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TESIS DOCTORAL POR COMPENDIO DE PUBLICACIONES

BÚSQUEDA DE BIOMARCADORES EN EL SINDROME DE FRAGILIDAD

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Dr. D. Omar Cauli, Profesor Titular del Departamento de Enfermería de la Facultad de Enfermería y Podología de la Universidad de Valencia, CERTIFICA:

Que Dña. Rut Navarro Martínez, Diplomada en Enfermería por la Universidad de Valencia, ha realizado bajo su dirección la presente Tesis Doctoral, titulada: "*Búsqueda de biomarcadores en el síndrome de fragilidad*", y autoriza su presentación para optar al Grado de Doctora en Enfermería.
Y para que conste a efectos oportunos, firma la presente certificación.

Fdo D. Omar Cauli

Valencia, 2017

A Otto

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He sido muy afortunada de poder contar en esta tesis doctoral con un director que desde el inicio han estado a mi lado, compartiendo con gran paciencia y generosidad todos sus conocimientos, y que me ha guiado con el máximo rigor científico. El doctor Omar Caulí, mi amigo, un referente en investigación y un ejemplo para mí.

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RESUMEN

En los próximos años, de mantenerse las tendencias demográficas de los ultimos años, se prevé un intenso proceso de envejecimiento. Las personas mayores son el grupo poblacional más heterogéneo en comparación con cualquier otro grupo de edad, y aunque un porcentaje accede al denominado "*envejecimiento exitoso*", otros acumulan multimorbilidad y diversos deterioros asociados a la edad, con la consecuente disminución de la esperanza y la calidad de vida. Tradicionalmente, la mayor parte de la investigación del envejecimiento se ha centrado en la supervivencia y en las posibles intervenciones para prolongar la esperanza de vida. Sin embargo, la tendencia actual es considerar más importante la prevención de la discapacidad que el simple aumento de la longevidad. En este sentido, la relevancia del concepto de fragilidad ha aumentado considerablemente en los últimos años, pues parece existir cierto consenso en que ésta constituye un estado que antecede a la discapacidad. Por tanto, el diagnóstico de la fragilidad es de gran importancia al permitir identificar a las personas con mayor riesgo de desarrollar discapacidad.

Una de las características más importantes de la fragilidad es que se trata de una condición dinámica, puede mejorar o empeorar con el tiempo e incluso invertirse. Por eso, el objetivo principal de la detección de la fragilidad es la intervención precoz y multidisciplinar con el fin de prevenir el deterioro funcional y la dependencia.

Actualmente, el diagnóstico de fragilidad se base principalmente en la medición de parámetros funcionales, que tienen utilidad clínica limitada, consumen tiempo, en ocasiones difíciles de realizar y a veces no están validados o suficientemente estandarizados. Por ello, en los últimos años, la búsqueda de marcadores sanguíneos de fragilidad que puedan identificar o al menos contribuir a la identificación precoz de las personas frágiles de manera sencilla, a la vez que se solicita un análisis rutinario, ha cobrado especial importancia.

Con este estudio se pretende identificar posibles marcadores sanguíneos de fragilidad, lo cual puede contribuir al desarrollo de nuevas herramientas clínicas con mayor poder diagnóstico y, por consecuencia, a mejora las decisiones terapéuticas con el fin de minimizar su progresión hacia la discapacidad, lo que ayudaría a disfrutar de una vejez más saludable y libre de discapacidad.

Para alcanzar este objetivo se evaluaron, en una población geriátrica institucionalizada, sin discapacidad ni deterioro cognitivo, las relaciones existentes entre los subtipos de leucocitos, la vitamina D y el factor neurotrófico derivado del cerebro (BDNF) con la fragilidad y, en particular, con alguno de los cinco criterios de fragilidad descritos por Fried (pérdida involuntaria de peso, baja energía o agotamiento, movilidad lenta, debilidad muscular y baja actividad física).

Nuestros resultados mostraron en relación con las subpoblaciones de leucocitos, una correlación positiva y significativa entre la puntuación de la escala de fragilidad de Fried y el porcentaje de neutrófilos ($p < 0,05$). Igualmente pudimos observar una correlación significativa entre el porcentaje de linfocitos con el número de criterios de Fried ($p < 0,05$), pero, en este caso, la relación fue en dirección opuesta. Estas asociaciones tambien fueron significativas para dos de los cinco criterios de fragilidad de Fried, baja fuerza muscular y baja actividad física ($p < 0,05$).

La relación entre fragilidad y los niveles de BDNF no mostró ninguna asociación significativa, sin embargo, se observó una correlación positiva y significativa entre las concentraciones de BDNF con el índice de Barthel, una medida de la capacidad para realizar las actividades básicas de la vida diaria ($p = 0,03$), la subcategoría de concentración medida con mini examen del estado mental (Mini Mental State Examination (MMSE)) ($p= 0,01$) y el recuento de eosinófilos en sangre ($p = 0,01$). Además, el BDNF se correlacionó de manera inversa y significativa con la concentración de colesterol total ($p = 0,04$) y de colesterol de lipoproteínas de alta densidad (HDL-c) ($p = 0,04$).

En nuestro estudio los sujetos frágiles, en comparación con los individuos robusto, presentaron una disminución significativa de las concentraciones séricas de vitamina D ($p < 0,01$), sin embargo, esta disminución no se correlacionó significativamente con la gravedad del síndrome de fragilidad ni con ninguno de los criterios individuales de la fragilidad.

Como conclusión nuestros resultados muestran la existencia de posibles biomarcadores de fragilidad relacionados con el sistema inmune y hormonal, los cuales no sólo contribuyen a la detección temprana de la fragilidad, sino también, nos permiten conocer los mecanismos biológicos que contribuyen a su desarrollo, con la consiguiente posibilidad de desarrollar o adaptar intervenciones de enfermería adecuadas para tratar, prevenir e incluso revertir el síndrome frágil.

ABSTRACT

In the coming years, if the demographic trends of the last decades are maintained, an intense process of aging is foreseen. Older people are the most heterogeneous population group in comparison with any other age group and, although a percentage accede to the so-called "successful aging", others accumulate multimorbidity and various age-related impairments, with the consequent decrease of hope and quality of life. Traditionally, most aging research has focused on survival and possible interventions to extend life expectancy. However, the current trend is to consider prevention of disability as more important than simply increasing longevity. In this sense, the clinical and scientific relevance of the concept of fragility has increased considerably in recent years, since there seems to be some consensus that it constitutes a state that precedes disability. Therefore, the diagnosis of fragility is of great importance, allowing identifying the people with greater risk of developing disability.

One of the most important characteristics of fragility is that it is a dynamic condition; it can improve or worsen over time. Therefore, the main objective of the detection of fragility is early and multidisciplinary intervention in order to prevent functional deterioration and dependence.

At present, the diagnosis of fragility is based mainly on the measurement of functional parameters, which have limited clinical utility, are time consuming, sometimes difficult to perform and may not be validated or sufficiently standardized. Thus, in recent years, the search for blood markers of fragility that can identify or at least contribute to the early identification of fragile individuals in a simple way, while requesting a routine analysis, has gained special importance.

This study aims to identify possible blood markers of fragility, which may contribute to the development of new clinical tools with greater diagnostic power, and consequently to improve therapeutic decisions in order to minimize their progression towards disability, which would help enjoy a healthier and disability-free old age.

To achieve this objetive, the relationships between leukocyte subtypes, vitamin D (measured as total 25-hydroxyvitamin D, 25 (OH) 2 D3) and brain-derived neurotrophic factor (BDNF) with brittleness were evaluated (involuntary weight loss, low energy or exhaustion, slow mobility,

muscle weakness and low physical activity) in an institutionalized geriatric population with no disability or cognitive impairment.

Our results showed a positive and significant correlation between the Fried fragility scale score and the neutrophil count ($p < 0.05$) in relation to the leukocyte subpopulations. We also observed a significant correlation between the lymphocyte counts with the number of Fried fragility criteria ($p < 0.05$), but, in this case, the relationship was in the opposite direction. These associations were significant only for two of the five Fried fragility criteria, low muscle strength and low physical activity ($p < 0.05$).

The study of the relationship between brittleness and BDNF levels did not show any significant association, however, a significant positive correlation was observed between plasma BDNF concentrations and the Barthel index, a measure of individuals' ability to perform ($p = 0.03$) and the subcategory of concentration measured with Mini Mental State Examination (MMSE) $p = 0.01$). In addition, plasma BDNF correlated inversely and significantly with blood eosinophil counts ($p = 0.01$), total cholesterol concentration ($p = 0.04$), and high density lipoprotein cholesterol ($p = 0$, 04).

In our study, fragile subjects had a significant decrease in serum concentrations of 25 (OH) 2 D 3 compared to robust individuals ($p < 0.01$), but this decrease was not significantly correlated with the severity of the fragility syndrome nor with any of the individual fragility criteria.

In conclusion, our results show the existence of possible biomarkers of fragility related to the immune and hormonal systems, which not only contribute to the early detection of fragility but also allows us to know the biological mechanisms that contribute to its development with the patient possibility of developing or adapting appropriate nursing interventions to treat, prevent and even reverse the fragility syndrome.

PREÁMBULO

En virtud del nuevo Reglamento sobre depósito, evaluación y defensa de la tesis doctoral, aprobado en cumplimiento de lo que dispone el Real Decreto 99/2011, de 28 de enero, por el que se regulan las enseñanzas oficiales de doctorado, así como el artículo 136 de los Estatutos de la Universitat de València; se presenta esta Tesis Doctoral con título "*Búsqueda de biomarcadores en el síndrome de fragilidad"* en la modalidad de compendio de publicaciones. He optado por este formato de Tesis Doctoral por dos razones fundamentales, en primer lugar, y coincidiendo con Luciano Devis (2007), las Tesis Doctorales por compendio de publicaciones permiten la comunicación y difusión de los resultados obtenidos a la comunidad científica de forma prácticamente inmediata, no teniendo que esperar varios años a que finalice el proyecto de tesis para enviar a publicar los trabajos realizados, lo cual puede provocar que éstos pierdan originalidad e interés. Y en segundo lugar, porque actualmente, uno de los principales criterios por los que se mide la calidad de un trabajo científico, es a través del nivel de las revistas en que éste es publicado.

Así, y teniendo en cuenta los requisitos expuestos en el punto 1 del artículo 8 del citado Reglamento, el presente documento, en lugar de adoptar el formato tradicional, incluye una amplia introducción general, que presenta los trabajos compendiados justificando su unidad temática, así como un resumen global de los resultados obtenidos, una discusión general de estos resultados y unas conclusiones finales.

Asimismo, la normativa de dicha modalidad obliga incluir únicamente un mínimo de 3 artículos publicados o admitidos para su publicación en revistas de reconocido prestigio. En este sentido, la totalidad de los trabajos que conforman la presente Tesis doctoral han sido publicados en revistas indexadas en Social Science Citation Index (SSCI) del Journal Citation Reports (JCR) de la Web of Knowledge, tras estrictas revisiones llevadas a cabo por al menos dos expertos independientes, lo cual es un importante indicador de la calidad del trabajo realizado.

A continuación, se muestran, por orden cronológico de publicación, las referencias de los 3 artículos compendiados en la presente Tesis doctoral, así como el factor de impacto de las mismos, pudiendo ser consultados en el apartado de Anexos (Anexo A) de este mismo documento:

Artículo 1:

Fernández-Garrido, J [1]., Navarro-Martínez, R [1]., Buigues-González, C., Martínez-Martínez, M., Ruiz-Ros, V., Cauli, O. (2014). The value of neutrophil and lymphocyte count in frail older women. *Exp Gerontol*, 54:35-41.

[1]Estos autores contribuyen igualmente en este trabajo.

- Factor de impacto en Journal Citation Reports (JCR), 2014: 3.485.
- Categoría y posición: Geriatrics & Gerontology, 12/50 (Q1).

Artículo 2:

Navarro-Martínez, R., Fernández-Garrido, J., Buigues, C., Torralba-Martínez, E., Martinez-Martinez, M., Verdejo, Y., Mascarós, M.C., Cauli, O. (2015). Brain-derived neurotrophic factor correlates with functional and cognitive impairment in non-disabled older individuals. *Exp Gerontol*, 72:129-37.

- Factor de impacto en Journal Citation Reports (JCR), 2015: 3.350.
- Categoría y posición: Geriatrics & Gerontology, 12/49 (Q1).

Artículo 3:

Navarro-Martínez, R., Fernández-Garrido, J., Buigues, C., Martínez-Martínez, M., Cantero-Díaz, L., Santamaría-Carrillo, Y., Serra-Catalá, N., Peris, C., Cauli, O. (2016). Serum vitamin D and functional impairment in octogenarian women. *Appl Nurs Res*, 30:e10-4.

- Factor de impacto en Journal Citation Reports (JCR), 2016: 1.379.
- Categoría y posición: Nursing, 36/116 (Q2).

Aunque figuro como primera autora de todos los artículos compendiados en el presente trabajo, cumpliendo así con la normativa vigente, quisiera subrayar que el conjunto de los mismos representa la labor colectiva realizada por el grupo de investigación emergente "Frailty Organized Research Group" (F.R.O.G.), del que soy miembro integrante, gracias al cual, la producción científica enfermera de la Universidad de Valencia se ha visto incrementada, tal y como lo demuestran, no solo las publicaciones que conforman este documento sino también, otros trabajos realizados en el marco de la misma línea de investigación. Una copia completa de cada uno de ellos también se halla en el apartado de anexos (Anexo B) de este mismo documento.

Finalmente, es importante destacar que estos trabajos han sido realizados con el apoyo de dos proyectos de investigación, el primero de ellos, el proyecto de investigación precompetitivo denominado: "Deterioro cognitivo en la población frágil: búsqueda de biomarcadores" (nº referencia: UV.INV.PRECOMP 13-115500); financiado por el servicio de investigación de la Universidad de Valencia. Y el segundo, el proyecto I+D denominado: "Búsqueda de biomarcadores para el síndrome de fragilidad" (nº referencia: GV/2014/043); financiado por la Consellería de Educación, Cultura y Deporte, de acuerdo a la siguiente convocatoria: Orden 79/2013 de 30 de julio de (DOCV núm. 7018, de 2 de agosto 2013).

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1,25- (OH)$_2$ D$_3$: 1,25- hidroxivitamina $_2$ D$_{3.}$

5-MTR: 5-metiltetrahidrofolato-homocisteína metiltransferasa.

8-OHdG: 8 hidroxi 2'deoxiguanosina .

25-(OH) D: 25-hidroxivitamina D.

± DE: ± Desviación estándar.

± SEM: ± Error estándar de la media.

ABVD: Actividades básicas de la vida diaria.

AIVD: Actividades instrumentales de la vida diaria.

AVD: Actividades de la vida diaria.

ALT: Alanina transaminasa.

AST: Aspartato transaminasa.

BDNF: Factor neurotrófico derivado del cerebro.

BTRC: Beta-transducina repeat containing.

CASP8: Caspasa 8.

CHS: Cardiovascular Health Study.

CMV: Citomegalovirus.

CREBBP: CREB- binging proteín.

CSHA: Canadian Study of Health and Aging.

CT: Colesterol total.

DE: Diagnóstico enfermero.

DHEA-S: Dehidroepiandrosterona sulfato.

ECV: Enfermedad/ades cardiovasculares.

EESE: Encuesta Europea de Salud en España.

ENS: Encuesta Nacional de Salud.

EEPA: Energy expenditure in physical activity.

ETES: Estudio Toledo para un Envejecimiento Saludable.

FRADEA: Fragilidad y Dependencia en Albacete.

GGT: Gamma-glutamil transferasa.

GH: Hormona del crecimiento.

GOT: Transaminasas oxaloacético glutámico.

GPT: Ttransaminasa pirúvico glutámico.

HDL-c: Lipoproteínas de alta densidad.

IF: Índice de fragilidad.

IGF-I: Factor de crecimiento similar a la insulina de tipo I.

IL: Interleuquina.

IMC: Índice de masa corporal.

InCHIANTI: Invecchiare in the Chianti.

INE: Instituto Nacional de Estadística.

KAT2B: lysina acetiltransferasa 2B.

LDL-c: Colesterol de lipoproteínas de baja densidad.

MILTPAQ: Minnesota leisure time physical activity questionnaire.

MMSE: Mini Mental State Examination.

MSSSI: Ministerio de Sanidad, Servicios Sociales e Igualdad.

NANDA-I: Asociación Norteamericana de Diagnósticos Enfermeros Internacional.

NGF: Factor de crecimiento nervioso.

NHANES III: Tercera Encuesta Nacional de Salud y Nutrición.

NPI-Q: Neuropsychiatric Inventory Questionaire.

NT-1: Neurotrofina-1.

NT-3: Neurotrofina-3.

NT-4: Neurotrofina-4.

OMS: Organización Mundial de la Salud..

PTH: Parathormona.

RI: Resistencia a la insulina.

SEGG: Sociedad Española de Geriatría y Gerontología.

SNC: Sistema nervioso central.

SNPs: Single nucleotide polymorphism.

SOF: Study de Osteoporotic Fractures.

TGD: Triglicéridos.

TSH: Hormona estimulante del tiroides.

TUG: Timed get up and go test.

VGI: Valoración geriátrica integral.

WHI-OS: Estudio de Salud-Iniciativa de Observación de la Mujer.

AVD: actividades de la vida diaria;.

BLSA: *Beijing Longitudinal Study of Aging*.

CHS: *Cardiovascular Health Study*.

CNPHS: *Canadian National Population and Health Survey*.

CSHA: *Canadian Study of Health and Agin*;.

H-EPESE: *Hispanic Stablished Populations for Epidemiologic Studies of the Elderly*.

ILSA: *Italian longitudinal estudy on aging*;.

NO: no significativo.

PEP: *Precipitating Events Project*.

RMAP: *Rush Memory and Aging Project*.

SHARE: *Survey of Health, Aging and Retirement in Europe*.

SI: asociación significativa.

SOF: *Study osteoporotic fractures*.

WHAS I y II: *Women's Health and Aging Study*.

WHI-OS: *Women's Health Initiative Observational Study*.

3-CITIES*: estudio de las 3 ciudades.*

CAPÍTULO I- INTRODUCCIÓN

"Una bella ancianidad es, ordinariamente, la recompensa de una bella vida."

Pitágoras

1. ENVEJECIMIENTO

1.1. El envejecimiento de la población

El creciente envejecimiento de la población es uno de los fenómenos demográficos más significativo de las últimas décadas (Abades y Rayón, 2012). Naciones Unidas, en su informe de la Segunda Asamblea Mundial sobre el Envejecimiento, señalaba que nuestro mundo está sufriendo cambios demográficos sin precedentes (Tinao Martín-Peña, 2005).

En España, desde hace años, la población viene atravesando un intenso proceso de envejecimiento, debido principalmente a un aumento significativo de la esperanza de vida y a una disminución importante de la fecundidad (Orueta Sánchez et al., 2008). Así, tal y como se observa en la siguiente tabla (véase Tabla 1), en las últimas décadas, se ha ido produciendo un rápido y progresivo trasvase de efectivos jóvenes hacia las edades adultas. De manera que la población joven entre 0 y 14 años ha pasado de representar, en 1970, el 27,79 % de la población total, al 15 %, en 2016. Al mismo tiempo se ha producido un aumento, igualmente importante, de la población de 65 y más años que, en el mismo periodo de tiempo, ha pasado del 9,7% al 18,4% de la población total (Instituto Nacional de Estadística (INE), Cifras de población 1970-2016). Es decir, en poco más de medio siglo el porcentaje de personas mayores se ha duplicado (Abellán García y Ayala García, 2012).

Este envejecimiento tiene dos rasgos característicos. Por una parte, se ha producido un envejecimiento de la población ya envejecida, fenómeno conocido como "*envejecimiento del envejecimiento*" (González y San Miguel, 2001). Los octogenarios son el grupo de edad que más rápido ha creciendo dentro del grupo de personas mayores de 65 años (Abellán García y Pujol Rodríguez, 2016). Actualmente, en España existen 2.046.5554 personas octogenarias, que suponen el 6% de la población total y el 32% de la población mayor de 65 años (INE, Cifras poblacionales 1970-2016). De mantenerse las tendencias demográficas de las últimas décadas, en 2066 el número de personas de 65 y más años ascenderá a más de 15 millones, es decir, el 38,7 % de la población total, donde las personas octogenarias constituirán el 13,1 % de la población española y el 44 % de la población de 65 y más años (INE, Proyecciones de población 2016-2066).

Tabla 1
Evolución de la población española 1970-2016.

Año	De 0-14 años		De 15-64 años		De 65 y más		De 80 y más	
	Absoluto	(% del total)	Absoluto	(% del total)	Absoluto	(% del total)	Absoluto	(% del total)
1970	9.459.640	27,7	21.290.338	62,5	3.290.679	9,7	523.661	1,5
1981	9.685.729	25,7	23.760.908	63,1	4.286.721	11,4	725.131	1,9
1991	7.532.668	19,4	25.969.348	66,8	5.370.252	13,8	1.147.868	3
2000	5.863.669	14,4	27.816.058	68,7	6.862.746	16,9	1.555.235	3,8
2010	6.936.340	14,8	32.153.527	68,4	7.931.164	16,9	2.303.206	4,9
2016	6.990.863	15	30.908.400	66,4	8.657.705	18,6	2.778.928	6
2031	5.443.421	11,9	28.717.658	62,6	11.725.208	25,5	3.650.244	8
2066	4.652.564	11.3	22.040.684	53,7	14.193.394	34,6	7.400.377	18

Fuente: Elaboración propia a partir de datos del INE. Cifras de población 1970-2016.

Por otra parte, las cifras de envejecimiento entre hombres y mujeres no se reparten de modo uniforme. Dado que las mujeres viven más que los hombres, el porcentaje de mujeres aumenta entre la población de mayor edad (Abellán García y Pujol Rodríguez, 2016). Por tanto, es posible hablar de un proceso de "*feminización de la vejez"* (González y San Miguel, 2001). En la actualidad, las mujeres representan el 57% de la población de 65 años y más. Entre los mayores de 80 años de edad, las mujeres prácticamente duplican el número de hombres y representan el 65% de la población de este grupo de edad (INE, Cifras de población 1970-2016). Dentro de pocos años, con las actuales tendendias demográficas, casi siete de cada diez españoles de 65 años o más será una mujer (INE, Proyecciones de población 2016-2066).

1.2. Significado de envejecimiento

Resulta difícil establecer con precisión el concepto de envejecimiento. De hecho, existen numerosas definiciones pero ninguna aceptada de manera universal. Diversos autores coinciden en tratarlo como un proceso dinámico, multifactorial e inherente a todos los seres vivos (Alvarado García y Salazar Maya, 2014).

Asimismo, de la mayoría de las definiciones recogidas sobre el envejecimiento, se puede extraer que se trata de un proceso de deterioro funcional progresivo por la suma de todas las modificaciones que, a nivel biológico, psicológico y social, se producen en el organismo con el paso del tiempo (Hernández Martínez-Esparza et al., 2006). En términos biológicos, estos cambios se traducen en una pérdida progresiva de la capacidad de adaptación y de la reserva funcional del organismo, lo que aumenta las posibilidades de padecer enfermedades y discapacidad y, en último término, de morir (Fernández-Garrido, 2009). Por ello, aunque debemos tener claro que el envejecimiento no es una enfermedad, los cambios que experimenta el organismo con el paso del tiempo aumentan las posibilidades de padecerlas (Pérez y Sierra, 2009). Así, según la Organización Mundial de la Salud (OMS), el envejecimiento se define como: *"El deterioro progresivo y generalizado de las funciones, que produce una pérdida de respuesta adaptativa al estrés y un mayor riesgo de sufrir enfermedades"* (OMS, 2002).

Para algunos autores es posible distinguir entre envejecimiento primario y envejecimiento secundario (Papalia y Wendkos, 1998). El envejecimiento primario, también llamado intrínseco, es responsable del conjunto de cambios biológicos vinculados al simple paso de los años. Está condicionado por la carga genética de cada individuo. Es por tanto, un proceso inevitable, universal y, que no está afectado por la influencia de enfermedades o del propio entorno, ocurre incluso en personas con buena salud. Actualmente no existe posibilidad de intervención sobre los mecanismos que determinan este deterioro, aunque en un futuro, el mejor conocimiento de las llamadas teorías del envejecimiento y el acceso a técnicas que permitan la manipulación genética, deje algún margen para la actuación.

El llamado envejecimiento secundario, o extrínseco, involucra a los procesos de deterioro que se relacionan con las propias enfermedades y factores del entorno, como el estilo de vida e influencias ambientales. En este proceso de envejecimiento es posible la intervención, especialmente a través de medidas preventivas, por tanto, es un envejecimiento evitable y no universal. Actuar a este nivel supone aumentar la esperanza de vida y mejorar su calidad, es decir, vivir más y mejor.

Para muchos autores, el envejecimiento intrínseco nunca puede escapar totalmente de la influencia del extrínseco (De Miguel Negredo, 2001). En último término ambas formas de envejecer se entrelazan y se superponen en mayor o menor grado. Esto hace que la población mayor de 65 años no sea una población homogénea pues, la simple observación de las personas mayores pone de relieve que, existen personas que llegan a edades avanzadas de la vida con buena salud mientras que otros presentan un gran deterioro (Fontecha Diazma, 2013). Esta heterogeneidad, que resulta de interacciones complejas entre los factores genéticos y ambientales, lleva a una variabilidad individual de edad fisiológica que no coincide exactamente con la edad cronológica (Alvarado García y Salazar Maya, 2014; De Calvo, 2007).

Esto hace que debamos distinguir distintas formas de envejecer que Rowe y Khan (1997) redujeron, sintéticamente, a tres: envejecimiento "*patológico*", "*usual*" y "*con éxito*". Según estos autores, mientras que el envejecimiento patológico sería el resultado de un organismo quebrantado por la enfermedad y los factores ambientales adversos, el envejecimiento usual es aquel que cursa sin enfermedades definidas pero acompañado de diversos deterioros asociados a la edad, los cuales no interfieren en el mantenimiento de una vida normal, independiente y de calidad. Aunque el envejecimiento usual no es patológico, se le considera de alto riesgo para una morbimortalidad elevada cuando se le compara con el envejecimiento exitoso, es decir, aquel con baja probabilidad de enfermar y de discapacidad asociada a un alto funcionamiento cognitivo y capacidad física funcional, donde además existe un activo compromiso con la vida. El modelo del envejecimiento exitoso se representa en la siguiente figura (veáse Figura 1).

Figura 1. **Modelo de envejecimiento exitoso.**
Fuente: Elaboración propia a partir de Rowe y Khan (1997).

La propia OMS a finales del siglo anterior, con la intención de transmitir un mensaje más completo que el de envejecimiento exitoso, introdujo el concepto de envejecimiento activo, definiéndolo como el proceso en que se optimizan las oportunidades de salud, participación y seguridad, a fin de mejorar la calidad de vida de las personas a medida que envejecen (Zunzunegui y Béland, 2010). Permite a las personas aprovechar al máximo su potencial de bienestar biopsicosocial y participar en la sociedad de acuerdos a sus necesidades, deseos y capacidades, mientras se les proporciona seguridad, protección y cuidados adecuados cuando lo necesiten. Un envejecimiento activo, desde el punto de vista de la sanidad, supone mantener la salud y conseguir que la persona mayor permanezca autónoma e independiente el mayor tiempo posible (Martin-Lesende et al., 2007).

1.3. Envejecimiento y gasto sanitario

España ha experimentado en las últimas décadas un aumento considerable del gasto sanitario (Aguado et al., 2012). Detrás de esta evolución se encuentran factores muy diversos que incluyen una demanda de servicios que aumenta con el desarrollo económico, así como, factores de oferta relacionados, por ejemplo, con el efecto del cambio tecnológico (Getzen, 2000). No obstante, la demografía, y especialmente el envejecimiento de la población, puede tener un impacto importante sobre el gasto sanitario (Casado et al., 2009).

Resulta un hecho comúnmente aceptado que el gasto sanitario aumenta con la edad (Alemayehu y Warner, 2004; Aguado et al., 2012; Hernández de Cos y Ortega Regato, 2002). En nuestro pais, los informes para el análisis del gasto sanitario muestran como, a partir de los 5 años el gasto sanitario per cápita se eleva lentamente a lo largo de la vida adulta, y aumenta de manera exponencial después de los 55 años de edad, llegando casi a triplicarse en los mayores de 75 años (véase Figura 2) (Hernández de Cos y Moral-Benito, 2011). Este mismo patrón se reproducen en el resto de paises de nuestro entorno, donde el gasto sanitario de la población mayor de 65 años es 3,6 veces superior al del resto de individuos (Molina Morales et al., 2012).

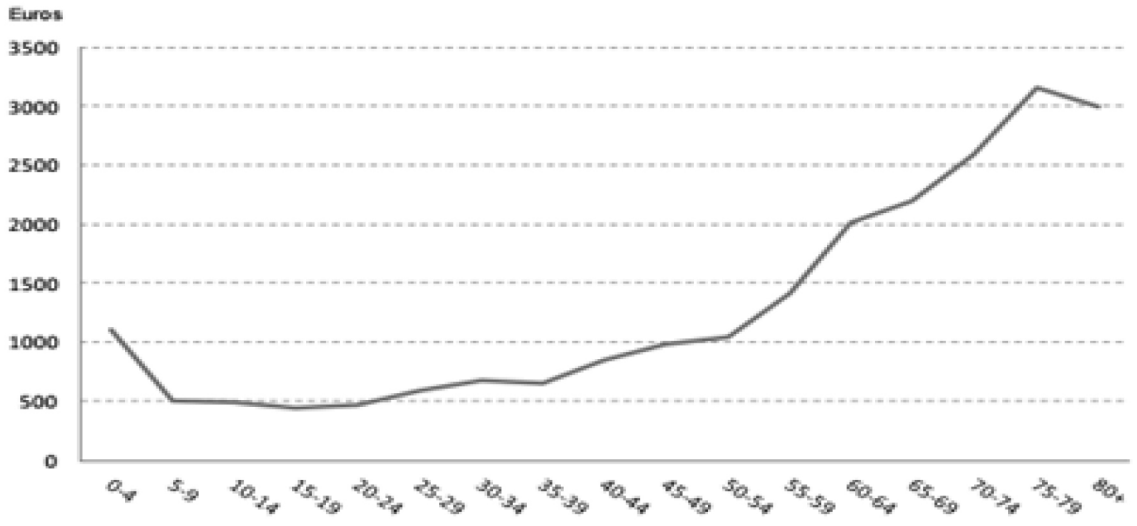

Figura 2. **Gasto sanitario per cápita por grupos de edad. España 2005.**
Fuente: Tomada de Instituto de Hernández de Cos y Moral-Benito (2011).

El mayor gasto sanitario de las personas mayores respecto a otros grupos de la población está relacionado con un mayor consumo de recursos sanitarios (Ahn et al., 2003). Los datos de la Encuesta Europea de Salud en España (EESE) del 2014 y la Encuesta Nacional de Salud (ENS) referida al 2012, muestran una mayor utilización de los servicios sanitarios y del consumo de medicamentos en el grupo de edad de 65 y más años con respecto a la población adulta (véase Tabla 2).

Tabla 2
Utilización de servicios sanitarios y consumo de medicamentos. Porcentajes por grupos de edad.

	Consulta médico/a de familia *		Consulta médico/a especialista *		Ingreso hospitalario **		Consumo de medicamentos *** (sólo recetados)	
	ENS	EESE	ENS	EESE	ENS	EESE	ENS	EESE
Población de 15-64 años	28.74	29.03	14.40	14.21	7.91	8.73	56.01	44.79
Población de 65-74 años	41.98	42.52	20.31	19.50	10.49	11.81	80.38	74.00
Población de 74-85 años	50.28	52.26	22.65	19.87	16.89	16.05	85.83	81.84
Población de > 85 años	47.50	50.45	16.28	15.49	18.32	18.75	87.58	84.33

Nota.* En las últimas 4 semanas ;** en los últimos 12 meses ;*** en las últimas 2 semanas.
Fuente: Elaboración propia a partir de datos de la EESE (2014) y la ENS (2012).

No obstante, para Casado Marín (2001), buena parte del impacto que puede tener el envejecimiento sobre el gasto y el uso de recursos sanitarios reside en la proximidad al momento de la muerte. Diversos estudios realizados acerca de cómo se distribuye el gasto sanitario a lo largo del ciclo vital, indican que el nivel más alto de gasto sanitario que realiza una persona a lo largo de su vida, con independencia de su edad, se concentra en sus últimos años de vida (Zweifel et al., 1999). Un fenómeno que confirma que no es la edad cronológica en sí misma, sino el estado de salud de los individuos, lo que incrementa el gasto sanitario (Seshamani y Gray, 2004).

1.4. Estado de salud de la población mayor

Hoy en día, en los países desarrollados, llegar a una edad avanzada ha dejado de ser algo excepcional, sin embargo, muchas personas no logran envejecer con una buena calidad de vida (Apiazu et al., 2002). En España, mientras que la esperanza de vida media al nacer se sitúa en 80,4 años para hombres y 85,9 años para mujeres, la esperanza de vida en buena salud se reduce a 64,7 y 63,9 años respectivamente (INE, Esperanza de vida 2016; INE,Esperanza de vida en buena salud 2015) (véase Tabla 3).

Tabla 3
Esperanza de vida global y en buena salud por sexo. España.

	Hombre	Mujer	Brecha hombre-mujer
Esperanza de vida media*	80,4	85,9	5,5
Esperanza de vida en buena salud **	64,1	63,9	- 0,8
Brecha de esperanza de vida media y en buena salud	16,3	22,0	5,7

Fuente: Elaboración propia a partir de datos del INE 2016* y 2015**.

En la última ENS (2012) la autovaloración positiva del estado de salud decrece con la edad, siendo las mujeres quienes perciben peor su salud. Este hecho es debido a que las mujeres son más propensas a enfermedades crónicas e incapacitantes, mientras que los hombres, por su estilo de vida, sufren enfermedades con mayor riesgo de muerte precoz (Morcillo Cebolla et al., 2014).

Aunque son múltiples los factores asociados al buen o mal estado de salud percibido, en las perosnas mayores la salud debe medirse en terminos de función y no de enfermedad, pues es aquella la que determina la expectativa de vida en buena salud (Fortin et al., 2006; Séculi et al., 2001). Por tanto, la situación funcional previa al desarrollo de la discapacidad y dependencia es uno de los mejores indicadores del estado de salud y resulta un mejor predictor de discapacidad que la propia morbilidad (Silguero et al., 2014). Un acercamiento a ello es el concepto de fragilidad.

2. FRAGILIDAD

2.1. Qué entendemos por fragilidad

El término fragilidad surgió hace varias décadas y con los años se ha ido convirtiendo en un concepto fundamental de la Geriatría, tanto desde el punto de vista de la atención clínica a las personas mayores, como de la investigación sobre el envejecimiento (Bergman et al., 2004). Surge de la necesidad de comprender la heterogeneidad del declive funcional que, en ausencia de enfermedad relevante, se observa con el envejecimiento cronológico (Sánchez Jurado, 2013).

La fragilidad es un concepto muy empleado pero difusamente definido (Ávila-Funes et al., 2008). Las similitudes e interrelaciones entre los procesos biológicos del envejecimiento y la fragilidad hacen que una definición conceptual definitiva sea complicada (Fulop et al., 2011). Sin embargo, entre los profesionales de la salud, una definición fiable y válida del concepto de fragilidad es necesaria para poder identificar a las personas mayores frágiles (Morley et al., 2013; Theou y Kloseck, 2008). Asimismo, la propia Unión Europea ha insistido en la importancia de una definición conceptual de la fragilidad debido a su vinculación con el alto consumo de recursos comunitarios, residenciales y hospitalarios, y asumiendo que una intervención precoz en las personas frágiles mejoraría la calidad de vida y disminuiría los costes de los cuidados (Sociedad Española de Geriatría y Gerontología (SEGG), 2010).

Aunque, hasta el momento, no existe un consenso claro sobre la definición de la fragilidad (Lally y Crome, 2007), generalmente se acepta que la fragilidad representa una mayor vulnerabilidad en el que un "*mínimo*" estrés puede provocar un mayor deterioro funcional (Clegg et al., 2013). Este estado de "*mayor*" vulnerabilidad se muestra de forma esquemática en la siguiente figura (véase Figura 3), donde la línea verde representa una persona mayor que, después de un estrés leve, como una infección urinaria, experimenta un pequeño deterioro de la función y luego regresa a la homeostasis. La línea roja representa a una persona mayor frágil que, después de un estrés similar, experimenta un mayor deterioro funcional, que puede manifestarse como dependencia funcional y que luego no vuelve a la homeostasis basal.

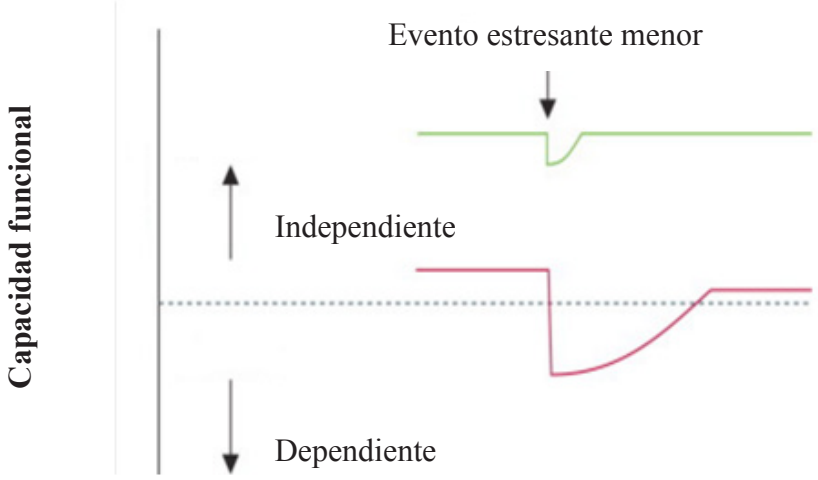

Figura 3. **La vulnerabilidad de las personas frágiles ante un estresor menor.**
Fuente: Elaboración propia a partir de Clegg et al. (2013).

En este sentido, el estado de fragilidad permite identificar a un subgrupo de personas mayores que conservan su independencia de manera inestable y que se encuentran en situación de mayor riesgo de pérdida funcional (Abizanda Soler et al., 2010). Asimismo, esta mayor pérdida funcional predispone a eventos adversos de salud (De Lepeleire et al., 2009). De manera que los adultos mayores frágiles, en relación a las personas no frágiles de su misma edad cronológica, presentan un mayor riesgo de desarrollar, entre otros resultados adversos de salud, discapacidad, hospitalización y muerte (Ensrud et al., 2009). Por lo tanto, la condición de fragilidad se relaciona más con la edad biológica que con la edad cronológica de las personas (Mitnitski et al., 2002; Wahlin et al., 2006).

Hoy en día también existe consenso de que la fragilidad, más que una enfermedad, es un síndrome geriátrico independiente con múltiples manifestaciones y vías de presentación (De Lepeleire et al., 2009). Los síndromes se refieren a condiciones de salud multifactoriales originadas por el acúmulo de déficits en distintos sistemas fisiológicos (Bergman et al., 2007). En este sentido, la mayoría de los autores consideran que la fragilidad puede ser vista como un cuadro clínico observable, caracterizado por una disminución de la capacidad de reserva funcional debido a un declive en diferentes e interrelacionados sistemas o dominios de funcionamiento (Fried et al., 2001). Por tanto, debemos considerarla como un proceso multidimensional que tiene en cuenta la compleja

interacción de diversos factores de dominio físico, psicológico y social (Markle-Reid y Browne, 2003; Sieber, 2016) (véase Figura 4).

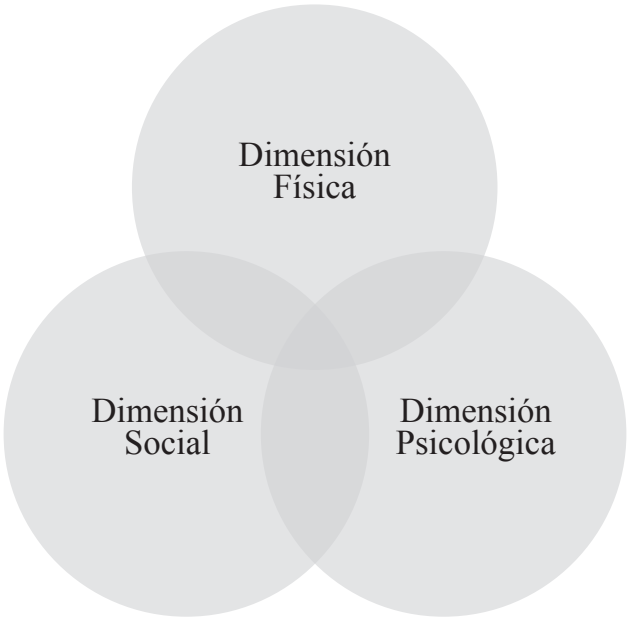

Figura 4. **Las dimensiones del síndrome de fragilidad.**
Fuente: Elaboración propia a partir de Sieber (2016).

Igualmente, en la actualidad, existe acuerdo en que es necesario distinguir la discapacidad y la enfermedad del síndrome de fragilidad (Fried et al., 2004). De hecho, dicho síndrome puede existir tanto en presencia como en ausencia de discapacidad y/o enfermedad (Fried et al, 2001; Teou et al., 2012; Walston et al., 2006).

Por último, la mayoría de los autores también coinciden en que el síndrome de fragilidad no sigue la ley del todo o nada, es decir, en lugar de pensar que la persona es o no frágil, ésta debe ser colocada en un continuo que se extiende desde el adulto mayor saludable hasta aquel extremadamente vulnerable con alto riesgo de morir (Castelblanque y Cuñat, 2002; Fernández-Garrido et al., 2014a). Por tanto, no todas las personas mayores son frágiles en la misma medida. Además, también es probable que cualquier adulto mayor no permanezca igual de frágil por largos periodos de tiempo, así pues, el síndrome de fragilidad es una condición dinámica que puede mejorar o empeorar con el tiempo e incluso invertirse (Markle-Reid y Browne, 2003; Puts et al.,

2005). De acuerdo con lo anterior, Gill et al. (2006) demostraron que las transiciones entre los estados de mayor a menor fragilidad son posibles, y que la probabilidad de hacer una transición de frágil a no frágil, aunque ésta es muy baja, también existe (véase Figura 5). Asimismo, la naturaleza dinámica del síndrome de fragilidad da lugar a la posibilidad de modificar positivamente la expresión esperada de dicho síndrome, mediante intervenciones terapéuticas y rehabilitadoras adecuadas (Bortz, 2002; Chen et al., 2014).

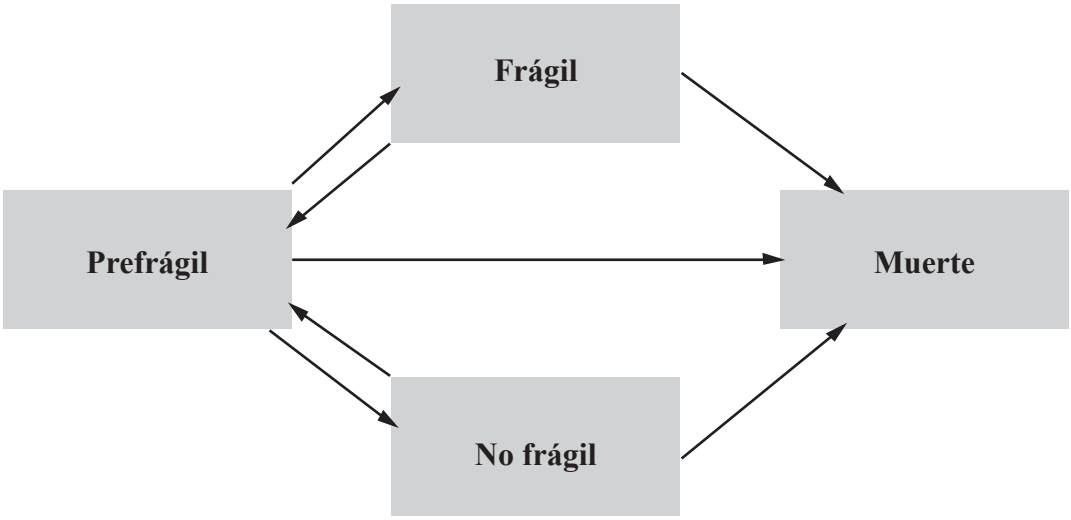

Figura 5. **Posibles transiciones entre estados de fragilidad.**
Fuente: Elaborada a partir de Gill et al. (2006).

En conclusión, y en base a lo anterior, una definición del síndrome de fragilidad como un concepto multidimensional y dinámico, que excluye la enfermedad y la discapacidad y se relaciona con resultados adversos en materia de salud sería la más satisfactoria.

Sin embargo, aunque se han propuesto varias definiciones conceptuales, Gobbens et al. (2010) afirmaron que ninguna cumple con los criterios de una definición exitosa, y acabaron proponiendo la siguiente definición: ”*La fragilidad es un estado dinámico que afecta a un individuo que experimenta pérdidas en uno o más dominios del funcionamiento humano (físico, psicológico, social) que son causadas por la influencia de una serie de variables y que aumenta el riesgo de los resultados adversos de salud*”.

2.2. Constructos de fragilidad

Una definición teórica de la fragilidad es ampliamente aceptada, sin embargo, en la práctica clínica, ésta es muy inespecífica puesto que no permite identificar en términos precisos (medibles) a las personas frágiles (Romero Cabrera, 2010). Dadas las dificultades para definir la fragilidad de manera operativa, Rockwood (2005) sugirió que cualquier definición científica trasladable a la práctica clínica debería cumplir con el conjunto de criterios propuestos en la siguiente tabla (véase Tabla 4).

Tabla 4
Criterios necesarios para una definición válida de fragilidad.

