracture, given its multiple levels. This is the first study to assess the effect of a multifactorial intervention that includes tele-rehabilitation based on physical exercise on the recovery of hip fracture patients. If our findings align with our expectations, a possible new pathway and therapeutic protocol after hip fracture could be developed and implemented.

6.2. Trial status

The trial commenced recruitment on 1 June 2022 and is currently open for recruitment. Recruitment will cease when 174 participants have been randomized. It is anticipated that this target will be reached by December 2025.

7. ETHICS AND DISSEMINATION

Ethics statement {24}

This study was approved by the Navarre University Hospital Research Ethics Committee (PI_2022/7) on 25 April 2022. At the point of screening and enrollment, we will acquire written consent from participants or their legal representatives using two distinct documents. To guarantee participant understanding, we will employ meticulous and comprehensive explanations while securing consent during both the screening and enrollment processes.

Modification of the protocol {25}

Any adjustments made to the protocol that could influence the implementation of the study, potential benefits to the patient, or jeopardize patient safety - including alterations to the study objectives, design, patient population, sample sizes, study procedures, or significant administrative aspects - will necessitate a formal amendment to the protocol. This amendment will be agreed upon by the research team and approved by the Ethics Committee before implementation.

Availability of data and materials {29}

Within 12 months of the conclusion of the study, we will publicly release the de-identified participant-level data used for analyzing research questions through an online data repository.

SUPPLEMENTARY MATERIAL: Supplementary Material 1: Spirit Checklist. Supplementary Material 2: Items from the World Health Organization Trial Registration Dataset. Supplementary Material 3: Informed Consent.Table

Conflicts of Interest {28}: Dr Cedeno-Veloz reports receiving lecture fees from Amgen, Grünenthal, Italfarmaco, Nutricia, Angelini and Abbott, and grant support from Amgen and Abbott. The authors declare that the research will be conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: BC-V, IC-M, ARG and LL-V will develop the protocol in consultation with FZ-F, AMHO, RR-V, MI and NM-V; BC-V, IC-M, ARG and MGL will be involved in the recruitment and evaluation of the patients, and all authors listed will make an intellectual contribution to the work and approved it for publication.

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