Validez de contenido	Incluye múltiples determinantes Es dinámica Su validez hace que pueda sustituir a definiciones previas Se puede emplear con éxito en diferentes contextos Puede ser empleada en programas computacionales
Validez de constructo	Es más común en mujeres que en hombres Es más común a edades avanzadas Se relaciona con la discapacidad Se relaciona con la comorbilidad y la autopercepción de salud
Validez de predictiva	Predice la mortalidad Predice otros eventos adversos (delirium, caídas, disminución de la funcionalidad…) Predice una edad a la cual todo el mundo podría ser frágil Aplicable tanto en estudios básicos de laboratorio como en clínicos poblacionales

Fuente: Elaboración propia a partir de Rockwood (2005).

Así, y partiendo de los criterios sugeridos por Rockwood (2005), en la literatura actual, la definición operativa para diagnosticar el síndrome de fragilidad se debate con ardor entre dos modelos, el modelo de déficits acumulados o índice de fragilidad y el modelo del fenotipo físico o síndrome clínico de fragilidad (Afilalo, 2016; Liotta et al., 2016). Cada uno de estos dos modelos deriva de un punto de vista teórico diferente de cómo se desarrolla y cómo se manifiesta el síndrome frágil en los adultos mayores. Si bien, se han propuestos otras definiciones operativas del síndrome de fragilidad, en su mayor parte, han sido sobre la base de uno de estos dos modelos básicos y por tanto, podrían incluirse en uno de ellos.

El modelo de déficits acumulados o índice de fragilidad se desarrolló como parte del *Canadian Study of Health and Aging* (CSHA), estudio de cohortes prospectivo de 5 años de duración, diseñado para investigar la epidemiología e impacto de la demencia en población de adultos mayores de Canadá (Rockwood y Ministki, 2007). Estos autores proponen detectar la fragilidad mediante la cuantificación de un amplio rango de enfermedades, síndromes geriátricos, discapacidades, factores psicosociales, etc. Este modelo considera el estado de fragilidad como una acumulación de diferentes déficits de salud relacionados con el envejecimiento que, aunque de manera individual no suponen una amenaza evidente para el desarrollo de la fragilidad, su acúmulo conjunto contribuye de forma significativa (Abizanda Soler et al., 2010). En este modelo cualquier déficit puede incluirse siempre que esté relacionada con la edad, sea perjudicial y no se sature demasiado pronto, es decir, que no esté presente en la totalidad o la mayoría de las personas adultas. Por ejemplo, la degeneración macular relacionada con la edad no sería considerada como déficit, ya que es un déficit benigno y casi universal a los 55 años de edad.

Puesto que el rango de los déficits es amplio e incluye diferentes dimensiones, el modelo es multidimensional y abarca la naturaleza multisistémica de la fragilidad. Sin embargo, la inclusión de afecciones o condiciones que pueden no estar relacionados por nexos fisiopalógicos comunes lo hacen poco útil para la identificación de los determinantes fisiopatológicos de la fragilidad (Song et al., 2010).

El modelo de déficits acumulados actualmente coexiste con el modelo del fenotipo físico o síndrome clínico de fragilidad. Este modelo fue desarrollado por Fried et al. (2001) a partir de un análisis secundario de los datos del *Cardiovascular Health Study* (CHS), estudio prospectivo diseñado para determinar la importancia de los factores de riesgo de las enfermedades cardiovasculares (ECV) en los adultos mayores. Estos autores propusieron para la identificación de la fragilidad un fenotipo físico específico centrado fundamentalmente en la presencia de cinco manifestaciones funcionales, que incluyen debilidad muscular, disminución de la energía vital, lentitud al caminar, baja actividad física y pérdida de peso no intencionada. Estos cinco criterios fenotípicos, conocidos como criterios o escala Fried, además de satisfacer los requisitos de un

síndrome clínico, representan los componentes clave de un hipotético "*ciclo de fragilidad*" cuyas bases fisiopatológicas son la malnutrición, la sarcopenia y el disbalance energético (véase Figura 6) (Abizanda Soler, 2010).

Según el modelo propuesto por Fried et al. (2001), la fragilidad es un fenómeno biológico que afecta a múltiples sistemas interrelacionados con unas bases fisiopatologías bien establecidas, lo que permite no solo identificar a los factores que predicen el síndrome frágil, sino también, formular intervenciones dirigidas a frenar el riesgo de convertirse en frágil (Castell Alcalá et al., 2010). Sin embargo, su carácter oligodimensional, basado exclusivamente en componentes físicos con ausencia de elementos esenciales como la salud mental y las comorbilidades, es algo controvertido (Rodríguez-Mañas et al., 2013). No obstante, es la definición operativa más ampliamente utilizada en la literatura científica y, actualmente, podría considerarse como un estándar de oro (Gill et al., 2006).

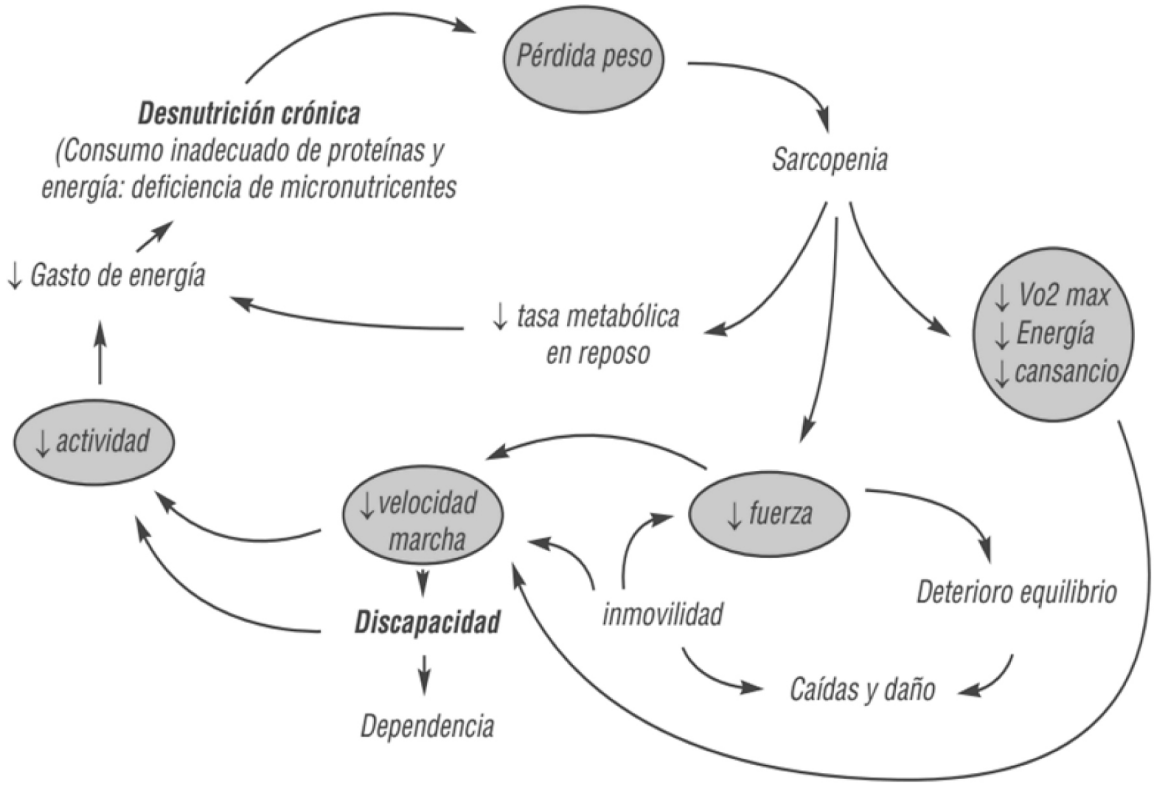

Figura 6. **Ciclo de la fragilidad según Fried.**
Fuente: Tomada de Abianza Soler (2010).

2.3. Prevalencia del síndrome de fragilidad

En su conjunto, la prevalencia del síndrome de fragilidad aumenta con la edad cronológica, es mayor en mujeres y en presencia de comorbilidad y discapacidad (Fried et al., 2001; Shamliyan et al., 2013). Sin embargo, no existe un consenso general sobre su tasa de prevalencia global, como así lo demuestran la gran variedad de cifras encontradas por Collard et al. (2012) en los principales estudios epidemiológicos publicados en diferentes contextos y países (véase Figura 7). Estos autores, estiman la prevalencia media del síndrome de fragilidad en adultos mayores de 65 años residentes en la comunidad en un 10,7%, con un amplio rango que varía de un 4,0% (Cawthon et al., 2007) a un 59,1% (Metzelthin et al., 2010).

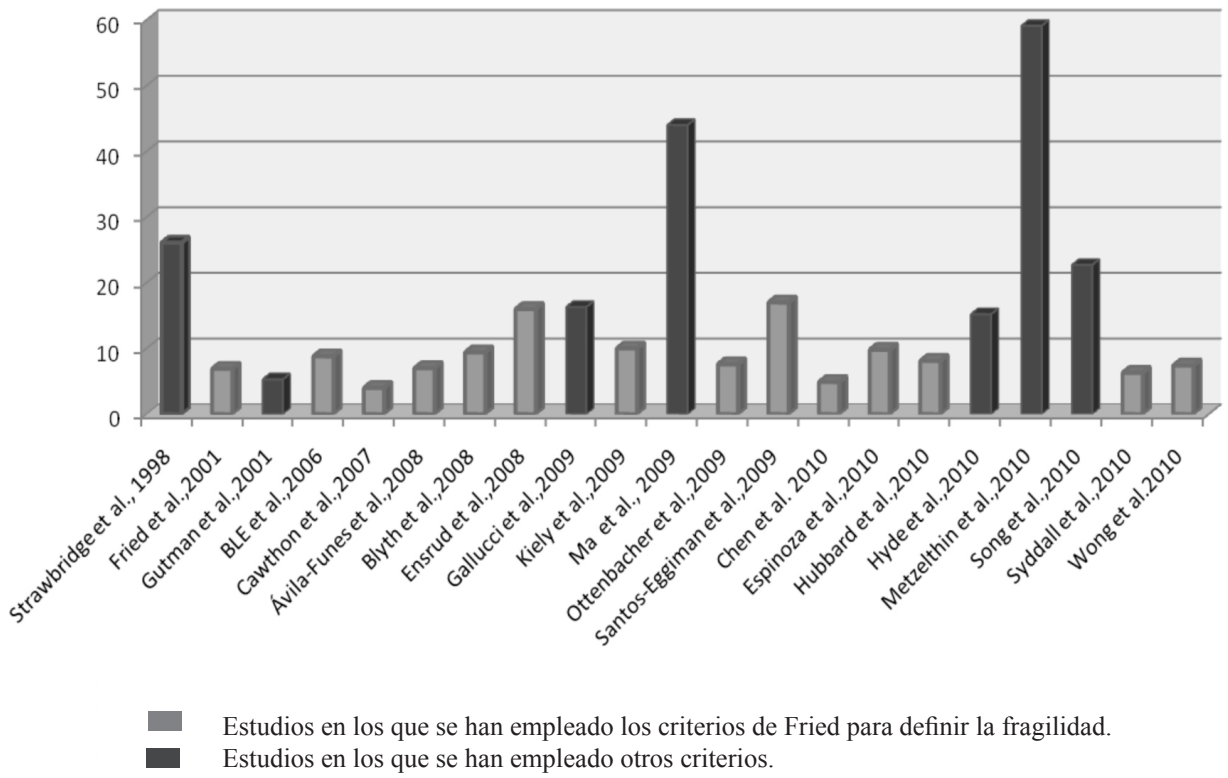

Figura 7. **Prevalencia del síndrome de fragilidad en adultos mayores que viven en la comunidad según los principales estudios epidemiológicos internacionales.**
Fuente: Adaptado de Collard et al. (2012).

Las características no homogéneas de las poblaciones, así como, los diferentes criterios utilizados para definir la fragilidad podrían explicar estas discrepancias. De hecho, en esta misma revisión, cuando se analizaron los diferentes estudios de acuerdo a la definición de fragilidad utilizada (véase Figura 8), el rango de prevalencia disminuye en aquellos que comparten la perspectiva de fragilidad según el fenoripo de Fried, pasando a ser de un 4% (Cawthon et al., 2007) a un 17% (Santos-Eggiman et al., 2009). Esto podría reflejar una definición más fiable de la fragilidad o un mayor consenso entre investigadores, motivo por el cual es la definicón más ampliamente utilizada.

No obstante, los datos, aunque en menor medida, continúan siendo dispares. Esta disparidad puede deberse a posibles modificaciones en la manera de medir los criterios de Fried (Abianza, 2013; Theou et al., 2015). Por tanto, si no se perfilan bien los instrumentos de medida, los datos de prevalencia variarán proporcionalmente al sesgo de interpretación. Este hecho advierte la necesidad de aclarar mejor los parámetros a partir de los cuales se define el síndrome, dado que podemos estar infradiagnosticando a quienes se beneficiarían de programas de prevención, o sobrediagnosticando a los que están en riesgo de enfermar, con el innecesario coste añadido (Collard et al., 2012).

Los estudios españoles que han empleado para su definición el fenotipo de Fried muestran unas cifras de prevalencia que oscilan entre el 8,4 % (García-García et al., 2011) y el 20,4% (Fernández-Bolaños et al., 2008). En el *Estudio Toledo para un Envejecimiento Saludable* (ETES) se estima una prevalencia de un 8,4% para mayores de 64 años, con un incremento sustancial en la población por encima de los 75 años, llegando al 20% en la población entre 80 y 84 años y al 27,3% en la población de más de 84 años (García-García et al., 2011). En el estudio *Fragilidad y Dependencia en Albacete* (FRADEA) la prevalencia alcanza el 15,2% para mayores de 69 años no institucionalizados (Abizanda et al., 2013). En el *Estudio de Peñagrande* se sitúa en el 10,5% en mayores de 64 años y en un 19,1% en mayores de 74 años (Castell et al., 2013). El *Estudio de Leganés* estima una prevalencia para la población mayor de 74 años en un 20,4% (Fernández-Bolaños et al., 2008), mientras que el *FRALLE* de Lérida, para ese rango de edad la estima en el 9,6% (Jürschik et al., 2012). El estudio *OCTABAIX*, en mayores de 85 años estudiados en atención primaria de salud, muestra un 20% de prevalencia del síndrome de fragilidad (Ferrer et al., 2013).

Los estudios sobre prevalencia del síndrome frágil en personas de edad avanzada institucionalizadas son escasos. En una revisión sistemática, publicada recientemente, se estimó la prevalencia global del síndrome de fragilidad en adultos mayores institucionalizados en un 52,3% (Kojima, 2015), mucho mayor en comparación con el 10,7% de las personas mayores residentes en la comunidad (Collard et al., 2012). Reflejando la asociación, ya demostrada, del síndrome de fragilidad con la institucionalización (Rockwood et al., 2006).

Puesto que el síndrome frágil aumenta con la edad, esta mayor prevalencia se puede argumentar por una mayor edad promedio en los participantes institucionalizados que en los residentes en la comunidad (80,3 años Vs 74,9 años) (Fried et al., 2001; Shamliyan et al., 2013). Sin embargo, al comparar la prevalencia en cada uno de los grupos de edad, ésta continua siendo mayor en los adultos mayores institucionalizados (véase Figura 8). Por tanto, esta mayor prevalencia puede ser debida a que la mayoría de los factores que se atribuyen a su desarrollo son muy comunes en esta población (Bell et al., 2015; Kaehr et al., 2015; Morley et al., 2014). Asimismo, se debe tener en cuenta que los adultos mayores institucionalizados son una población heterogénea, que incluye tanto adultos mayores válidos como dependientes (De la Rica-Escuín et al., 2014).

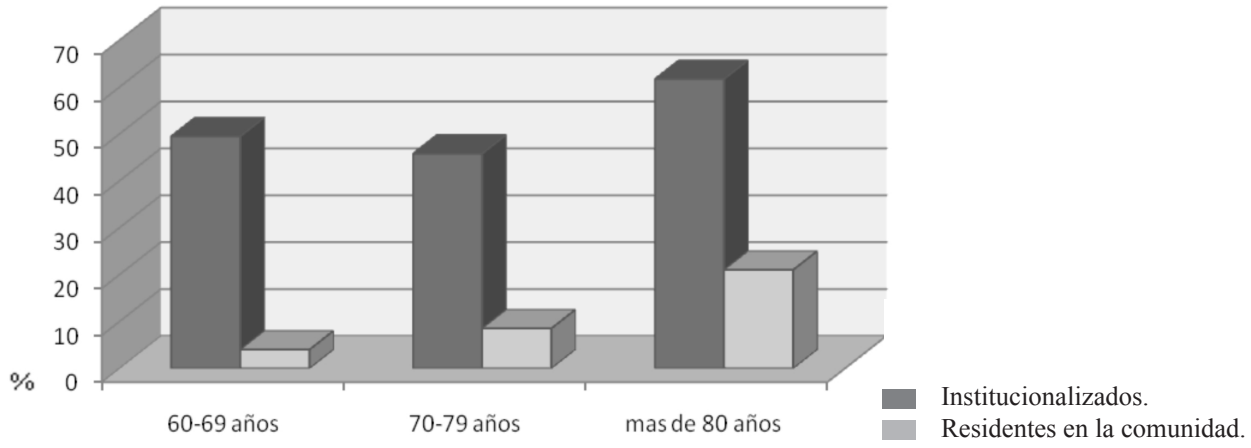

Figura 8. **Prevalencia global del síndrome de fragilidad por grupos de edad en adultos mayores institucionalizados Vs residentes en la comunidad.**

Fuente: Elaboración propia a partir de los datos tomados de las cohortes incluidas en Collard et al. (2012) y Kojima (2015).

2.4. Fisiopatología del síndrome de fragilidad

Se han llevado a cabo muchas investigaciones para comprender los mecanismos fisopatológicos que subyacen al síndrome frágil (Abizanda Soler, 2010). Aunque los aspectos sociales y psicológicos del síndrome de fragilidad son muy importantes, estas investigaciones se han centrado principalmente en sus aspectos fisiológicos (Walston et al., 2006).

Se considera que el deterioro fisiológico multisistémico asociado al envejecimiento podría ser el origen central del síndrome frágil (Ávila-Funes et al., 2008; Jaureguim y Rubin, 2012). Sin embargo, en la fragilidad esta disfunción multisistémica es mayor, lo que aumenta el riesgo de episodios adversos para la salud (Clegg et al., 2013). Por tanto, una cuestión clave es determinar si existe un umbral crucial de disfunción fisiológica asociada al envejecimiento, a partir del cual dicho síndrome se hace evidente.

En este sentido, Fried et al. (2009) mostraron, con independencia de la edad y la comorbilidad, una relación no lineal entre el número de sistemas fisiológicos alterados y la probabilidad de ser frágil. Estos resultados sugieren la existencia de un umbral de disfunción multisistémica más allá del cual se puede afectar la capacidad de adaptación homeostática , y en última instancia, contribuir a la mayor vulnerabilidad observada en el síndrome de fragilidad. Aunque es importante destacar que el número de sistemas alterados fue más predictivo de fragilidad que la alteración de cualquier sistema en particular, el sistema neurondocrino, inmunológico y muscular han sido los sistemas fisiológicos que más se han estudiado en el desarrollo del síndrome frágil (Walston et al., 2006).

Del mismo modo, éste síndrome también se ha asociado con la pérdida de reserva fisiológica en el sistema respiratorio (Vaz Fragoso et al., 2012), cardiovascular (Afilalo et al., 2009), renal (Abadir, 2011), óseo (Coelho et al., 2015), hematopoyético (Chaves et al., 2005) y de coagulación (Walston et al., 2002). Igualmente el estado nutricional y la inactividad pueden ser factores mediadores del síndrome de fragilidad (Clegg et al., 2013) (véase Figura 9).

Dado que muchos de los sistemas fisiológicos evaluados están fisiológicamente interrelacionados, la alteración de uno de ellos no puede ser independiente del resto. Esto sugiere que los sistemas fisiológicos alterados pueden interactuar sinérgicamente en un mayor riesgo de fragilidad.

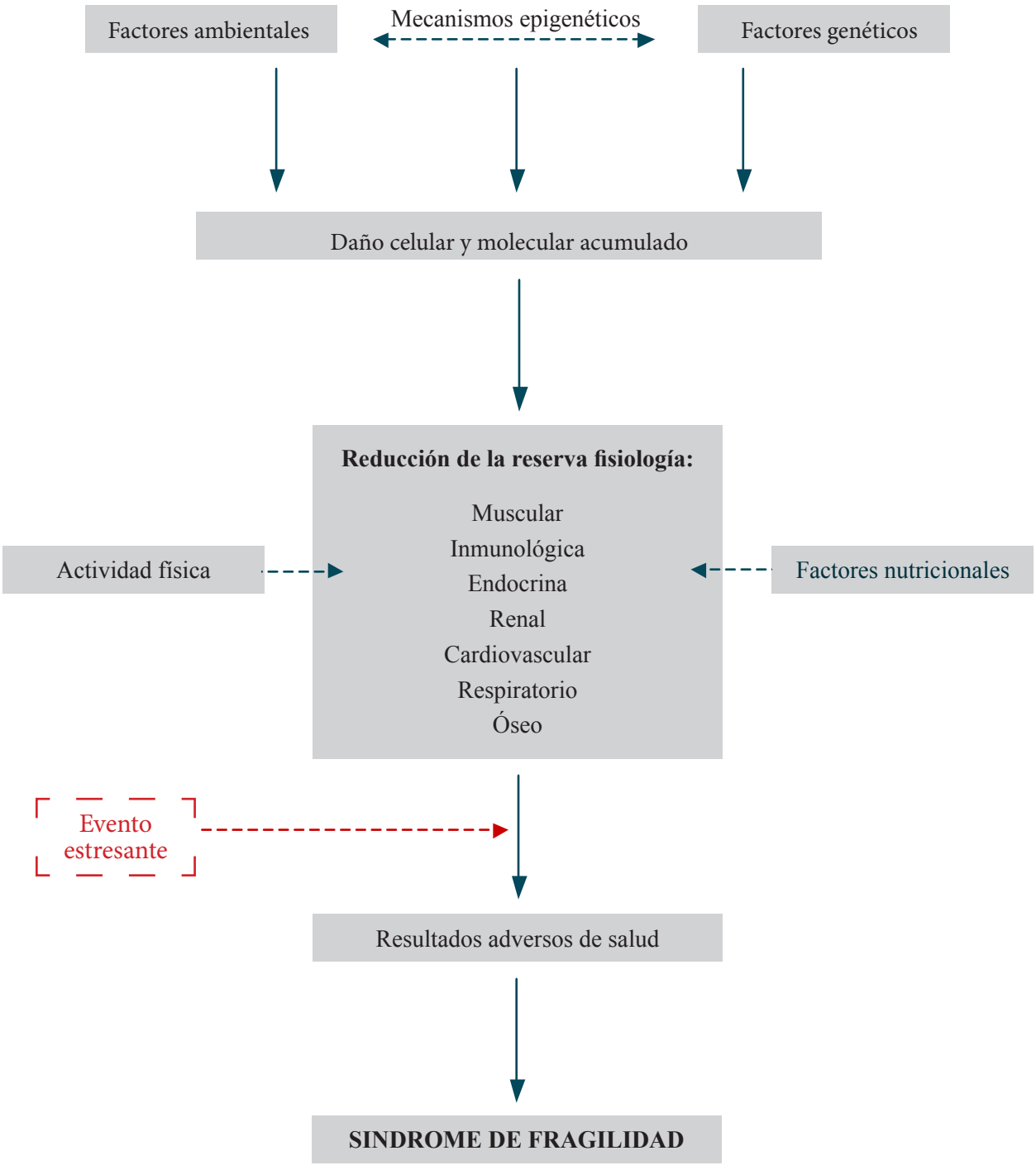

***Figura 9*. Representación esquemática de la fisiopatología del síndrome frágil.**
Fuente: Clegg et al. (2013).

2.4.1. El papel potencial del sistema muscular.

La Fragilidad y la sarcopenia se consideran condiciones interconectadas con respecto a la independencia funcional (Ávila-Funes et al., 2008). Por ello, durante mucho tiempo, se ha planteado la hipótesis de que la sarcopenia es un componente integral de la fragilidad (Rosenberg, 1997). De hecho, diversos estudios sugieren que la pérdida de músculo esquelético relacionada con el envejecimiento es mayor en las personas mayores frágiles que en las no frágiles (Theou et al., 2008). Lo que sugiere que la sarcopenia podria estar relacionada con la declinación funcional característica del síndrome frágil.

Entre los mecanismos señalados en la génesis de la sarcopenia se incluyen las alteraciones endocrinas con disminución de las hormonas con efecto anabólico; el escaso ingreso nutricional, principalmente de aminoácidos; la pérdida de la función neuromuscular por denervación o reinervación inapropiada; el desuso por la disminución de la actividad física regular y la presencia de enfermedades especificas (Volpi et al., 2004). Asimismo, el descenso de la masa muscular que acontece a lo largo del proceso de envejecimiento no es un fenómeno aislado, sino que está fuertemente ligado a un aumento paralelo del contenido de lípidos intramuscular, fenómeno conocido como obesidad sarcopenica (Delmonico et al., 2007). Esta grasa intramuscular presenta características metabólicas similares a la grasa visceral, por lo que tendrá actividades endocrinas y paracrinas incluyendo la secreción de hormonas y citoquinas inflamatorias (Garcia-Garcia, 2011). Estos depósitos grasos metabólicamente activos pueden inducir un estado pro-inflamatorio crónico que a su vez contribuye a la aparición de la sarcopenia (Blaum et al., 2005).

Puesto que la sarcopenia implica la disfunción de distintos sistemas fisiológicos interrelacionados, es concebible que los mecanismos que conducen a la sarcopenia a menudo se superponen con los del sindrome fragil (Ávila-Funes et al., 2006). En este sentido, el déficit nutricional, el declive en la señalización hormonal anabólica y la inflamación crónica están interconectados y han sido sugeridos como contribuyentes tanto de la sarcopenia como de la fragilidad (Wilson et al., 2017; Evans et al., 2010).

2.4.2. El papel potencial del sistema inmune.

Durante el envejecimiento a menudo se produce una desregulación de los mecanismos que regulan la respuesta inflamatoria, dando lugar a un estado de inflamación crónica de bajo grado conocido como "*inflamm-aging*" (Evans et al., 2010). Este fenómeno se caracteriza por altas concentraciones de citocinas pro-inflamatorias, así como de proteínas de fase aguda (Franceschi et al. 2000). Diversos estudios sugieren que este estado de inflamación crónica contribuye a la aparición del síndrome de fragilidad (Blaum et al., 2005; Clegg et al., 2013; Leng et al., 2007; Yao et al., 2011). De hecho, varios mediadores inflamatorios se han asociado de manera independiente con la condición de ser frágil (Barzilay et al., 2007; Hubbard et al., 2009; Leng at al., 2007).

Hay varias explicaciones posibles para la asociación entre la sarcopenia, el síndrome de la fragilidad y un estado de inflamación crónica. La activación de citoquinas pro-inflamatorias promueve la anorexia y el catabolismo proteico del músculo esquelético, lo que podría contribuir a un empeoramiento del estado nutricional y una reducción de la masa muscular y, consecuentemente, a la pérdida de peso y debilidad muscular, dos características comunes del síndrome frágil (Clegg et al., 2013). Además, este estado de inflamación crónica induce la aparición de disfunción endotelial, resistencia a la insulina (RI) y fenómenos procoagulantes, todos ellos promotores de la arteriosclerosis (Abizanda, 2010).

Diversos investigadores han considerado la aterosclerosis como un componente importante del síndrome de fragilidad (Morley et al., 2002). La alteración en la perfusión conduce a una disminución de la irrigación de nervios y músculos, lo que agrava la sarcopenia, componente clave del sindrome de la fragilidad. Asimismo, la cardiopatía isquémica tambien favorece la aparicion de sarcopneia al disminuir el gasto cardiaco, y por tanto, el consumo máximo de oxígeno. Por su parte, la enfermedad vascular cerebral puede favorecer el deterioro cognitivo que ha sido asociado al síndrome de fragilidad.

2.4.3. El papel potencial del sistema endocrino.

Está demostrado que en el envejecimiento se produce una disfunción en el eje hipotálamo-hipofisario-suprarrenal. En primer lugar, esta disfunción se expresa con una disminución en la síntesis de la hormona de crecimiento (HG), con la consecuente reducción en la producción del factor de crecimiento similar a la insulina de tipo I (IGF-I) (Payette et al., 2003). Dichas hormonas mejoran la actividad anabólica en muchas células, y por tanto, juegan un papel muy importante en el desarrollo y mantenimiento de la masa muscular, así, sus bajos niveles promueven la pérdida de masa y fuerza muscular, lo que puede contribuir al desarrollo de la fragilidad. Hay también evidencia de interacción entre IGF-1 e interleuquina- 6 (IL-6), lo que sugiere que la inflamación puede conducir a niveles bajos de IGF-1 o regular a la baja su sensibilidad (Leng et al., 2004).

En segundo lugar, la actividad de las células adrenocorticales, productoras del mayor precursor de esteroides sexuales (dehidroepiandrosterona sulfato (DHEA-S)), disminuye con la edad, y a menudo, junto con un aumento gradual de la liberación de cortisol (Evans et al., 2010). Diversos estudios han encontrado una asociación entre una elevación de cortisol y el desarrollo de la fragilidad, ya que los valores persistentemente elevados de cortisol, aunque amortiguan en parte la inflamación, se asocian con anorexia y un aumento del catabolismo celular, lo que lleva a la pérdida de peso y de masa y fuerza muscular, principales características clínicas del síndrome de fragilidad (Varadhan et al., 2008). Además, se ha sugerido que la DHEA-S suprime la inflamación, por lo tanto, niveles más bajos de DHEA-S pueden contribuir a la inflamación crónica y en última instancia al síndrome frágil (Romero-Cabrera et al., 2013).

Por último, y en tercer lugar, el estradiol y testosterona disminuyen igualmente con la edad (Evans et al., 2010). La disminución de su secreción es el principal factor asociado con la pérdida de masa y fuerza muscular tanto en hombres como en mujeres mayores. Además, hay evidencia de que determinadas citoquinas inflamatorias, tales como IL-6, pueden interferir con la función anabólica de los esteroides sexuales y a la inversa, es decir, también es probable que la pérdida de estrógenos, y tal vez de testosterona, conduce a una mayor producción de IL-6 y otros mediadores de la inflamación en varios tipos de células, favoreciendo un estado de inflamación crónica que

contribuye al desarrollo de sarcopenia y del síndrome de fragilidad (Romero-Cabrera et al., 2013).

Asimismo, con la edad también se produce un aumento significativo de la RI (Abbatecola et al., 2005). Estudios recientes han demostrado que la RI relacionada con la edad puede ser un factor determinante tanto de la sarcopenia como de la fragilidad (Evans et al., 2010). Una menor actividad de la insulina periférica genera cambios en la calidad del tejido muscular, mediante una reducción de su anabolismo y una mayor infiltración de grasa pericelular, lo que conduce a su vez, a la pérdida de masa y fuerza muscular (Abbtecola y Paolisso, 2008). Igualmente la RI, también, genera cambios en la eficiencia de la contracción de las fibras musculares, especialmente de las fibras tipo I, las más representativas en el músculo de las personas mayores, mediante una disminución en la captación y metabolismo de la glucosa intracelular (Song et al., 1999; Staron et al., 2000). Por otro lado, la RI a nivel cerebral favorece un aumento de las placas de amiloide y de los ovillos neurofibrilares, lo que podría contribuir al deterioro cognitivo asociado al síndrome de fragilidad (Buchman et al., 2008).

2.4.4. El papel potencial de la ingesta nutricional.

Se ha sugerido que con el envejecimiento se produce una pérdida fisiológica del apetito, fenómeno conocido como "*anorexia del envejecimiento*" (Di Francesco et al., 2007). En la actualidad es bien sabido que este fenómeno puede desempeñar un papel importante en la disminución de la función en las personas mayores (Morley, 2001). El escaso ingreso nutricional, principalmente de proteínas y de algunos micronutrientes, conduce a una disminución de la masa y fuerza muscular, puesto que la sarcopenia juega un papel importante en el inicio de la fragilidad, esta malnutrición selectiva está implicada en el desarrollo del síndrome de fragilidad (Visser et al., 2003).

La anorexia fisiológica en personas de edad avanzada es causada por la disminución de las funciones quimiosensoriales, el deterioro de las funciones psicológicas, los cambios ambientales y la disminución de las secreciones de las hormonas que regulan el apetito (Malafarina et al., 2013). En relación a esto último, y como respuesta al déficit de andrógenos que se produce durante el envejecimiento, con la edad se incrementa la secreción de leptina (Romero-Cabrera et al.,

2013). La leptina favorece la anorexia e incrementa la tasa metabólica, lo cual disminuye el aporte de alimentos, promueve los mecanismos catabólicos y puede llevar a desnutrición, sarcopenia y fragilidad (Hubbard et al., 2008; Morley, 2001). Otra hormona relacionada con el apetito, la grelina, también disminuye con la edad y sus bajos niveles basales se han asociado, en personas mayores no institucionalizadas, con un empeoramiento del estado nutricional y con la consecuente pérdida de peso, lo que también puede implicar sarcopenia y fragilidad (Morley, 2013). Por último, la adiponectina también juega un papel potencial en la regulación central de la ingesta y gasto de energía y sus altos niveles, en las personas de edad avanzada, se han asociado con la pérdida de peso y sarcopenia, lo que puede inducir a la aparición del síndrome frágil (Dridi y Taouis 2009).

Por otro lado, la evidencia sugiere que las citocinas pro-inflamatorias asociadas al envejecimiento implican una alteración en las señales de saciedad y hambre, dando lugar a la anorexia y caquexia (Hubbard et al., 2008). Asimismo, la desnutrición puede favorecer el deterioro de la respuesta inmunológica, lo cual se suma a los cambios inespecíficos del sistema inmunitario asociados al envejecimiento (Lesourd, 2004). En la siguiente figura (véase Figura 10) se muestra los diversos mecanismos fisiológicos interconectados relacionados con la edad que unen a la sarcopenia y al sindrome de fragilidad.

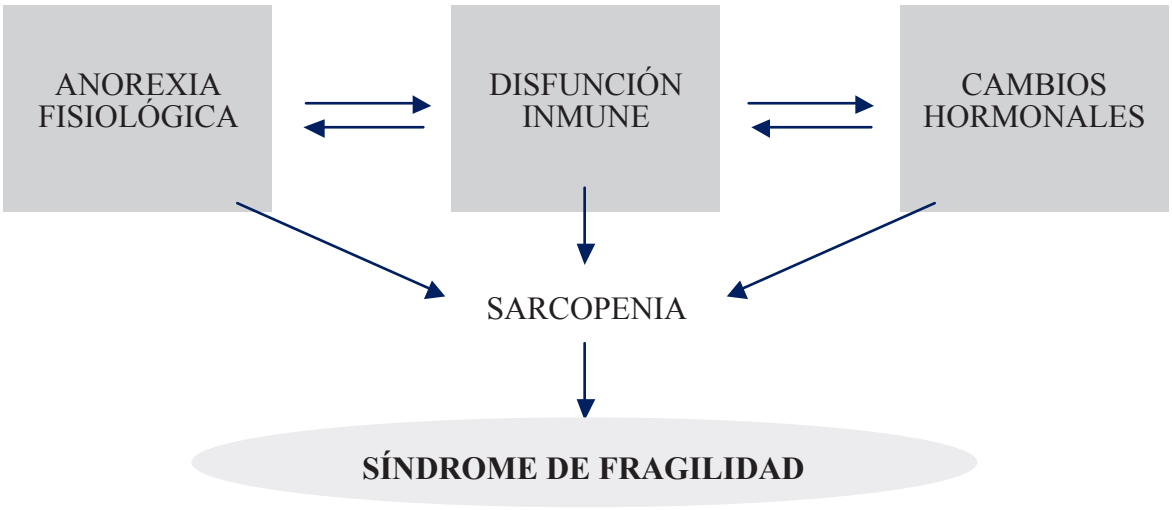

Figura 10. **Cambios fisiológicos relacionados con la edad que unen al síndrome de fragilidad y la sarcopenia.**
Fuente: Adaptado de Evans et al. (2010).

2.5. Determinantes del síndrome de fragilidad

El conocimiento de los factores determinantes que predicen el síndrome de fragilidad puede ayudar a reconocer, de una forma más eficaz, a las personas mayores en riesgo de fragilidad y a formular medidas de prevención e intervención temprana, con el fin de tratar este síndrome y prevenir resultados adversos relacionados (Mello et al., 2014).

Como se ha sugerido, el síndrome de fragilidad es multifactorial, y por tanto, diversos factores influyen en su aparición y desarrollo. En la siguiente tabla (véase Tabla 5) se resumen los principales determinantes del síndrome de fragilidad descritos en la literatura.

Tabla 5
Factores predictivos del síndrome de fragilidad.

Factores demográficos y socieconómicos	
Mayor edad	Sexo femenino
Menor nivel educacional	Bajos ingresos económicos
Raza/Etnia	
Condiciones de salud	
Comorbilidad/enfermedades	Mala autopercepción de la salud y calidad de vida
Polimedicación	Estado funcional
Caidas	Hospitalizacion y reingresos
Factores ambientales o estilo de vida	
Ingesta de alcohol	Ser fumador/a
Inactividad física	
Estado nutricional	
Pérdida de peso u obesidad	Malnutrition
Factores psicológicos	
Deterioro cognitivo	Alteraciones afectivas
Factores sociales	
Soporte social deficitario	Vivir solo o viudo

Fuente: Elaboración propia a partir de Ding et al. (2017).

Numeros estudios demuestran la relación entre la fragilidad y distintos aspectos demográficos y socioeconómicos. La prevalencia del sindrome de fragilidad aumenta con la edad, y más significativamente después de los 80 años (Fried et al., 2001; Walston et al., 2002). Una hipótesis para esta relación se encuentra en el estrés oxidativo celular que se acumula con los años, lo que lleva al daño del ADN. Tal daño, induce alteraciones sistémicas por desregulaciones en los procesos de apoptosis y transcripción celular, dando lugar a diversas condiciones adversas, como la fragilidad (Mulero et al., 2011).

Igualmente se ha demostrado que el sexo femenino tiene una marcada influencia sobre la prevalencia de la fragilidad (Gordon et al., 2016). Las mujeres poseen características intrínsecas que favorecen el desarrollo de la sarcopenia, un componente integral del sindrome frágil (Espinoza y Fried, 2007). Además de tener menores niveles basales de masa muscular, debido a factores hormonales, sufren una mayor pérdida durante el envejeciendo, probablemente por el menor consumo calórico y la menor actividad físca asociada al sexo femenino (Gobbens et al., 2010). Por otro lado, diversos estudios han sugerido que las mujeres toleran durante más tiempo esta condición, como lo demuestran sus menores tasas de mortalidad (Gordon et al., 2016). Una posible explicación de esta paradoja de salud y supervivencia entre hombres y mujeres, puede ser debida en primer lugar, a que algunos déficits de salud parecen estar asociados con un menor riesgo de mortalidad (Shi et al., 2014). Y en segundo lugar, es posible que las mujeres toleren la acumulación de déficit mejor que los hombres, al poder presentar éstos últimos una menor reserva fisiológica (García-González et al., 2009). Sin embargo, esta mayor supervivencia de la mujer no se traduce en un mejor estado de salud (Gorman y Read, 2006). De hecho, las mujeres tienden a tener más comorbilidad, mayores niveles de discapacidad y peor autopercepción de la salud (Hubbard y Rockwood, 2011).

Asimismo, algunos autores han relacionado el síndrome de fragilidad con la raza/etnia, encontrando una mayor prevalencia en los afroamericanos e hispanos (Fried et al., 2001; Woods et al., 2005). Se cree que la raza es un marcador de polimorfismo genético que influye en la aparición de la fragilidad (Hirsch et al., 2006).

La asociación entre el bajo nivel socioeconómico y la mala salud ha sido bien documentada. Tener una mala situación soocioeconómica, con frecuencia, coexiste con una atención sanitaria inaccesible y desnutrición, entre otros factores de riesgo (Alvarado et al., 2008). En este sentido, la literatura también es consistente en la relación entre la fragilidad y el menor nivel socioeconomico (Castell et al., 2010; Szanton et al., 2009; Woo et al., 2005; Woods et al., 2005). Éste, aunque no actúan directamente en la fisiopatología del síndrome, al interferir en el estilo y la calidad de vida del individuo puede influir en el desarrollo de la fragilidad (Ding et al., 2017).

Investigaciones actuales sugieren que la presencia de enfermedad, tanto si se mide como una enfermedad específica o como una acumulación de varias enfermedades (comorbilidad), puede contribuir al desarrollo de la fragilidad (Abizanda et al., 2005). Fried et al. (2001) revelaron que el 67.7% de los frágiles presentaban comorbilidad y el promedio de enfermedades crónicas de un adulto mayor frágil fue de 2.1, comparado con 1.4 de los no frágiles (véase Figura 11). Asimismo, estos autores observaron que existía un mayor riesgo de fragilidad en aquellos que presentaban enfermedades crónicas y que éste aumentaba de manera significativa con el número de afecciones.

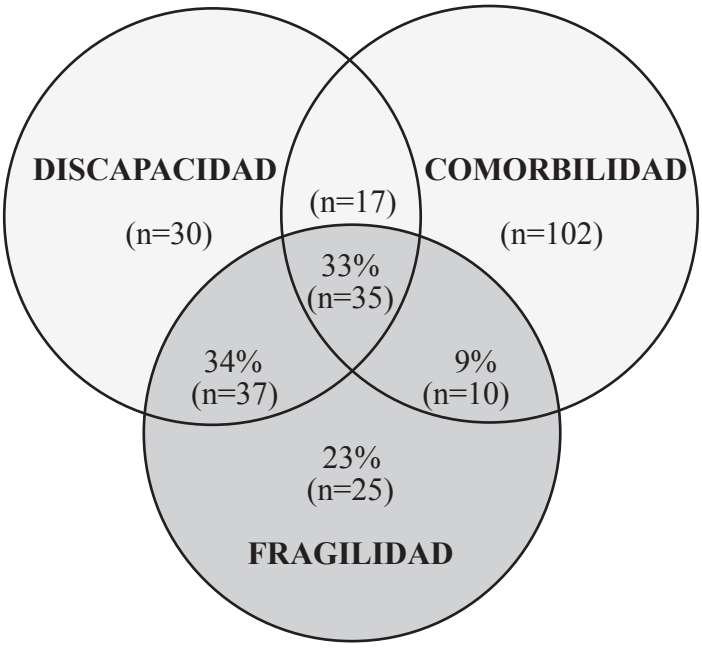

Figura 11. **Relación entre fragilidad, discapacidad y comorbilidad.**
Fuente: Elaboracion propia a partir de Fried et al. (2001).

El desarrollo de enfermedades puede precipitar la fragilidad al movilizar las capacidades de reservas del organismo, con la posibilidad subsiguiente del agotamiento de las reservas fisiológicas (Lluis Ramos, 2013). Entre otras enfermedades se han asociado con el síndrome de fragilidad la diabetes mellitus, la patología cardiovascular, respiratoria, osteoarticular, renal, neurológica, el cáncer y la anemia (Cesari et al., 2006; Chen et al., 2010; García-García et al., 2011; Snih et al., 2009; Woods et al., 2005). Aunque no existe una única enfermedad vinculante, diversos estudios han encontrado que la presencia simultánea de dos enfermedades, como la anemia y enfermedad cardiovascular o anemia y depresión o anemia y enfermedades pulmonares, aumenta el riesgo de fragilidad por un posible efecto sinérgico entre ellas (Chang et al., 2009; Chaves et al., 2005). Por otra parte, los resultados de varios estudios documentan que la fragilidad asociada con las enfermedades crónicas favorece la progresión y empeora el pronóstico de estas últimas, debido a una disminución de la capacidad de reaccionar ante las mismas. Asi, un estudio realizado por Hubbard et al. (2010) mostró que las personas mayores frágiles con diabetes tuvieron una menor expectativa de vida y 2.62 veces más posibilidades de tener complicaciones de la diabetes, que los adultos mayores diabéticos no frágiles. Igualmente, la fragilidad incrementa las tasas de hospitalizacion y de mortalidad por ECV, en particular de la insuficiencia cardiaca y la enfermedad coronaria (Afilalo et al., 2009; Lupón et al., 2009). También se ha documentado que la severidad de la enfermedad de Parkinson es mayor en los paciente frágiles que en los no frágiles (Ahmed et al., 2008). Por último, Rozzini et al. (2005) demostraron que los adultos mayores frágiles con enfermedades crónicas cuando fueron hospitalizados presentaron un alto riesgo de mortalidad. Por tanto, se puede afirmar que la fragilidad y las enfermedades crónicas, aunque pueden ocurrir en paralelo, están interrelacionadas en cuanto a mecanismos patogénicos, curso y pronóstico (Romero Cabrera, 2011).

Además de las influencias de la enfermedad o comorbilidad en la fragilidad, la mayoría de autores también están de acuerdo en que el número de medicamentos que se consumen diariamente predice este síndrome (Coelho et al., 2015). Los efectos adversos de la polimedicación y su relación directa con el nivel de comorbilidades podrían explicar esta asociación.

Una asociación directa también se ha observado entre la fragilidad y el estado funcional, medido principalmente por la capacidad de realizar actividades de la vida diaria (AVD). De hecho, una parte importante de personas mayores frágiles presenta incapacidad funcional (Alcalá et al, 2010; Fried et al, 2001). Sin embargo, para distintos autores es posible una causalidad inversa, al sugerir que el síndrome de fragilidad es un precursor de la discapacidad (Al Snih et al., 2009; Martin-Lesende et al., 2010; Wong et al., 2010) (vease Figura 12). Por tanto, para estos autores, prevenir la discapacidad actuando sobre el síndrome de fragilidad es posible, dado que este último se puede detectar y es susceptible de intervención.

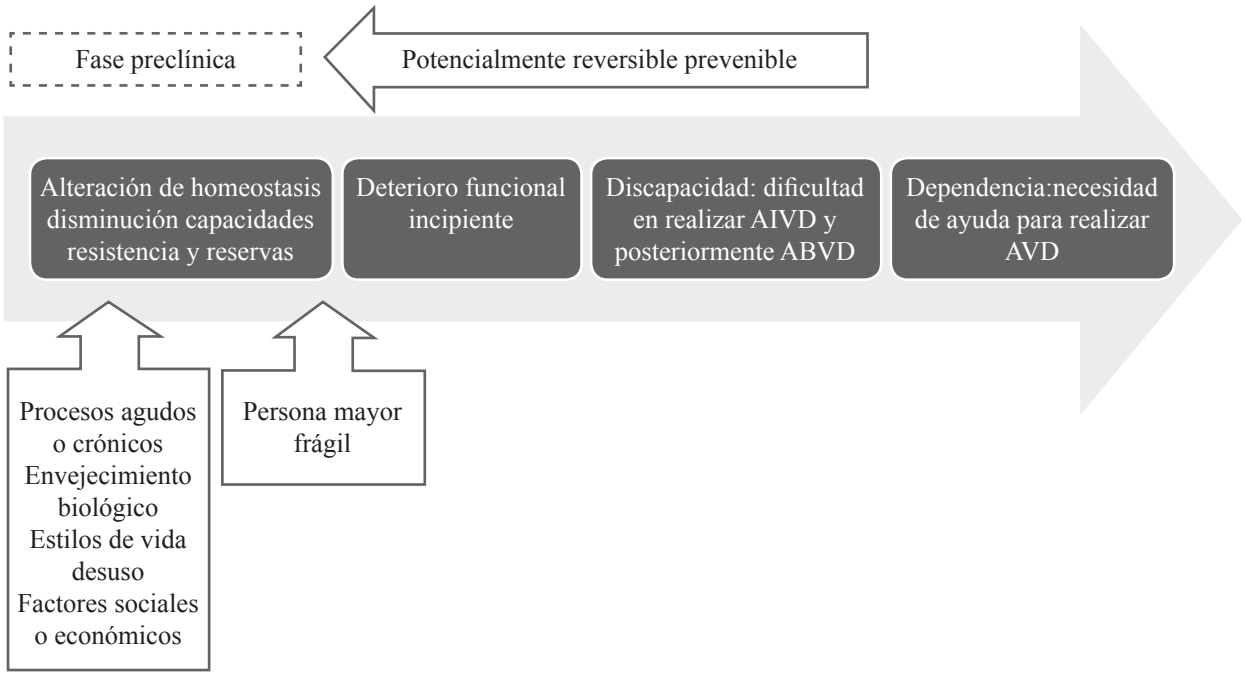

***Figura* 12. Desarrollo de la pérdida de función hasta la dependencia y la persona mayor fráil.**
Nota**.** AIVD: actividades instrumentales vida diaria ; ABVD: actividades básicas vida diaria; AVD: actividades vida diaria.
Fuente: Elaboración propia a partir de Martín-Lesende et al. (2010).

En general, las personas frágiles presentan una peor autopercepción de su estado de salud y de su calidad de vida (Kanauchi et al., 2008). Esto puede ser debido a la mayor susceptibilidad a resultados adversos de salud y a la pérdida funcional que presentan las personas frágiles, condiciones que pueden poner en peligro una vida autónoma e independiente (Morley et al., 2013).

Esto, a su vez, corrobora el impacto negativo que tiene la pérdida de la funcionalidad, autonomía e independencia, en la autopercepción de la salud y la satisfacción con la vida (Sabatini, 2014).

Un estilo de vida poco saludable tambien parece influir de manera independiente en el riesgo de ser frágil. En este sentido, diversos estudios han mostrado que las personas mayores con un consumo moderado de alcohol, en comparación con los no bebedores, presentan una menor probabilidad de ser frágil (Ortolá et al., 2015; Seematter-Bagnoud et al., 2014; Woods et al., 2005). Son varios los mecanismos que podrían explicar la reducción del riesgo de fragilidad asociada con el consumo de alcohol. Existe evidencia de que una mayor ingesta de alcohol se asocia con niveles más altos de colesterol de lipoproteínas de alta densidad (HDL-c) y adiponectina, con niveles más bajos de fibrinógeno y con la mejora de los marcadores del metabolismo de la glucosa (Brien et al., 2011; Galán et al., 2014). Como consecuencia, el consumo moderado de alcohol se ha relacionado con un menor riesgo de ECV y de diabetes, enfermedades asociadas con un mayor riesgo de fragilidad (Baliunas et al., 2009; Newman et al., 2001; Morley et al., 2014; Ronksley et al., 2011). Asimismo, diferentes estudios longitudinales, en adultos mayores, han asociado el consumo moderado de alcohol con un menor riesgo de desarrollar limitaciones funcionales o discapacidad (Karlamangla et al., 2009; Lin et al., 2011).

En contraste con el consumo de alcohol, el tabaquismo se ha relacionado de manera positiva con el riesgo de ser frágil (Guessous et al., 2014 ; Hubbard et al., 2009b; Wang et al., 2013; Woods et al., 2005). Los fumadores, en relación a los no fumadores, presentan un mayor riesgo de ser frágil. El tabaquismo activo puede inducir daño muscular y sarcopenia en la edad avanzada (Steffl et al. 2014). Igualmente, los resultados del *Hallym Aging Study* indican que el tabaquismo se asocia con una disminución de la fuerza de agarre en los varones mayores de 65 años (Quan et al., 2013). Asimismo, algunos estudios prospectivos de cohortes han demostrado que el hábito de fumar es un fuerte predictor de discapacidad (Rist et al., 2014; Kim et al., 2013; Ropponen et al., 2013; Wong et al., 2015).

Igualmente, una vida sedentaria aumenta el riesgo de fragilidad. En este sentido, Peterson et al. (2009), utilizando datos *Health, Aging and Body Composition Study,* encontraron, después de 5 años de seguimiento, que los adultos mayores que practicaban regularmente actividad física, en

comparación con aquellos que llevaron una vida sedentaria, fueron menos propensos a desarrollar fragilidad. Estos autores, también evidenciaron que el ejercicio físico atenuó la transición a la fragilidad severa en adultos mayores moderadamente frágiles. Del mismo modo, Savela et al. (2013) en una cohorte de sexo masculino, tras 26 años de seguimiento, mostraron que una mayor actividad física con carácter regular en mitad de la vida estaba fuertemente asociada con un menor riesgo de fragilidad en la vejez. Las vías por las que la actividad física puede prevenir o atenuar la fragilidad son varias. Diversos estudios han demostrado que la actividad física reduce el riesgo de varias características comunes de la fragilidad, como la sarcopenia, carga alostática, deterioro cognitivo y enfermedades crónicas (Geffken et al., 2001; Sofi et al., 2011; Song et al., 2015; Willis et al., 2012). Igualmente un mejor estado físico se ha asociado con una mayor capacidad cardiorrespiratoria o fuerza muscular, lo que predice un mejor pronóstico cardiovascular y supervivencia (Artero et al., 2012; McAuley et al., 2012). Por último, la actividad física también se ha demostrado que tienen un fuerte efecto protector ante la discapacidad, caídas y mortalidad (Paterson et al., 2010; Savela et al., 2010; Sherrington et al., 2008).

En base a lo anterior, la fragilidad se ha asociado con déficits sensoriales (auditivos o visuales), alteraciones en la marcha o equilibrio y antecedentes o riesgo de caídas, situaciones que conllevan una pérdida de autoconfianza en la independencia funcional, lo que origina una reducción en la actividad física que favorecer la aparición de la fragilidad (López et al., 1995). Asimismo, una caída puede tener diversos resultados adversos incluyendo las hospitalizaciones. Diferentes estudios señalan que la hospitalización reciente y los reingresos en las personas mayores pueden desencadenar o aumentar el riesgo de fragilidad (Hervás y García de Jalón, 2005). La hospitalización resulta frecuentemente en una declinación irreversible del estado funcional y un cambio en la calidad y estilo de vida, lo que a su vez se asocia con una mayor prevalencia de fragilidad (Solano et al., 1997).

Centrándonos en las variables nutricionales, en comparación con los adultos mayores bien nutridos, los sujetos desnutridos o que están en riesgo de desnutrición son más propensos a desarrollar el síndrome de fragilidad (Bollwein et al., 2013; Chang, 2016). La desnutrición puede

actuar sobre la vía subyacente relacionada con la fragilidad. Asi, Masel et al. (2009) encontraron que el bajo peso en las personas mayores, según el índice de masa corporal (IMC), se relaciona con el síndrome de fragilidad. La asociación entre el síndrome de fragilidad y el bajo peso puede estar relacionada con la pérdida de masa y fuerza muscular común en las personas con pérdida de peso involuntaria, lo que conduce a la sarcopenia y a una disminución de la función motora, condiciones que contribuyen al desarrollo de la fragilidad (Biolo et al., 2014). Woods et al. (2005), a pesar de encontrar la misma asociación, mostraron que el síndrome de fragilidad también podría estar asociado con el sobrepeso y la obesidad. Estos autores argumentaron que esta relación, entre el síndrome de fragilidad y el sobrepeso y la obesidad, podría ser debida a la presencia de la "*obesidad sarcopenica*", es decir, a la pérdida de masa muscular concurrente con el aumento de masa grasa. Este exceso de grasa puede estar asociado con la activación de los procesos inflamatorios que desencadenan alteraciones sistémicas, lo que a su vez, puede influir en la aparición del síndrome de fragilidad (Espinoza y Fried, 2007; Jauregui et al., 2012). Además de la malnutrición, varios estudios han asociado el síndrome frágil con una menor ingesta de energía (≤ 21Kcal/Kg/día), de proteínas y con bajos niveles séricos de determinados micronutrientes (Bartali et al., 2006; Beasley et al., 2010; Semba et al., 2006; Smit et al., 2013). También se ha sugerido que la calidad general de la dieta, en comparación con los componentes individuales, disminuye el riesgo de ser frágil (Shikany et al., 2014). De manera que la dieta mediterránea, con alto contenido en verduras, frutas, legumbres, cereales sin refinar, frutos secos y pescado, se ha asociado con una menor probabilidad de desarrollar el síndrome de fragilidad (Bollwein et al., 2013; Talegawkar et al., 2012).

Más allá del dominio físico, el deterioro cognitivo y los síntomas depresivos son las condiciones psicológicas que confieren mayor riesgo de fragilidad (Yew et al., 2017). Probablemente los adultos mayores con deterioro cognitivo tengan mayores dificultades para comer, hacer ejercicio y caminar (Kang et al., 2016). Esto puede conducir a una menor masa muscular, y consecuentemente, a la pérdida de peso y disminución de la función motora, lo que favorece la aparición del síndrome frágil (Fried et al., 2001). Por otro lado, Samper-Ternent et al. (2008) sugieren que el deterioro cognitivo y la fragilidad podría tener vías etiológicas comunes. En este sentido, el proceso inflamatorio crónico

asociado al envejecimiento podría mediar la relación entre el síndrome de fragilidad y el deterioro cognitivo. Las citoquinas pro-inflamatorias periféricas además de promover reacciones catabólicas que disminuyen la masa corporal magra, aumentan los niveles de citoquinas pro-inflamatorias centrales que generan neurotoxicidad, lo que conduce a la fragilidad y al deterioro cognitivo respectivamente (Halil et al., 2015). Del mismo modo, los mecanismos neuropatológicos de la enfermedad de Alzheimer conducen al deterioro cognitivo y también afectan a la fragilidad. De hecho, Buchman et al. (2008) encontraron que el síndrome de fragilidad empeora con el aumento de la gravedad de la enfermedad de Alzheimer. Por último, Halil et al. (2015) mostraron que las puntuaciones más altas de la escala de depresión geriátrica se asociaron a una mayor disminución en la función cognitiva. Asimismo, Mezuk et al. (2012) encontraron una correlación positiva entre la fragilidad y la depresión en los adultos mayores. En base a los resultados de estos estudios, podemos suponer que la depresión también podría mediar la correlación entre la fragilidad y la cognición.

En relación a los síntomas depresivos, los resultados de un artículo de revisión, llevado a cabo por nuestro propio grupo de investigación, muestran una relación bidireccional entre la depresión y la fragilidad (Buigues et al., 2015). Esto no es sorprendente dado que la depresión comparte muchos síntomas, factores de riesgo y consecuencias con la fragilidad (Lohman et al., 2016). La depresión en los adultos mayores puede conducir a la inactividad física, debilidad y pérdida de masa muscular, alteraciones que a su vez también pueden estar presentes en las personas frágiles (Stieglitz et al., 2014). Del mismo modo, los síntomas depresivos son predictivos de muchos de los mismos episodios adversos que la fragilidad, incluyendo caídas, fracturas, discapacidad y mortalidad (Laursen et al., 2007; San Onge et al., 2014; Whooley et al., 1999). Por otro lado, se han descrito alteraciones fisiopatológicas comunes entre la fragilidad y la depresión como la enfermedad cerebrovascular subclínica, la desregulación neuroendocrina y un estado pro-inflamatorio (Buigues et al., 2012; Paulson and Lichtenberg, 2013). Finalmente, el envejecimiento celular acelerado, medido por el acortamiento de la longitud del telómero, también podría estar relacionado con la depresión y la fragilidad (Saum et al., 2014; Wolkowitz et al., 2011).

Desde el punto de vista social, la fragilidad se ha asociado con la muerte de un ser querido (Coelho et al., 2015). Teniendo en cuenta el impacto físico y psíquico del duelo es comprensible que la viudez pueda conducir a la aparición de este síndrome (Stroebe et al., 2017). Este acontecimiento puede contribuir, en parte, a que las mujeres sean más frágiles que los hombres, puesto que estos últimos al morir antes, hacen que las mujeres vivan solas durante más años. Además vivir solo también favorece el desarrollo de la fragilidad, lo que puede explicarse por el hecho de que los adultos mayores aislados con mayor frecuencia desarrollan síntomas depresivos (Bilotta, 2014). Igualmente, diferentes autores han observado que la falta de participación social favorece al desarrollo de la fragilidad (Duppen et al., 2017). El sindrome fragil también se ha asociado con la situación familiar del anciano. La fragilidad es menor en aquellos adultos mayores que cuentan con el apoyo familiar que demanda sus necesidades (Peek et al., 2012).

Por último, se debe tener en cuenta que los predictores de la fragilidad no actúan como factores de riesgo aislados, sino que será la interacción entre ellos los que predigan con mayor probabilidad el futuro desarrollo de la fragilidad en adultos mayores.

2.6. Riesgos del síndrome de fragilidad

La principal relevancia de este síndrome es que funciona como un importante predictor de eventos adversos para la salud a corto, medio y largo plazo (Abellan van Kan et al., 2010). De hecho, cada vez más estudios sustentan que la fragilidad, en la población mayor, es mejor predictor de episodios adversos de salud que la propia edad cronológica (Clegg y Young, 2011). De esta manera, este término permite hacerse una idea del estado de salud del individuo, proporcionando una cuantificación más precisa de la vulnerabilidad, que si sólo se atiende a la propia edad cronológica.

Diversos estudios epidemiológicos han identificado, aunque de forma heterogénea, una clara asociación longitudinal entre el síndrome de fragilidad y un mayor riesgo de caídas, fracturas, pérdida de movilidad, discapacidad para AVD, hospitalización, institucionalización y muerte (Abianza Soler et al., 2013). En la siguiente tabla (véase Tabla 6) se exponen los episodios adversos para la salud evaluados en diferentes estudios internacionales.

Tabla 6
Asociación del síndrome de fragilidad y episodios adversos para la salud en estudios internacionales.

	Caídas	Fracturas	Perdida movilidad	Discapacidad AVD	Hospitalización	Institucionalización	Mortalidad
CHS	NO		**SI**	**SI**	**SI**		**SI**
SOF	**SI**	**SI**		**SI**			**SI**
WHAS I-II	NO			**SI**	NO	**SI**	**SI**
WHI-OS		**SI**		**SI**	**SI**		**SI**
CNPHS							**SI**
H-EPESE	**SI**			**SI**	**SI**		**SI**
RMAP				**SI**			**SI**
3-CITIES			NO	**SI**	**SI**		NO
CSHA						**SI**	**SI**
SHARE				**SI**			
BLSA							**SI**
PEP	**SI**			**SI**		**SI**	**SI**
ILSA				**SI**			**SI**

Nota. AVD: actividades de la vida diaria; BLSA: *Beijing Longitudinal Study of Aging*; CHS: *Cardiovascular Health Study*; CNPHS: *Canadian National Population and Health Survey*; CSHA: *Canadian Study of Health and Agin*; H-EPESE: *Hispanic Stablished Populations for Epidemiologic Studies of the Elderly*; ILSA: *Italian longitudinal estudy on aging*; NO: no significativo; PEP: *Precipitating Events Project*; RMAP: *Rush Memory and Aging Project*; SHARE: *Survey of Health, Aging and Retirement in Europe*; SI: asociación significativa; SOF: *Study osteoporotic fractures*; WHAS I y II: *Women's Health and Aging Study*; WHI-OS: *Women's Health Initiative-Observational Study*; 3-CITIES*: estudio de las 3 ciudades.*
Fuente: Elaboración propia a partir de Romero Rizosy Abianza Soler (2013).

En estos estudios, la mortalidad y la discapacidad para AVD han sido los principales efectos adversos analizados. Casi todos los estudios, excepto el *estudio de las tres ciudades* (3-CITIES), mostraron que, en el seguimiento de hasta 10 años, este síndrome frágil fue un factor predictor independiente de muerte con un riesgo relativo entre 1,63 y 6,03. Asimismo, la totalidad de los estudios que evaluaron la relación entre el síndrome de fragilidad y la discapacidad encontraron una asociación significativa entre ambos, con un riesgo relativo entre 1,79 y 15,74 para las ABVD (actividades básicas de la vida diaria) y entre 2,79 y 10,44 para las AIVD (actividades instrumentales

de la vida diaria) (Bandeen- Roche et al., 2006; Ensrud et al., 2008; Fried et al., 2001).

En nuestro país el estudio *FRADEA* ha puesto de manifiesto, en una población mayor de 70 años, que el síndrome de fragilidad, medido mediante los criterios de Fried, supone un riesgo ajustado de mortalidad 5,5 veces mayor, un riesgo de pérdida de movilidad 2,7 veces mayor y un riesgo de discapacidad 2,5 para ABVD y 1,9 para AIVD. El estado de fragilidad se asoció de manera significativa con la mortalidad, la discapacidad y la deficiencia de movilidad, siendo la lentitud de la marcha el criterio de fragilidad que presentó una mayor asociación con todos ellos (véase Tabla 7) (Abizanda et al., 2013; Romero Rizos y Abizanda Soler, 2013).

Tabla 7
Incidencia de episodios adversos asociados a criterios específicos del fenotipo de fragilidad en FRADEA.

	Deficiencia de movilidad OR IC 95%	Discapacidad ABVD OR IC 95%	Discapacidad AIVD OR IC 95%	Mortalidad HR IC 95%
Velocidad de la marcha	2,5 (1,6-3,9)	2,0 (1,1-3,1)	1,5 (1,0-2,2)	3,2 (1,4-7,2)
Actividad física	2,0 (1,2-3,5)	1,9 (1,1-3,3)	1,0 (0,6-1,8)	3,7 (1,8-7,6)
Pérdida de peso	1,0 (0,6-2,1)	1,9 (1,1-3,4)	1,7 (1,1-2,7)	3,3 (1,7-6,4)
Cansancio	1,5 (1,0-2,4)	1,5 (0,9-2,1)	1,6 (1,0-2,3)	1,6 (0,8-3,0)
Debilidad	1,0 (0,6-1,5)	1,6 (1,0-2,5)	0,8 (0,6-1,1)	1,4 (0,7-2,8)

Nota. ABVD: actividades básicas de la vida diaria; AIVD: actividades instrumentales de la vida diaria; HR: Hazard ratio; IC 95%: intervalo de confianza del 95%; OR: Odds ratio
Fuente: Elaboración propia a partir del estudio FRADEA (Abianza et al., 2013).

Estas asociaciones implican que el objetivo de la atención geriátrica debe ser la detección de la fragilidad, entendida como un síndrome previo a la discapacidad sobre el que implementar tratamientos específicos que retarden su aparición y las consecuencias derivadas del deterioro funcional.

2.7. Evaluación del síndrome de fragilidad

Dado que la fragilidad es el paso previo a la discapacidad, la identificación precoz de los adultos mayores frágiles es un tema prioritario (Abizanda Soler et al., 2010). Así, con el fin de retrasar o prevenir el deterioro funcional y la dependencia, el objetivo principal de su detección es la intervención precoz y multidisciplinar, pues la evidencia sugiere que la fragilidad podria ser reversible (Bouillon et al., 2013; Pons Raventos et al., 2016).

Los adultos mayores muestran características heterogéneas y en especial, en la forma de presentación de la enfermedad (Goméz Pavón, 2006). Así, mientras muchas personas mayores presentan problemas médicos concretos, otras presentan problemas menos definidos que se escapan de la valoración clínica tradicional. Por ello, estos pacientes más complejos a menudo necesitan una valoración geriátrica integral (VGI).

La VGI se define como un proceso de diagnóstico multidimensional e interdisciplinario que permite identificar y cuantificar los problemas clínicos, funcionales, sociales y psíquicos de las personas mayores, y en base a ellos, elaborar un plan de tratamiento y seguimiento coordinado e integral con el fin de optimizar recursos, preservar el estado funcional así como, retrasar o reducir al mínimo el riesgo de resultados adversos para la salud (Domínguez-Ardila y García-Manrique, 2014). La revisión de la literatura muestra múltiples beneficios de la VGI en la atención al anciano (véase Figura 13) (Ariño y Benavent, 2001).

Debido a que la VGI tiene un coste, por su carácter interdisciplinar y el tiempo que se tarda en obtener datos relevantes, es importante identificar a aquellos adultos mayores que son más susceptibles de beneficiarse de esta valoración, y por tanto, del uso preferente de recursos más especializados y del diseño de intervenciones adecuadas (Baena et al., 2007). Teniendo en cuenta que la VGI parece mostrar una mayor efectividad cuando se selecciona a personas mayores vulnerables o con riesgo elevado de presentar efectos adversos para la salud, la identificación de adultos mayores frágiles debe ser el paso previo para su realización (Martín-Lesende y Gorroñogoitia Iturbe, 2009). Sin embargo, esta aspiración se ve frustrada por la falta de herramientas simples y fiables que permitan identificar de forma estandarizada la fragilidad (Casado Marín, 2005).

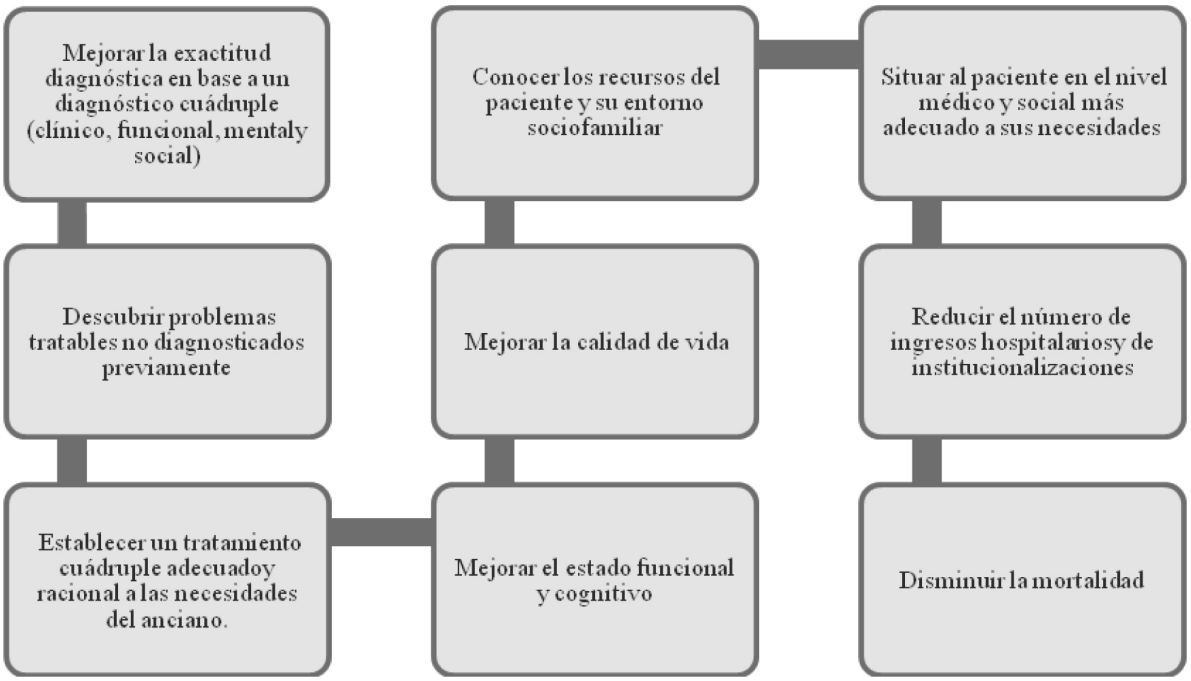

Figura 13. **Beneficios de la valoración geriátrica integral en la atención a la persona mayor.**
Fuente: Elaboracion propia a partir de González Montalvo (2001).

En la práctica clínica, la labor de detección del paciente frágil queda relegada casi exclusivamente a los profesionales sanitarios del ámbito de la atención primaria y las enfermeras gestoras de casos hospitalarios (Pons Raventos et al., 2016). La cercanía y el conocimiento global que estos profesionales tienen de los pacientes pueden ayudar en gran medida a detectar precozmente la fragilidad (Pérez Cárceles et al., 2006). Sin embargo, dicha labor es necesario desarrollarla también en el ámbito hospitalario (Baztán-Cortés et al., 2000). Así pues, cualquier profesional sanitario que atiende a personas mayores debe estar implicado en su detección (Baztán-Cortés et al., 2000).

En este sentido, la creación de un diagnóstico enfermero (DE) que permita la detección y prevención de la fragilidad por parte de enfermería es una necesidad. Es importante resaltar que el desarrollo de un DE en torno al síndrome frágil mejoraría la continuidad de los cuidados de dichos pacientes, al favorecer la utilización de un lenguaje enfermero estandarizado y permitir una buena comunicación con otros profesionales. Asimismo, la incorporación de este DE ayudaría a los gestores a planificar de forma más eficaz los recursos necesarios para el abordaje integral y cuidado de estos pacientes (Jiménez-Ruiz et al., 2017).

2.7.1. Sindrome de fragilidad como diagnóstico enfermero.

En base a lo anterior, y según los datos más actuales sobre niveles de evidencia y práctica de enfermería, la Asociación Norteamericana de Diagnósticos Enfermeros Internacional (NANDA-I), una sociedad científica de enfermería cuyo objetivo es estandarizar el DE, en su último manual publicado, "*International Nursing Diagnoses. Definitions & Classification 2015-2017*", ha incorporado, en relación a la edición anterior, 25 nuevos DE (3 de ellos propuestos por España). De entre los nuevos DE incorporados se encuentran dos diagnósticos de síndrome, el Síndrome del anciano frágil (002571) y el Riesgo de síndrome del anciano frágil (00231), uno real y otro de riesgo, ambos validados por enfermeras españolas que bien merecen ser nombradas, las enfermeras Margarita Garrido Abejar, María Dolores Serrano Parra y Rosa María Fuentes Chacón.

El mencionado Sindrome del anciano frágil, incluido dentro del Dominio 1 (Promoción de la salud) en la clase 2 (Mantenimiento de la salud) de la Taxonomía-III, ha sido definido como: "*Estado dinámico de equilibrio inestable que afecta al anciano que experimenta deterioro en uno o más dominios de la salud (física, funcional, psicológica o social) que produce un aumento de la susceptibilidad a efectos adversos en la salud, en particular a la discapacidad*" (Herdman y Kamitsuru, 2015).

Un síndrome, definido por NANDA-I, es un juicio clínico sobre un agrupamiento específico DE que suceden conjuntamente y que, además, se identifican mejor juntos y son tratados con intervenciones similares. Por tanto, para hacer un diagnóstico de síndrome deben estar presentes, entre las características definitorias, dos o más DE (Jiménez-Ruiz et al., 2017). De este modo, las características definitorias que identifican al citado diagnóstico son una serie de DE relacionados en torno a la persona mayor con dificultades en el área física, funcional, psicosocial o ambiental. Dificultades que le generan un determinado nivel de discapacidad (véase Figura 14). Entre los factores relacionados para este diagnóstico se encuentra la alteración de la función cognitiva, antecedentes de caídas, enfermedad crónica, enfermedad psiquiátrica, hospitalización prolongada, malnutrición, obesidad sarcopénica, sarcopenia, sedentarismo y vivir solo (Herdman y Kamitsuru, 2015).

Figura 14. **Características definitorias del síndrome del anciano frágil.**
Fuente: Elaborado a partir de Herdman y Kamitsuru (2015).

Cuando la fragilidad viene determinada en forma de riesgo potencial, la NANDA-I propone el uso del DE riesgo de síndrome del anciano frágil, encuadrado también dentro del Dominio 1 (Promoción de la salud) en la clase 2 (Mantenimiento de la salud) de la Taxonomía-III, y definido como*:"Riesgo de desequilibrio inestable en el estado dinámico, que afecta a la persona de edad avanzada, además de existir deterioro en uno o más dominios de la salud (física, funcional, psicológica o social), que conduce a un aumento de la susceptibilidad y a efectos adversos para la salud, en particular la discapacidad"* (Herdman y Kamitsuru, 2015) . Asimismo, los factores causales identificados para este DE incluyen, entre otros, la actividad física diaria menor de la recomendada al género y edad, el agotamiento, la alteración en la función cognitiva, en el proceso de coagulación (p. ej., Factor VII, D-dímeros), en equilibrio y movilidad, el apoyo social insuficiente, la anorexia, la ansiedad, los antecedentes de caídas, el aislamiento social, los bajos recursos económicos, el déficit sensorial y la depresión.

2.7.2. Instrumentos para la evaluación del síndrome de fragilidad.

Se han descrito numerosas formas de medir el síndrome frágil. Sin embargo, sigue existiendo un debate sobre la mejor manera de medirlo, tanto en el ámbito clínico como en el de la investigación. Parece que hay un acuerdo general en que el instrumento para la medición del síndrome de la fragilidad debe ser multidimensional, que permita captar su estado dinámico, no incluya la discapacidad o comorbilidad, tenga validez predictiva de resultados adversos para la salud y sea factible (Buta et al., 2016; Gobbens et al., 2010; Hogan et al., 2003).

La mayoría de los autores consideran que el síndrome frágil es un concepto multidimensional (Walston et al., 2006). La fragilidad se caracteriza por una disminución de la capacidad de reserva en diferentes dominios de funcionamiento (Fried et al., 2001). Por tanto, su evaluación debe abarcar otras dimensiones más allá de la física, como la psicológica y la social (Gobbens et al., 2007; Markle-Reid y Browne, 2003). Revisiones sistemáticas recientes sobre instrumentos de evaluación de la fragilidad han puesto de relieve que una parte sustancial de los instrumentos sólo tienen en cuenta los aspectos físicos de la fragilidad (Gobbens et al., 2012; Gray et al., 2016; Pialoux et al., 2012). Sin duda, para muchos clínicos es más útil identificar la fragilidad sobre la base de factores físicos, ya que estos son más tangibles, más objetivos y más propensos a ser tratados por medios médicos (García-García y Alfaro Acha, 2010). Sin embargo, un enfoque en el que el foco no está exclusivamente en los problemas físicos, sino que también incorpora problemas psicosociales, permite una visión integral y multidisciplinar de las personas frágiles. Por lo que un enfoque exclusivamente basada en componentes físicos, pondrá en peligro la atención de la persona como un todo, dando lugar a la fragmentación y, con ello, a una reducción en la calidad de la atención prestada (Gobbens et al., 2010). Igualmente, Bergman et al. (2002) evidenciaron que la atención integral de las personas mayores frágiles mediante la coordinación de los servicios sociosanitarios, además de optimizar los recursos disponibles y, como consecuencia, reducir costes, puede tener un impacto importante en la salud y calidad de vida de estas personas. Además, una medida de la fragilidad que incluya diversos dominios de funcionamiento es un mejor predictor de institucionalización y muerte (Rockwood et al., 2006).

Sabemos que el nivel real de fragilidad puede cambiar en cualquier dirección, lo que significa que con el tiempo uno puede llegar a ser más o menos frágil e incluso invertirse (Markle-Reid y Browne, 2003; Puts et al., 2005). Por tanto, cualquier instrumento de medición de fragilidad debe ser capaz de distinguir varios niveles de fragilidad y, por ende, medir un cambio de nivel en el tiempo para permitir monitorizar la evolución o los resultados de las intervenciones. Así pues, con el fin de capturar la naturaleza dinámica de la fragilidad es preferible un sistema de puntuación continuo o de puntuación ordinal en múltiples niveles (Mitnitski et al., 2001). La mayoría de los instrumentos de evaluación de la fragilidad, identificados en una revisión sistemática llevada a cabo por Gobbnes et al. (2012), utilizaron un sistema de puntuación dicotómica, donde una persona se clasifica como frágil o no frágil.

Tal y como hemos comentado con anterioridad, el síndrome de fragilidad puede estar presente en combinación con la discapacidad y/o la comorbilidad, pero también es probable que se presente de manera individual (Fried et al., 2004; Fulop et al., 2010) (Ir a Figura 11). En base a ello, algunos autores han argumentado que los instrumentos de evaluación del síndrome de fragilidad no deben incluir elementos de comorbilidad y/o discapacidad (Gobbens et al., 2010). Sin embargo, en una revisión realizada por Bouillon et al. (2013), más de la mitad de los instrumentos de fragilidad evaluados incluyeron medidas de discapacidad o comorbilidad.

También, como se aplica a cualquier instrumento de medición, éste tiene que mostrar propiedades clinimétricas. En este sentido, la fiabilidad y validez son los indicadores de calidad más importantes de cualquier instrumento de medida (Terwee et al., 2007). La validez predictiva de episodios adversos se ha demostrado entre la mayoría de los instrumentos de evaluación de la fragilidad (Bouillon et al., 2013; de Vries et al., 2011). De hecho, la evaluación de la fragilidad se usa comúnmente como un factor de riesgo de episodios adversos para la salud (Romero Rizos y Abizanda Soler, 2013). Igualmente el instrumento de evaluación de la fragilidad debe ser factible. Sin embargo, en el ambito clinico, restricciones relacionadas con el tiempo, la financiación, los recursos y el espacio a menudo están presentes (Pialoux et al., 2012).

Teniendo en cuenta que no se ha determinado un método definitivo para realizar el cribado de fragilidad. Sternberg et al. (2011) llegaron a la conclusión de que las necesidades y los objetivos del estudio o la clínica pueden determinar el instrumento más adecuado para evaluar la fragilidad. Según Buta et al. (2016), de los 67 instrumentos de fragilidad que identificaron, los más ampliamente utilizados fueron el Fenotipo de Fried y el IF de Rockwood.

El Fenotipo de Fried fue descrito y validado en el *CHS* por Fried et al. (2001). Dichos autores elaboraron un fenotipo físico de fragilidad en base a cinco criterios centrados en manifestaciones funcionales: debilidad, baja resistencia al esfuerzo, lentitud, baja actividad física y pérdida de peso no intencionada (véase Tabla 8). Los valores del quintil más bajo se utilizaron para definir la ausencia o presencia de estos criterios. Las personas con tres de los cinco criterios se consideran frágiles, aquellos con uno o dos prefrágiles y con ninguno robustos o no frágiles. Asimismo, establecieron que la pérdida de peso involuntaria y el cansancio autoreferido son los criterios de riesgo más importantes para el desarrollo de fragilidad. Sin embargo, la debilidad fue, de los cinco criterios, el más común entre las mujeres que desarrollaron fragilidad (Xue et al., 2008).

Tabla 8
Criterios del síndrome de fragilidad según Fried.

Pérdida de peso no intencionada
Igual o mayor a 4,6 kg o igual o mayor al 5% del peso corporal en el último año
Debilidad
Fuerza prensora en el percentil (P) 20 inferior, ajustado por sexo e índice de masa corporal
Baja energía y resistencia
Cansancio autorreferido identificado por 2 preguntas de la escala Center Epidemiological Studies-Depression. Se demuestra asociado a consumo máximo de oxígeno en pruebas de evaluación del ejercicio
Lentitud en la marcha
Velocidad para caminar 15 pies (4,5 metros) en el P 20 inferior ajustado para sexo y altura
Bajo grado de actividad física
Cálculo de kilocalorías consumidas semanalmente, según la información dada por el paciente, en el quintil inferior ajustado por sexo (originalmente evaluado por el Minnesota Leisure Time Activity Questionnaire)

Fuente: Elaboración propia a partir Fried et al. (2001).

Esta escala es el instrumento más utilizado en el ámbito investigador, lo que permite realizar comparaciones entre diversos estudios. Sin embargo, a pesar de su amplio uso, tiene algunos inconvenientes al traspasarlo a la práctica clínica habitual (Abizanda Soler, 2010). Por un lado, es necesario un equipamiento especifico (dinamómetro) y por otro, los puntos de corte de algunos criterios, como la fuerza de prensión, la velocidad en la marcha y la actividad física, requieren de valores referenciales apropiados a la población de estudio, que pueden no estar disponibles, siendo necesarios estudios poblacionales que proporcionen referencias estandarizadas (García-García y Alfaro Acha, 2010). Asimismo, otros factores potencialmente importantes, tales como el deterioro cognitivo o sintomas depresivos, condiciones altamente prevalentes asociadas con la fragilidad, no se incluyen como componentes del fenotipo Fried (Yew et al., 2017). En respuesta a este desafío, los estudios recientes han examinado los componentes Fried y han añadido la cognición y el estado de ánimo a la escala de Fried (García-García y Alfaro Acha, 2010). No obstante, a pesar de estas críticas, el constructo de Fried ha sido validado de forma independiente como un fuerte predictor de eventos adversos para la salud (Romero Rizos y Abizabda Soler, 2013).

Otras escalas han adoptado esta escala con algunas variaciones, por ejemplo, la *escala FRAIL*. Esta escala cuenta con 5 items (fatiga, comorbilidad, incapacidad para subir un tramo de escalera o para caminar una cuadra y pérdida de peso ≥ 5%) donde cada respuesta afirmativa es valorada con 1 punto, se considera prefragilidad 1 o 2 puntos y fragilidad puntuaciones ≥ a 3 (Morley et al., 2012). Asimismo, el "*Instrumento de fragilidad para la atención primaria de la encuesta de salud, envejecimiento y Jubilación en Europa: SHARE-FI*", también basado en los criterios de Fried, es aplicable en el ámbito de la atención primaria para pacientes no residentes en instituciones. A través de la web http: //www. biomedcentral.com/1471-2318/10/57 se encuentran calculadoras de acceso gratuito diferenciadas por sexo y la versión española está disponible en https://sites. google.com/a/tcd.ie/share-frailty-instrument-calculators/. Otra escala más sencilla derivada de los criterios Fried es la escala del *Study de Osteoporotic Fractures* (SOF), ésta incluye 3 items (fatiga, incapacidad para levantarse de una silla 5 veces sin utilizar los brazos y pérdida de peso ≥ 5%), 1 punto se considera prefrágil y fragilidad puntuaciones ≥ 2 (Ensrud et al., 2008).

El IF fue descrito y validado en *CSHA* por Rockwood y Ministki (2007), quienes, para medir la fragilidad evaluaron cerca de 92 déficits, y el nivel de fragilidad se definió como la proporción de los déficits presentes (p.e, alguien con 40 déficit de 92 tiene un IF de 0,43). En base a estos déficits, estos autores, elaboraron una escala de fragilidad con 7 grados progresivos: 1) robusto; 2) bien, sin enfermedad; 3) bien, con enfermedad controlada; 4) vulnerable aparentemente; 5) leve dependencia en AIVD; 6) ayuda en AIVD y ABVD y 7) dependiente. Esta escala ha demostrado capacidad predictiva de mortalidad e institucionalización (Romero Rizos y Abizabda Soler, 2013). Estudios posteriores han conseguido reducir la cifra inicial de 92 déficits a una cantidad de 30 sin pérdida de capacidad predictiva (SEGG, 2010).

Este IF permite una valoración multidimensional y al utilizar un sistema de puntuación continua cuantifica la fragilidad más allá de su presencia o ausencia. Sin embargo, debido a su extensión es poco aplicable en la clínica. Asimismo, el hecho de integrar medidas de comorbilidad lo hace poco sensible al cambio, ya que son entidades poco reversibles, y la inclusión de déficit funcional la aleja del concepto de fragilidad como un estado precursor de discapacidad (Abizanda Soler, 2010).

Desde una perspectiva funcional, la fragilidad se ha relacionado con la pérdida de funcionalidad (Álcala et al., 2010). En base a esta relación, las pruebas destinadas a cuantificar la limitación funcional incipiente son un método potencial para identificar a las personas mayores frágiles (Abianza Soler, 2010). Entre las diferentes opciones para cuantificar la pérdida de funcionalidad precoz se encuentran las pruebas de ejecución. Hasta un 17.7 % de los estudios utilizan dichas pruebas funcionales para el cribado de la fragilidad (Stenberg, 2011). Son pruebas objetivas, breves y sencillas que valoran la marcha, el equilibrio y la movilidad. Muestran buen rendimiento en cuanto a fiabilidad test-retest e interobservador así como, validez predictiva de eventos adversos (Shimada et al., 2010; Studenski et al., 2011). Su buena concordancia con otras pruebas funcionales para el cribado de la fragilidad y factibilidad de uso en la clínica diaria, las convierten, probablemente, en el mejor método para detectar la fragilidad en atención primaria (Martin- Lesende et al., 2009). En nuestro entorno entre las más utilizadas está la velocidad de la marcha y el test de levántate y anda cronometrado (Timed Get Up and Go test “TUG”).

La velocidad de la marcha, además de ser uno de los criterios de Fried, es la prueba de ejecución más utilizada para la evaluación de la limitación funcional (Abizanda Soler et al., 2011). Consiste en medir el tiempo que tarda una persona en recorrer, a su ritmo habitual, una distancia preestablecida (2,4;4;5;6 o 10 m) (Schwenk et al., 214) (véase Figura 15). Los test más utilizados y mejor validados son los que calculan la velocidad en distancias de 4 m (Perera, 2006). Los puntos de corte con mejor valor predictivo de fragilidad suele situarse entre 1 y 0,7 m/seg, siendo esta última cifra la más extendida en los diferentes estudios y recomendaciones de consenso, por tanto, es considerada como marcador de fragilidad (Apostolo et al., 2017).

La prueba TUG ha sido validada como herramienta diagnóstica de fragilidad (Sawa et al., 2013). Esta prueba mide el tiempo que tarda la persona en levantarse de una silla, caminar 3 m, girarse, regresar a la silla y volver a sentarse sin utilizar los brazos como apoyo (véase Figura 15) (Podsiliadlo y Richardson, 1991). Se considera normal si se realiza en un tiempo ≤ a 10 seg, un tiempo entre 10-20 seg es criterio para detectar la fragilidad y cuando es ≥ de 20 seg se considera que la persona tiene riesgo de caídas (Mir Sanchez, 2016). En este sentido, también esta validada para evaluar el riesgo de caídas (Bellanco y Benitez, 2014).

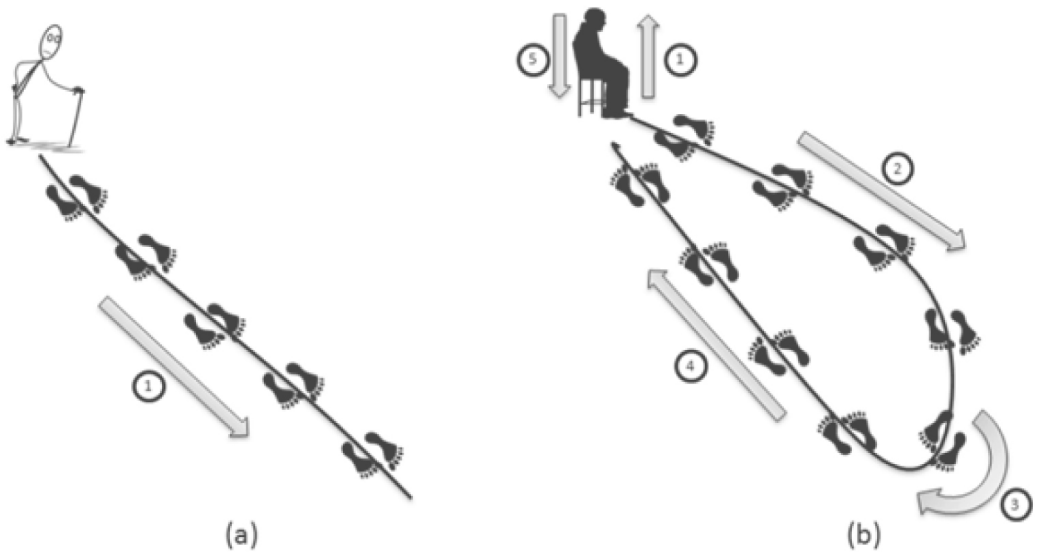

Figura 15. Valoración de la marcha (a) Tinetti (marcha), (b) Times Get-Up and Go test.
Fuente: Tomada de Fontecha (2013).

2.7.3. La importancia de los biomarcadores en el síndrome de fragilidad

Un biomarcador es aquella sustancia utilizada como indicador de un estado biológico. Debe poder medirse objetivamente y ser evaluado como un indicador de un proceso biológico normal o patológico, o de respuesta a una intervención terapéutica (Biomarker definitions Working Group, 2001). Según la información que proporcionan podemoos distinguir biomarcadores de riesgo, de diagnóstico o de pronóstico (Fougère et al., 2015). Los primeros informan sobre la predisposición de padecer una patología, los de diagnóstico dan información sobre si un paciente padece una enfermedad o si ha estado expuesto a algún tóxico o patógeno y por último, los de pronóstico informan sobre la progresión de la enfermedad, es decir, si la enfermedad mejora o empeora tras el tratamiento correspondiente. El biomarcador ideal deber proporcionar información diagnóstica, pronóstica y terapéutica adicional a la que se obtiene a partir de los datos clínicos del paciente.

En este contexto, la búsqueda de biomarcadores de fragilidad es un campo muy interesante si realmente se consiguiera encontrar en un futuro un marcador biológico (o varios) que fuera lo suficientemente sensible y específico como para identificar individuos frágiles (Erusalimsky et al., 2016; Ingles de la Torre, 2014). Asimismo, los últimos avances permiten que los biomarcadores puedan detectar de manera precoz ciertas enfermedades, incluso antes de que se produzcan los síntomas (Ortega-Ortiz de Apodala, 2010). Dado que la evidencia sugiere que el deterioro fisiológico del estado de fragilidad empieza a ser evidente en una fase preclínica (Fernández Garrido et al., 2014a), la identificación de un biomarcador específico de fragilidad ayudaría no solo a mejorar la precisión diagnóstica del síndrome frágil en la práctica clínica, sino también, a identificar de manera precoz a pacientes en riesgo de ser frágiles, y que pudiesen ser subsidiarios de intervenciones de prevención primaria. Además, su monitorización permitiría evaluar el pronóstico de fragilidad en términos de discapacidad y de otros resultados adversos así como, la respuesta a las intervenciones aplicadas (Lippi et al., 2015). Por esta razón, en los últimos años, varios estudios han relacionado la fragilidad con diferentes biomarcadores involucrados en la fisiopatología del deterioro fisiológico característico del síndrome de la fragilidad (Collerton et al., 2012; Kalyani et al., 2012a; Schoufour et al., 2015).

Si atendemos a la definición previa de la fragilidad, ésta se origina por la alteración de múltiples e interrelacionados sistemas fisiológicos. En el *MacArthur Study of Successful Aging*, estudio longitudinal sobre el envejecimiento exitoso, se evidenció en una muestra de 1.192 adultos mayores de 70 años, una asociación entre la carga alostática, un índice de desregulación fisiológica multisistémica, y el desarrollo de la fragilidad. La carga alostática se determinó de acuerdo con la alteración de diferentes biomarcadores de una amplia gama de sistemas fisiológicos, incluidos el sistemas cardiovascular, metabólico, inflamatorio y neuroendocrino (Gruenewald et al., 2009). Por tanto, estos biomarcadores podrían servir como una señal de alerta para el desarrollo de la fragilidad. Sin embargo, a pesar de que varias medidas bioquímicas muestran una fuerte asociación con la fragilidad, actualmente no existe ningún biomarcador que puede identificar adecuadamente al síndrome frágil (Abizanda Soler, 2010). Asimismo, y dada la naturaleza multisitémica de la fragilidad, es probable que se requieran para su mediccón una combinación de medidas bioquímicas en lugar de un único biomarcador (Homlett et al., 2014). En la siguiente figura (véase Figura 16) se muestra la vía de la fragilidad y el papel de los biomarcadores propuestos hasta el momento.

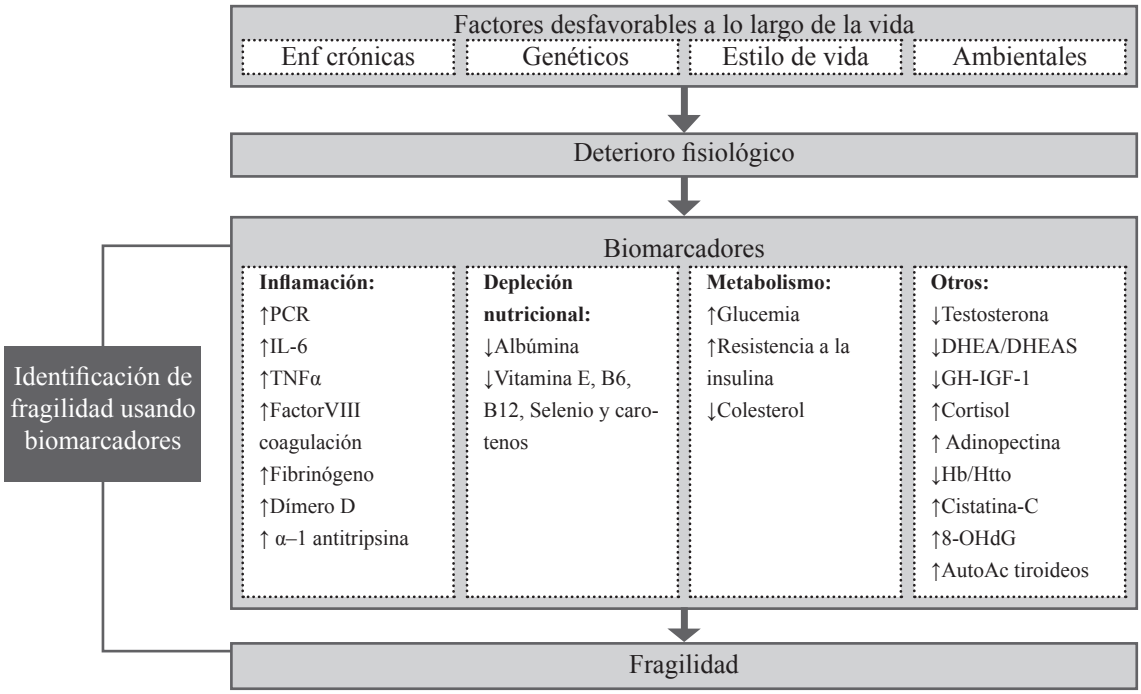

***Figura 16*. La vía de la fragilidad y el papel de los biomarcadores.**
Fuente: Elaborada a partir de Schoufour et al. (2015).

- ***Biomarcadores de inflamación.***

La inflamación crónica ha sido considerada como uno de los componentes más importantes que contribuyen al desarrollo de la fragilidad (Ershler y Keller, 2000). De hecho, los niveles de citoquinas pro-inflamatorias y otros marcadores de la inflamación han sido ampliamente estudiados.

En este sentido, la IL-6, una citocina importante que modula el sistema inmunológico, se ha asociado de manera significativa con el síndrome de fragilidad (Bandeen-Roche et al., 2009; Collerton et al., 2012; Fernández-Garrido et al., 2014a; Hubbard et al., 2009; Leng et al., 2007; Schmaltz et al., 2005). Así, una reciente revisión sistemática de 32 estudios transversales mostró que las personas mayores frágiles, en comparación con las robustos, presentaban unos niveles más altos de IL-6 (Soysal et al., 2016). Curiosamente, en un estudio transversal llevado a cabo en mujeres mayores que viven en la comunidad, se ha observado que la infección asintomática crónica por citomegalovirus (CMV) se asocia con el síndrome de fragilidad y que tiene un importante efecto multiplicador sobre los niveles de IL-6, lo que sugiere una relación sinérgica entre la infección crónica asintomática por CMV y la IL-6 sobre el estado de fragilidad (Schmaltz et al., 2005).

Mientras que la IL-6 se ha asociado de manera consistente con el estado de fragilidad, los resultados para otros mediadores de la inflamación como la PCR y TNF-alfa son contradictorios (Collerton et al., 2012). Así, mientras que Hubbard et al. (2009), Collerton et al. (2012) y Serviddio et al. (2009) observaron una relación positiva entre el TNF-alfa y el síndrome de fragilidad, el *InCHIANTI Study* (Bandeen-Roche et al., 2009) y el llevado a cabo por Leng et al. (2004) no encontraron ninguna asociación entre la concentración de TNF-alfa y el riesgo de ser frágil. Asimismo, diversos autores han detectado una asociación significativa entre el riesgo de ser frágil y el incremento de los valores de la PCR (Barzilay et al., 2007; Collerton et al., 2012; Hubbard et al., 2009; Walston et al., 2002; Wu et al. 2009). Sin embargo, tanto el *Women´s Health Initiative Observational Study* (WHI-OS) como el *InCHIANTI Study* no observaron ninguna diferencia significativa entre los niveles de PCR de las personas frágiles y las no frágiles o robustas (Bandeen-Roche et al., 2009; Reiner et al., 2009).

Por otro lado, Ronning et al. (2010) encontraron mayores niveles de IL-6, TNF-alfa y PCR en sujetos mayores frágiles oncológicos, lo que sugiere que estos marcadores inflamatorios podrán desencadenar el síndrome de fragilidad en estos pacientes.

- ***Biomarcadores de coagulación y fibrinolisis***

Puesto que las citoquinas inflamatorias estimulan la liberación de factores procoagulantes por distintos tipos de células, diversos estudios han asociado, con independencia de posibles factores de confusión, el síndrome de fragilidad con un aumento de los biomarcadores de coagulación y fibrinolisis, como el factor VIII, fibrinógeno, dímero D, activador del plasminógeno tisular y factor XI activo α–1 antitripsina (Barzilay et al., 2007; Kanapuru y Ershler, 2009; Reiner et al., 2009). A su vez, la conexión entre coagulación y fibrinólisis con la fragilidad se ha visto apoyada por un estudio reciente, en el cual, los adultos mayores frágiles que viven en la comunidad presentaron un incremento moderado del riesgo de desarrollar una tromboembólia venosa idiopática, en comparación con los no frágiles (Folsom et al., 2007).

- ***Biomarcadores metabólicos***

Varios marcadores metabólicos como la glucemia y los niveles séricos de lípidos también han sido estudiados en su relación con la fragilidad. Así, un estudio llevado a cabo por Zaslavsky et al. (2016) refleja que, en adultos mayores con o sin diabetes, los altos niveles de glucemia en ayunas y 2 horas después de la ingestión oral de 75 g de glucosa, incluso dentro del rango normal, pueden ser un factor de riesgo de fragilidad. Del mismo modo, Kalyani et al. (2012a) evidenciaron, en una población de mujeres mayores no diabéticas, que los altos niveles de hemoglobina glicosilada (HbA1c) aumentan el riesgo de fragilidad y de limitación de la movilidad de las extremidades inferiores. Igualmente, diversos estudios han mostraron una asociación entre la fragilidad y la RI, evaluada mediante el test HOMA (Homeostatic model assesment) (Barzalay et al., 2007; Kalyani et al., 2012b).

Asimismo, Kalyani et al. (2012c) sugieren que el estado de fragilidad, además de asociarse con una alteración en la dinámica de la glucosa e insulina, también se asocia con una desregulación de otras hormonas circulantes del metabolismo energético, como la ghrelina. De hecho, estos autores encontraron que las mujeres mayores frágiles, en comparación con las no frágiles, presentaban unos menores niveles de ghrelina en ayunas y a los 120 minutos después de la ingesta oral de 75gr de glucosa.

Con respecto al metabolismo lípidico, Hwang et al. (2015), con el fin de evaluar la asociación entre el riesgo cardiometabólico y la fragilidad, observaron que los bajos niveles de HDL-c se asociaron significativamente con el síndrome de fragilidad. Del mismo modo, Ramsay et al. (2015), en una muestra representativa de ancianos británicos sin ECV establecida, evidenciaron que la fragilidad se asocia con una gama de factores de riesgo cardiovascular incluyendo los bajos niveles de HDL-c. Este tipo de asociaciones, entre la fragilidad y los niveles de HDL-c o triglicéridos (TGD), también han sido observadas en estudio previos (Bastos-Barbosa et al., 2012; Chang et al., 2012). Por otro lado, en el *Longitudinal Aging Study Amsterdam*, estudio longitudinal centrado en los determinantes, las trayectorias y las consecuencias del funcionamiento físico, cognitivo, emocional y social de una población de adultos mayores en los Países Bajos, se observó que los bajos niveles de colesterol total (CT) pueden aumentar el riesgo de deterioro funcional, uno de los componentes claves de la fragilidad (Schalk et al., 2004).

- ***Biomarcadores endocrinos***

Se ha teorizado que las alteraciones en las hormonas anabólicas contribuyen al desarrollo de la fragilidad (Evans et al., 2010). En este sentido diversos estudios han analizado la relación entre la fragilidad y los niveles séricos del eje GH- IGF-1, DHEA/DHEA-S y testosterona. Así, Leng et al. (2004b) evidenciaron que los sujetos mayores frágiles presentaban unos niveles de IGF-I y DHEA-S más bajos que los no frágiles. Del mismo modo, la mayoría de estudios relacionan la fragilidad con bajos niveles de testosterona, especialmente con los bajos niveles

de testosterona libre (Cappola et al., 2009; Wu et al., 2012). Asimismo, O'Connell et al. (2011) sugieren que la carga absoluta de los déficits de las hormonales anabólicas es un predictor más fuerte de fragilidad que un tipo específico de déficit hormonal.

También se ha estudiado la posible relación entre la fragilidad y los niveles de cortisol. La activación del eje hipotálamo-hipofisario-suprarenal para producir cortisol es una respuesta endocrina fundamental para situaciones que amenazan la homeostasis (Fernández- Garrido et al., 2014a). Por lo que se ha sugerido que el cortisol podría funcionar como mediador de los procesos endocrino metabólicos implicados en el desarrollo de la fragilidad (Abizanda Soler, 2010). En este contexto, varios estudios han demostraron que los altos niveles de cortisol así como, una débil variación circandiana, pueden estar involucrados en el desarrollo de la fragilidad (Johar et al., 2014; Varadhan et al., 2008). En el marco de la *Women's Health and Aging Study II* (WHAS II), Varadhan et al. (2008) encontraron una asociación significativa y positiva entre la carga de la fragilidad y los niveles de cortisol durante el día, pero no así con los niveles matinales. Según estos autores, estos resultados sugieren que el síndrome frágil es el resultado de un bloqueo en el descenso fisiológico de cortisol que acontece durante el día.

La concentración de adinopectina, una hormona sintetizada por el tejido adiposo, ha sido estudiada en los adultos mayores frágiles debido a su papel en regulación de la homeostasis energética. En un estudio realizado por Tsai et al. (2013) los niveles de adiponectina en plasma se correlacionan positivamente con un mayor número de componentes de la fragilidad, pero solo en los hombres de edad avanzada. Para estos autores esta diferencia entre hombres y mujeres sugiere que ciertos mecanismos específicos ligados al sexo pueden afectar a la asociación entre los niveles de adiponectina y la fragilidad.

- ***Biomarcadores nutricionales***

Dado que el estado nutricional es un factor a considerar en el desarrollo de la fragilidad, su relación con las deficiencias de micronutrientes ha sido investigada. En este sentido, los resultados de diversos estudios muestran como las concentraciones séricas B6 y B12, selenio

y carotenoides totales (suma de β-caroteno, β-criptoxantina, luteína / zeaxantina y licopeno) fueron más bajas en adultos mayores frágiles que no frágiles (Bartali et al., 2006; Benedetta et al., 2005; Matteini et al., 2008; Michelon et al., 2006; Smit et al., 2012). Asimismo, Ble et al. (2006), utilizando datos del *InCHIANTI Study*, observaron que los bajos niveles plasmáticos de vitamina E, uno de los componentes más importantes del sistema antioxidante del organismo, aumentan el riesgo de fragilidad.

- ***Otros biomarcadores***

La albúmina sérica es la proteína sanguínea más abundante en los seres humanos y sus bajos niveles han sido asociados con la desnutrición, la enfermedad y la inflamación, por tanto, su alteración puede reflejar complicaciones en múltiples sistemas (Schoufour et al., 2016). En este sentido no es sorprendente que la fragilidad, caracterizada por una alteración multisistémica, se asocie con los bajos niveles séricos de albúmina. De acuerdo con esto último, diversos estudios han informado de una disminución de los niveles séricos de albumina, incluso dentro del rango normal, en sujetos mayores frágiles, con independencia de los marcadores inflamatorios y del estado nutricional (Bianca et al., 2005; Schalk et al., 2005).

Puesto que las consecuencias de los bajos niveles de hemoglobina (por ejemplo, fatiga o baja fuerza muscular) se observan con frecuencia en individuos frágiles, Leng et al. (2010) observaron, en presencia de niveles elevados de IL-6, que las personas mayores frágiles, en comparación con las no frágiles, presentaban menores concentraciones de hemoglobina. Asimismo, en una muestra de 1.622 hombres británicos de edad avanzada, con independencia de marcadores inflamatorios, la anemia se asoció con la fragilidad (Ramsay et al., 2015). Por otro lado, Chang et al. (2012), utilizando datos de las cohortes de *WHAS I y II,* y tras evaluar si un mayor recuento de enfermedades relacionadas con la inflamación aumentaba la probabilidad de fragilidad, observaron que la anemia, en combinación con otras enfermedades, aumentaba la probabilidad de ser frágil.

La enfermedad renal se asocia con cambios fisiológicos que pueden predisponer a la fragilidad. Así, en los participantes del *Modification of Diet in Renal Disease Study* (1989-1993) se observó una asociación positiva entre la fragilidad autoreportada y los niveles séricos de cistatina C y creatinina (Delgado et al., 2015). Igualmente, Hart et al. (2013), utilizando datos del *Osteoporotic Fractures in Men Study* (MrOS), encontraron que los altos niveles de cistatina C, pero no los de creatinina, aumentaban el riesgo de fragilidad (Hart et al., 2013). Asimismo, entre los biomarcadores que predicen la fragilidad, en pacientes de edad avanzada tras un síndrome coronario agudo, se encuentran unos niveles séricos de cistatina-C ≥1,2 mg /L (Sanchís et al. 2015). Por otro lado, en una cohorte de 4.562 adultos mayores de 50 años, los niveles de cistatina C sérica se relacionaron inversamente con un peor rendimiento funcional, objetivado por una menor la velocidad de la marcha, TUG y fuerza de agarre (Canney et al., 2017). La disminución del estado funcional es uno de los componentes claves de la fragilidad.

La relación entre los parámetros sanguíneos de la función hepática y la fragilidad también ha sido estudiada por Le Couteur et al. (2010). De los distintos parámetros sanguíneos estudiados (alanina transaminasa (ALT), aspartato transaminasa (AST) y gamma-glutamil transferasa (GGT)) solo aquellos participantes con niveles de ALT por debajo de la media fueron más propensos a ser frágiles.

Con la finalidad de valorar el papel de la autoinmunidad en la desregulación multisistémica que caracteriza al síndrome frágil, Wang et al. (2010) estudiaron, en una muestra de 641 mujeres mayores de edad avanzada, la posible relación entre los autoanticuerpos tiroideos y la fragilidad. Sus resultados mostraron, con independencia del estado de la función tiroidea, que las mujeres seropositivas para autoanticuerpos tiroideos eran menos propensas a ser frágil que las seronegativas. Aunque estos resultados sugieren que la fragilidad se asocia a una inmunodeficiencia que afecta a la producción de autoanticuerpos, el hecho de que en esta misma población de mujeres, los anticuerpos antinucleares (ANA), un marcador sistémico de autoanticuerpo, no disminuyesen la probabilidad de ser frágil indica que los autoanticuerpos tiroideos podrían tener un efecto beneficioso en la patogénesis de la fragilidad.

Varios estudios han observado una asociación entre el estrés oxidativo y la fragilidad, demostrando que la fragilidad se asocia con el daño oxidativo del ADN. Así, un estudio con 1.919 participantes de edad avanzada observó que los niveles de isoprostanos y la masa de lipoproteína fosfolipasa A2 (LpPLA2), dos marcadores de estrés oxidativo relacionados con la ECV, se asociaron con un mayor riesgo de fragilidad (Liu et al., 2016). Igualmente, otros estudios, con tamaños de muestra más pequeños, también han observado una asociación entre el estrés oxidativo y la fragilidad. Así, en una muestra de 742 adultos mayores, se observó una asociación entre la fragilidad y los altos niveles séricos de malondialdehído y de los carbonilos de proteínas, los cuales se regulan positivamente durante el estrés oxidativo (Ingles et al., 2014). Asimismo, en los estudios llevados a cabo por Wu et al. (2009) y Serviddio et al. (2009) se evidenció que las personas frágiles de edad avanzada tenían mayores niveles séricos de fragmentos de glutatión oxidado y de 8-hidroxi-2'-desoxiguanosina (8-OHdG), subproductos del daño oxidativo. Sin embargo, en cada uno de estos estudios la muestra era inferior a un centenar de personas. En contraste con estos hallazgos, en un estudio transversal con 552 adultos mayores, los isoprostanes no se asociaron con la fragilidad (Collerton et al., 2012). Según sus autores, el tamaño de la muestra y la evaluación de los niveles de isoprostanos podrían justificar la ausencia de relación significativa.

Por último, puesto que la literatura muestra que tanto la fuerza del músculo esquelético como la activación de la vía inflamatoria son rasgos hereditarios (de Craen at al., 2005; Ershler y Kellerr, 2000; Evans et al., 2010; Frederiksen et al., 2002), varios autores han estudiado la asociación entre la fragilidad y los genes implicados en la biosíntesis de las hormonas esteroideas o en las vías de la inflamación. Así, mientas que Watson et al. (2005) no encontrando ninguna relación significativa entre cualquier polimorfismo de nucleótido simple (SPNs) del gen de IL-6 y la fragilidad, Almeida et al. (2012), mostraron que los individuos que llevan el polimorfismo 1846G>A del gen de PCR presentaban una mayor probabilidad de ser frágil. Igualmente, en un estudio reciente llevado a cabo por Melki et al. (2016) se evidenció la implicación del SPNs del gen de TNF (rs1800629) en el fenotipo de fragilidad. Asimismo,

el mayor estudio realizado hasta la fecha, en términos de número de SNPs investigados, ha encontrado asociaciones entre la fragilidad y las variantes genéticas de los genes implicados en las vías relacionadas con la apoptosis y la regulación de la transcripción, como el 5-metiltetrahidrofolato-homocisteína metiltransferasa (MTR), Caspasa 8 (CASP8), CREB-binging proteín (CREBBP), lysina acetiltransferasa 2B (KAT2B), y los beta-transducina repeat containing (BTRC loci) (Ho et al., 2011). Sin embargo, estos resultados no sobrevivieron a la corrección para pruebas múltiples.

Otros posibles biomarcadores relacionados con el síndrome de fragilidad, y que son objeto de la presente tesis, son el factor neurotrófico derivado del cerebro (BDNF), el recuento de leucocitos y la vitamina D (Fernández- Garrido et al., 2014b; Navarro-Martínez et al., 2015; Navarro- Martínez et al., 2016).

CAPÍTULO II- RESUMEN GLOBAL DE LOS RESULTADOS

"Los números hablan por sí mismos en términos de todo lo que hemos hecho"

Paul Rand.

ARTÍCULO 1

THE VALUE OF NEUTROPHIL AND LYMPHOCYTE COUNT IN FRAIL OLDER WOMEN

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1- Características demográficas y clínicas de la población a estudio

Este estudio piloto transversal se realizó en el año 2013. La población a estudio estuvo constituida por 42 mujeres con perfil residencial, pertenecientes a 4 centros privados de atención residencial para personas mayores (*GeroResidencias La Saleta)* ubicados en la provincia de Valencia (España). De los datos demográficos, obtenidos mediante un cuestionario estructurado a través de una entrevista personal, se determinó que la totalidad de las participantes del estudio eran de raza caucásica, ninguna de ellas fumaba y casi la mitad de las mismas eran viudas (45%). Su edad oscilaba entre 70 y 99 años, con una edad media (± desviación estándar (DE)) de 84,2 (± 6,5) años.

En relación a sus características clínicas, según los datos revisados en las historias clínicas, se observó que las participantes del estudio presentaron una alta comorbilidad. La media (± DE) de las enfermedades crónicas que padecían fue de 4,1 (± 1,6). La enfermedades crónicas más prevalentes fueron la hipertensión arterial (38%), la enfermedad inflamatoria crónica (35%) y la hiperlipemia (28%). Destacar que casi la mitad de las mujeres de la muestra (n=15) presentaron enfermedades inflamatorias crónicas, entre las cuales destacaron la osteoartritis general (n=11), la

osteoartritis de la rodilla (n=2), la polimiositis (n=1) y la artritis reumatoide (n=1). Asimismo, con un consumo medio (± DE) de 7,3 (± 3,2) fármacos al día por persona, el uso de medicamentos por este grupo de población también fue elevado.

En lo que se refiere al diagnóstico gerontológico integral de las participantes, las escalas de AVD mostraron que las participantes presentaron, con unas puntuaciones medias (± DE) en el índice de las ABVD de Barthel de 65,0 (± 25,7) puntos (valor normal = 60-100) y para el índice de AIVD de Lawton y Brody de 4,2 (± 1,4) puntos (valor normal = 8), una independencia para la realización de las ABVD y una dependencia moderada para la realización de las AIVD, y la totalidad de las mismas presentaban capacidad de deambular de forma independiente. Desde el punto de vista afectivo, las participantes mostraron ausencia de sintomatología depresiva, con una media (± DE) en la puntuación de la escala de depresión geriátrica Yesavage de 3,4 (± 3,3) puntos (valor normal ≤ 5). De igual manera, con una puntuación media (± DE) en el mini examen del estado mental (Mini Mental State Examination (MMSE)) de 28,3 (± 5,6) puntos (valor normal > 27), las participantes mostraron ausencia de deterioro cognitivo. La media (± DE) de la puntuación del cuestionario de inventario neuropsiquiátrico (Neuropsychiatric Inventory Questionaire (NPI-Q)) para la evaluación clínica de la sintomatología neuropsiquiátrica en las enfermedades que cursan con demencia fue de 2,9 (± 4,5) (valor normal = 0). La valoración realizada con la escala Norton mostró ningún o un mínimo riesgo de desarrollar ulceras por presión, al obtener una puntuación media (± DE) en dicha escala de 16,5 (± 2,2) puntos (valor normal > 14). Finalmente, en la determinación del riesgo de caídas, de acuerdo con unas puntuaciones medias (± DE) de 6,4 (± 3,7) puntos para la escala de la marcha de Tinetti (valor normal= 12) y de 8,1 (± 4,5) para el índice de equilibrio de Tinetti (valor normal = 16), las participantes presentaron un alto riesgo de sufrir caídas. Las características clínicas y demográficas de todas las participantes incluidas en el estudio, incluyendo la edad, las puntuaciones de las escalas de valoración geriátrica, el número de medicamentos que tomaban a diario así como, el tipo y número de comorbilidades, se muestran en la siguiente tabla (véaseTabla 9).

Tabla 9
Características demográficas y clínicas de las participantes incluidas en el estudio con la media (± DE) y el rango para cada valor / escala.

	Media (± DE)	Rango
Edad (años)	84,2 ± 6,5	70 - 99
Índice de equilibrio de Tinetti	8,1 ± 4,5	0 - 14
Índice de la marcha de Tinetti	6,4 ± 3,7	0 -12
Norton	16,5 ± 2,2	13 - 20
MMSE	28,3 ± 5,6	17-35
NPI-Q	2,9 ± 4,5	0-20
Yesavage	3,4 ± 3,3	0-9
Índice de Barthel	65,0 ± 25,7	25-100
Índice de Lawton y Brody	4,2 ± 1,4	2-7
Número de medicamentos	7,3 ± 3,2	-
Número de todas las comorbilidades	4,1 ± 1,6	-
	%	
Diabetes	14	
Hipertensión	38	
Insuficiencia cardiaca congestiva	7	
Hiperlipemia	28	
Obesidad	3	
Enfermedad inflamatoria	35	

Nota. La edad se expresa en años. La valoración geriátrica se evaluó mediante una batería de escalas de evaluación geriátrica: el índice Tinetti de la marcha y del equilibrio para determinar el riesgo de sufrir caídas, la escala de Norton para medir el riesgo de desarrollar úlceras por presión, el mini examen del estado mental (MMSE) para detectar el deterioro cognitivo, el cuestionario de inventario neuropsiquiátrico (NPI-Q) para la evaluación clínica de la sintomatología neuropsiquiátrica para las enfermedades que cursan con demencia, la escala Yesavage para la depresión geriátrica, índice de Barthel para medir la capacidad para realizar las ABVD y el índice de Lawton y Brody para realizar las AIVD. La distribución de las comorbilidades más comunes se expresan como porcentaje de sujetos que tienen la enfermedad.

Cuando analizamos el síndrome frágil mediante la escala Fried, tan sólo dos de las mujeres incluidas en el estudio no cumplieron ninguno de los criterios de fragilidad (5% de la muestra). En relación al porcentaje de cada uno de los cinco criterios de la escala Fried,el más representado en la totalidad de la muestra fue el bajo nivel de actividad física (91%), seguido de la lentitud en la marcha (88%), la debilidad o baja fuerza muscular (69%), del agotamiento o baja energía (28%) y por último, de la pérdida de peso no intencionada en el último año (13%).

2- Evaluación de la relación entre las variables demográficas y clínicas de las participantes con la fragilidad de Fried

Dado que las variables se distribuyeron normalmente, y con el fin de determinar la posible correlación entre el número de criterios de fragilidad de Fried con las distintas variables demográficas y clínicas de las participantes, se calculó el coeficiente de correlación lineal de Pearson. Los resultados obtenidos mostraron que la severidad del síndrome de fragilidad no se asoció ni con la edad ni con el estado de salud general de las participantes de nuestro estudio, al no observase diferencias estadísticamente significativas entre el número de criterios de la escala Fried y la edad ($r = 0,22$, $p = 0,23$) o las puntuaciones obtenidas en las escalas de evaluación geriátrica (índice de marcha de Tinetti: $r = - 0,14$, $p = 0,44$; índice de equilibrio de Tinetti: $r = - 0,23$, $p = 0,21$; índice de Barthel: $r = 0,11$, $p = 0,54$; MMSE: $r = 0,14$, $p = 0,44$; escala Yesavage: $r = 0,33$, $p = 0,08$; y escala de Norton: $r = - 0,31$, $p = 0,09$). Tampoco la severidad del síndrome de fragilidad se asoció de manera significativa con el número de medicamentos diarios ($r = 0.13$, $p = 0.48$) o con el número de comorbilidades ($r = 0.23$, $p = 0.20$) de las participantes.

3- Evaluación de la relación entre el porcentaje diferencial de leucocitos con criterios de fragilidad de Fried

Tal y como se ha explicado en el capittulo I de la presente tesis, la etiología del síndrome de fragilidad se ha asociado con déficits en multiples e interrelacionados sistemas fisiológicos, incluyendo el sistema inmunológico (Walston et al., 2002). En relación con los mediadores celulares de la

inflamación, la evidencia sugiere que los niveles elevados en los recuentos totales de glóbulos blancos contribuyen al desarrollo de este síndrome (Leng et al., 2009). Sin embargo, la correlación de las subpoblaciones de leucocitos específicos con el síndrome de fragilidad es controvertida y escasa (Fernández-Garrido et al., 2014b). Por tanto, con el objetivo de esclarecer el papel potencial de las subpoblaciones de leucocitos en la patogénesis de la fragilidad evaluamos en primer lugar, la posible relación entre los porcentajes individuales de leucocitos y la puntuación de la escala de fragilidad de Fried. Y en segundo lugar, nos planteamos si podría existir una asociación entre los porcentajes diferenciales de glóbulos blancos con cada uno de los cinco criterios de la escala Fried, pues hasta donde sabemos dicha relación todavía no ha sido estudiada.

Para ello, como en el caso anterior, se aplicó el análisis de correlación lineal de Pearson. Sin embargo, en este caso, los resultados mostraron una relación positiva y significativa entre el número de criterios de fragilidad con el porcentaje de neutrófilos ($r = 0,38$, $r^2 = 0,144$, $p < 0,05$) (véase Figura 17A), de forma que las participantes con mayores porcentajes de neutrófilos obtuvieron una mayor puntuación en la escala Fried, y por tanto, presentaron un mayor grado de fragilidad. Del mismo modo, los resultados del análisis estadístico también mostraron una relación significativa entre la severidad del síndrome de fragilidad y el porcentaje de linfocitos ($r = - 0,37$, $r^2 = 0,140$, $p < 0,05$) (véase Figura 17B), pero en este caso, la asociación fue en dirección opuesta, de modo que, las participantes con un mayor número de criterios de Fried fueron quienes presentaron los porcentajes de linfocitos más bajos.

Por el contrario, el análisis de la correlación de Pearson entre la puntuación de la escala Fried y el porcentaje de monocitos, eosinófilos y basófilos no mostró diferencias significativas (monocitos: $r = - 0,07$, $r^2 = 0,144$; eosinófilos: $r = 0,027$, $r^2 = 0,001$; y basófilos: $r = - 0.007$, $r^2 = 0,001$) (véase Figura 18A-C) y por tanto, dichas subpoblaciones de leucocitos no se asociaron con la severidad del síndrome de fragilidad en esta población.

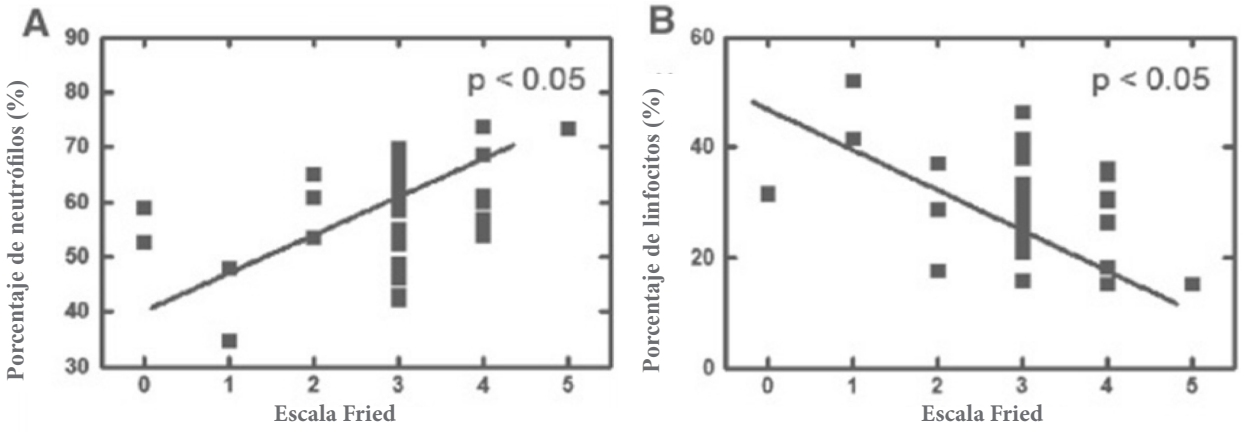


***Figura 17*. Correlación entre los porcentajes de neutrófilos y de linfocitos con de la puntuación de la escala de fragilidad de Fried.**

Nota. La fragilidad se midió de acuerdo con los cinco criterios de Fried. Los porcentajes de neutrófilos (%) (A) y de linfocitos (%) (B) se midieron en sangre y se representaron frente a la puntuación de la escala Fried de cada participante inscrita en el estudio. El nivel de significación se indica en cada gráfico de dispersión.

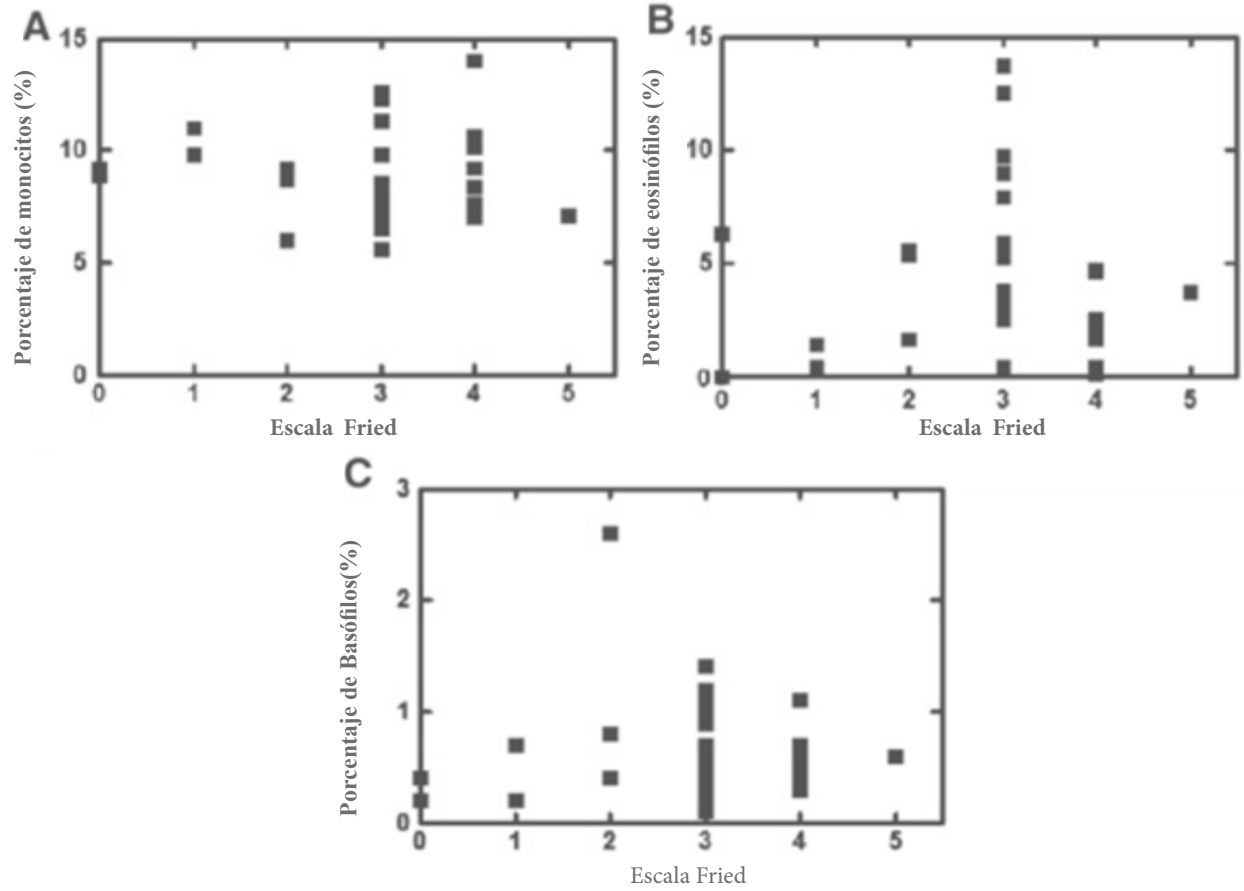


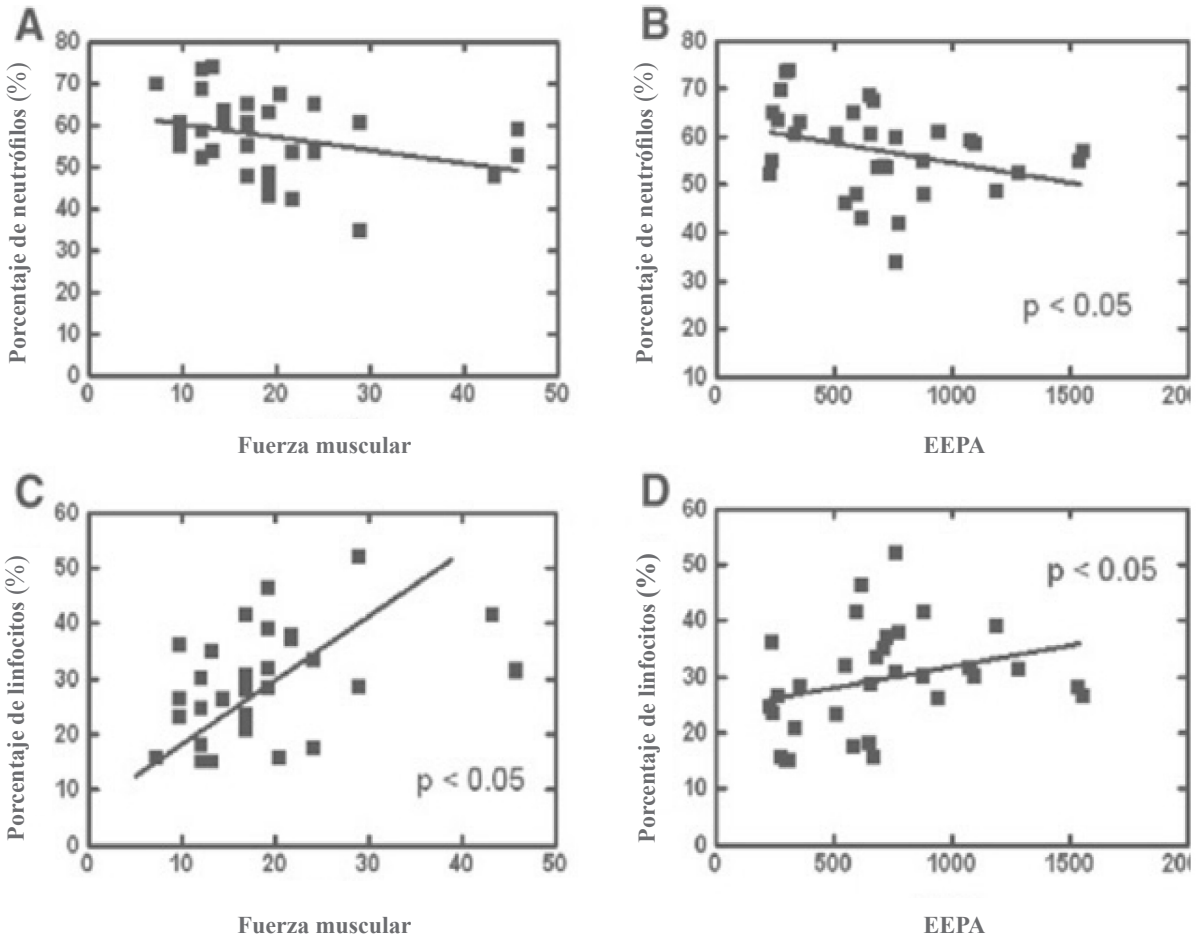
***Figura 18.* Correlación entre los porcentajes de monocitos, eosinófilos y basófilos con la puntuación de la escala de fragilidad de Fried.**

Nota. La fragilidad se midió de acuerdo con los cinco criterios de Fried. Los porcentajes de monocitos (%) (A), eosinófilos (%) (B) y basófilos (%) (C) se midieron en sangre y se representaron frente a la puntuación de la escala Fried de cada participante inscrita en el estudio. Ninguna de las correlaciones fue significativa.

Puesto que la identificación de las subpoblaciones especificas de leucocitos como biomarcadores para un único criterio de la fragilidad puede ser muy útil en el conocimiento de los mecanismos específicos responsables del desarrollo de la fragilidad, evaluamos la posible relación entre los porcentajes de neutrófilos y de linfocitos con cada uno de los criterios de fragilidad de la escala Fried, a excepción de la pérdida de peso no intencional en el último año, debido a que este criterio sólo se obtuvo en 3 de las 32 mujeres incluidas en el estudio (7 % de la muestra).

En este sentido, el análisis de correlación de Pearson mostró que los porcentajes de neutrofilos se relacionaron de manera inversa y significativa con el nivel de actividad física, de manera que, las participantes con mayores niveles de neutrofilos presentaron un menor nivel de actividad física y, por tanto, un menor gasto energético total diario en la actividad física en el tiempo de ocio (energy expenditure in physical activity, EEPA total), medido con la adaptación española del cuestionario de Minnesota (Minnesota leisure time physical activity questionnaire, MLTPAQ) ($r = - 0,37$, $r^2 = 0,18$, $p < 0,05$) (véase Figura 19B). Igualmente, pudimos observar como las participantes con mayores porcentajes de neutrofilos presentaron una menor fuerza de presión manual, es decir, una mayor debilidad muscular. Sin embargo, está asociación no llegó a alcanzar los niveles de significación estadística ($r = - 0,342$, $r^2 = 0,1$, $p = 0,05$) (véase Figura 19A). Tampoco se observó ninguna correlación estadísticamente significativa entre los porcentajes de neutrófilos con otros criterios de Fried como el agotamiento y la velocidad de la marcha (datos no mostrados).

A diferencia de lo observado con los niveles de neutrofilos, los porcentajes de linfocitos se correlacionaron de manera significativa con la fuerza de prensión manual (Pearson: $r = 0,38$, $r^2 = 0,15$, $p < 0,05$) (véase Figura 19C), de modo que, las participantes con los niveles más bajos de linfocitos mostraron una menor fuerza de presión manual o mayor debilidad muscular. También, al igual que los porcentajes de neutrofilos, los de linfocitos se correlacionaron de manera significativa con EEPA (Pearson: $r = 0,3$, $r^2 = 0,1$, $p < 0,05$) (véase Figura 21D), sin embargo, en este caso, las participantes con mayores porcentajes de linfocitos fueron las que presentaron mayores niveles de actividad física. Tampoco los porcentajes linfocitarios se correlacionaron de manera significativa con el agotamiento o baja energía y la velocidad de marcha (datos no mostrados).



Figuras 19. **Correlación entre los porcentajes de neutrófilos y de linfocitos con la fuerza muscular y la actividad física.**

Nota. La fragilidad se midió de acuerdo con los cinco criterios Fried. La fuerza muscular se midió mediante la evaluación de la fuerza de agarre de mano con un dinamómetro. La actividad física se midió como el gasto de energía en la actividad física en el tiempo libre (energy expenditure in physical activity, EEPA total), la adaptación española del cuestionario de Minnesota (Minnesota leisure time physical activity questionnaire, MLTPAQ). Los porcentajes de neutrófilos (%) (A-B) y linfocitos (%) (C-D) se midieron en la sangre y se representaron frente a la fuerza muscular (A-C) o la actividad física (B-D) de cada participante inscrita en el estudio. El nivel de significación estadística se indica en cada panel.

Asimismo, con el fin de evaluar si estos cambios se encontraban dentro del rango fisiológico normal o patológico, se analizaron los porcentajes de neutrófilos y linfocitos tanto en la totalidad de la muestra como en los subgrupos de las participantes clasificadas, según la severidad de la fragilidad, como robustas (mujeres con 0 criterios Fried), pre-frágiles (mujeres con 1 o 2 criterios Fried) y frágiles (mujeres con 3,4 o 5 criterios Fried). Los resultados de dicho análisis mostraron que los valores medios de los porcentajes de neutrófilos y linfocitos estaban dentro del rango fisiológico

normal y no se observaron diferencias significativas entre los grupos (véase Tabla 10). Se debe destacar que la única participante que representó un porcentaje de neutrófilos y linfocitos fuera del rango de referencia normal, curiosamente, fue aquella que cumplió con los cinco criterios de Fried.

Tabla 10
Los valores de los porcentajes de neutrófilos y de linfocitos en toda la muestra y en las participantes clasificadas como robustas, pre-frágiles y frágiles.

	Porcentaje de neutrofilos	Porcentaje de linfocitos
Muestra total	57,2 ± 1,6 Rango normal (40-75)	29,4 ± 1,6 Rango normal (20-45)
Robustas (mujeres con 0 criterios Fried)	55,9 ± 3,3	31,7 ± 0,2
Pre-frágiles (mujeres con 1 o 2 criterios Fried)	52,4 ± 5,3	35,0 ± 5,3
Frágiles (mujeres con 3, 4 o 5 criterios Fried)	58,3 ± 1,5	28,1 ± 1,6

Nota. La fragilidad se midió de acuerdo con los cinco criterios de Fried. Las mujeres con tres o más de los cinco criterios Fried se definieron como frágiles, aquellas con uno o dos componentes como pre-frágiles y como no frágiles aquellas con ningún criterios Fried. Los porcentajes de neutrófilos (%) y de linfocitos (%) se midieron ensangre y se expresaron como porcentajes del total de leucocitos de la sangre.

A continuación, y dado que los mediadores celulares inflamatorios influyen en múltiples sistemas fisiológicos, nos planteamos si la presencia de enfermedades en las participantes podía tener alguna influencia en los resultados obtenidos, es decir, si la comorbilidad, y no la condición de fragilidad, podría ser responsable de los cambios observados en los porcentajes de estas subpoblaciones de los leucocitos. En este sentido, el análisis de regresión lineal mostró que las asociaciones entre los porcentajes de neutrófilos y linfocitos (como variables dependientes) con la severidad de la fragilidad, la baja actividad física y la pobre resistencia muscular (como variables independientes), se mantuvieron significativas después de ajustar por el número y el tipo de comorbilidades de las participantes (datos no mostrados).

4- **Evaluación de la relación entre los recuentos individuales de leucocitos con los resultados de las escalas de valoración geriátrica**

Para explorar si la asociación entre los porcentajes de neutrofilos o de linfocitos con la fragilidad y la actividad física o fuerza muscular se vio afectada por el estado de salud general de las participantes, se analizaron las correlaciones entre los porcentajes de neutrófilos y de linfocitos con las puntuaciones obtenidas en cada una de las escalas de evaluación geriátrica, mediante el índice de correlación de Pearson, no encontrándose ninguna correlación estadísticamente significativa (índice de la marcha de Tinetti: r = - 0,23; p = 0,21 para los porcentajes de neutrófilos y r = 0,14; p = 0,45 para el porcentaje de linfocitos; índice del equilibrio de Tinetti: r = - 0,13; p = 0,49 para los porcentajes de neutrófilos y r = 0,01; p = 0,98 para el porcentaje de linfocitos; índice de Barthel: r = 0,50; p = 0,78 para los porcentajes de neutrófilos y r = - 0,11, p = 0,55 para el porcentaje de linfocitos; MMSE: r = 0,14; p = 0,45 para el porcentaje de neutrófilos y r = - 0,10; p = 0,57 para el porcentaje de linfocitos; escala Yesavage: r = - 0,17; p = 0,35 para los porcentajes de neutrófilos y r = 0,21; p = 0,25 para el porcentaje de linfocitos; la escala de Norton: r = - 0,20; p = 0,28 para los porcentajes de neutrófilos y r = 0,12; p = 0,52 para el porcentaje de linfocitos). Estos resultados sugieren que los cambios observados en las subpoblaciones de leucocitos no se deben a la disminución general de las funciones físicas o psicológicas relacionadas con el envejecimiento. Igualmente, el análisis de Pearson mostró que la edad de las participantes de la muestra tampoco influyó en los cambios observados en los porcentajes de neutrófilos o linfocitos, al no encontrarse diferencias estadisticamente significativas entre dichas subpoblaciones de leucocitos con la edad (r = 0,26; p = 0,16 para los porcentajes de neutrófilos y r = - 0,32; p = 0,08 para el porcentajes de linfocitos).

5- **Evaluación de la relación entre la fragilidad y otros parámetros analíticos**

Finalmente, dado el caracter multisitemico del sindrome frágil, se exploraron la existencia de otros biomarcadores de fragilidad. En este sentido, mediante el coeficiente de correlación de Pearson, se establecieron correlaciones bivariadas entre los distintos parámetros hematológicos y el número de

criterios de Fried Los resultados obtenidos no mostraron ninguna asociación significativa entre la puntuación de la fragilidad y el recuento de plaquetas ($r = - 0,06$, $r^2 = 0,004$, véase Figura 20A), de eritrocitos ($r = 0,013$, $r^2 = 0,017$, véase Figura 20B) o la concentración de hemoglobina ($r = 0,112$, $r^2 = 0,013$, véase Figura 20C). Los valores medios de los recuentos de plaquetas y de eritrocitos se encontraban dentro del rango fisiológicos normal (recuento de plaquetas: $218 \pm 13,7\ 10^3/\mu L$ (rango normal; $130 - 450\ 10^3/\mu L$); recuento de eritrocitos: $3,75 \pm 0,09\ 10^6/\mu L$ (rango normal ($3.7 - 5.2\ 10^6/\mu L$)). En contraste, la concentración media de hemoglobina se encontraba por debajo del rango normal: $11,3 \pm 0,3$ g/dL (rango normal 12-16 g/dL), aunque se debe tener en cuenta que este hallazgo es muy común entre la población geriátrica.

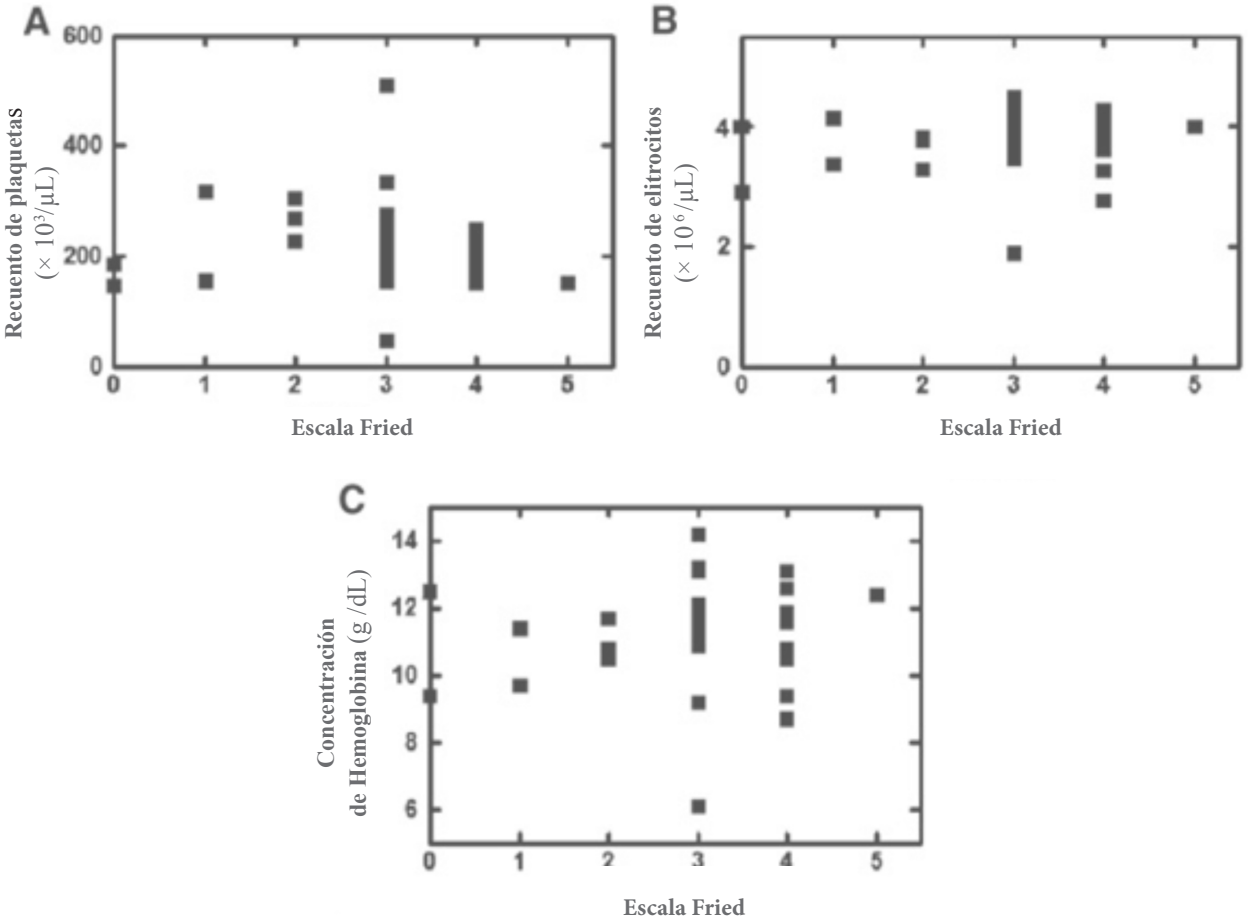


Figura 20. **Correlación entre el recuento de plaquetas, de eritrocitos y la concentración de hemoglobina con el síndrome de fragilidad.**

Nota. La fragilidad se midió de acuerdo con los cinco criterios de Fried. El recuento de eritrocitos ($\times 10^6/\mu L$) (A), de plaquetas ($\times 10^3/\mu L$) (B) y la concentración de hemoglobina (g /dL) (C) se midieron en sangre y se representaron frente a la puntuación de la escala de Fried por cada participante inscrita en el estudio. Ninguna correlación fue significativa.

Del mismo modo, en el análisis de correlación de Pearson, tampoco se observó ninguna correlación estadísticamente significativa entre el numero de criterios Fried y la mayoría de los parámetros bioquímicos medidos en la sangre, como la glucosa, urea, ácido úrico, CT, TGD, creatinina, transaminasas oxaloacético glutámico (GOT) y GPT (glucosa: $r = 0,10$, $p = 0,59$; urea: $r = - 0,2$, $p = 0,92$; ácido úrico: $r = 0,17$, $p = 0,37$; creatinina: $r = 0,10$, $p = 0,58$; CT: $r = 0,50$, $p = 0,80$; TGD: $r = 0,30$, $p = 0,86$; GOT: $r = 0,20$, $p = 0,28$; GPT: $r = 0,15$; $p = 0,42$). Por el contrario, como era de esperar, la concentración de PCR se correlacionó de forma positiva y significativa con la puntuación de la escala Fried ($r = 0,39$, $r^2 = 0,15$, $p < 0,05$). Sin embargo, no se observaron correlaciones significativas entre los valores de PCR con ninguno de los cinco criterios de fragilidad.

ARTÍCULO 2

BRAIN-DERIVED NEUROTROPHIC FACTOR CORRELATES WITH FUNCTIONAL AND COGNITIVE IMPAIRMENT IN NON-DISABLED OLDER INDIVIDUALS

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1- Características clínicas y demográficas de la población a estudio

En este estudio clínico de diseño transversal, realizado durante los años 2013-2014, se incluyeron un total de 75 personas mayores no discapacitadas, 84% mujeres y 16% hombres, pertenecientes a 5 centros privados de atención residencial (*GeroResidencias La Saleta)* ubicados en la provincia de Valencia (España). Todos los sujetos eran de raza caucásica, no fumaban y cerca del 67% eran viudos o viudas. Su edad oscilaba entre 61 a 99 años con una edad media de 83 años (rango intercuartil: 78-89). Con respecto a los niveles plasmáticos de BDNF, la mediana fue de 64 pg/ mL (rango intercuartil: 42,10-64,00). Según la escala Fried, el 79% de los sujetos fueron clasificados como frágiles (3, 4, o 5 criterios Fried) y el 21% como pre-frágiles (1 o 2 criterios Fried). El IMC medio (calculado a partir del peso y la altura de pie) fue 28,05 kg/m ^{2} (rango intercuartil: 24,22-32,80). En relación con el número de medicamentos diarios, el 54,9% de las personas incluidas en el estudio consumía diez o más medicamentos al día, con una mediana de 10 (rango intercuartil: 6-13). Los resultados también mostraron una alta comorbilidad, con una mediana de 4 enfermedades (rango intercuartil: 3-5), el 3,2% de los sujetos tenía una enfermedad, el 9,7% dos, el 16,1% tres, el 38,7% cuatro, el 12,9% cinco, seis el 12,9%, siete el 3,2% y ocho el 3,2%.

A continuación, en la siguiente tabla (véase Tabla 11) se muestran las características clínicas y demográficas de las personas incluidas en el estudio incluyendo la edad, el género, los niveles de BDNF en plasma (pg /ml), los resultados de los criterios de Fried, el IMC (kg/m ^{2}), el número de comorbilidades y de medicamentos que toman a diario.

Tabla 11
Las características clínicas y demográficas de los sujetos incluidos en el estudio con la mediana y el rango intercualtil para cada valor.

	Valor de la Mediana	Rango intercuartil
Edad (años)	83	78-89
IMC (Kg / m ^{2})	28,05	24,22-32,80
Criterios de Fried	3	2-4
Niveles plasmáticos de BDNF (pg/ ml)	64	42,10-64
Número de todas las comorbilidades	4	3-5
Número de medicamentos	10	6-13

Nota. La edad se expresa en años, el IMC se calculó a partir del peso y la altura de pie (Kg / m ^{2}), la fragilidad se midió según los cinco criterios propuestos por Fried y los niveles de BDNF (pg / ml) se midieron en el plasma.

Una vez realizado el análisis descriptivo de las características clínicas y demográficas de los sujetos que constituyen la muestra, con el fin de conocer los factores que influyen en las concentraciones plasmáticas de BDNF de los y las participantes del estudio se analizaron las asociaciones entre el BDNF plasmático y el resto de variables, mediante test paramétrico de Spearman o prueba de Mann-Whitney U, tras considerar sus características distribucionales. En este sentido, en nuestros resultados no se observaron relaciones estadisticamente significativas entre la edad y las concentraciones de BDNF en plasma de los y las participantes (Spearman r = 0,22, p = 0,23). Sin embargo, a diferencia de lo observado con la edad, los valores plasmáticos de BDNF se relacionaron con el género, de manera que los hombres presentaron unos niveles de BDNF promedio en plasma más altos que las mujeres, 98,00 (± 11,69) pg /ml frente a 68,54

(± 5,07) pg /ml respectivamente ($p < 0,05$, prueba de Mann-Whitney U). Similar a la edad, ni el IMC (Spearman: r = - 0,85, $p = 0,47$), ni el número de comorbilidades (Spearman: r = 0,25, $p = 0,16$) o de medicaciones que tomaban a diario (Spearman: r = 0,78, $p = 0,33$) se correlacionaron significativamente con los niveles plasmáticos de BDNF. Finalmente, en cuanto a la posible relación entre los distintos feneotipos de fragilidad (frágil, pre-frágil y robusto)y el BDNF plasmático, los análisis estadísticos no mostraron diferencias estadísticamente significativas (Spearman: r = -0,72, $p = 0,53$). Tampoco los resultados de la relación entre cada uno de los cinco criterios de Fried y las concentraciones plasmáticas de BDNF mostraron significación estadística.

Las puntuaciones de las escalas de valoración geriátrica de los sujetos estudiados se resumen en la siguiente tabla (véase Tabla 12). En relajación con sus características funcionales, como resultado de la mediana de las puntuaciones de las escalas de ABVD de Barthel de AIVD de Lawton y Brody, la totalidad de la muestra presentó un nivel de independencia para la realización de las ABVD y un nivel de dependencia moderado para las AIVD. Del mismo modo, la mediana del MMSE, así como la de cada una de sus subescalas (orientación espacio-temporal, fijación de la memoria inmediata, memoria diferida, funcionamiento del lenguaje y capacidad de concentración y cálculo), no reveló deterioro cognitivo. Asimismo, desde el punto de vista del estado de ánimo, los sujetos del estudio mostraron ausencia de sintomatología depresiva. Finalmente, las medianas de las puntuaciones de las escalas de Norton y Tinneti mostraron que, la mayoría de la muestra, no presentó ni riesgo de caídas ni de desarrollar ulceras por presión.

Tabla 12
Resultados de las escalas de valoración geriátrica de los sujetos incluidos en el estudio, con la mediana y el rango intercualtil para cada escala.

	Valor de la Mediana	Rango intercuartil
Índice de Barthel	80	59,50-87,50
Índice de Lawton	4	3-5
La prueba MMSE	1	0,91-1,17
- **Orientación espacio-temporal**	9	7-10
- **Fijación o memoria inmediata**	3	3-3
- **Memoria diferida**	2	1-3
- **Lenguaje**	10	8,75-11
- **Capacidad de concentración y cálculo**	6	4-8
Yesavage	3	0-7
El índice Tinetti total	19	12,50-24
- **El índice Tinetti para el equilibrio**	10	6-14
- **El índice Tinetti para la marcha**	9	6-10
La escala de Norton	18	15,50-19

Nota. La valoración geriátrica se evaluó mediante las siguientes escalas validadas: el índice de Barthel para medir la capacidad para realizar las ABVD, el índice de Lawton para medir la capacidad para realizar las AIVD, el mini examen del estado mental (MMSE) para detectar el deterioro cognitivo, la escala de Yesavage para la depresión geriátrica, el índice Tinetti del equilibrio y de la marcha para determinar el riesgo de caídas, la escala de Norton para medir el riesgo de desarrollar úlceras por presión.

2- Evaluación de la relación entre los niveles plasmáticos de BDNF con las escalas de evaluación geriátrica

Una vez realizado el diagnóstico gerontológico integral, mediante el coeficiente de correlación de Sperman analizamos las asociaciones entre los niveles de BDNF en plasma con las puntuaciones de cada una de las escalas de la valoración geriátrica. Tras el análisis, los resultados mostraron una relación significativa y positiva entre los niveles de BDNF en plasma y las puntuaciones del índice de Barthel que miden las ABVD ($r = 0,28$, $p = 0,04$) (véase Figura 21).

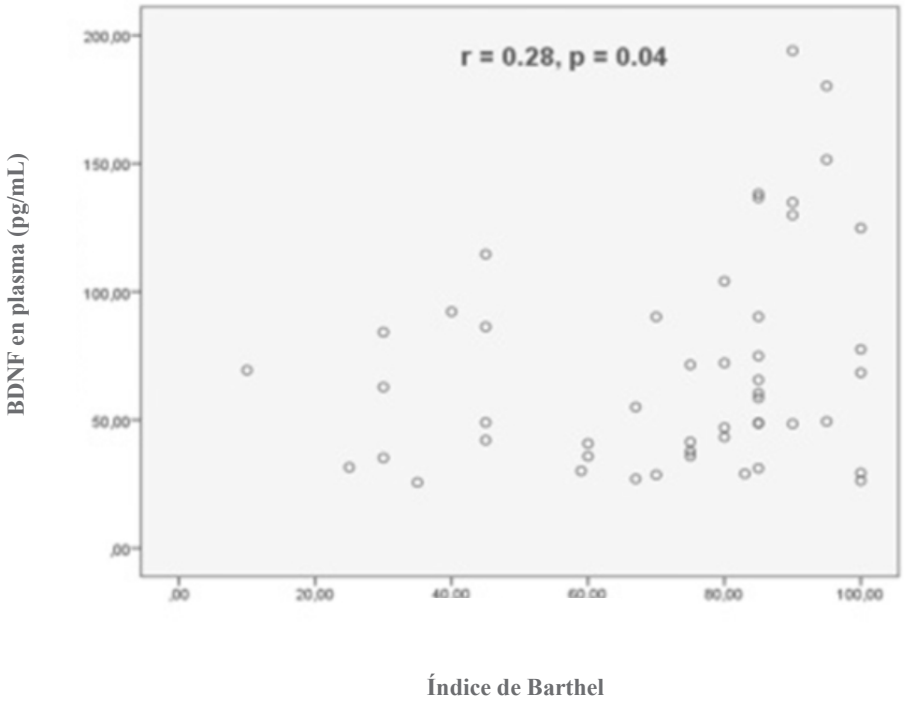

Figura 21. **Correlación entre la concentración de BDNF y la puntuación del índice de Barthel para las ABVD.**
El índice de Barthel mide el nivel de autonomía en las siguientes 10 ABVD: alimentarse, bañarse, vestirse, asearse, defecar, orinar, el uso del baño, las transferencias (por ejemplo, desde el sillón a la cama), caminar y subir escaleras. El índice tiene un rango de puntuación de 0-100, donde 0 es la dependencia total y 100 corresponde a la independencia total. Los niveles de BDNF (pg / ml) se midieron en plasma y se representaron frente al índice de Barthel para cada participante inscrito/a en el estudio. El nivel de significación se indica en la figura.

Asimismo, aunque no hubo una diferencia significativa entre la concentración plasmática de BDNF y la puntuación total de la prueba MMSE (r = 0,17, p = 0,14) (véase Figura 22A), cuando analizamos la relación entre los valores de BDNF en plasma con cada uno de los subdominios de la prueba MMSE (orientación espacio-temporal, fijación de la memoria inmediata, memoria diferida, funcionamiento del lenguaje y capacidad de concentración y cálculo) solo observamos una relación significativa y positiva entre los niveles plasmaticos de BDNF con la subescala de concentración (Sperman: r = 0,27, p = 0,01) (váse Figura 22D). Estas asociaciones estadísticamente significativas, entre la concentración de BDNF en el plasma y el índice de Barthel y subescala concentración de MMSE, se mantuvieron después de controlar por covariables como el género, edad, IMC y nivel educativo (p = 0,03 y p = 0,01, respectivamente). Por el contrario, no se encontraron asociaciones significativas entre la concentración de BDNF en el plasma y las otras subescalas, incluso después de controlar por covariables (orientación: Sperman r = 0,06, p = 0,57 (véase Figura 22B); fijación: Sperman r = 0,19, p = 0,09) (véase Figura 22C); memoria: Sperman r = 0,08, p = 0,54 (véase Figura 22E); idioma: Sperman r = 0,06, p = 0,55 (véase Figura 22F)).

Los niveles de BDNF en plasma tampoco se asociaron de manera significativa con las puntuaciones de las otras escalas de evaluación geriátrica, utilizando la prueba de Spearman en todos los casos e incluso después de controlar por covariables (Tinetti total (r = 0,89, p = 0,56), el índice de la marcha Tinetti (r = 0,79, p = 0,59) y el índice de equilibrio Tinetti (r = 0,10, p = 0,50) para determinar el riesgo de caídas, la escala Yesavage para la depresión geriátrica (r = 0,13, p = 0,35), la escala de Norton para la evaluación de las ulceras por presión (r = 0,15, p = 0,29), o el índice de Lawton de las AIVD (r = - 0,48, p = 0,79)).

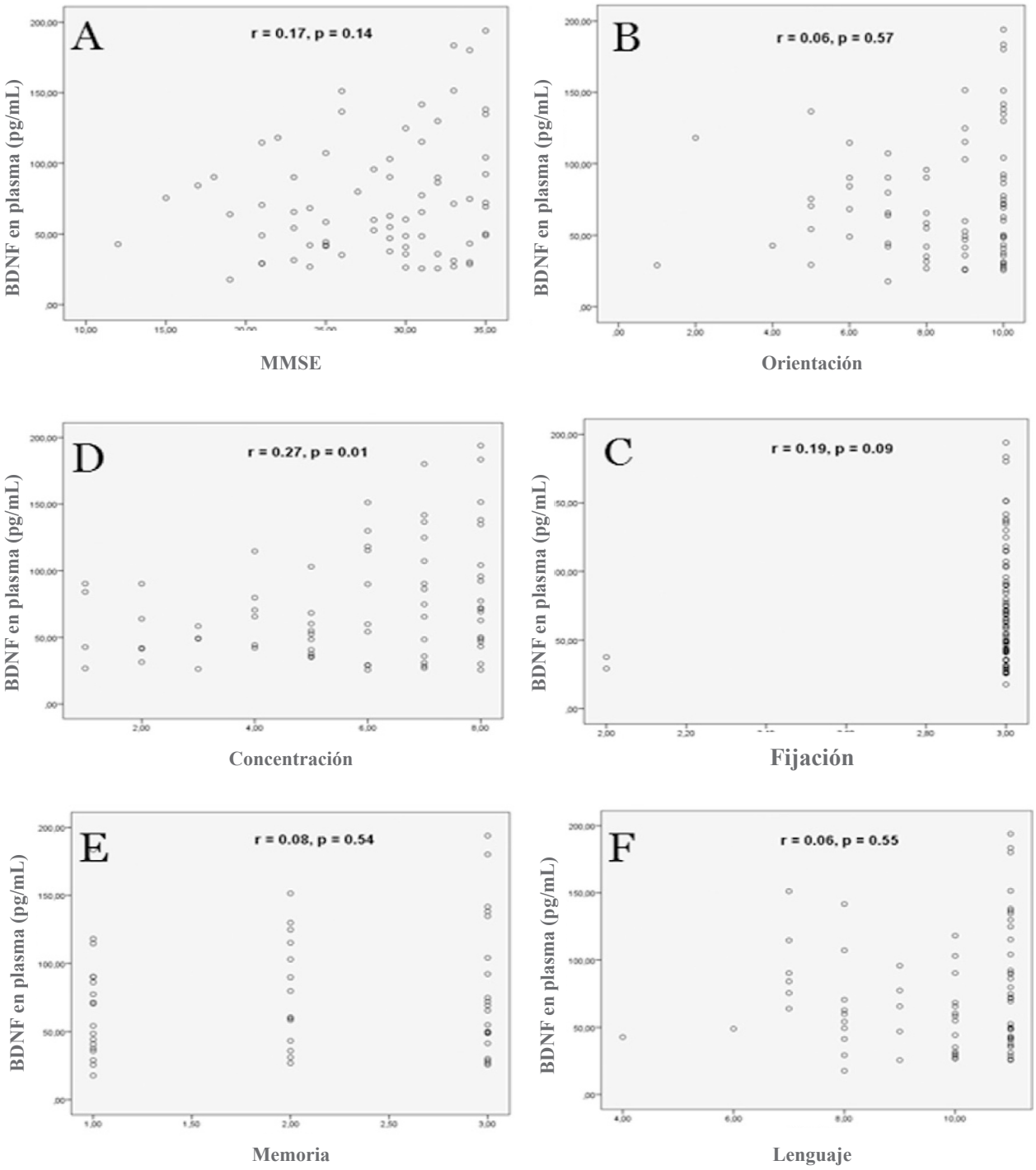

Figura 22. **Correlación entre la concentración de BDNF y la puntuación de la prueba del MMSE y sus subescalas.**

Nota. El mini examen del estado mental (MMSE) evalúa diferentes elementos agrupados en cinco sunescalas: orientación, memoria inmediata, atención y cálculo, memoria diferida, el lenguaje y la concentración; tiene un rango de puntuación de 0-30 y las puntuaciones más altas indican un mejor rendimiento. Los niveles de BDNF (pg /ml) se midieron en plasma y se representaron frente a las puntuaciones del MMSE (A), y sus subescalas: orientación (B), fijación (C), concentración (D), memoria (E) e idioma (F) para cada participante inscrito/a en el estudio. El nivel de significación se indica en la figura correspondiente.

2- Evaluación de la relación entre los niveles plasmáticos de BDNF con otros parámetros analíticos

En las muestras de sangre de los sujetos del estudio,con el fin de encontrar alguna correlación significativa con la concentración de BDNF en el plasma, también se midieron otros parámetros analíticos. Sin embargo, el análisis de correlación de Sperman no mostró correlaciones significativas entre la concentración plasmática BDNF y el recuento de plaquetas ($r = 0,08$, $p = 0,52$), el recuento de leucocitos ($r = 0,15$, $p = 0,26$; (véase Figura 23A), el recuento de eritrocitos ($r = - 0,26$, $p = 0,85$) o la concentración de hemoglobina ($r= 0,03$, $p = 0,81$) en sangre. Los valores de la mediana (rango intercuartil) para los recuentos de plaquetas, leucocitos y eritrocitos se encontraban dentro del intervalo normal de referencia (recuento de plaquetas: 205×10^3 / l, rango intercuartil: 176-248 (rango normal: $130\text{-}450 \times 10^3$ / l) ; recuento de leucocitos: $6,35 \times 10^3$ / l, rango intercuartil: 5,20 a 8,47 (rango normal: 4,o-1o,5 $\times 10^3$ / l), y recuento eritrocitos: $3,88 \times 10^6$ / µl, rango intercuartil: 3,57 a 4,24 (rango normal : 3.7 a 5.2 $\times 10^6$ / l)). En contraste, la mediana de concentración de hemoglobina se encontraba por debajo del rango fisiológico normal (hemoglobina: 11,60 g / dl, rango intercuartil: 10,82-12,97 (rango normal: 12,5-16 g / dL par amujeres y 13,5-18 g / dL para hombres)). Sin embargo, se debe destacar que este es un hallazgo fisiológico común en los pacientes geriátricos.

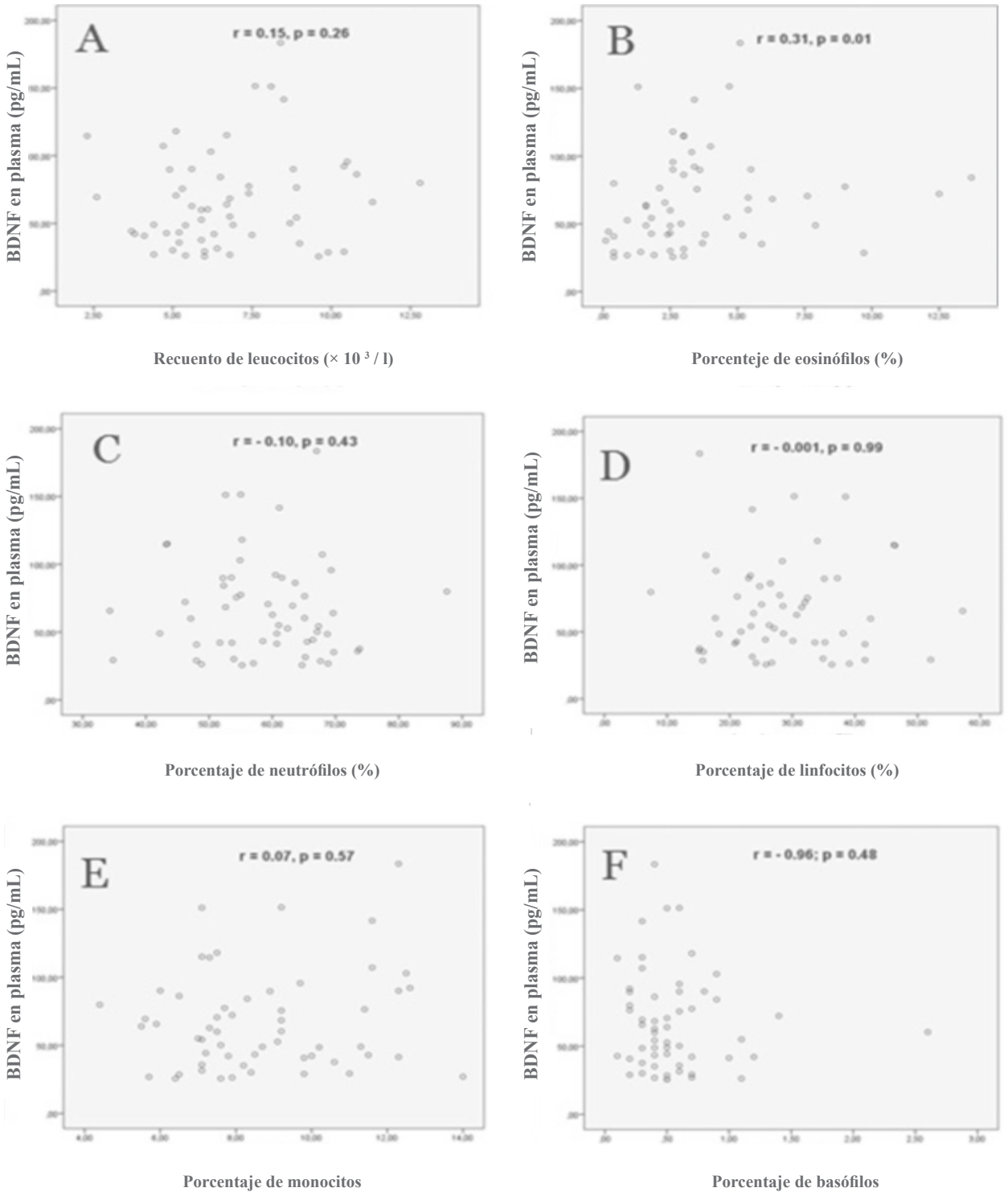

Figura 23. **Correlación entre la concentración de BDNF y el recuento total y diferencial de leucocitos.**
Nota. Los niveles de BDNF (pg / ml) se midieron en plasma. Los recuento de leucocitos (× 10 ³ / l) (A) y los porcentajes de eosinófilos (%) (B), de neutrófilos (%) (C), de linfocitos (%) (D), de monocitos (%) (E) y de basófilos (%) (F) se midieron en sangre y reprentaron frente a los niveles plasmáticos de BDNF para cada participante inscrito/a en el estudio. El nivel de significación se indica en la figura correspondiente.

El análisis de correlación de Sperman mostró, tras asociar los niveles de BDNF en plasma con cada una de las subpoblaciones leucocitarias como los neutrófilos, linfocitos, monocitos, basófilos y eosinófilos, una correlación positiva y significativa con el recuento de eosinófilos (eosinófilos: Spearman r = 0,31, p = 0,01 (véase Figura 23B). Por el contrario, no se encontraron asociaciones significativas entre la concentración de BDNF en el plasma y el resto de las subpoblaciones leucocitarias (neutrófilos: Spearman r = - 0,10, p = 0,43 (véase Figura 23C); linfocitos: Spearman r = - 0,001, p = 0,99 (véase Figura 23D); monocitos: Spearman r = 0,07, p = 0,57, (véase Figura 23E); basófilos: Spearman r = - 0,96; p = 0,48 (véase Figura 25F)).

Todas las correlaciones significativas se realizaron tomando covariables, debido al potencial de varianza, es decir, se tuvieron en cuenta el género, la edad, el IMC y el nivel educativo, dado lugar a resultados similares (datos no mostrados). Los valores de la mediana (rango intercuartil) para cada subpoblación de leucocitos estaban dentro del rango fisiológico normal (recuento de neutrófilos: 60,25%, rango intercuartil: 52,60-66,17 (rango normal: 40-75%); recuento de linfocitos: 26,90%, rango intercuartil: 22,10-35,05 (rango normal: 20-45%); recuento de monocitos: 8,25%, rango intercuartil: 7.10-10.15, (rango normal: 2-10%); recuento de basófilos: 0,50%, rango intercuartil: 0,30 a 0,67 (intervalo normal: 0-2%); recuento de eosinófilos: 2,95%, rango intercuartil: 1,80 a 4,67 (intervalo normal: 1-6%)). Del mismo modo, tampoco se observó una correlación significativa entre los niveles de BDNF en plasma y otros parámetros bioquímicos básicos como la glucosa, urea, ácido úrico, TGD, LDL-c, creatinina, GOT y GPT, Na, K, Ca, Fe, Ferritina, B12, proteínas totales o hormona estimulante del tiroides (TSH) (glucosa: r = 0,05, p = 0,97; urea: r = 0,02, p = 0,91; ácido úrico: r = - 0,08, p = 0,55; creatinina: r = 0,2, p = 0,85; TGD: r = - 0,12, p = 0,36, (véase Figura 24B); HDL-c: r = - 0,27, p = 0,70, (véase Figura 24C); GOT: r= - 0,18, p = 0,43; GPT: r = - 0,22, p = 0,10; Na: r = - 0,19, p = 0,26; K: r = - 0,84, p = 0,54; Ca: r = - 0,04, p = 0,97; Fe: r = - 0,21, p = 0.14; Ferritina: r = 0,008, p = 0,95; B12: r = - 0,10, p = 0,45; proteínas totales: r = 0,03, p = 0,82; TSH: r = - 0,20, p = 0,15, todo evaluado mediante la prueba de Spearman). En contraste, una correlación inversa y significativa surgió entre los niveles de BDNF en plasma y el CT (Spearman r = - 0,26, p = 0,04; (véase Figura 24A)) y HDL-c (Spearman r = - 0,31, p =

0,04; (véase Figura 24D)). Los valores de la mediana (rango intercuartil) de estos parámetros bioquímicos también se encontraban dentro del intervalo de referencia normal. Los datos de los parámetros analíticos de la población a estudio se muestran en la siguiente tabla (véase Tabla 13).

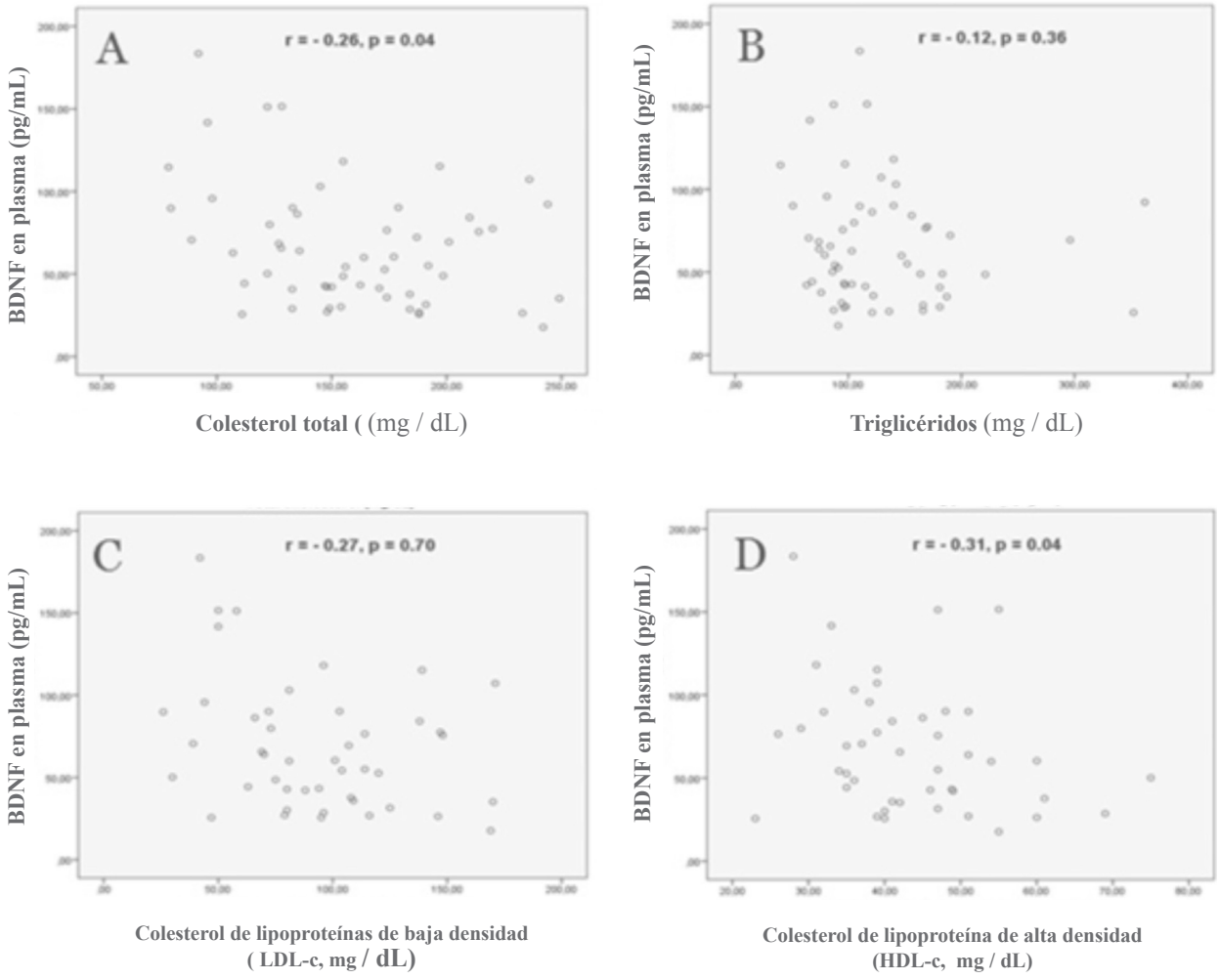

Figura 24. **Correlación entre la concentración de BDNF y el perfil lipídico.**
Los niveles de BDNF (pg / ml) se midieron en plasma. Los niveles de colesterol total (CT) (mg / dL) (A), de triglicéridos (TGD) (mg / dL) (B), de colesterol de lipoproteínas de baja densidad (LDL-c; mg / dL) (C) y de colesterol de lipoproteína de alta densidad (HDL-c mg / dL) (D) se midieron en la sangre y se representaron frente a los niveles plasmáticos de BDNF para cada participante inscrito/a en el estudio. El nivel de significación se indica en las figuras correspondientes.

Tabla 13
Resultados de los parámetros hematológico y bioquímicos de la poblacion a estudio con la mediana, el rango intercualtil y rango normal para cada valor.

	Valor de la Mediana	Rango intercuartil	Rango normal
Recuento de plaquetas (10^3/µL)	205	176-248	130-450
Recuento de eritrocitos (10^6/µL)	3.88	3.57-4.24	3.7-5.2
Concentración de hemoglobina (g/dL)	11.60	10.82-12.97	12-16
Recuento de leucocitos (10^3/µL)	6.35	5.20-8.47	3.8-10.8
Porcentaje de neutrofilos (%)	60.25	52.60-66.17	40-75
Porcentaje de linfocitos (%)	26.90	22.10-35.05	20-45
Porcentaje de monocitos (%)	8.25	7.10-10.15	2-10
Porcentaje de basofilos (%)	2.95	1.80-4.67	0-2
Porcentaje de eosinofilos (%)	0.50	0.30-0.67	1-6
Glucosa (mg/dL)	82	73.50-99	74-106
Urea (mg/dL)	47.20	37.05-70.65	17-60
Ácido úrico (mg/dL)	5.40	4.20-6.10	2.6-6
Trigliceridos (mg/dL)	110	87-159.80	50-150
Lipoproteina de baja densidad (mg/dL)	94	67.5-115	0-130
Creatinina (mg/dL)	0.94	0.73-1.10	0.51-0.95
GOT (U/L)	17.40	14-21.55	10-31
GPT(UL)	14	8-22.70	10-34
Na (mEq/L)	140.95	139.37-142.67	136-146
K (mEq/L)	4.50	4.20-4.77	3.5-5.1
Ca (mg/dL)	9.10	8.80-9.50	8.8-10.6
Fe (ug/dL)	54.50	42.25-67.75	60-180
Ferritina (ug/L)	126.20	54.90-193	10-120
Vitamina B12 (pg/mL)	281.50	218-418.75	180-914
Hormona estimulante del tiroides (uUI/ml)	1.72	1.17-3.18	0.34-5.6

	Valor de la Mediana	Rango intercuartil	Rango normal
Colesterol total (mg/dL)	155	128.07-188	100-200
Lipoproteínas de alta densidad (mg/dL)	41	35.50-50	40-200
Proteínas totales (g/dL)	6.35	6.17-6.82	6.6-8.3

Nota. Todos los valores de los parámetros bioquímicos y hematológicos se expresaron en unidades convencionales.

Finalmente se realizó un análisis de regresión lineal, utilizando los niveles plasmáticos de BDNF como variable dependiente y como variables independientes, los factores que fueron significativos en el análisis anterior o aquellos con evidencia de que pudiesen tener un efecto sustancial. Así, los resultados de dicho análisis mostraron que el género ($r= 0,26$, $r^2= 0,07$, $p = 0,02$), la subescala de concentración de la prueba MMSE ($r= 0,30$, $r^2 = 0,09$, $p = 0,009$) y el CT ($r= 0,32$, $r^2= 0,10$, $p= 0,01$) fueron factores independientes asociados con los niveles plasmáticos de BDNF. En contraste, el índice de Barthel ($r= 0,26$, $r^2 = 0,07$, $p = 0,06$), los recuentos de eosinófilos ($r= 0,15$, $r^2 = 0,02$, $p = 0,25$), y los niveles de HDL-c ($r= 0,29$, $r^2= 0,08$, $p= 0,053$) no fueron significativos.

ARTÍCULO 3

SERUM VITAMIN D AND FUNCTIONAL IMPAIRMENT IN OCTOGENARIAN WOMEN

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1- **Características clínicas y sociodemográficas de la población a estudio**

Este estudio de diseño transversal se llevó a cabo durante el año 2015 en una población de 104 mujeres no osteoporóticas de edad avanzada, todas ellas pertenecientes a 4 centros privados de atención residencial para personas mayores (*GeroResidencias La Saleta),* ubicados en la provincia de Valencia (España). De sus datos sociodemográficos, obtenidos mediante un cuestionario estructurado a través de una entrevista personal, destacamos que el 82% de las participantes eran viudas y que su edad oscilaba entre 75 y 99 años, con una edad media de 84 años. La valoración geronto-geriátrica integral, realizada mediante escalas validadas, reveló, en lo referente a las características funcionales de la totalidad de la muestra, un nivel de independencia para la realización de las ABVD y un nivel de dependencia moderado para la realización de las AIVD, con unas puntuaciones medias de 60 (rango: 25-100) puntos (valor normal = 60-100 puntos) para la escala de ABVD de Barthel y de 4 (rango: 2-7) puntos (valor normal = 8 puntos) para la escala de AIVB de Lawton y Brody. La media del estado cognitivo, evaluado a través de la prueba MMSE, fue de 24 (rango: 21-30) puntos (valor normal > 27 puntos), lo que puso de manifiesto que el grado de deterioro cognitivo de las participantes fue leve. Asimismo, con una puntuación media en la

escala Norton de 15 (rango: 13-20) puntos (valor normal > 14 puntos) se verificó que, la muestra en su totalidad no presentó riesgo de desarrollar ulceras por presión. Finalmente, las participantes incluidas en el estudio presentaron un riesgo leve de sufrir caídas, ya que las puntuaciones medias en la escala Tinetti de la marcha y del equilibrio fueron de 6 (rango: 0-12) puntos (valor normal = 12 puntos) y de 9 (rango: 0-14) puntos (valor normal = 16 puntos) respectivamente. Los resultados de la edad y de las diferentes evaluaciones geriátricas se muestran en la siguiente tabla (véase Tabla 14).

Tabla 14
Edad y resultados de las escalas de evaluación geriátricas de las participantes incluidas en el estudio con la media y el rango para cada valor / escala.

	Media	Rango
Edad (años)	84	75 - 99
Índice de equilibrio de Tinetti	9	0 - 14
Índice de la marcha de Tinetti	6	0 - 12
Escala Norton	15	13 - 20
Test MMSE	24	21 - 30
Índice de Barthel	60	25 - 100
Índice de Lawton y Brody	4	2 - 7

Nota. La edad se expresa en años. La valoración geriátrica se evaluó mediante escalas validadas:el índice Tinetti de la marcha y del equilibrio para determinar el riesgo de caídas, la escala de Norton para medir el riesgo de desarrollar úlceras por presión, el mini examen del estado mental (MMSE) para detectar el deterioro cognitivo, el índice de Barthel para medir la capacidad de realizar las ABVD y el índice de Lawton y Brody para medir la capacidad de realizar las AIVD.

Una vez realizado el diagnóstico gerontológico integral, se midió el síndrome de fragilidad de acuerdo con los cinco criterios propuestos por Fried (Fried et al., 2005). Se dispuso de todos los criterios de fragilidad de Fried para la totalidad de la muestra. En relación al número de criterios de Fried, el 20 % de las participantes no presentó ninguno de los cinco criterios, el 5 % sólo uno de ellos, el 18 % dos, el 49 % tres, el 20% cuatro y ninguna de las participantes cumplió con los cinco criterios de fragilidad de Fried (véase Figura 25A). Asimismo, la escala Fried, identificó

como frágil (mujeres con ≥ 3 criterios Fried) al 58% de la muestra, como prefragil (mujeres con 1 o 2 criterios Fried) al 21% y como robusta o no frágil (mujeres con ningún criterio Fried) al 21% restante (véase Figura 25B).

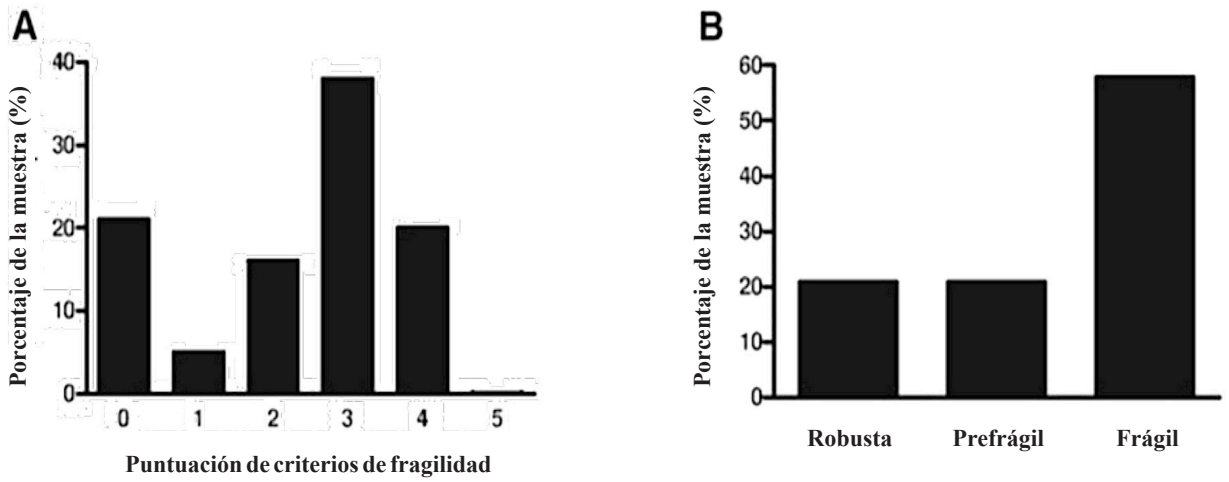

Figura 25. **Evaluación del síndrome de fragilidad en la muestra de estudio.**
Nota. La fragilidad se midió según los cinco criterios propuestos por Fried. (A) El número o la puntuación de criterios de fragilidad de Fried para el total de la muestra, expresado como porcentaje de la población total. (B) Porcentaje de las categorías de la fragilidad, se expresó de la siguiente manera: los participantes que cumplieron con tres o más criterios fueron clasificados como frágiles, los que cumplían uno o dos como prefrágiles, y aquellas que no cumplieron con ninguno de los criterios como no frágiles (o robustas).

Cuando se analizaron los criterios de Fried de forma aislada, el más representado en la totalidad de la muestra fue la baja actividad física en el tiempo libre (93%), seguido de la lenta velocidad en la marcha (85%), de la debilidad o baja fuerza muscular (71%), de la fatiga crónica autorreferida o baja energía (36%) y de la pérdida de peso involuntaria en el último año (9%).

Una vez caracterizada la población de estudio, se procedió a determinar la posible correlación entre la puntuación de la escala de Fried con la edad y los resultados obtenidos en las escalas de valoración geriátrica. Las correlaciones se analizaron mediante el coeficiente de correlación de Spearman. Los resultados obtenidos en el análisis bivariado no mostraron diferencias estadísticamente significativas entre el número de criterios de Fried y las puntuaciones de las escalas de evaluación geriátrica, lo que sugiere que la severidad de la fragilidad no se correlacionó con el estado de salud general de las participantes. Por el contrario, el coeficiente de correlación

de Spearman, mostró que la edad de las mujeres frágiles fue mayor que la edad de las mujeres robustas, sin embargo, no se observaron diferencias significativas entre la edad de las mujeres pre-frágiles y frágiles.

2- Evaluación de la relación entre los niveles séricos de vitamina D con los criterios de fragilidad de Fried y el metabolismo fosfocalcico

Diversos estudios han descrito que la deficiencia de vitamina D se asocia con la fragilidad, sin embargo, estos excluían a la población institucionalizada (Fernández- Garrido et al., 2014a). Así, con el objetivo de determinar la posible correlación entre los niveles séricos de vitamina D y la fragilidad en personas mayores institucionalizadas, en primer lugar, se medimos la concentración sérica de vitamina D (medida como 25-hidroxivitamina D (25 (OH) D_3)) en las muestras de sangre de todas las mujeres incluidas en el estudio. La media (± error estándar de la media (SEM)) de la concentración sérica de vitamina D fue de 46 (± 6) ng /ml en mujeres robustas, de 29 (± 4) ng /ml en las pre-frágiles y de 28 (± 3) ng /ml en las frágiles (véase Figura 28A). Se debe destacar que, los valores medios de la concentración sérica de vitamina D en los tres grupos de mujeres estudiadas (robustas, pre-frágiles y frágiles) se encontraban dentro de los niveles recomendados para nuestra población de estudio, puesto que, los tres grupos presentaron una concentración media de 25 (OH) D_3 > a 20 ng / ml (o 50 nM).

Posteriormente, y una vez determinados los niveles medios de 25 (OH) D_3 en suero para cada uno de los tres grupos de mujeres estudiadas, analizamos, mediante la prueba no paramétrica de Kruskal-Wallis, la posible existencia de diferencias significativas entre las mujeres robustas, prefragiles y frágiles. Los resultados mostraron diferencias estadísticamente significativas entre las mujeres robustas y las mujeres pre-frágiles y frágiles ($p < 0,01$), de manera que, tanto las mujeres pre-fragiles como las frágiles tenían concentraciones de 25 (OH) D_3 en suero significativamente más bajas en comparación con las mujeres robustas (véase Figura 28A). Sin embargo, no se observaron diferencias significativas en las concentraciones séricas de 25 (OH) D_3 entre las mujeres prefrágiles y frágiles, y por tanto, en nuestro estudio, el nivel de 25 (OH) D_3 en suero no parece

estar asociado con la severidad de la fragilidad. Igualmente, los resultados de las correlaciones bivariadas entre los niveles séricos de 25 (OH) D_3 con la puntuación de la escala Fried, también sugieren que, en nuestras participantes, el nivel de vitamina D en suero no es un biomarcador de la severidad del síndrome de fragilidad. Tampoco, el análisis de correlación entre los niveles séricos de vitamina D y cada uno de los cinco criterios de la escala Fried mostró diferencias estadísticamente significativas.

La vitamina D es necesaria para mantener una adecuada concentración de calcio y fósforo en la sangre, de hecho, los niveles bajos de 25 (OH) D_3 en suero disminuyen la absorción del calcio y fósforo, asimismo, los bajos niveles séricos de calcio y fósforo elevan la síntesis de parathormona (PTH) con el fin de estimular la producción renal de la 1,25- $(OH)_2$ D_3, con el fin de aumentar los niveles séricos de calcio y fósforo. Por tanto, para descartar que el papel de la vitamina D en el síndrome frágil no estuviese relacionado con el deterioro del metabolismo fosfocálcico, también medimos en las muestras de sangre de todas las participantes del estudio, la concentración sérica de calcio, fósforo y PTH, para posteriormente verificar, mediante la prueba no paramétrica de Kruskal-Wallis, posibles diferencias entre los grupos estudiados.

La concentración media (± SEM) de calcio en suero fue de 7,9 (± 0,5) mg /dl en las mujeres robustas, de 8,2 (± 0,8) mg /dl en las pre-frágiles y de 8,0 (± 0,4) mg /dl en las frágiles (véase Figura 26B), y al igual que en el caso anterior, los valores medios de calcio sérico en todas las muestras de sangre estaban dentro del rango fisiológico normal en los tres grupos estudiados. Sin embargo, a diferencia de lo observado en la vitamina D, en los resultados obtenidos mediante la prueba de Kruskal-Wallis, no se observaron diferencias estadísticamente significativas en la concentración de calcio entre las mujeres frágiles, pre-frágiles y robustas ($p = 0,9$) (véase Figura. 26B).

De manera similar al calcio, los resultados hallados mediante la prueba de Kruskal-Wallis, tampoco mostraron diferencias estadísticamente significativas en la concentración de fósforo y PTH entre las mujeres robustas, pre-frágiles, y frágiles. En este caso, los valores medios de PTH y fósforo en suero se también encontraban dentro de los rangos de referencia normales en los tres grupos estudiados. Por lo tanto, estos resultados en su conjunto sugieren que, en nuestra población

de estudio, el papel de la vitamina D en el síndrome de fragilidad no se encuentra influenciado por el deterioro del metabolismo fosfocálcico.

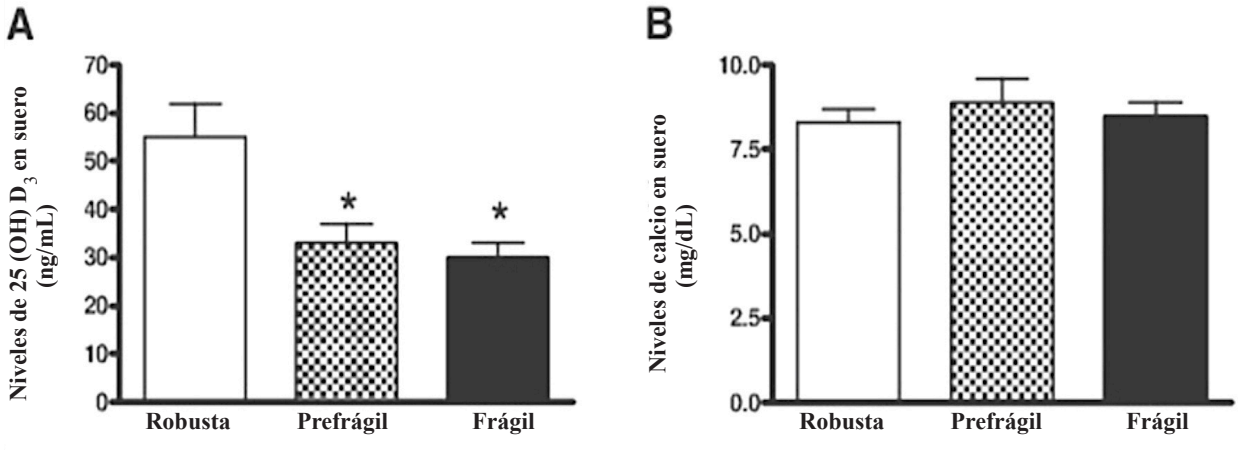

***Figura 26*. La concentración sérica de 25 (OH) D_3 (ng/dL) (A) y Calcio (mg/dL) (B) de las participantes robustas, pre-frágiles y frágiles.**
Nota. Los datos se expresan como la media ± error estándar de la media (SEM) para cada grupo * $p < 0{,}05$ en comparación con participantes robustas.

3- Evaluación de la relación entre los niveles séricos de vitamina D con los resultados de las escalas de evaluación geriátrica

En cuanto a la relación entre las concentraciones séricas de 25 (OH) D_3 con las puntuaciones obtenidas en las escalas de evaluación geriátrica, los resultados hallados mediante el coeficiente de correlación de Spearman no mostraron diferencias significativas entre las mujeres robustas, pre-frágiles y frágiles. Estos resultados sugieren que los bajos niveles séricos de 25 (OH) D_3 tampoco fueron influidos por el estado de salud general de las participantes del estudio.

4- Evaluación de la relación entre los niveles séricos de vitamina D con otros parámetros analíticos

En las muestras de sangre de las participantes del estudio, además de los niveles de 25 (OH) D_3, calcio, fósforo y parathormona, también se midieron otros parámetros analíticos como los niveles de eritrocitos, hemoglobina, hematocrito, plaquetas, glucosa, urea, úrico ácido, CT, TGD,

creatinina, GOT y GPT, con el objetivo de explorar la posible influencia de los mismos sobre el papel de la vitamina D en el síndrome frágil. Los resultados obtenidos, mediante el coeficiente de correlación de Spearman, no mostraron ninguna asociación significativa entre los niveles séricos de 25 (OH) D_3 y cada uno de los parámetros analíticos previos.

CAPÍTULO III- DISCUSIÓN GENERAL

Razonar y convencer, ¡qué dificil, largo y trabajoso!¿Sugestionar?¡Qué fácil, rápido y barato!

Santiago Ramón y Cajal

DISCUSIÓN

Los principales resultados de la presente tesis demuestran cómo, en una población de personas mayores institucionalizadas, determinados biomarcadores hematológicos como la concentración sérica de vitamina D y los porcentajes de neutrofilos y linfocitos se asocian con la condición de fragilidad, definida como la presencia de tres de los cinco criterios propuestos por Fried (pérdida de peso involuntaria, bajo nivel de actividad física, debilidad, agotamiento, velocidad lenta de la marcha) (Fried et al., 2001). Asimismo observamos que el BDNF plasmático, aunque no se asoció con el síndrome de fragilidad, se relaciona con algunas características clínicas típicas de este grupo de población.

La longevidad, con frecuencia, lleva aparejada la pérdida de capacidades físicas y cognitivas que implican una mayor susceptibilidad a episodios adversos de salud. En este contexto, investigaciones recientes están destacando el papel de la fragilidad en la identificación de personas mayores en riesgo (Walston et al., 2006). La fragilidad, en su forma más simple, es considerada como un estado de mayor vulnerabilidad que aumenta el riesgo de desarrollar efectos adversos para la salud (Abizanda et al., 2013). Asimismo, la propia Unión Europea ha insistido en la necesidad de identificar a las personas frágiles debido a su vinculación con el alto consumo de recursos comunitarios, residenciales y hospitalarios y, a continuación, siempre que sea posible, a través de una intervención precoz mejorar la calidad de vida y disminuir los costes de los cuidados (SEGG, 2010).

Por otro lado, estudios previos han evidenciado que la prevalencia de fragilidad aumenta de manera exponencial con la edad, desde un 3,2% a los 65 años, a un 16,3% en mayores de 80 años y un 23,1% a los 90 años (Fried et al., 2001). Del mismo modo, la fragilidad es más prevalente en el sexo femenino (Gordon et al., 2017; Syddall et al., 2010; Woods et al., 2005). Asimismo, la fragilidad también ha sido asociada con la institucionalización (Rockwood et al., 2006). En este sentido, los resultados globales de un reciente meta-análisis mostraron como, aproximadamente,la mitad de los adultos mayores que viven en residencias son frágiles en comparación con el 10,7%

de las personas mayores que viven en la comunidad (Kojima, 2015). En base a lo anterior, nos propusimos estudiar el síndrome de fragilidad en un grupo de personas de edad avanzada, preferentemente mujeres e institucionalizadas en residencias geriátricas.

En España, a pesar de la escasez de datos, se considera que el 3,3% de la población de edad avanzada vive en residencias para personas mayores, estando a dos puntos porcentuales de esa media todas las comunidades autónomas españolas (1,3-5,3 %) (Acevedo et al., 2014). Aunque se trata de una proporción más bien baja, el progresivo envejecimiento de nuestra población supone una creciente institucionalización de las personas mayores, especialmente aquellas de mayor edad o que presentan alguna limitación o discapacidad (Damian et al., 2004). De hecho, un 5,2% de los hombres y un 8,7% de las mujeres de 75 y más años viven en alojamientos colectivos, a partir de los 85 años son un 9,2% y un 14,1% respectivamente (Abellán García y Pujol Rodríguez, 2014).

Por otro lado, a pesar de que los adultos mayores institucionalizados son una población muy vulnerable, por presentar peores condiciones físicas y psicosociales (De la Rica-Escuín et al., 2014), sorprende que los estudios que investigan la fragilidad en dicha población son muy escasos. Sólo cuatro estudios en España (Abizanda Soler et al., 2011; De la Rica-Escuín et al., 2014; Garrido et al., 2012; González-Vaca et al., 2014), dos en Canadá (Freihet et al., 2011; Rockwood et al., 2007b) y uno en Polonia (Matusik et al., 2012) han examinado la fragilidad en adultos mayores institucionalizados. Sin embargo, ante el progresivo envejecimiento de la población y la creciente institucionalización de las personas mayores, es necesario considerar que la valoración de la fragilidad es un aspecto fundamental a tener en cuenta en los centros residenciales, ya que la identificación de los residentes relativamente vulnerables a un mayor riesgo de efectos adversos para la salud, y si se combina con intervenciones eficaces, ofrecería mayores oportunidades para maximizar su independencia y su calidad de vida (Freihet et al., 2011).

Actualmente, la evaluación de la fragilidad se basa principalmente en la medición de parámetros funcionales, que tienen utilidad clínica limitada (Sanchís et al., 2015). Por ello, en los últimos años la búsqueda de biomarcadores de fragilidad, rentables y fácilmente disponibles, que proporcionen información adicional a la que se obtiene a partir de los datos clínicos, ha cobrado

especial importancia (Blodgett et al., 2014; Howlett et al., 2014). De hecho, varios estudios han sugerido que la inclusión de los datos de laboratorio en los índices de fragilidad podría mejorar su poder pronóstico (Sanchís et al., 2015). Así, en una cohorte del *CSHA,* un índice de fragilidad en base a datos de laboratorio identificó a los adultos mayores con mayor riesgo de muerte (Howlett et al., 2014). Por otro lado, los últimos avances permiten que los biomarcadores de laboratorio, además de proporcionar información diagnóstica y pronóstica, puedan detectar de manera muy precoz ciertas enfermedades, incluso antes de que se produzcan los síntomas, o la predisposición de llegar a desarrollar la enfermedad (Ortega-Ortiz de Apodala, 2010). Dado que la evidencia sugiere que el deterioro fisiológico asociado a la fragilidad empieza a ser evidente en una fase preclínica (Fernandéz- Garrido et al., 2014a), la identificación de un (o varios) marcador biológico lo suficientemente sensible y específico del síndrome frágil o de aspectos concretos del mismo, permitiría mejorar la exactitud de su diagnóstico en la práctica clínica y predecir el riesgo de fraglidad (Butler et al., 2004; Inglés de la Torre, 2014; Lippi et al., 2015). Asimismo, su monitorización permitiría evaluar el pronóstico de la fragilidad en términos de discapacidad y de otros resultados adversos, así como la respuesta a las intervenciones aplicadas (Lippi et al., 2015).

Igualmente, para poder lograr un diagnóstico precoz que salve al sujeto de sufrir esta dolencia, es clave conocer la fisiopatología de la enfermedad. La fisiopatología permite la comprensión de los mecanismos por los cuales se originan las distintas enfermedades, de lo cual se desprende la forma específica de tratarlas, por tanto, el desconocimiento de estos mecanismos lleva a que los síntomas sean tratados de forma empírica, limitándose solo al control de los síntomas sin hacer nada por la causa que los está originando (Universidad de Valencia, 2015). Por esta razón, en los últimos años, varios estudios han relacionado el síndrome frágil con diferentes biomarcadores involucrados en la fisiopatología del deterioro fisiológico característico del síndrome frágil (Schoufour et al., 2016).

Los mecanismos fisiopatológicos que subyacen a la aparición y desarrollo de la fragilidad siguen siendo complicados y poco conocidos, no obstante, diversos estudios sugieren que la inflamación sistémica crónica puede contribuir de manera directa o indirecta, a través de otras desregulaciones fisiológicas, al desarrollo del síndrome de fragilidad (Barzilay et al., 2007; Boxer et al., 2008; Chen

et al., 2014; Ferrucci et al., 2002; Paganelli et al., 2006). De hecho, varios mediadores inflamatorios se han asociado de manera consistente con la fragilidad (Barzilay et al., 2007; Hubbard et al., 2009; Leng et al., 2007). Igualmente, en términos de marcadores celulares de la inflamación se ha demostrado que un aumento en los recuentos totales de leucocitos está asociado con la fragilidad, sin embargo, esta asociación es difícil de interpretar, ya que los recuentos totales de leucocitos incluyen varios tipos de células con papeles potencialmente distintos, por lo que su efecto sobre la fragilidad puede depender de un subtipo específico (Leng et al., 2007). En este sentido, y con el fin de esclarecer el papel de los subtipos de leucocitos en la fisiopatología del síndrome de fragilidad, diversos estudios han investigado el impacto de los recuentos diferenciales de leucocitos sobre la fragilidad, con resultados poco concluyentes (Collerton et al., 2012; De Fanis et al., 2008; Desquilbet et al., 2007; Gibson et al., 2009; Leng et al., 2009; Semba et al., 2005). Por lo tanto, una evaluación exhaustiva de los recuentos individuales de glóbulos blancos en la fragilidad es necesaria. Asimismo, si existe una asociación entre los recuentos totales y diferenciales de leucocitos con los criterios individuales de la escala Fried (Fried et al., 2001), ésta, todavía no ha sido investigada en poblaciones geriátricas frágiles.

En este contexto, y con el fin de avanzar en el conocimiento sobre las bases celulares del estado inflamatorio asociado a la fragilidad, hemos investigado las asociaciones entre los recuentos totales de leucocitos y los porcentajes de los subtipos de leucocitos tanto con la fragilidad como con cada uno de los cinco criterios la escala Fried y, consecuentemente, nuestros resultados confirman la importancia de las células del sistema inmune en la fragilidad, al demostrar que los porcentajes de neutrófilos y de linfocitos están asociados significativamente con la fragilidad en una población de mujeres mayores institucionalizadas. Asimismo, este estudio también ha demostrado, y por primera vez, la evidencia de que dos de los cinco criterios de Fried, la actividad física y la fuerza muscular, están asociados significativamente con los porcentajes de neutrófilos y de linfocitos. Curiosamente, estas asociaciones tuvieron lugar dentro del rango fisiológico normal de las dos subpoblaciones de leucocitos y persistieron después de ajustar los resultados por factores de confusión como la edad, el estado de salud general y la comorbilidad de las participantes del estudio.

Aunque en este estudio no se han observado asociaciones significativas entre los recuentos totales de leucocitos y la fragilidad, hemos encontrado que ésta aumenta significativamente a medida que disminuyen los porcentajes de linfocitos y aumentan los de neutrófilos. Hasta ahora este hallazgo sólo había sido descritos en la población geriátrica que vive en la comunidad y no en personas mayores institucionalizadas (Collerton et al., 2012; Leng et al., 2009). Así, un estudio encontró que un incremento en los recuentos de neutrófilos y de monocitos, pero no de linfocitos, se asoció positivamente con la puntuación de la fragilidad en las mujeres mayores que viven en comunidad, con un amplio rango de edad y una alta prevalencia de incapacidad funcional, sin embargo, estas asociaciones no alcanzaron significación estadística al ajustar por edad (Leng et al., 2009). Asimismo, en un estudio llevado a cabo en una cohorte de personas mayores de 85 o más años que viven en la comunidad, tras ajustar por posibles factores de confusión, la fragilidad se relacionó con una disminución en el recuento de linfocitos y un aumento en el recuento de neutrofilos (Collerton et al., 2012).

El hecho de que las participantes con mayores puntuaciones de fragilidad presentasen unos porcentajes de neutrófilos más altos, puede deberse a que los neutrófilos juegan un papel importante en la inflamación y la inmunidad innata mediante la producción de metabolitos de oxidación, radicales libres y citoquinas pro-inflamatorias, mediadores inflamatorios que causan daño oxidativo en múltiples sistemas orgánicos (Hubbard et al., 2009; Leng et al , 2004; Wu et al., 2009). Por consiguiente, los neutrófilos pueden estar implicados en la desregulación multisistémica característica del síndrome frágil (Romero-Cabrera et al., 2013).

El nivel de severidad de la fragilidad también parece estar asociado con los porcentajes de linfocitos, de manera que, aquellas participantes con menores recuentos linfocitarios presentaron las puntuaciones más altas en la escala de Fried. Estos resultados sugiere que la inmunosenescencia, definida como las alteraciones del sistema inmune relacionadas con el envejecimiento, puede estar asociada con la fragilidad, ya que se cree que un bajo recuento de linfocitos es un marcador característico de la inmunosenescencia (Falandry et al., 2013). De hecho, es bien conocido que los recuentos de linfocitos disminuyen con la edad (Comptéa et al., 2014; Jiao et al., 2009; Yang

y Reckelhoff, 2011). Asimismo, los bajos recuentos de linfocitos también se han asociado con un mayor riesgo de mortalidad en personas mayores sanas (Bender et al., 1986; Izaks et al., 2003). La asociación negativa entre los recuento de linfocitos y mortalidad puede atribuirse a la disminución de la vigilancia inmunológica y a la senescencia replicativa (Izaks et al., 2003). La vigilancia inmune describe el reconocimiento y la eliminación de células anormales por el sistema inmune, fundamentalmente por los linfocitos (Burnet, 1970). De acuerdo con esta hipótesis, las personas de edad avanzada pueden ser más vulnerables a las enfermedades y a la muerte si los recuentos de linfocitos son bajos. La senescencia replicativa se refiere a un estado en que las células limitan su proliferación en respuesta al estrés (López-Diazguerrero et al., 2005). Este estado celular se ha asociado al envejecimiento y a diversas patologías (Izaks et al., 2003). Por tanto, un bajo recuento de linfocitos no sólo puede indicar un agotamiento de la línea celular linfoide, sino también, podría ser un marcador de la disminución en otras funciones fisiológicas. De acuerdo con esto último, un bajo recuento de linfocitos podría reflejar la desregulación multisistémica asociada a la fragilidad. Es evidente que el papel y las relaciones entre los linfocitos y la fragilidad deben ser investigados con más detalle.

Es importante destacar que las relaciones de la fragilidad con los porcentajes de neutrófilos y linfocitos fueron en direcciones opuestas. Este hecho, en conjunto, podría ser debido al efecto inmunosupresor de las citoquinas inflamatorias producidas por los neutrófilos sobre los linfocitos T citotóxicos (el-Hag et al., 1987; Petrie et al., 1985). Confirman esta teoría, los resultados de un estudio reciente donde el índice neutrófilo-linfocito, definido como el recuento absoluto de neutrófilos dividido por el recuento de linfocitos, se ha correlacionado positivamente con la fragilidad en pacientes de edad avanzada con cáncer, de manera que, los pacientes con un alto índice neutrofilo-linfocito fueron significativamente más frágiles (Nishijima et al., 2017). Este resultado necesitará ser investigado en poblaciones geriátricas sin cáncer, pues creemos que el índice neutrófilo-linfocito es un marcador más sólido para la fragilidad que los recuentos individuales de neutrófilos o linfocitos, ya que combina los dos marcadores celulares que están asociadas con la fragilidad y que podrían reflejar la fisiopatología subyacente de la misma.

Asimismo, en nuestro estudio los porcentajes de neutrófilos y linfocitos también se han relacionado de manera significativa con una baja actividad física y una menor fuerza muscular. Estas dos medidas reflejan de manera directa o indirecta una disminución de la actividad muscular. El principal componente del deterioro muscular relacionado con la edad es la sarcopenia, ésta, a su vez, es considerada un componente clave en la fisiopatología del síndrome de fragilidad en adultos mayores (Evans et al., 2010; Kamel, 2003; Marzetti et al., 2012; Rolland et al., 2008). De hecho, diversos estudios sugieren que la sarcopenia, o la pérdida de masa y fuerza muscular relacionada con el envejecimiento, es mayor en las personas mayores frágiles que en las no frágiles (Theou et al., 2008). Dado que la sarcopenia, al igual que el síndrome de fragilidad, implica la disfunción de distintos sistemas fisiológicos interrelacionados, es concebible que los mecanismos que conducen a la sarcopenia a menudo se superpongan con los del síndrome frágil (Ávila-Funes et al., 2008). En este sentido, diversos estudios poblacionales han implicado a la inflamación sistémica crónica en el desarrollo tanto de la fragilidad como de la sarcopenia (Evans et al., 2010).

El mecanismo por el cual los bajos porcentajes de linfocitos se asocian con un bajo nivel de actividad física puede estar relacionado con el efecto del ejercicio físico sobre el sistema inmune. En este sentido, diversos estudios han asociado un mayor grado de actividad física con un incremento en los recuentos de linfocitos en adultos mayores sanos (Nieman et al., 1993; Shinkai et al., 1995; Shinkai et al., 1998). Los mecanismos subyacentes son multifactoriales e incluyen factores neuroendocrinos y metabólicos (Perdersen and Toft, 2000). Sin embargo, hasta donde sabemos, esta relación entre el ejercicio y los recuentos de linfocitos no ha sido demostrada en los adultos mayores frágiles, como en nuestra población de estudio (Kapasi et al., 2003). Se ha argumentado que este hecho puede ser debido a que los adultos de edad avanzada, una vez se han vuelto frágiles, no pueden ser capaces de realizar el nivel de ejercicio suficiente como para generar cambios en la inmunidad o bien, a que el ejercicio no puede revertir los cambios inmunológicos asociados a dicho estado (Senchina y Kohut, 2007). Sin embargo, es difícil extraer conclusiones, dado que los datos que se disponen son demasiado limitados. Por tanto, el mecanismo por el cual los bajos recuentos de linfocitos se asocian con unos menores niveles de actividad física en adultos

mayores frágiles no ha sido identificado. Se necesitan estudios adicionales, incluyendo estudios funcionales de los subtipos de linfocitos específicos, con el fin de evaluar con más detalle el papel de los linfocitos sobre la actividad y fuerza muscular, o viceversa, en los adultos mayores frágiles.

La baja actividad física también se ha asociado con los altos niveles de neutrófilos. Los neutrófilos, tal y como se ha comentado, pueden causar daño en múltiples tejidos, incluido el tejido muscular, al inducir una respuesta inflamatoria crónica mediante la producción de radicales libres y citoquinas pro-inflamatorias (Hubbard et al., 2009; Leng et al., 2004; Wu et al., 2009). Hay varias explicaciones posibles para la asociación entre la sarcopenia, fragilidad y un estado de inflamación crónica inducido por los neutrófilos. La activación de citoquinas pro-inflamatorias promueve la anorexia y el catabolismo proteico del músculo esquelético, lo que podría contribuir a un empeoramiento del estado nutricional y una reducción de la masa muscular y, consecuentemente, a la pérdida de peso y a la debilidad muscular, dos características comunes del síndrome frágil (Clegg et al., 2013). Además, este estado de inflamación crónica induce la aparición de disfunción endotelial, RI y fenómenos procoagulantes, todos ellos promotores de la arteriosclerosis (Abizanda, 2010). La arteriosclerosis conduce a una alteración en la perfusión y, por tanto, a una disminución de la irrigación de los músculos, lo que disminuye la disponibilidad del oxígeno en los músculos y agrava la sarcopenia (Morley et al., 2002). Por tanto, la sarcopenia inducida por las citoquinas pro-inflamatorias liberadas por los neutrófilos podría ser la causa de una menor actividad física en las personas mayores frágiles. Sin embargo, el mecanismo molecular exacto necesita ser explorado, dado que otros criterios de Fried, como el agotamiento y la baja velocidad en la marcha, relacionados también con la sarcopenia, no han sido asociados con los porcentajes de neutrófilos.

En la literatura un estado de inflamación crónica de bajo grado también ha sido asociado con las condiciones geriátricas (Comptéa et al., 2015), sin embargo, su relación con los recuentos totales e individuales de leucocitos no se conoce. Por tanto, los resultados de nuestro estudio muestran, y también por primera vez, que el estado de salud general de las participantes, evaluado mediante una batería exhaustiva de escalas validadas en personas mayores, no se correlaciona ni

con los porcentajes de neutrófilos ni con los de linfocitos. Esta falta de correlación, sugiere que los cambios observados en los porcentajes de neutrófilos y de linfocitos no están influenciados por la disminución general de las funciones físicas o psicológicas relacionadas con el propio proceso de envejecimiento. Por tanto, dichos cambios están selectivamente asociados con la fragilidad y con el bajo nivel de actividad física o de fuerza muscular. Asimismo, la falta de asociaciones significativas entre la edad de las participantes incluidas en el estudio y los subtipos de leucocitos o las puntuaciones de fragilidad corroboran este resultado.

Asimismo se sabe que las células del sistema inmune, al influir en múltiples sistemas fisiológicos, son un factor de riesgo para determinadas enfermedades, como por ejemplo las enfermedades cardiovasculares, de manera que, la presencia de comorbilidades podría haber influido en nuestros resultados (Danesh et al., 2000; Danesh et al., 2008; Pinto et al., 2004; Friedman et al., 1974). Sin embargo, tras el ajuste de nuestros resultados por número y tipo de las principales comorbilidades de las participantes de la muestra, tales como la diabetes, las ECV y las inflamatorias, observamos que dichas asociaciones continuaron siendo significativas. Esto sugiere que los cambios en estas subpoblaciones de leucocitos no se asociaron con la presencia de determinadas comorbilidades. Apoyando este resultado, destacamos que las dos participantes del estudio que no cumplieron con ninguno de los cinco criterios de fragilidad, tenían el mismo número de comorbilidades que la mayoría de las mujeres que cumplían con tres de los cinco criterios de fragilidad. Además, ninguna de las mujeres de nuestro estudio, excepto una, presentó porcentajes de linfocitos o neutrófilos fuera del rango fisiológico normal, lo que sugiere que las correlaciones que encontramos tampoco son atribuibles a ninguna otra patología que pueda modificar estos subtipos de leucocitos. Curiosamente, la única participante que presentó los porcentajes de neutrófilos y linfocitos anormales, fue aquella que cumplía los cinco criterios de Fried.

Por último, hay que destacar que no sabemos si, en las participantes de nuestro estudio, el bajo nivel de actividad física y de fuerza muscular es una causa o más bien una consecuencia de la institucionalización. De hecho, la evidencia sugiere que la institucionalización se asocia con un mayor deterioro funcional (Fuente Sanz et al., 2012; Rojas Ocaña et al., 2006). Sin embargo,

el estado de salud de los residentes es muy heterogéneo y, por tanto, es obvio que este deterioro funcional no se produce en la totalidad de la población mayor institucionalizada. Además, los estudios poblacionales realizados en España han demostrado que, si la edad y las comorbilidades son similares, el nivel de actividad física de las personas mayores no difiere en relación al contexto en el que habitan (Fernández-Ballesteros, 1998; Fernández-Ballesteros et al., 1998). También se debe tener en cuenta la presencia de diversas instalaciones y la elevada proporción de personal que existe en los centros privados de atención residencial (por ejemplo, gimnasios, participación en programas de actividad física, animación sociocultural, fisioterapeutas y terapia ocupacional), así como la reducción de las barreras arquitectónicas (por ejemplo, la falta de escaleras) y la prestación de los factores ambientales positivos (por ejemplo, jardines o espacios verdes), factores que pueden influir positivamente y promover la actividad física diaria. Estos hallazgos sugieren que los cambios observados en la actividad física de las mujeres mayores frágiles de nuestro estudio, probablemente, son debidos al deterioro físico observado en el síndrome de fragilidad y no la institucionalización en sí o a los procesos generales de envejecimiento.

Las relaciones entre los porcentajes de neutrófilos y linfocitos con la fragilidad y la baja actividad fisca o baja fuerza muscular sugieren el papel potencial del sistema inmune en la patogénesis de la fragilidad, sin embargo, como hemos comentado previamente, el síndrome frágil se caracteriza por el declive en múltiples e interrelacionados sistemas fisiológicos (Clegg et al., 2013). Por tanto, la alteración de uno de ellos no puede ser independiente de la de los otros. De manera que, el estado inflamatorio que acontece en la fragilidad, no es un fenómeno aislado, sino que está interconectado con el declive en otro u otros sistemas fisiológicos. De hecho, es importante destacar que un estado inflamatorio ha sido relacionado con una disminución de la concentración de vitamina D y, a su vez, ambas situaciones han sido sugeridas como contribuyentes independientes del síndrome de fragilidad (Collerton et al., 2012; Enrusd et al., 2011; Hirani et al., 2013; Leng et al., 2007; Lips, 2006; Van Etten y Mathieu, 2005).

En la actualidad, la deficiencia de vitamina D se considera una epidemia mundial. Afecta a personas de cualquier edad, pero es en las personas de edad avanzada donde su prevalencia alcanza

mayor proporción, especialmente entre las institucionalizadas (Holick, 2007; Mateo-Pascual et al., 2014; Sem et al., 1987; Pilz et al., 2012; Toss et al., 1980). En nuestro país, los diferentes estudios realizados en la población mayor de 60 años muestran un rango de prevalencia de hipovitaminosis (definida como niveles de 25(OH) D_3 en suero < 20 ng/mL(o 50 nM)) entre un 79% y un 86%, incluso llegando a un 91% en personas mayores que viven en residencias (Almirall et al., 2010; Niño y Pérez-Castrillón, 2008; Pérez-Llamas et al., 2008).Otra condición común en las personas mayores institucionalizadas es la fragilidad (Kojima y Tanabe, 2016).

Por otro lado, los diversos estudios que han investigado la relación entre ambas condiciones, las concentraciones de 25(OH) D_3 en suero y el síndrome de fragilidad, muestran resultados heterogéneos (Fernández-Garrido et al., 2014a; Morley et al., 2013; Smit et al., 2012; Wilhelm-Leen et al., 2010). Las diferencias en las poblaciones de estudio, en el tamaño de la muestra, en los métodos para medir la 25 (OH) D_3, en los puntos de corte utilizados para definir la deficiencia de 25 (OH) D_3, en las definiciones de síndrome de fragilidad o la adecuación de ajuste para posibles factores de confusión pueden explicar los resultados discrepantes (Wong et al., 2015). Así, en *el Osteoporotic Fractures in Men Study* realizado en EE.UU. sobre una población de hombres mayores de 65 años residentes en la comunidad, los niveles séricos de 25(OH) D_3 < 20 ng/mL se asociaron de manera independiente con un incremento de la fragilidad (Enrusd et al., 2011). Resultados similares se encontraron en un estudio australiano, donde los hombres de edad avanzada con niveles de 25(OH) D_3 < 16 ng/mL, en relación con aquellos con niveles > 27,6 ng/mL, presentaron un mayor riesgo de fragilidad (Hirani et al., 2013). Wilhelm-Leen et al., (2010) utilizando datos de la *Tercera Encuesta Nacional de Salud y Nutrición* (NHANES III) en EE.UU., encontraron que la deficiencia de 25 (OH) D_3, definida como la concentración sérica < 15 ng / ml, se asoció con un riesgo de fragilidad 3,7 veces mayor entre los participantes blancos y cuatro veces más riesgo en las personas de raza negra. En contraste con estos resultados, Enrusd et al., (2010), en una población de mujeres ≥ 69 años de edad, encontraron una asociación no lineal en forma de U entre los niveles de 25(OH) D_3 en suero y la fragilidad, con el riesgo más bajo entre el grupo mujeres con concentraciones séricas de 25(OH) D_3 entre 20,0 a 29,9 ng / ml. En un estudio utilizando datos

del estudio italiano *InCHIANTI* se observó que los niveles de 25 (OH) D_3 < 20 ng/mL se asociaron con la fragilidad en hombres pero no en mujeres (Shardell et al., 2009). Sin embargo, la mayoría de estos estudios han sido realizados en muestras de personas mayores con un amplio rango de edad (65 o más años) que viven en la comunidad. Por tanto, hasta donde sabemos, no existen estudios acerca de la deficiencia de vitamina D y la fragilidad en las personas mayores institucionalizadas.

En este contexto, nuestros resultados muestran que, en las mujeres mayores institucionalizadas que no recibían suplementos de vitamina D en combinación con calcio, los bajos niveles séricos de 25 (OH) D_3 se asociaron con la fragilidad, de forma que, las participantes frágiles y pre-frágiles en comparación con las robustas presentaron unas concentraciones de 25 (OH) D_3 en suero significativamente más bajas. Estos resultados, por un lado, sugieren un papel potencial de la vitamina D en la fisiopatología de la fragilidad y por otro, ponen de relieve la importancia de evaluar la concentración sérica de vitamina D en los adultos mayores institucionalizados, con el fin de poder ayudar a identificar a las personas frágiles. Sin embargo, el hecho de que los niveles de 25 (OH) D_3 en suero fueran similares entre las mujeres pre-fragiles y frágiles, indica que, en nuestra población seleccionada, el nivel de 25 (OH) D_3 en suero no es un biomarcador para expresar la severidad del síndrome de fragilidad. Corroboran esta hipótesis la ausencia de relaciones estadísticamente significativas entre las concentraciones séricas de 25 (OH) D_3 con las puntuaciones de la escala de fragilidad de Fried. Estos dos últimos resultados ponen de manifiesto la posible existencia de un nivel umbral por debajo del cual, y una vez desencadenada la fragilidad, los niveles de de 25 (OH) D_3 en suero no influyen en la progresión de la fragilidad.

El vínculo entre los niveles de 25 (OH) D_3 y la fragilidad es complejo. Existen varias vías fisiológicas a través de las cuales los bajos niveles de 25 (OH) D_3 en suero aumentan el riesgo de fragilidad incluidos los efectos sobre el hueso, el músculo y la inmunidad. Los efectos de la vitamina D en la salud ósea son bien conocidos (Lips, 2006). Así, una menor densidad mineral ósea ha sido observada en sujetos con deficiencia de vitamina D (Kruavit et al., 2012). Los bajos niveles de 25 (OH) D_3 producen una disminución de los valores plasmáticos de calcio, con el consiguiente hiperparatiroidismo secundario que conduce a la resorción ósea y a la osteoporosis, causando a

su vez un aumento del riesgo de caídas y, por tanto, de fracturas. Estas condiciones, junto con la falta de equilibrio, pueden conducir al miedo a caer, lo que contribuye al sedentarismo, situación que pueden acelerar el proceso de fragilidad (Shardell et al., 2009). Sin embargo, esta vía no es compatible con nuestros resultados, dado que en nuestra muestra, aunque no disponemos de datos sobre la medición cuantitativa del contenido mineral óseo de las participantes, las concentraciones medias de calcio, fósforo y PTH en los tres grupos de mujeres estudiadas, se encontraban dentro del rango fisiológico normal. Este hecho sugiere que, en términos del metabolismo fosfocálcico, las participantes de nuestro estudio no presentaban alteración del metabolismo óseo. Asimismo, tampoco presentaron ni riesgo de caídas ni deterioro funcional (véase valores medios del índice de Tinnetti así como del índice de Barthel para ABVD y Lawton y Brody para AIVD).

Por otro lado, los bajos niveles de 25 (OH) D_3 también pueden conducir a la fragilidad a través de sus efectos sobre la modulación de la respuesta inmune. La vitamina D tiene un efecto antiproliferativo y regula a la baja los marcadores inflamatorios (Lips, 2006). El metabolito activo 1,25- (OH) $_2$ D_3 inhibe la secreción de IL-12 por las células presentadoras de antígeno. Dado que la IL-12 inhibe el desarrollo de las células T helper $CD4^+$ subtipo 1 y estimula el desarrollo de T helper $CD4^+$ subtipo 2, el metabolito activo 1,25- (OH) $_2$ D_3 da como resultado una inhibición de las citoquinas pro-inflamatorias (IL-2, interferón-γ, TNFα, IL-9, IL-22) y una estimulación de las citoquinas antiinflamatorias (IL-3, IL-4, IL-5, IL-10) (Lips, 2006; Van Etten y Mathieu, 2005). En base a esto, es fácil entender que un déficit de vitamina D pueda contribuir al desarrollo y empeoramiento de la fragilidad al promover un estado inflamatorio, pues tal y como indicamos previamente, un estado inflamatorio crónico ha sido asociado de manera consistente con la fragilidad (Hubbard et al., 2009; Leng et al., 2007). Por tanto, aunque en nuestro estudio no hemos analizado la relación entre los niveles séricos de 25 (OH) D_3con los recuentos totales y diferenciales de leucocitos y, por ello, no podemos explicar el papel que desempeña la vitamina D en la patogénesis de la fragilidad a través de su efecto inmunomodulador, parece evidente que las relaciones entre vitamina D, mediadores celulares de la inflamación y la fragilidad deben ser investigadas con más detalle.

Por último, los bajos niveles de 25 (OH) D_3 también pueden afectar a la fragilidad a través de sus efectos sobre la función muscular. La vitamina D regula el desarrollo y la contracción muscular estimulando la síntesis proteica y la absorción de calcio en las células musculares, influyendo así en la masa y fuerza muscular (Gómez de Tejada Romero, 2014). Igualmente, los efectos de los bajos niveles de vitamina D en el músculo esquelético pueden estar mediados por una elevación de las citoquinas pro- inflamatorias, las cuales, tal y como se ha comentado previamente, pueden afectar negativamente a la fuerza y masa muscular (Bautmans et al., 2005; Cesari et al., 2004). Por otro lado, y en consonancia con lo anterior, diversos estudios han demostrado que los suplementos de vitamina D mejoran considerablemente la fuerza muscular, especialmente en la población anciana con hipovitaminosis (Bunout et al., 2006). En base a ello, diversas investigaciones sugieren que la concentración sérica de vitamina D, de manera directa o indirecta, puede desempeñar un papel en el desarrollo de la sarcopenia (Kim et al., 2017; Shardell et al., 2009). La sarcopenia se asocia con una disminución funcional, lo que a su vez puede conducir al desarrollo de la fragilidad (Bruyère et al., 2017). Por tanto, la relación entre el estado de vitamina D y la fragilidad, en parte, puede estar mediada por el desarrollo de la sarcopenia. Sin embargo, en nuestra población seleccionada, esta vía tampoco puede explicar el papel potencial de la vitamina D en el desarrollo de la fragilidad. Puesto que, los estudios que sugieren la implicación de la vitamina D en la fragilidad a través de esta vía fisiológica, han relacionado los bajos niveles de 25 (OH) D_3 en suero con los criterios del síndrome de fragilidad altamente dependientes del sistema muscular como la debilidad muscular, la baja actividad física, agotamiento y la lentitud en la marcha (Gutiérrez-Robledo et al., 2016; Hirani et al., 2013; Shardell et al., 2009). De hecho, las personas con debilidad, baja actividad, agotamiento y lentitud probablemente son físicamente inactivas e incapaces de mantener un equilibrio adecuado de proteínas en los músculos (Jeejeebhoy, 2012; Rosen y Manson, 2010). Sin embargo, en nuestro estudio, aunque el criterio de Fried más representado entre las mujeres pre-frágiles y frágiles fue la baja actividad física (93%), seguido de la lenta velocidad en la marcha (85%) y de la debilidad muscular (71%), ninguno de estos criterios de fragilidad han sido asociados con los bajos niveles séricos de 25 (OH) D_3. Esta falta de asociación, entre las bajas concentraciones de 25 (OH) D_3 en

suero con la aparición de posibles efectos adversos en la función muscular, puede ser debida a la existencia de unos niveles de corte superiores a los establecidos para el mantenimiento de la salud ósea.

Aunque es biológicamente razonable que los bajos niveles de 25 (OH) D_3 en suero pueden contribuir al síndrome de fragilidad, la dirección de la causalidad también podría estar en la dirección opuesta. Las personas frágiles, debido a la inactividad, son más propensas a limitar sus actividades al aire libre, lo que lleva a una disminución de la exposición al sol y, por tanto, a una menor producción de 25 (OH) D_3 (Rolland et al., 2008). Sin embargo, y aunque desconocemos las horas de exposición solar de las participantes del estudio, no hemos encontrado ninguna asociación entre el bajo nivel sérico de 25 (OH) D_3 y baja actividad física (o un menor gasto energético). Igualmente las participantes del estudio deambulaban y eran capaces de realizar AVD (véase valores índice de Barthel y Lawton)

Aunque nuestros resultados sugieren que la vitamina D puede desempeñar un papel en la etiología de la fragilidad, el mecanismo por el cual los bajos niveles de 25 (OH) D_3 se asocian con la fragilidad en mujeres institucionalizadas de edad muy avanzada no ha sido identificado. Se necesitan estudios adicionales, incluyendo estudios longitudinales, con el fin de evaluar con más detalle el papel de la vitamina D sobre la fragilidad o viceversa.

Por otro lado, aunque las mujeres pre-fragiles y frágiles en comparación con las no frágiles presentaron unas concentraciones séricas de 25 (OH) D_3 significativamente más bajas, sorprendentemente, y en contrate con la mayoría de estudios en este campo, éstas se encontraban dentro del rango fisiológico recomendado en los tres grupos de mujeres estudiadas (≥ a 20 ng / ml (o 50 nM)). Únicamente, la *NHANES,* realizada en una población de personas mayores de EE.UU., aportó resultados similares a los nuestros, con unos niveles séricos medios de 25 (OH) D_3 en los participantes frágiles, pre-frágiles y robustos dentro del rango fisiológico recomendado (Smit et al., 2012). Sin embargo, se debe tener en cuenta que, a diferencia de nuestra muestra de estudio, se trataba de una cohorte mixta, más joven (mayores de 60 años) y que vive en la comunidad. El hecho de que el valor medio de los niveles séricos de 25 (OH) D_3 de nuestras participantes no

se encontrase dentro de los niveles catalogados como deficiencia vitamínica, puede explicarse, en primer lugar, por los niveles de exposición a la luz solar. La mayor parte de la vitamina D en el organismo humano se deriva principalmente de la radiación solar (Wong y Flicker, 2015). Aunque no disponemos de las horas de exposición al sol de las participantes, el hecho de que este estudio se llevó a cabo en un área geográfica donde la luz solar es abundante durante todo el año, garantiza que las participantes de nuestra muestra, en comparación con las participantes de los estudios realizados en otros países, tuviesen una mayor exposición solar. Además, las muestras de sangre se recogieron en primavera (abril-mayo), cuando hay una mayor cantidad de luz solar al día y más estímulos para salir al exterior. Igualmente, la latitud del área geográfica donde viven las participantes de la muestra también influye en la capacidad de síntesis de vitamina D por la piel. Latitudes por encima de los 40 grados causan un descenso de la síntesis cutánea de vitamina D, principalmente en invierno, como consecuencia de la inclinación de la radiación ultravioleta (Niño y Perez-Castrillón, 2008). Nuestra región se encuentra a 39.47 grados, lo que explica que las participantes de nuestro estudio presenten, en relación a otros estudios realizados en otros países, una mayor capacidad de síntesis cutánea de vitamina D y, por tanto, unos mayores niveles séricos de 25 (OH) D_3. Asimismo, es posible que la población de personas mayores que vive en la comunidad, y en relación a las personas institucionalizadas, pueden haber disminuido la exposición al sol debido a las dificultades económicas, a la soledad, a la escasa realización de actividades al aire libre o bien, al deterioro funcional y cognitivo asociado a la fragilidad.

En segundo lugar, la dieta también puede jugar un papel importante en este hallazgo. Si bien, en nuestra población no se han recogido datos dietéticos en relación a la ingesta de vitamina D en la dieta, es probable que, en general, los residentes institucionalizados en relación con las personas mayores que viven en la comunidad estén mejor nutridos, ya que las comidas están cuidadosamente planificadas por profesionales (médicos, enfermeras y nutricionistas) con el fin de proporcionar una dieta equilibrada. Esto probablemente asegure una adecuada ingesta de vitamina D. Por contra, los individuos que viven en comunidad pueden tener concentraciones séricas de vitamina D por debajo del nivel recomendado pues podrían no ser capaces de planificar, comprar

o preparar las comidas adecuadamente debido al deterioro funcional y cognitivo asociado a la fragilidad. Además, aunque no lo hemos tenido en cuenta, la ingesta de alimentos fortificados con vitamina D (por ejemplo, leche o yogurt) puede variar entre los países y las personas, dando lugar a posibles variaciones de los niveles de vitamina D en sangre, lo que puede sesgar los resultados (Adams y Hewison, 2010; Rizzoli et al., 2013).

Por otra parte, independientemente de los mecanismos y la dirección de la causalidad, la asociación entre la fragilidad y los bajos niveles séricos de 25 (OH) D_3 se mantuvo estadísticamente significativa después de ajustar por edad, comorbilidad y estado de salud general de las participantes. La mayoría de los estudios indican que los niveles de vitamina D descienden a medida que aumenta la edad (Ginde et al., 2009; Holick, 2007; Mithal et al., 2009). Con la edad disminuye la capacidad de síntesis cutanea de vitamina D, de manera que, a partir de los 50 años hay una reducción del 50% que desciende a un 25% a partir de los 70 años (Fernández Del Buey et al., 2016). Así, para igual dosis de exposición solar, una persona de 70 años produce un 75% menos de vitamina D que un joven de 20 años (Holick, 2007). En contraste con estos resultados, en nuestro estudio, aunque se trataba de una población con una edad muy avanzada (edad media 84 años), los niveles de 25 (OH) D_3 en suero no disminuyeron con la edad. No obstante, en un estudio realizado en Australia sobre una cohorte de hombres entre 70-97 años que viven en la comunidad, encontraron una disminución de los niveles de 1,25- (OH) $_2$ D_3 con el aumento de la edad, la cual no se encontró para los niveles de 25 (OH) D_3 (Hirani et al., 2013). Esto, en parte, es debido a una disminución fisiológica de la eficiencia de la conversión renal de 25(OH) D_3 a la forma activa, 1,25- (OH) $_2$ D_3, que se produce con el envejecimiento, incluso en presencia de niveles séricos de 25(OH) D_3 dentro de los rangos normales (Solvik et al., 1981). Este hallazgo, no nos permite descartar la influencia de la edad en el papel potencial de la vitamina D en el síndrome de fragilidad, al no disponer de los niveles séricos de 1,25- (OH) $_2$ D_3 y, por tanto, sugiere una interesante línea futura de investigación.

Asimismo, la formación renal de 1,25- (OH) $_2$ D_3 no solo puede ser restringida por los procesos generales del envejecimiento sino también, por una función renal alterada o por la presencia de enfermedades crónicas (Hirani et al., 2013). Igualmente, la literatura ha demostrado que la deficiencia

sérica de 25 (OH) D_3 se asocia con un mayor riesgo de enfermedades extraóseas incluyendo cáncer, infecciones, diabetes mellitus tipo 2, enfermedades autoinmunes y cardiovasculares (Aranow, 2011; Pilz et al., 2012). La enfermedad puede ser el comienzo de un círculo vicioso entre el déficit de 25 (OH) D_3 en suero y la fragilidad. La gente enferma sale al exterior con menos frecuencia, lo cual conduce una menor exposición a la luz solar y, por tanto, a una disminución de 25 (OH) D_3 (Pabst et al., 2015). Asimismo, las enfermedades crónicas también se han asociado con el desarrollo de la fragilidad en adultos mayores. La menor actividad a consecuencia de la enfermedad puede ser el comienzo de la fragilidad (Ronland et al, 2012). En base a ello, estudiamos como posibles mediadores de la relación entre la vitamina D y la fragilidad, la presencia de enfermedades crónicas tales como la diabetes mellitus, ECV e inflamatorias. Sin embargo, no se pudo demostrar ningún efecto mediador de esta condición sobre la asociación entre la vitamina D y fragilidad, al no observarse relaciones estadísticamente significativas entre los niveles de séricos de 25 (OH) D_3 y la presencia de dichas enfermedades. Esto sugiere que los niveles séricos de 25 (OH) D_3 tenían un efecto independiente sobre la fragilidad. Pese a ello, es posible que la presencia de otras enfermedades crónicas, no incluidos en este estudio, puedan intermediar la relación, lo que puede llevar a una subestimación del papel de la comorbilidad como un factor que contribuye a la relación de los niveles de séricos de 25 (OH) D_3 con la fragilidad.

Del mismo modo, tampoco el estado de salud general de las participantes, evaluado mediante las escalas de evaluación geriátrica (descritas anteriormente), influyó en la relación entre la fragilidad y la vitamina D, al no observarse relaciones estadísticamente significativas entre las puntuaciones de las escalas incluidas en la evaluación geriátrica y la condición de ser frágil. Sin embargo, aunque en nuestro estudio la reducción en la concentración de vitamina D en la sangre no se asoció con ningún aspecto específicos del envejecimiento, en la literatura se ha sugerido que los bajos niveles de 25 (OH) D_3 en suero pueden disminuir las funciones físicas y cognitivas relacionadas con el propio proceso de envejecimiento, al asociarse de manera significativa con una pérdida de independencia de las AVD y un mayor deterioro cognitivo en las personas mayores no discapacitadas y sin demencia (Di Monaco et al., 2010; Van der Schaft et al., 2013). De hecho, los receptores de

la vitamina D además de encontrarse en el músculo esquelético, también existen en la mayoría de las aéreas del cerebro responsables del desarrollo de la memoria y de las funciones cognitivas (Bischoff et al., 2001; Olsson et al., 2017). Asimismo, el sistema nervioso central (SNC) tiene la capacidad de activar la 1,25-$(OH)_2 D_3$, la forma biológicamente activa de la vitamina D (Annweiler et al., 2010). La 1,25 $(OH)_2 D_3$ en el SNC se asocia con cambios en la producción y liberación de factores neurotróficos, también llamados neurotrofinas (Wrzosek et al., 2013). La familia de las neurotrofinas está formada por el factor de crecimiento nervioso (NGF), la neurotrofina-1 (NT-1), la neurotrofina-3 (NT-3), la neurotrofina-4 (NT-4) y el BDNF. Más concretamente, esta última neurotrofina además de ser responsable del desarrollo, la supervivencia y el mantenimiento de las neuronas (Henderson, 1996; Nurjono et al., 2012 Tapia-Arancibia et al., 2004), se considera el principal mediador molecular de la plasticidad cerebral (Arancio y Chao, 2007; Schinder y Poo, 2000; Tanaka et al., 2008). Por otra parte, el BDNF puede reducir la inflamación en modelos de ratón (Makar et al., 2008), lo que sugiere un papel regulador negativo del BDNF en la inflamación. Sobre la base de estos resultados, y dado que nuestros hallazgos anteriores apoyan el papel potencial de la inflamación y del déficit de la vitamina D en la patogénesis de la fragilidad, parece razonable formular la hipótesis de que la disminución de los niveles de BDNF pueda estar implicada en los mecanismos que conducen a la fragilidad.

Así, y con el fin de analizar el papel potencial de esta neurotrofina en el síndrome frágil, se asociaron las concentraciones plasmáticas de BDNF con los distintos fenotipos de la fragilidad (frágil, pre-frégil y robusto) en una población de personas mayores institucionalizadas, no discapacitadas y sin demencia. En este sentido, nuestros resultados mostraron como los niveles de BDNF en plasma, aunque no se relacionaron con la condición de ser frágil, disminuyeron significativamente con el deterioro funcional y con algunos aspectos de la función cognitiva. Estos hallazgos sugieren que el BDNF podría jugar un papel en la fisiopatología del deterioro funcional y cognitivo en las personas mayores frágiles.

En este estudio hemos optado por medir las concentraciones de BDNF en plasma y no en suero, puesto que la medición plasmática de BDNF probablemente sea la mejor estimación de los niveles

de BDNF periféricos. Se sabe que los niveles medios de BDNF en suero son 100 veces más altos que los niveles plasmáticos (Radka et al., 1996). Esta diferencia se debe a la desgranulación de las plaquetas durante el proceso de coagulación, pues la cantidad de BDNF encontrado en los lisados de plaquetas lavadas es casi idéntica a la observada en el suero (Fujimura et al., 2002; Lommatzsch et al., 2005). Por tanto, la diferencia entre los niveles de BDNF en suero y plasma parece reflejar la cantidad de BDNF almacenada en las plaquetas circulantes. Así, la medición plasmática del BDNF es el mejor reflejo de la situación de su estado estacionario y, en consecuencia, de la cantidad de BDNF biodisponible, al reducir la influencia de la liberación de BDNF a partir de plaquetas (Krabbe et al., 2009).

En la literatura los bajos niveles de BDNF han sido asociados con la fragilidad. Así, Coelho et al. (2012), en una población de mujeres de edad avanzada que viven en la comunidad, encontraron que los niveles circulantes de BDNF fueron significativamente menores en las mujeres pre-frágiles en comparación con las mujeres no frágiles. Del mismo modo, Inglés et al. (2016), en una cohorte de 2.488 individuos del *Estudio Toledo para el Envejecimiento Saludable*, obtuvieron resultados similares entre los sujetos frágiles y no frágiles. No obstante, en ninguno de los dos estudios anteriores, se observaron diferencias significativas en los niveles de BDNF entre las personas frágiles y pre-frágiles. Este hallazgo puede explicar el motivo por el cual, en nuestro estudio, los niveles plasmáticos de BDNF no se asociaron con ninguno de los fenotipos de fragilidad, dado que un elevado porcentaje de la población estudiada fue clasificada como frágil (79%) o como pre-frágil (21%) y tan solo un 10% como no frágil. Igualmente, este hecho, a su vez, puede justificar que las concentraciones plasmáticas de BDNF de nuestro estudio (mediana: 73,26 pg/ml) fuesen inferiores a las obtenidas en otros estudios previos llevados a cabo en adultos mayores sin demencia o deterioro cognitivo grave (Krabbe et al., 2009; Pereira et al., 2013). Igualmente, se ha sugerido que las concentraciones plasmáticas de BDNF disminuyen significativamente con la edad (Krabbe et al., 2009; Lommatzsch et al., 2005; Tapia-Arancibia et al., 2008; Yasui-Furukori et al., 2013; Ziegenhorn et al., 2007). Hecho que podría explicar los menores valores de BDNF hallados en nuestro estudio. Sin embargo, aunque la mediana de edad de nuestros participantes fue de 83

años (rango intercuartil 78-89), ésta no se correlacionó con los niveles de BDNF en plasma. Por tanto, esta falta de asociación apoya la hipótesis de que los bajos niveles de BDNF observados en nuestro estudio podrían ser atribuidos a la alta condición de fragilidad de nuestros participantes.

A diferencia de lo observado con la fragilidad y la edad, los niveles de BDNF en plasma se correlacionaron con el sexo, de manera que los hombres en comparación con las mujeres presentaron unos mayores niveles plasmáticos de BDNF, hecho que contrasta con los resultados de otros estudios previos que muestran el efecto contrario (Krabbe et al., 2009). Esta diferencia de género aunque podría estar justificada, en parte, por los cambios hormonales que experimentan las mujeres con la edad, ya que el estradiol puede inducir la expresión de BDNF (Frye y Rhodes, 2005; Gibbs, 1999; Sohrabji y Lewis, 2006), la mujer más joven de nuestra muestra tenía 62 años de edad y, por tanto, todas ellas eran posmenopáusicas y además ninguna estaba recibiendo terapia de reemplazo hormonal. De acuerdo con nuestros resultados, Bus et al. (2012a) encontraron una disminución en los niveles sistémicos de BDNF con la edad, pero sólo en las mujeres. Por lo tanto, no se debe descartar una diferencia de género en la liberación de BDNF a la circulación sanguínea.

Otra posible explicación de la diferencia de género en los niveles periféricos de BDNF podría ser la asociación recientemente propuesta entre la depresión y la disminución de las concentraciones de BDNF (Molendijk et al., 2014; Pereira et al., 2013), asociación que incluso puede extenderse a la gravedad de los síntomas de la depresión (Gervasoni et al., 2005). Igualmente, Lang et al. (2004) asociaron los bajos niveles de BDNF con rasgos de personalidad relacionados con la depresión. Es posible que estos hallazgos pueda estar relacionados con el hecho de que las mujeres posmenopáusicas a menudo muestran un mayor comportamiento depresivo (MacQueen y Chokka, 2004). Precisamente, los resultados de nuestro estudio mostraron, aunque no de manera significativa, que las mujeres en relación con los hombres obtuvieron las puntuaciones más altas en la escala de Yesavage (escala de depresión geriátrica ad hoc) y, por tanto, una mayor tendencia a la depresión que los hombres, hecho que puede explicar los menores niveles de BDNF observados en las mujeres de nuestra muestra. Sin embargo, el hecho de que nuestra muestra no fuese seleccionada específicamente para estudiar el papel de BDNF en la depresión, es probable

que varios factores tales como la heterogeneidad de la medicación psicotrópica, la duración de los diferentes esquemas de tratamiento antidepresivos, así como la presencia de síntomas depresivos no diagnosticados y, por lo tanto, la ausencia de tratamiento antidepresivo adecuado, al no estar adecuadamente controlados pueden haber afectado significativamente los resultados estadísticos.

Dado que varios estudios previos han demostrado que la concentración de BDNF en sangre disminuye durante el envejecimiento (Krabbe et al., 2009; Lommatzsch et al., 2005; Tapia-Arancibia et al., 2008; Yasui-Furukori et al., 2013; Ziegenhorn et al., 2007), es posible que la reducción de los niveles plasmáticos de BDNF puede correlacionarse con algunos aspectos específicos del mismo. En base a ello, analizamos las posibles asociaciones entre los niveles de BDNF en el plasma y las escalas de evaluación geriátrica incluidas en VGI (descritas anteriormente). En este sentido, nuestros resultados mostraron una correlación positiva entre los niveles plasmáticos de BDNF y el índice de Barthel para las ABVD y una correlación negativa con la subescala de concentración de la prueba MMSE.

La novedosa correlación entre los niveles de BDNF en plasma y el índice de Barthel para las ABVD de nuestros participantes sugiere que los bajos niveles plasmáticos de BDNF se asocian con una mayor dependencia funcional en las personas mayores. Tal vez, este hallazgo esté vinculado con la asociación también observada entre los bajos niveles de BDNF en plasma con la subescala de concentración del MMSE. De hecho, aunque existen otras fuentes potenciales de BDNF circulante tales como las células inmunes, células endoteliales y células musculares lisas, las neuronas se consideran la principal fuente celular de BDNF periférico (Donovan et al., 1995; Kerschensteiner et al., 1999; Lommatzsch et al., 1999; Nakahashi et al, 2000; Noga et al., 2003; Papathanassoglou et al., 2014). Asimismo, diversos estudios han mostrado que el BDNF puede cruzar la barrera hematoencefálica en ambas direcciones. Este hallazgo sugiere que las mediciones de los niveles de BDNF sangre reflejan los niveles de BDNF del tejido cerebral y que el BDNF periférico podría influir en el cerebro, desencadenando una mayor plasticidad neuronal y contribuyendo así no sólo a una mejora de la función cognitiva sino también, a una mejora del estado funcional (Klein et al., 2011; Pan et al., 1998; Sartorius et al., 2009). Pues, varios estudios han correlacionado de

manera positiva el deterioro cognitivo asociada con la edad con diversas limitaciones funcionales, de manera que, la disminución de la capacidad cognitiva en personas de edad avanzada afecta a su capacidad para realizar las AVD, produciéndose así una pérdida de la independencia y una necesidad constante de ayuda (Giovannetti et al., 2008; Sitjas Molina et al., 2003). Por tanto, aunque todavía hay controversia en cuanto a la relación entre el deterioro cognitivo y el déficit funcional, es posible que los cambios en los procesos de plasticidad cerebral causen este declive funcional (Mahncke et al., 2006). Así, dado que el BDNF se asocia con la plasticidad neuronal y un mejor funcionamiento cognitivo en la vejez (Calero y Navarro, 2007), y considerando la interdependencia entre el envejecimiento cognitivo y funcional, nuestros hallazgos sugieren que el BDNF de algún modo está relacionado con un mayor nivel de independencia y autonomía funcional en la vejez. En consecuencia, los bajos niveles plasmáticos de BDNF pueden estar asociados con un mayor riesgo funcional, lo cual es especialmente útil, puesto que el deterioro funcional en las personas mayores es predictivo de malos resultados de salud y de mortalidad, independientemente de su diagnóstico (Trigas-Ferrín et al., 2011).

Varios estudios anteriores han demostrado que los niveles de BDNF en sangre están asociados con el deterioro cognitivo en las personas mayores (Komulainen et al., 2008; Shimada et al., 2014). Igualmente, las altas concentraciones séricas de BDNF parecen ser un factor de protección contra la enfermedad de Alzheimer, como se muestra en un estudio longitudinal de gran alcance realizado en el marco de la *Framingham Heart Study* (Weinstein et al., 2014). Asimismo, existe evidencia de que muchas funciones cerebrales relacionadas con la cognición son dependientes del BDNF y, puesto que éste es un importante mediador molecular de la plasticidad cerebral (Arancio y Chao, 2007, Bird y Burgess, 2008; Schinner y Poo, 2000; Tanaka et al., 2008), parece razonable afirmar que las funciones cognitivas óptimas están ligadas a una plasticidad neuronal eficiente (Tapia-Arancibia et al., 2008). Sin embargo, aunque la plasticidad cerebral se produce durante toda la vida, ésta disminuye con la edad (Tapia-Arancibia et al., 2008; Burke y Barnes, 2006). Por tanto, aunque con una amplia variabilidad interindividual, la mayoría de las personas mayores sin demencia generalmente muestran un declive gradual en las funciones cognitivas (Cabras, 2012).

Concretamente, las funciones cerebrales asociadas con la corteza o el hipocampo, dos regiones altamente plásticas y cruciales para el aprendizaje y la memoria, son las más susceptibles al deterioro de la función cerebral relacionado con la edad (Lee et al., 2012; Lister y Barnes, 2009). En este contexto, investigamos la posible asociación entre los niveles plasmáticos de BDNF con los resultados de las pruebas del MMSE. Curiosamente nuestro análisis mostró una relación positiva y significativa entre las concentraciones plasmáticas de BDNF y la subescala de concentración del MMSE. Asimismo, numerosos estudios han sugerido que el trastorno depresivo en las personas adultas está asociado con déficits cognitivos, en particular con aquellos relacionados con el dominio de la atención (Hasselbalch et al., 2012; Talarowska et al., 2015). En línea con estos resultados, nuestro estudio muestra una correlación significativa entre la escala de Yesavage y la subescala de la concentración del MMSE, pero no con las otras subescalas del MMSE. Esto significa que los sujetos del estudio con problemas de concentración podrían tener un trastorno subyacente del estado de ánimo o al menos algunos síntomas depresivos, los cuales son altamente prevalentes en la población geriátrica (Sjöberga et al., 2017).

Otro de los hallazgos relevantes del presente estudio es la relación observada entre los niveles plasmáticos de BDNF y los recuentos de eosinófilos. Curiosamente, esta asociación significativa tuvo lugar dentro del intervalo fisiológico normal de los recuentos de eosinófilos. Aunque, no podemos descartar que otras células sanguíneas como los leucocitos, incluyendo los linfocitos, macrófagos y monocitos, así como las plaquetas pueden actuar como fuentes potenciales de BDNF periférico, nuestros resultados, como mínimo, sugieren que los eosinófilos de la sangre pueden ser una fuente de producción de BDNF circulante (Amadio et al., 2016; Barouch et al., 2001; Fujimura et al., 2002; Kerschensteine et al., 1999; Rost et al., 2005). Hasta la fecha, sólo un único estudio anterior ha demostrado que este subtipo de leucocitos es capaz de producir, almacenar y liberar a la circulación periférica esta neurotrofina (Noga et al., 2003). Asimismo, dado que los eosinófilos son las principales células que median los procesos inflamatorios alérgicos, diversos estudios han observado un aumento de los niveles de BDNF circulante en sujetos con enfermedades alérgicas, lo que sugiere un papel regulador del BDNF en la inflamación (Bonini et al., 1996; Marka et al.,

2008; Namura et al., 2007; Noga et al., 2001; Raap et al., 2005). La inflamación está fuertemente asociada con la fragilidad. Por tanto, es sensato pensar que estos niveles periféricos de BDNF puedan estar vinculados con la fragilidad.

Los efectos del BDNF periférico en las células no neuronales todavía siguen sin estar claros (Fujimura et al., 2002). No obstante, se ha descrito que el BDNF circulante a nivel del músculo esquelético está implicado en el desarrollo y diferenciación de los mioblastos y fibras musculares así como, en la regulación de la supervivencia de las neuronas motoras, la liberación presináptica de neurotransmisores y el mantenimiento de la región postsináptica de las fibras esqueléticas (Raschke y Eckel, 2013; Sakuma et al., 2015). Asimismo, un estudio reciente demostró que el ejercicio aeróbico induce un aumento de los niveles periféricos de BDNF y como este aumento se correlacionó con un incremento de la fuerza muscular (Tsai et al., 2015). En este contexto, nuestros resultados mostraron una correlación positiva y significativa entre los recuentos de eosinófilos y la baja fuerza muscular, uno de los cinco criterios de fragilidad de Fried. Esta asociación sugiere que el BDNF liberado por los eosinófilos puede estar involucrado en la actividad muscular y, dado que la fragilidad se caracteriza por una pérdida de masa y fuerza muscular, es posible que unos menores recuentos de eosinófilos puedan estar relacionados con los mecanismos implicados en el síndrome de la fragilidad. Por tanto, la influencia de los recuentos de los eosinófilos sobre la fuerza muscular en los sujetos frágiles podría ser un tema interesante para futuras investigaciones.

Existe un creciente interés científico por el posible papel del BDNF en la regulación metabólica. Recientemente, el BDNF se ha implicado en la regulación del apetito, el metabolismo y la homeostasis energética (Fargali et al., 2012; Rothman et al., 2012). En este sentido, nuestros resultados muestran una correlación inversa y significativa entre el BDNF plasmático y los niveles de CT y HDL-c. Asimismo, aunque no de manera significativa, los niveles de BDNF en plasma se asociaron positivamente con los niveles de LDL-c e inversamente con TGD. Estos hallazgos apoya el papel potencial del BDNF en el metabolismo de los lípidos y están en consonancia con los resultados de otros estudios en los que el BDNF circulante se correlaciona con los niveles de TGD, CT y LDL-c (Golden et al., 2010; Jung et al., 2011). No obstante, a pesar de las correlaciones observadas en

nuestro estudio, no podemos explicar ninguna conclusión sobre la naturaleza específica de estas asociaciones. Sin embargo, es posible que esta neurotrofina pueda estar relacionada con algún aspecto fisiopatológico de la ECV, o que la disminución de los niveles de BDNF en plasma puede representar una respuesta compensatoria a una ECV subyacente. De hecho, se ha informado que los bajos niveles de BDNF circulante están asociados con un aumento de la incidencia de eventos cardiovasculares (Jiang et al., 2011; Pikula et al., 2013). Así, el BDNF plasmático, aunque en nuestro estudio no se presenta como un biomarcador candidato de la fragilidad, podría ser un marcador viable de las comorbilidades metabólicas que a menudo se asocian con la fragilidad.

A pesar de que nuestros hallazgos proporcionan un mayor conocimiento sobre los mecanismos subyacentes que contribuyen al desarrollo de la fragilidad en las personas de edad muy avanzada institucionalizadas en centros residenciales, éstos resultados deben ser interpretados con precaución, ya que nuestro estudio presenta varias limitaciones.

En primer lugar, el tamaño de la muestra. Sin embargo, en nuestra opinión, el tamaño de nuestra muestra es suficiente por tratarse de un estudio piloto cuya finalidad es crear futuras líneas de investigación en este campo. Los estudios piloto son importantes a fin de evitar errores significativos antes de implementar estudios a gran escala; su objetivo es poner a prueba la viabilidad y obtener datos preliminares que se pueden utilizar para diseñar una investigación relevante a gran escala, económica y estadísticamente adecuada (Thabane et al., 2010; Van Teijlingen y Hundley, 2002). Sin embargo, el hecho de que el diseño, el contenido y los resultados de la mayoría de los estudios piloto permanezcan inéditos es, en nuestra opinión, lamentable por dos razones. La primera, porque su publicación puede evitar que otros investigadores comentan errores metodológicos similares, y, por tanto, desperdiciar los escasos recursos disponibles en la investigación. Y la segunda, porque también puede evitar a otros investigadores tener que evaluar la viabilidad de determinados aspectos de los estudios propuestos.

Otra limitación del estudio es que nuestra muestra no es representativa de toda la población de adultos mayores institucionalizados, lo que limita la generalización de nuestros resultados. Por un lado, en nuestro país, las residencias de personas mayores son muy heterogéneas. Existen centros

públicos y privados con diferentes recursos humanos y materiales. Por lo tanto, para generalizar las conclusiones acerca de la fragilidad en esta población, sería necesario estudios que incluyan todo tipo de instituciones. En este sentido, los centros incluidos en nuestro estudio son centros privados con altos recursos humanos (geriatra, enfermeras, fisioterapeutas, terapeutas ocupacionales, trabajador social) y materiales (centro de día, gimnasio, unidad especial para residentes enfermos). Por otro lado, se debe tener en cuenta que los adultos mayores institucionalizados son una población heterogénea, que incluye tanto adultos mayores válidos como dependientes (Rica-Escuín et al., 2014). La utilización de los criterios de Fried, como método de evaluación de la fragilidad, al exigir la exclusión de aquellas personas con deterioro cognitivo (véase valores MMSE), que no deambulen o incapaces de realizar las AVD (véase valores índice de Barthel para ABVD e índice de Lawton y Brody para AIVD), características de la mayor parte de la población institucionalizada con este rango de edad, no solo limitó el tamaño de nuestra muestra, sino que también, la redujo a aquellas personas institucionalizadas con buena salud general. Es importante destacar que este subgrupo de personas institucionalizadas representa el 25-30% del total personas residentes en centros para mayores (alrededor de 600.000 personas en España) (MSSI, 2015). Sin embargo, esta limitación podría entenderse como una fortaleza del estudio, ya que la evidencia sugiere que el deterioro fisiológico asociado a la fragilidad empieza a ser evidente en una fase preclínica (Fernández-Garrido et al., 2014a). Así, la identificación de marcadores hematológicos específicos del síndrome de fragilidad en la población institucionalizada con buen estado de salud, ayudaría a detectar de manera precoz a personas frágiles o que pueden estar en riesgo de serlo, con la consiguiente posibilidad de poner en marcha intervenciones para enlentecer su progresión y prevenir sus complicaciones. Asimismo, el hecho de que los sujetos estudiados presenten características homogéneas, al incluir solo a adultos mayores válidos, asegura una menor dispersión en los resultados.

Aunque la fragilidad se ha conceptualizado como un síndrome geriátrico multidimensional (Walston et al., 2006), nosotros la hemos evaluado mediante los criterios propuestos por Fried et al. (2001), criterios muy vinculados a la condición física. Sin embargo, la escala Fried, de acuerdo

con numerosos estudios observacionales y clínicos, tanto nacionales como internacionales, es el instrumento más aceptado y usado en el ámbito investigador, lo que nos permite realizar comparaciones entre diversos estudios (Abizanda et al., 2011; Avila Funes et al., 2009; Ble et al., 2006; Gill et al., 2006; Ottenbacher et al., 2009; Szanton et al., 2009). Asimismo, ha demostrado un buen criterio y validez de constructo en diferentes cohortes de personas de edad avanzada (> de 65 años) (González-Vaca et al., 2013). Igualmente, posee varias ventajas que la hacen, desde nuestro punto de vista, adecuada para el ámbito clínico. Pues, se trata de una herramienta objetiva, breve y simple, al emplear medidas estandarizadas y de fácil manejo (García- García et al., 2010).

Finalmente, al tratarse de un estudio de sección transversal nos impide el establecimiento de la dirección de la asociación, es decir, no nos permiten distinguir si los cambios observados en los biomarcadores son una causa o más bien consecuencia de la fragilidad. Igualmente, la naturaleza transversal del estudio se opone a la determinación de la temporalidad de los marcadores hematológicos y la fragilidad, y por tanto, las asociaciones observadas pueden deberse a relaciones no causales (por ejemplo, factores de confusión no medidos). No obstante, en la actualidad, nuestro grupo de investigación F.R.O.G. está trabajando en la realización de diversos estudios de carácter longitudinal o de seguimiento, con el fin de poder conocer el valor predictivo de dichos biomarcadores sanguíneos en la detección del síndrome de fragilidad.

Sin embargo, pensamos que estas limitaciones no deben restar importancia a los resultados obtenidos, ya que éstos contribuyen a mejorar el conocimiento de los mecanismos fisiopatológicos subyacentes al desarrollo del síndrome frágil y de las características de las personas mayores frágiles, a la vez que apuntan las diferencias en la expresión y consecuencias de la fragilidad entre hombres y mujeres en las personas mayores institucionalizadas.

CAPÍTULO IV: CONCLUSIÓN

"Conclusión es el lugar donde llegaste cansado de pensar"

Anónimo

CONCLUSIÓN

Por último, aunque conscientes de las limitaciones de nuestro estudio, los resultados obtenidos nos permiten sacar conclusiones interesantes acerca de la existencia de posibles biomarcadores de fragilidad en las personas mayores institucionalizadas:

1 Se necesitan herramientas simples y fiables que permitan identificar de forma estandarizada a las personas mayores frágiles. Más allá de los tests de cribado tradicionales, en ocasiones difíciles de realizar o a veces poco estandarizados, parámetros analíticos tan habituales como los porcentajes de neutrofilos y linfocitos e incluso algo tan simple como la concentración sérica de vitamina D, pueden ser utilizados como biomarcadores sanguíneos para distinguir a las personas frágiles de los no frágiles. Las personas frágiles en relación con las no frágiles presentan unos porcentajes de neutrofilos más altos, unos menores porcentajes linfocitarios y unos niveles de vitamina D en suero más bajos. Si bien la utilización de estos parámetros analíticos no puede sustituir, de momento, a los índices de fragilidad tradicionales, su inclusión en los mismos mejorarían su exactitud diagnóstica, al proporcionan información adicional a la que se obtiene a partir de los datos clínicos, lo cual ayudaría a la identificación de la fragilidad, incluso de manera precoz. Además, su monitorización permitiría evaluar la progresión de la fragilidad o la respuesta a las intervenciones aplicadas.

2 La asociación de estos parámetros analíticos con el síndrome de fragilidad sugiere la existencia de una disfunción a nivel del sistema inmune y hormonal, lo que confirma, en parte, la desregulación multisistémica característica del síndrome de fragilidad.

3 La identificación de estos biomarcadores sanguíneos de fragilidad contribuye a mejorar el conocimiento de los mecanismos fisiopatológicos subyacentes al desarrollo de la fragilidad, al sugerir un papel potencial de la vitamina D, así como de los neutrofilos y linfocitos en la patogénesis de la fragilidad. Igualmente, las correlaciones

entre las alteraciones en estas subpoblaciones leucocitarias con dos de los cinco criterios de fragilidad descritos por Fried et al. (2001), la actividad física y la fuerza muscular, proporciona información útil para establecer posibles vínculos comunes entre alteraciones físicas específicas y los mecanismos específicos responsables del desarrollo de la fragilidad.

4 Integrar biomarcadores en la investigación de enfermería resulta inprescindible para ofrecer cuidados de calidad a la población general. La investigación de biomarcadores asociados a la fragilidad no solo contribuye a su identificación, sino también, nos permite conocer los mecanismos biológicos que contribuyen a su aparición y desarrollo. Del conocimiento de estos mecanismos se desprende la forma específica de tratar la fragilidad. En base a esto último, y dado que la enfermería tiene un papel relevante en el cuidado de las personas mayores institucionalizadas, la incorporación de biomarcadores de fragilidad en la investigación enfermera proporciona una poderosa herramienta para desarrollar o adaptar intervenciones de enfermería adecuadas para tratar, prevenir e incluso revertir el síndrome de fragilidad. Además, la medición de dichos biomarcadores al inicio y después de recibir la intervención nos permitirá evaluar la efectividad de la misma. Así, y conforme a los resultados obtenidos, en este grupo de población, se debe diseñar programas de actividad física o de actividades para evitar o disminuir la repercusión de situaciones estresantes como por ejemplo, la práctica de mindfullness, yoga, meditación, etc., con la finalidad de mejorar el funcionamiento del sistema inmune. Asimismo, para obtener las dosis recomendadas de vitamina D se debe garantizar un consumo adecuado de vitamina D, así como fomentar la exposición a la luz solar mediante la realización de actividades al aire libre. La asociación de la deficiencia de vitamina D con la fragilidad, sugiere que la administración de vitamina D podría ser útil para el tratamiento del síndrome de fragilidad, sin embargo, el hecho de que las concentraciones séricas medias de vitamina D en los tres grupos estudiados se encontrase dentro del rango fisiológico recomendado para dicha población, y dado que la vitamina

D es liposoluble, es necesario que la suplementación con vitamina D sea pautada de manera individualizada, pues su administración en cantidades suprafisiológicas puede conducir a efectos secundarios importantes.

5 En las personas mayores institucionalizadas los niveles plasmáticos de BDNF al asociase con las puntuaciones del índice de Barthel para ABVD y de la subescala de concentración de la pruebe MMSE, si bien no permiten identificar a las personas mayores frágiles, contribuyen a mejorar el conocimiento de los mecanismos fisiopatológicos del deterioro funcional y cognitivo asociado al envejecimeinto y, por tanto, a desarrollar o evaluar la efectividad de intervenciones de prevención o promoción de estilos de vida más saludables. Los niveles de BDNF en plasma aumentan con el ejercicio, por lo tanto, el diseño de programas de actividades físicas está posicionado para mejorar la condición física y la función cognitiva en las personas mayores.

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ANEXOS

ANEXO A: Artículo 1

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The value of neutrophil and lymphocyte count in frail older women

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ABSTRACT

Increasing evidence suggests that systemic inflammation is associated with many pathophysiological processes including frailty in older adults. We evaluated the relationships between white blood cell subtypes, geriatric assessment, and frailty syndrome and in particular, how they correlate with individual frailty criteria (involuntary loss of weight, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity) in frail older women. There was a significant and positive correlation between the frailty score and neutrophil count, but a significantly negative correlation was found when this score was compared to the lymphocyte count. These associations were significant only for two frailty criteria: poor muscular strength and low physical activity. Further investigation into the role of white blood cell subtypes in ageing and its associated adverse outcomes in older adults is warranted, in particular in the loss of muscular strength and for poor physical activity.

1. Introduction

Frailty is a geriatric syndrome describing the physical and functional decline that occurs as a consequence of certain diseases (e.g., cancer, chronic infection, etc.) but which can also occur in the absence of disease. Frailty is characterised by an increased risk of poor outcomes such as a higher incidence of falls or fractures, and increased disability, comorbidity, health care expenditure, and premature mortality (Fried et al., 2001; Fugate-Woods et al., 2005). The concept of frailty has grown in importance because of a need for a better understanding of research into the health trajectory of older people (Wyman and Henly, 2011) and a need to prevent, or at least to delay, the onset of late-life disability (Fried et al., 2001). On the basis of data derived from large cohorts of elderly individuals, Fried et al. offered an operational definition of frailty, incorporating the assessment of five specific criteria: weight loss, exhaustion, weak grip strength, slow walking speed, and low physical activity (Fried et al., 2001).

The aetiology of frailty is not well understood but it has been associated with changes in several physiological systems, including inflammation, coagulation, haematological, and endocrine systems, as well as affecting micronutrients and vitamins (Walston et al., 2002). White blood cells (WBCs) are an important cellular component of systemic inflammation, and high total WBC counts are associated with an increased risk of disability and mortality (de Labry et al., 1990; Furlan et al., 2013; Gillum et al., 1993; Grimm et al., 1985). Increases in neutrophil and decreases in lymphocyte counts have been associated with five-year mortality in community-dwelling older women (with moderate to severe disability) recruited to the Women's Health and Aging Studies (WHAS I; Leng et al., 2005), and a higher total WBC count is associated with an increased prevalence of frailty in community-dwelling older women (Leng et al., 2007). Moreover, one study found that an increase in neutrophil and monocyte counts, but not in lymphocyte counts, was positively associated with frailty although the association was not significant (Leng et al., 2009). However the correlation of white blood cells with frailty is controversial (Collerton et al., 2012; Desquilbet et al., 2007; Gibson et al., 2009; Semba et al., 2005). Three previous studies found no association, although these had relatively small sample sizes (De Fanis et al., 2008; Semba et al., 2005) and/or excluded men (Leng et al., 2009; Semba et al., 2005).

A study in the Women's Health and Ageing cohort reported an association with frailty and a low CD4/CD8 T-lymphocyte cell ratio but with a high $CD8^+/CD28^-$ lymphocyte subpopulation count (Semba et al., 2005). De Fanis et al. (2008) found an association with frailty and low CD4 as well as high CD8 T-cell counts. In the Multicentre Aids Cohort, low CD4 T-cell counts in 245 HIV-infected men were independently predictive of a frailty-related phenotype (Desquilbet et al., 2007). In contrast to these studies, Collerton et al. (2012) did not find any association between the CD4/CD8 T-lymphocyte ratio and frailty, as assessed by Fried criteria, although they did with the total lymphocyte count (Collerton et al., 2012). Hence a thorough evaluation of different

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WBC counts in frailty is necessary and will also shed light on the role of these cells and inflammatory phenotypes in this condition. Whether an association exists between differential WBC counts and individual frailty criteria has not yet been explored, but would ultimately allow possible links between specific physical alterations and common pathophysiological changes to be suggested.

Identifying differential WBC counts as biomarkers for single physical frailty criterion could be very helpful in pinpointing the specific mechanisms responsible for the development of frailty and may help tailor treatments or prevention strategies, or to promote healthier lifestyles in older individuals.

In this pilot study we addressed three main objectives:

(1) Evaluation of the relationship between individual WBC counts and each of the five frailty criteria.
(2) Evaluation of the relationship between individual WBC counts and other parameters on geriatric assessment scales.
(3) The relationship between individual WBC counts and other analytical parameters (erythrocytes, platelets, haemoglobin, glucose, urea, uric acid, cholesterol, triglyceride, creatinine, glutamic-oxaloacetic and glutamic-pyruvic transaminases [GOT and GPT], and C-reactive protein).

Frailty was assessed by Fried criteria. Geriatric assessment was evaluated by validated scales: the Tinetti gait and balance index to determine the risk for falls, the Norton scale of pressure ulcer assessment, the mini-mental score examination test (MMSE), behavioural assessment using the neuropsychiatric inventory questionnaire (NPI-Q), the Yesavage scale for geriatric depression, and the Barthel index to measure daily living and mobility activities. The clinical history was also carefully analysed in order to find evidence for any chronic inflammatory diseases which might be responsible for changes in WBC counts.

2. Materials and methods

2.1. Study population

A cross-sectional pilot study was performed in women with a residential profile, who were institutionalised in private long-stay centres for the elderly in the province of Valencia (Spain) in 2013 (*GeroResidencias La Saleta*, Valencia). The inclusion criteria were: ability to get up from a chair and walk 6 m and an age of 65 years or older. Exclusion criteria were: dementia, major psychiatric disease (schizophrenia, bipolar disorders, etc.) or blindness; acute infections or known cancer. Forty-two women, belonging to four centres, participated in the study. This research was compiled according to the requirements of the Declaration of Helsinki, and the entire study protocol was approved by the local ethical committee. Written informed consent was obtained from all participants. We measured the sociodemographic characteristics, and the five frailty criteria (involuntary weight loss, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity), and did geriatric assessments using the Tinetti index, MMSE test, Yesavage scale, NPI-Q questionnaire, Barthel index, and Norton index. The type and number of comorbidities, and the number of medications were also recorded.

2.2. Measurement of frailty criteria

Frailty was measured by evaluating the presence or absence of five measurable characteristics of the frailty syndrome (Fried criteria; Fried et al., 2001) which were defined and evaluated as follows:

1) Weight loss. Weight loss was defined as the unintentional loss of 4.5 kg or more in the past year.
2) Self-reported exhaustion. Exhaustion was indicated by subjects providing a positive response to either of two statements from the Center for Epidemiologic Studies—Depression Scale (Orme et al., 1985): "I felt that anything I did was a big effort at least three to four days a week," and "I felt that I could not keep on doing things at least three to four days a week." when referring to the past week. Or if the participant answered "Often" or "Most of the time" to the question "How often in the last week did you feel that everything you did was an effort?" also included in the Centre for Epidemiologic Studies—Depression scale (Radloff, 1977). All exhaustion criteria were considered as present.
3) Low physical activity (PA) level. Low PA was quantified by using the Spanish adaptation of the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) re-adapted for women (Elosua et al., 1994, 2000; Ruiz-Comellas et al., 2012). The MLTPAQ was administered by a trained interviewer who was provided with detailed instructions and a list of very clearly defined PAs. The participants were given a list of suggested activities and asked to mark those that they performed during the last year. To avoid memory bias as far as possible, the activities performed during the last week were collected first, followed by those performed in the last month, last quarter, and finally the last year, always including the former periods. For validation purposes only the information referring to the last year was used. Total energy expenditure from leisure time PA (EEPA) was then calculated based on this questionnaire and was used to quantify PA (Jacobs et al., 1993).
   This criterion was defined as participants who performed no physical activity, spent most of the time sitting, or rarely had a short walk (or other non-demanding physical activity).
4) Slowed motor performance. Slowness was based on the 4–6 metre walking speed test, adjusted for sex and height according to the standards of the Short Physical Performance Battery (Guralnik et al., 1994). A low walking speed was considered to correspond to the worst quintile for the sex and height of the group.
5) Weakness. To assess weakness, strength was measured with a Jaymar hydraulic dynamometer, according to the standards of the Hispanic Established Populations for the Epidemiologic Studies of the Elderly (Ottenbacher et al., 2002).

2.3. Measurement of haematology and biochemistry markers

Fasting blood tests were performed in the morning at the time of enrolment. White blood cells, haemoglobin concentration, erythrocytes, and platelets were measured on automated instruments at local haematology laboratories near each field centre. Biochemistry analyses included glucose, urea, urate, cholesterol, triglyceride, creatinine, and GOT and GPT measurements in serum. Blood serum was obtained by collecting 5 mL of blood in a BD Vacutainer tube and centrifuging it at 500 *g* for 10 min.

2.4. Statistical analysis

Bivariate correlations among variables were evaluated using the Pearson correlation test. Univariate and multiple (i.e., fully adjusted and stepwise backward methods) logistic regression analyses were performed to specify the association between changes in WBC counts and individual frailty criteria or values obtained in each of the geriatric assessment scale measurements. The number of chronic inflammatory diseases was used as potential confounders. P-values less than 0.05 were considered as statistically significant. All statistical analyses were performed using SPSS (version 15.0; SPSS, Inc., Chicago, IL).

3. Results

3.1. Study population, frailty score, geriatric assessment and comorbidities

All participants were Caucasian, all were non smokers, 45% of them were widows. Mean age (±SD) of the participants was 84.2 ± 6.5.

The Fried's criterion more represented in the sample was poor physical activity (91%) followed by slow walking speed (88%), weakness (69%), exhaustion (28%) and weight loss (13%). Two women did not meet any frailty criterion according to the Fried scale. Age and geriatric assessment scales of all study participants included in this study are summarised in Table 1. Frailty score was not significantly associated with age (Pearson r = 0.22, p = 0.23) nor with any of the geriatric assessment scales (Tinetti gait index: r = −0.14; p = 0.44; Tinetti balance index: r = −0.23; p = 0.21); Barthel index to measure the activities of daily living and mobility (r = 0.11; p = 0.54); mini-mental score examination test (MMSE) for cognitive impairment (r = 0.14; p = 0.44); Yesavage scale for geriatric depression (r = 0.33; p = 0.08); and Norton scale for pressure ulcer assessment (r = −0.31; p = 0.09).

The type and number of comorbidities and number of medication are shown in Table 2. Frailty score was not significantly associated with the number or type of comorbidity (Pearson r = 0.23, p = 0.20) or with number of medications (Pearson r = 0.13, p = 0.48).

3.2. Evaluation of the relationship between individual WBC counts with frailty's criteria

There was a significant and positive relationship between the number of fulfilled frailty criteria with neutrophil count (Pearson r = 0.38, R squared = 0.144 p < 0.05) (Fig. 1A) and a significant negative correlation with lymphocyte count (Pearson r = −0.37, R squared = 0.140 p < 0.05) (Fig. 1B). In order to assess whether these changes were within the normal range we analysed neutrophil and lymphocyte counts in the entire population and in the subgroup of participants with different severity of frailty syndrome. Neutrophil and lymphocyte counts were within the normal range and no significant differences were observed between groups (Table 3). The only participant that displayed neutrophil and lymphocyte count outside the normal range was the only one who fulfilled the 5 Fried criteria. An analysis of the correlation between frailty score and monocytes, eosinophil, basophil showed no significant differences (monocytes: Pearson r = −0.07, R squared = 0.144; eosinophil: Pearson r = 0.027, R squared = 0.001; and basophil: Pearson r = −0.007, R squared = 0.001 Fig. 2A–C).

3.3. Evaluation of the relationship between erythrocytes and platelets count with frailty's criteria

No significant correlation was observed between frailty score and platelet count (Pearson r = −0.06, R squared = 0.004, Fig. 3A) or erythrocyte count (Pearson r = 0.013, R squared = 0.017, Fig. 3B) or haemoglobin concentration in the blood (Pearson r = 0.112, R squared = 0.013, Fig. 3C). The mean values of platelet and erythrocyte counts were within normal interval (platelet count: 218 ± 13.7 10^3/μL (normal range; 130–450 10^3/μL); erythrocyte count: 3.75 ± 0.09 10^6/μL (normal range (3.7–5.2 10^6/μL). In contrast mean haemoglobin concentration was lower than the normal range: 11.3 ± 0.3 g/dL (normal range 12–16 g/dL).

Table 1
Age of participants enrolled in the study and results of geriatric scale assessment. Mean ± standard deviation and range for each value/scale. Geriatric assessment was evaluated by validated scales: Tinetti gait and balance index to determine the risk for falls, Norton scale of pressure ulcer assessment, mini-mental score examination test (MMSE), behavioral assessment—neuropsychiatric inventory questionnaire (NPI-Q), Yesavage scale for geriatric depression, Barthel index to measure the activities of daily living and mobility, Lawton index for instrumental activities of daily living.

	Mean value	Range
Age (years)	84.2 ± 6.5	70–99
Tinetti balance index	8.1 ± 4.5	0–14
Tinetti gait index	6.4 ± 3.7	0–12
Norton	16.5 ± 2.2	13–20
MMSE	28.3 ± 5.6	17–35
NPI-Q	2.9 ± 4.5	0–20
Yesavage	3.4 ± 3.3	0–9
Barthel index	65.0 ± 25.7	25–100
Lawton index	4.2 ± 1.4	2–7

Table 2
Distribution of the most common comorbidities expressed as percentage of subjects having the disease and mean number of daily medications.

Comorbidities and medications	
Diabetes (%)	14
Hypertension (%)	38
Congestive heart failure (%)	7
Hiperlipidemia (%)	28
Obesity (%)	3
Inflammatory disease (%)	35
Number of all comorbidities (mean ± SD)	4.1 ± 1.6
Number of medications (mean ± SD)	7.3 ± 3.2

3.4. Correlation between individual WBC counts with each of the five criteria of frailty

There was no statistical significant correlation between neutrophil count and hand grip strength although a clear tendency appeared (Pearson r = −0.342, R squared = 0.1, p = 0.05, Fig. 4A). There was an inverse significant correlation between neutrophil count and total energy expenditure from leisure time physical activity (EEPA) (Pearson r = −0.37, R squared = 0.18, p < 0.05, Fig. 4B). There was a significant positive correlation between lymphocyte count and hand grip strength (Pearson r = 0.38, R squared = 0.15, p < 0.05, Fig. 4C). There was a significant positive correlation between lymphocyte count and total energy expenditure from leisure time physical activity (EEPA) (Pearson

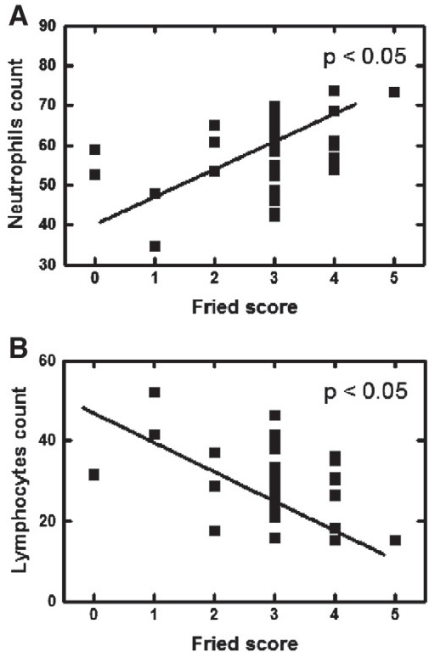

Fig. 1. Correlation between neutrophil and lymphocyte counts and frailty. Frailty was measured according to the Fried criteria as described in Materials and methods. Neutrophil (×10^3/μL) (A) and (×10^3/μL) lymphocyte (B) counts were measured in the blood and they were plotted against the Fried score for each participant enrolled in the study. The level of significance is indicated in each panel. N = 42.

Table 3
Values of neutrophil and lymphocyte counts (expressed as % of total white blood cells) in the entire population and in the population classified as non frail (0 Fried criteria), prefrail (1 or 2 Fried criteria) and frail (3, 4 or 5 Fried criteria).

	Neutrophil count	Lymphocyte count
Entire population	57.2 ± 1.6 Normal range (40–60)	29.4 ± 1.6 Normal range (20–40)
Non frail (Women with 0 Fried criteria)	55.9 ± 3.3	31.7 ± 0.2
Prefrail (Women with 1 or 2 Fried criteria)	52.4 ± 5.3	35.0 ± 5.3
Frail (Women with 3,4 or 5 Fried criteria)	58.3 ± 1.5	28.1 ± 1.6

$r = 0.3$, R squared $= 0.1$, $p < 0.05$, Fig. 4D). No significant correlation was observed between neutrophil or lymphocyte counts and the exhaustion or walking speed (data not shown). All lymphocyte or neutrophil counts in the studied population were within the interval of physiological range. The unintentional weigh loss criterion was met by only 3 women (13% of the sample) thus no statistical correlations were performed.

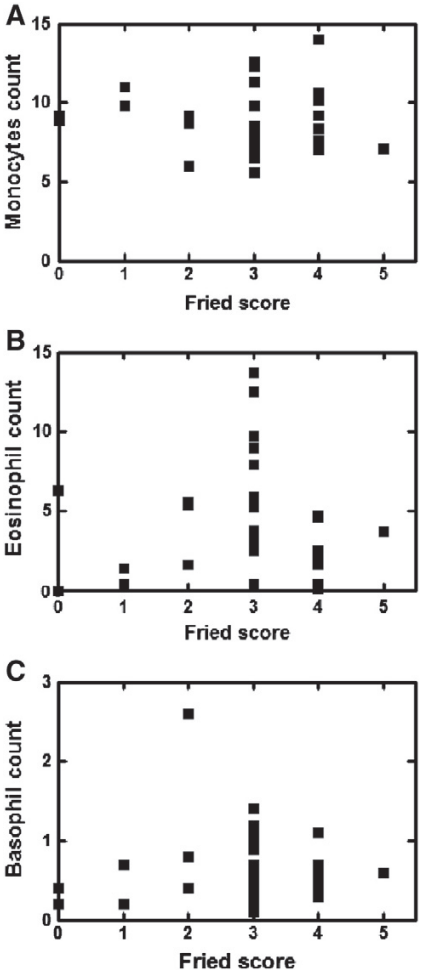

Fig. 2. Correlation between monocyte, eosinophil and basophil counts and frailty. Frailty was measured according to the Fried criteria as described in Materials and methods. Monocyte ($\times 10^3/\mu L$) (A), eosinophil ($\times 10^3/\mu L$) (B) and basophil ($\times 10^3/\mu L$) (C) counts were measured in the blood and they were plotted against the Fried score for each participant enrolled in the study. None of the correlations were significant. N = 42.

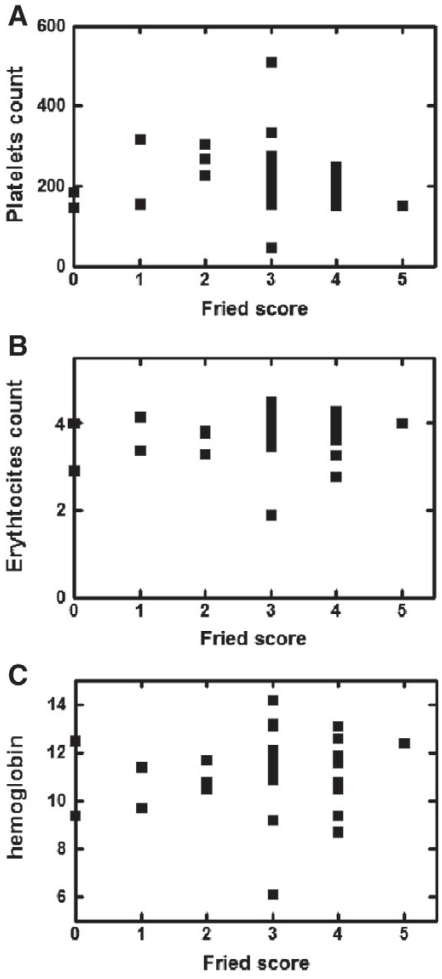

Fig. 3. Correlation between platelet and erythrocyte counts and haemoglobin concentration and frailty. Frailty was measured according to the Fried criteria as described in Materials and methods. Platelet counts ($\times 10^3/\mu L$) (A), erythrocyte counts ($\times 10^6/\mu L$) (B), and haemoglobin concentration (g/dL) (C) were measured in the blood and they were plotted against the Fried score for each participant enrolled in the study. None of the correlations were significant. N = 42.

Adjustment for number and type of comorbidities did not modify the significant associations found between neutrophil or lymphocyte counts and poor physical activity or poor muscular strength (data not shown).

3.5. Evaluation of the relationship of individual WBC counts with other parameters of geriatric assessment scales

No significant correlations were observed between neutrophil or lymphocyte count and each the scales of the geriatric assessment: Tinetti gait and balance indexes to determine the risk for falls (Tinetti gait index: $r = -0.23$; $p = 0.21$ for neutrophil counts and $r = 0.14$; $p = 0.45$ for lymphocyte count; $p = 0.778$; Tinetti balance index: $r = -0.13$; $p = 0.49$ for neutrophil counts and $r = 0.01$; $p = 0.98$ for lymphocyte count); Barthel index to measure the activities of daily living and mobility ($r = 0.50$; $p = 0.78$ for neutrophil counts and $r = -0.11$; $p = 0.55$ for lymphocyte count); Mini-mental score examination test (MMSE) for cognitive impairment ($r = 0.14$; $p = 0.45$ for

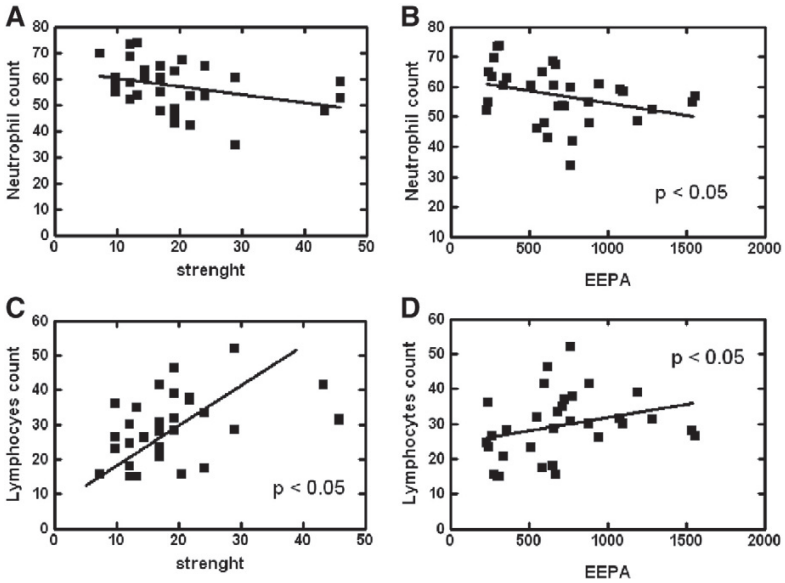

Fig. 4. Correlation between neutrophil or lymphocyte counts and muscular strength or physical activity. Frailty was measured according to the Fried criteria as described in Materials and methods. Muscular strength was measured by assessing the hand grip strength with a dynamometer as described in Materials and methods. Physical activity was measured as energy expenditure from leisure time physical activity (EEPA) as described in Materials and methods. Neutrophil ($\times 10^3/\mu L$) (A–B) and lymphocyte ($\times 10^3/\mu L$) (C–D) counts were measured in the blood and they were plotted against muscular strength (A–C) or physical activity (B–D) for each participant enrolled in the study. Level of statistical significance is indicated in each panel. N = 42.

neutrophil counts and r = −0.10; p = 0.57 for lymphocyte count); Yesavage scale for geriatric depression (r = −0.17; p = 0.35 for neutrophil counts and r = 0.21; p = 0.25 for lymphocyte count); Norton scale for pressure ulcer assessment (r = −0.20; p = 0.28 for neutrophil counts and r = 0.12; p = 0.52 for lymphocyte count). No significant correlations were observed between neutrophil or lymphocyte count and the age (r = 0.26; p = 0.16 for neutrophil counts and r = −0.32; p = 0.08 for lymphocyte count).

3.6. Evaluation of the relationship of frailty score with other analytical parameters

In blood samples we measured typical biochemical parameters such as haemoglobin, glucose, urea, urate, cholesterol, triglyceride, creatininine, GOT and GPT transaminases, C-reactive protein (CRP) in order to find any correlation with frailty score. No correlation was observed between frailty score and most biochemical parameters measured in the blood (glucose: r = 0.10; p = 0.59; urea: r = −0.2; p = 0.92; uric acid: r = 0.17; p = 0.37; creatinine: r = 0.10; p = 0.58; cholesterol: r = 0.50; p = 0.80; triglycerides: r = 0.30; p = 0.86; and transaminase GOT: r = 0.20; p = 0.28; transaminase GPT: r = 0.15; p = 0.42). The only exception concerned the concentration of CRP that was significantly and positively correlated with frailty score (Pearson r = 0.39, R squared = 0.15, p < 0.05). However no significant correlations were observed between CRP values and the individual Fried criterion.

4. Discussion

The new and significant findings in this report include the evidence that two physical frailty criteria described by Fried et al. (2001) are significantly associated with neutrophil and lymphocyte counts. Of note, these changes take place within the physiological range of the two WBC subpopulations. Low physical activity was significantly associated with higher neutrophil and with lower lymphocyte counts, whereas poor muscular strength (measured by assessing hand grip strength) was significantly correlated with lower lymphocyte counts. However, one study found that an increase in neutrophils and monocytes, but not lymphocytes, is positively associated with the frailty score of community-dwelling older women with a broad age range and high prevalence of functional disability (Leng et al., 2009). To the best of our knowledge, the relationship between individual WBC counts and single frailty criterion has not yet been explored. Our results show that the lymphocyte count is significantly and positively correlated with both hand grip strength and physical activity. These two measurements directly or indirectly reflect deterioration of muscular activity which is traditionally involved in the physiopathology of frailty syndrome (Evans et al., 2010; Marzetti et al., 2012; Rolland et al., 2008). A principle component of age-related weakness and frailty in women is sarcopenia (Kamel, 2003). This decrease in skeletal muscle mass is a progressive syndrome that affects the quality of elderly women's lives by decreasing their ability to perform many activities. The mechanism by which increased lymphocyte counts are positively correlated with better muscular strength and higher physical activity in older women is likely to be complex, however, increased lymphocyte counts have been positively associated with physical exercise in young people (Nielsen, 2003; Pedersen and Toft, 2000; Tauler et al., 2003); although this association has not been previously reported in older women with low physical activity such as in our sample group, these parallels probably share common physiological pathways.

Low lymphocyte counts either in the pathological or physiological range have also been associated with an increased risk of mortality in different pathologies (Heffernan et al., 2012; Reddan et al., 2003), and have been connected to an increased mortality risk in older people without any apparent disease (Bender et al., 1986; Izaks et al., 2003). It has been argued that a decrease in immune surveillance or the occurrence of replicative senescence can cause this negative association between lymphocyte count and mortality risk (Miller, 2001). According to this theory, a low lymphocyte count is an indicator of a general decline in physiological functions that eventually causes death. Clearly

the research field needs to be widened and the role and relationships between lymphocytes, muscular function, sarcopenia, and mortality clearly need to be explored in more detail.

Poor physical activity that we found to be positively associated with lymphocyte counts also appears to be significantly and inversely associated with neutrophil counts. In this case a general explanation can be easily postulated since neutrophils produce oxidation metabolites, cytokines, and free radicals that cause oxidative damage to multiple tissues including muscles and organ systems, and these processes are associated with frailty (Hubbard et al., 2009; Leng et al., 2004; Wu et al., 2009) and an increased risk of mortality (Babior, 1978; Fridovich, 1978). This may also provide a plausible explanation for the significant and positive correlation between neutrophil counts and both muscular strength and physical activity, although the exact molecular mechanism still needs to be elucidated, especially given that other frailty criteria such as fatigue or slow walking speed are unrelated to lymphocyte or neutrophil counts. In addition none of the women in our study, except one, showed lymphocyte or neutrophil counts outside the physiological range, suggesting that the correlations we found are not attributable to any pathology that modifies these individual WBCs. Only one participant showed abnormal neutrophil and lymphocyte counts and interestingly she was also the only one who fulfilled all five Fried criteria. Fifteen women enrolled in the study had chronic inflammatory diseases: general osteoarthritis (11 women), osteoarthritis of the knee (2 women), polymyositis (1 woman), and rheumatoid arthritis (1 woman). Adjusting the data to account for the presence of inflammatory and/or cardiovascular diseases or diabetes did not significantly change the correlations with lymphocyte or neutrophil counts and thus does not explain the correlations observed between these WBC subtypes and poor muscular strength or low physical activity.

Neutrophil and lymphocyte counts do not correlate with any of the geriatric evaluation criteria which comprise an exhaustive battery of validated scales represented by the Tinetti gait and balance index to determine the risk for falls, the Norton scale of pressure ulcer assessment, the mini-mental score examination test (MMSE), the neuropsychiatric inventory questionnaire (NPI-Q), the Yesavage scale for geriatric depression, or the Barthel index for measuring the activities of daily living and mobility. This lack of correlation suggests that the changes in neutrophil and lymphocyte counts are selectively related to muscular strength and low physical activity rather than to a general decline of physical or psychological functions due to ageing processes. Corroborating this result, no significant associations were found between age and WBC subtypes or frailty scores. Finally one important conclusion from our study is the association between lymphocyte or neutrophil counts within the normal physiological range and the reduction of physical activity and muscular strength in frail older women. Follow-up studies should evaluate if these two markers might be useful either in the prevention of these conditions or in other aetiological studies.

Other studies have shown that subclinical cardiovascular diseases are associated with frailty (Chaves et al., 2005; Newman et al., 2001), although it is known that cellular mediators of inflammation influence multiple physiological systems. Albeit this pilot study was designed to evaluate the relationship between WBC subtypes and each frailty criterion, according to the Fried criteria (Fried et al., 2001), we adjusted our findings according to the number and type of major comorbidities in our sample such as diabetes, cardiovascular and inflammatory disease, because there may be important intermediary factors in these associations. We found the same significant associations after such adjustments suggesting that they were not directly due to the presence or to the number of chronic diseases. Supporting this finding, two participants that did not fulfil any frailty criteria had the same number of comorbidities of the majority of women fulfilling three frailty criteria (data not shown). However these results should be interpreted with caution since the main limitation of our study is the small sample size although in our opinion, it was sufficient for a pilot study and to create future research directions in this interesting field. Pilot studies are important in order to avoid significant errors before implementing large scale studies; they aim to access feasibility, and to obtain preliminary data that can be used to design a relevant, economical and statistically adequate large scale study (Thabane et al., 2010; van Teijlingen and Hundley, 2002). However, the fact that the design, content, and results from most pilot studies remain unpublished is, in our opinion, unfortunate for two reasons. 1) The feasibility of the published data may prevent other researchers from making similar methodological mistakes, and thus wasting scarce research resources. 2) Making pilot study results available may allow others to avoid having to assess the feasibility of particular aspects of their proposed studies.

Despite the limitations in sample size and the fact that causality could not be determined, our results show a significant association between lymphocyte and neutrophil counts and two frailty criteria: low physical activity and hand grip strength. The study of frailty based on the Fried criteria has been extensively investigated in both living at home and institutionalised older individuals. We do not know whether reduced physical activity and muscular strength are the causes or rather the consequence of institutionalised living but they could clearly contribute to the increase of frailty defined as a state of reduced physiologic reserve associated with increased susceptibility to disability that is not manifested in the same way or with the same intensity by all institutionalised individuals as shown in our results. Population studies performed in Spain have demonstrated that no significant differences emerge when the amount of physical activity performed by older individuals living at home is compared with those that are institutionalised if the age and comorbidities of the two groups are similar (Fernández-Ballesteros, 1998; Fernández-Ballesteros et al., 1998). Such findings suggest that the changes we observed in physical activity in frail older women are likely due to the physical deterioration observed in frailty syndrome rather than to the institutionalisation itself or general ageing processes. It also should be kept in mind that the presence of various facilities and a high personnel ratio in private institutionalised centres (e.g. gyms, physiotherapists, participation in physical activity programmes, sociocultural animation, and occupational therapy) as well as a reduction in architectural and physical barriers, (e.g. the lack of stairs) and provision of positive environmental factors (e.g. gardens or green spaces) might in fact positively influence and promote daily physical activity.

We hope that these preliminary findings will help other researchers to shed some light on the molecular mechanisms underlying different components of frailty syndrome and will provide a basis for further investigation into the underlying inflammatory mechanisms that contribute to frailty in older women. We hope that this will facilitate the development of potential immunological interventional strategies in the future in order to improve muscular function and physical activity in frail individuals.

Conflict of interest statement

No conflict of interest has been declared by the authors.

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ANEXO B: Artículo 2

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Brain-derived neurotrophic factor correlates with functional and cognitive impairment in non-disabled older individuals

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ABSTRACT

We used a complete battery of geriatric and psychometric tests to evaluate whether plasma-borne brain-derived neurotrophic factor (BDNF), a master molecule in neuroplasticity, is associated with the severity of functional and cognitive impairment in non-disabled older individuals. There was a significant positive correlation between BDNF plasma concentrations and the Barthel index, a measurement of the ability of individuals to perform the activities of daily living ($p = 0.03$) and the concentration subcategory measured with the mini mental state examination (MMSE) test ($p = 0.01$). Furthermore, plasma BDNF inversely and significantly correlated with the blood eosinophil count ($p = 0.01$), the total cholesterol concentration ($p = 0.04$), and high-density lipoprotein cholesterol ($p = 0.04$). However, BDNF did not correlate with any other socio-demographic or clinical characteristics, other analytical parameters measured in the blood, or any other geriatric assessment scales. Our results suggest that BDNF may play a role in the pathophysiology of functional impairment in the elderly and in some aspects of cognitive function. However, more studies are needed to understand the relationship between circulating BDNF and functional impairment to determine if BDNF represents a candidate biomarker for this type of cognitive impairment.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a protein encoded by the BDNF gene which belongs to the neurotrophin family. It is produced inside the brain by different cell types and can be transported outside the brain through the blood–brain barrier (Iughetti et al., 2011; Mowla et al., 2001; Pan et al., 1998). BDNF has a protective effect on neuronal differentiation and growth during development and in neuronal survival and maintenance in adulthood (Henderson, 1996; Tapia-Arancibia et al., 2004), and is considered to be a master molecular mediator of brain plasticity (Arancio and Chao, 2007; Schinder and Poo, 2000; Tanaka et al., 2008). Several clinical studies have shown alterations in blood BDNF concentration in patients with neuropsychiatric disorders such as major depression (Karege et al., 2002; Shimizu et al., 2003), schizophrenia (Toyooka et al., 2002), and Alzheimer disease (Peng et al., 2005; Tapia-Arancibia et al., 2008). In addition, numerous physiological and environmental factors can increase the expression of BDNF, i.e. learning (Jones et al., 2006), living in an enriched environment (Brown et al., 2003), physical exercise (Van Praag et al., 1999), food restriction (Lee et al., 2002; Seroogy et al., 2002), long-term antidepressant drug treatment (De Foubert et al., 2004), and estrogen exposure (Matsuki et al., 2014). In contrast, stress (Givalois et al., 2001; Jacobsen and Mork, 2006), weight gain (Lommatzsch et al., 2005), and peripheral hormones such as glucocorticoids (Parque et al., 2015) downregulate its expression, and BDNF plasma concentrations also naturally decrease with age (Lommatzsch et al., 2005; Tapia-Arancibia et al., 2008). Moreover, Krabbe et al. (2009) showed that decreased plasma BDNF differentially increases the risk of mortality in women depending on their functional status, education, morbidity, and low-grade inflammation (but independently of age), further suggesting the involvement of this neurotrophin in the disease networks and multi-morbidity associated with the elderly. Recently, Coelho et al. (2013) suggested that circulating BDNF could somehow be linked to the phenotype of frailty in old age. Frailty has been defined as a multidimensional geriatric syndrome characterized by a decline in physiological reserves which results in increased vulnerability and a reduced ability to cope with stress factors (Fried et al., 2001). It is a common syndrome in older adults with a prevalence of frailty at around 11% (Collard et al., 2012) and prefrailty at around 40–50% (Fernández-Garrido et al., 2014) in community-dwelling adults aged 65 years or over.

Because BDNF can cross the blood–brain barrier in both directions, and given its crucial role in brain function and that several reports show that blood concentrations of BDNF decrease during aging, we hypothesized that reduced BDNF concentrations in blood might correlate

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with the severity of cognitive and functional impairment in older individuals.

The aging process in older individuals is very heterogeneous, but several aspects of aging-related impairments are linked to changes in different physiological systems. It is possible that the reduction in the BDNF concentration in blood is associated with some specific aspects of aging and does not necessarily reflect a general impairment in functional and cognitive functions. Therefore in this study we aimed to separately analyze the associations, if any, between blood BDNF concentrations and several other parameters related to functional and cognitive impairment, frailty, and neuropsychiatric symptoms in older individuals, which are included in the complete geriatric assessment.

Frailty was measured according to the five criteria proposed by Fried: involuntary weight loss, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity (Fried et al., 2001). The Barthel index was used as a tool to quantify functional impairment, and geriatric and psychological assessments were performed using a scale validated for older individuals: the Tinetti gait and balance index to determine the risk of falls, the Norton scale for pressure-ulcer risk, the mini-mental score examination test (MMSE) to assess cognitive impairment, and the Yesavage scale for geriatric depression. Moreover, we also explored the relationship between these tests and blood analytical parameters in order to find any alterations related to the concentration of BDNF.

2. Materials and methods

2.1. Design and study population

A cross-sectional study was conducted between 2013 and 2014 in older non-disabled individuals with a residential profile, who were institutionalized in one of the five long-stay centers for the elderly in the province of Valencia, Spain (GeroResidencias La Saleta, Valencia). Frailty was assessed using the Fried scale (Fried et al., 2001); we selected residents of either gender, aged 60 years or more, and with the ability to get up from a chair and walk six meters. Participants were excluded if they presented dementia, major psychiatric disease (schizophrenia, bipolar disorders, etc.), blindness, acute infections, or known cancer.

We measured frailty syndrome (using Fried criteria) in each participant. The functional, mental, and structural conditions of the participants were evaluated using the following validated geriatric assessment scales: the Barthel index, Lawton and Brody scale, MMSE, Yesavage scale, Tinetti Index, and Norton Index. We measured several hematological and biochemical parameters including blood plasma BDNF concentrations, as well as socio-demographic characteristics including the number of medications taken, and the type and number of any comorbidities.

2.2. Ethical considerations

According to the requirements of the Declaration of Helsinki, written consent was obtained from all of the selected subjects before beginning the study, after having informed them about the procedures involved and the purpose of the research. The whole study protocol was approved by the local ethical committee at the University of Valencia.

2.3. Measurement of frailty criteria

Frailty was measured according to the five Fried criteria (Fried et al., 2001), as previously reported by our group (Fernández-Garrido et al., 2014). Briefly the criteria were assessed as follows: 1) Unintentional body weight-loss (5% or 4.5 kg or more in the last year). 2) Self-reported chronic fatigue: they met the criteria if they answered "A few times", "Often", or "Most of the time" to the question "How often in the last week did you feel that everything you did was an effort?", included in the Center for Epidemiologic Studies depression scale (Radloff, 1977). 3) Low physical activity levels were measured using the Spanish adaptation of the Minnesota leisure-time physical activity questionnaire (Elosua et al., 1994, 2000; Ruiz-Comellas et al., 2012). 4) According to the standards of the short physical-performance battery (Guralnik et al., 1994), participants who walked 4.6 m in a longer time than the worst quintile of the gender and height-adjusted sample fulfilled the reduced walking-speed criterion. Our values were: men taller than 173 cm: ≥6 s, height <173 cm: ≥7 s; women taller than 159 cm: ≥ 6 s, height <159 cm: ≥ 7 s, 5) To measure muscle weakness, grip strength (Kg) was measured three times for each hand alternately with a hydraulic dynamometer (Jaymar) according to the standards of the Hispanic established-populations for epidemiological studies of the elderly (Ottenbacher et al., 2002). Participants were considered fragile if they met at least three criteria and prefrail if they met one or two. All measurements were performed by trained members of the Department of Nursing at the University of Valencia, using a questionnaire with detailed instructions.

2.4. Geriatric assessment

Functional status was evaluated using the Barthel index and Lawton and Brody scale. The Barthel index defines the ability to perform the basic activities of daily living (Mahoney and Barthel, 1965) and measures the level of independence in the following 10 items: feeding, bathing, dressing, grooming, defecating, urinating, toilet use, transfers (e.g. from armchair to bed), walking, and climbing stairs. The index has a score range 0–100, where 0 is total dependence and 100 corresponds to total independence. The ability to perform the instrumental activities of daily living was assessed using the Lawton and Brody scale (Lawton and Brody, 1969) which evaluates 8 items (ability to: use the telephone and transport systems, manage their own medications and money, and to do shopping, cooking, housekeeping, and laundry) and assigns them a value of 1 (independent) or 0 (dependent). The final score is the sum of all the response values. It ranges from 0 (full dependency) to 8 (total independence).

The MMSE test and Yesavage scale were used to assess the mental state of the participants. The MMSE test was used to detect cognitive impairment; it evaluates different items grouped into five sections: orientation, immediate memory, attention and calculation, delayed recall, and language and construction; it has a score range of 0–30 and the highest scores indicate better performance (Folstein et al., 1975). The Yesavage scale quantifies depressive symptoms in older adults; it consists of 15 items with a dichotomous response (yes or no) pattern. One point is given to each response suggestive of a depressive episode. An overall score of five or more indicates depression (Sheikh and Yesavage, 1986).

Finally the structural state was evaluated using the Norton index and Tinetti index. The Norton scale is used to measure the risk of developing pressure ulcers (Norton, 1987). It measures five items (general condition, mental state, activity, mobility, and incontinence) with a severity scale of 1 to 4; the lower the score, the higher the risk. Fall-risk was evaluated with the Tinetti index; it assesses balance and gait according to how the specific actions are implemented. The maximum score is 12 for gait and 16 for balance and the sum of both scores gives the risk of falls, which is high when the score is lower than 19 (Tinetti et al., 1986).

2.5. Brain-derived neurotrophic factor measurement and analytical parameters

Blood samples were obtained from each subject between 7:30 a.m. and 10 a.m. in the morning to avoid hormonal fluctuations and their potential influence on BDNF levels. Ten ml blood was collected was collected into two BD Vacutainer tubes containing EDTA. After extraction, the blood samples were allowed to stand for 15 min and were centrifuged at 1500 rpm for 10 min at room temperature. Subsequently the plasma supernatants were aliquoted and stored at −20 °C until analysis. After thawing, the samples were centrifuged at 1500 rpm for 10 min at

room temperature to completely remove all of the cells. The plasma samples were kept at −80 °C and were analyzed up to 3 months after collection. The plasma concentration of BDNF was measured using a commercial enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (ab99978; BDNF Human ELISA Kit, Abcam®) with minor modifications. Before measuring BDNF in plasma samples we conducted a pilot study in order to determine if the concentrations of BDNF were within the typical range of standard curve according to manufacture' instructions. In light of the values of BDNF obtained we used the range of standard curve between 20 and 2000 pg/mL for the determination of BDNF in our samples. All values of BDNF in our sample were included in this range. Calculation of BDNF concentration in the samples was determined by non-linear regression from standard curve include in each microplate. The assay sensitivity threshold was found at 23 pg/mL. The sample's coefficient of variance ranged between 2.6–7.1%. In order to evaluate the intra-assay variance we analyzed two samples on two different plates on the same day. The resulting coefficients of variance were 0.1 and 2.9% which were both below the level specified by the manufacturer. Measurements were performed in duplicated and averaged to give a mean value for each sample and expressed in pg/mL. Low and high concentration quality control pools were prepared by adding 1 and 100 ng to 5 mL aliquots of human serum. High affinity 96-well plates were used and the resulting absorbance was read in duplicate using a microplate reader (LT-5000MS Elisa reader, Labtech) at 450 nm. To minimize assay variance all the measurements were conducted on the same day and the results were expressed as the concentration of BDNF in pg/ml.

For all other analytical determinations, the residential center control blood extractions were used. Blood was obtained in the morning (after 8–10 h fasting) by collecting 10 mL of blood into two BD Vacutainer tubes. Hematological parameters (white blood cells, hemoglobin, erythrocytes, and platelets) and biochemical parameters (glucose, urea, urate, cholesterol, triglycerides, creatinine, glutamic oxaloacetic transaminase [GOT], and serum glutamic pyruvic transaminase [GPT]) were measured in clinical laboratories belonging to local public health centers.

2.6. Other measurements

Socio-demographic variables were collected using an interview based on a structured questionnaire, which was elaborated and completed by our research staff in the residential centers. This questionnaire included questions about age, gender, marital status, and education level. Clinical data (comorbidity, medication, and physical parameters) were provided by residential center staff from the medical records.

2.7. Statistical analysis

Descriptive statistics, including measurements of central tendency (median) and measures of dispersion (interquartile range) were used to describe all the quantitative variables. The normal distribution of each variable was estimated with the Kolmogorov–Smirnov test. Given that none of the variables were normally distributed, correlation analysis was done using the Spearman correlation coefficient. Linear regression analysis was used to specify the association between changes in the plasma concentration of BDNF and any variables which were significant in the previous analysis. All the statistical analyses were also performed taking covariates due to potential variance i.e. gender, age, body mass index, and educational level into account. BDNF values were controlled for basic covariates in all the analyses. Bonferroni correction was used for multiple comparisons. The non-parametric Mann–Whitney U test was performed to verify any possible differences in BDNF levels in participants in relation to psychotropic medication and gender. Statistical significance was set at $p < 0.05$ and statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Evaluation of the relationship between plasma brain-derived neurotrophic factor levels and demographic, frailty score, and medical variables

A total of 75 subjects, 84% women and 16% men, belonging to five residential care centers located in the Valencia province (Spain) were enrolled in the study. All the participants enrolled were Caucasian and approximately 67% were widows or widowers. Their age ranged from 61 to 99 years and the median age was 83 years (interquartile range: 78–89). With regard to BDNF plasma levels, the median values were 64 pg/mL (interquartile range: 42.10–64.00). According to the Fried scale 79% of the subjects were classified as frail (3, 4, or 5 Fried criteria) and 21% as prefrail (1 or 2 Fried criteria). The median body-mass index (calculated from weight and standing height) was 28.05 kg/m^2 (interquartile range: 24.22–32.80). In relation to the number of daily medications the participants took, 54.9% of them consumed ten or more medications daily, with a median of 10 (interquartile range: 6–13). The results also showed high comorbidity, with median of 4 diseases (interquartile range: 3–5); 3.2% of subjects had one disease, 9.7% two, 16.1% three, 38.7% four, 12.9% five, 12.9% six, 3.2% seven, and 3.2% eight.

The clinical and demographic characteristics of all participants enrolled in this study, including age (years), gender, plasma levels of BDNF (pg/mL), results of the Fried criteria, body-mass index (kg/m^2), number of comorbidities, and the number of daily medications are shown in Table 1. No correlation between age and plasma BDNF concentration was found ($r = 0.22$, $p = 0.23$, Spearman test). However, there was a significant gender difference in the BDNF values for men and women. Men had significantly higher average plasma BDNF levels than women, 98.00 ± 11.69 pg/ml versus 68.54 ± 5.07 pg/ml ($p < 0.05$ Mann–Whitney U test). Similar to age, there was no significant correlation between plasma BDNF levels and body-mass index ($r = -0.85$, $p = 0.47$), Fried criteria ($r = -0.72$, $p = 0.53$), the number of comorbidities ($r = 0.25$, $p = 0.16$), or the number of daily medications taken ($r = 0.78$, $p = 0.33$), all assessed using the Spearman test. The intake of psychotropic medication did not influence the analysis of variance results either for depressive symptoms or the plasma levels of BDNF.

3.2. Evaluation of the relationship between plasma brain-derived neurotrophic factor levels and geriatric assessment scales

Data from the geriatric assessment scales are summarized in Table 2. There was a significant and positive relationship between plasma BDNF levels and Barthel index scores which measure the basic activities of daily living ($r = 0.28$, $p = 0.04$, Spearman test; Fig. 1), however, there was no significant difference between the plasma concentration of BDNF and the total MMSE test score ($r = 0.17$, $p = 0.14$, Spearman test; Fig. 2A). When we analyzed the relationship between BDNF and each MMSE subdomain (concentration, orientation, fixation, short-

Table 1
Age, body mass index (BMI), number of fulfilled Fried criteria, levels of brain-derived neurotrophic factor (BDNF), and the number of comorbidities and daily medications of the 105 participants enrolled in the study. Data are expressed as the median and interquartile range for each value. Age was expressed in years, BMI was calculated from weight and standing height (Kg/m^2), frailty was measured as described in the materials and methods section, and the levels of BDNF (pg/mL) were measured in the plasma.

Clinical and demographic characteristics of participants	Median Value	Interquartile range
Age (years)	83	78–89
Body-mass index (kg/m2)	28.05	24.22–32.80
Fried's criterions	3	2–4
BDNF plasma (pg/mL)	64	42.10–64
Number of all comorbidities	4	3–5
Number of medications	10	6–13

Table 2
Results of geriatric-scale assessment of the all subjects included in the study. The median and interquartile range for each scale. Geriatric assessment was evaluated using the Barthel index to measure the activities of daily living, Lawton index for instrumental activities of daily living, mini-mental score examination test (MMSE), Yesavage scale for geriatric depression, Tinetti gait and balance index to determine the risk for falls, and Norton scale for pressure-ulcer rick assessment.

Geriatric assessment scales	Median value	Interquartile range
Barthel index	80	59.50–87.50
Lawton index	4	3–5
MMSE test	1	0.91–1.17
Orientation	9	7–10
Fixation	3	3–3
Memory	2	1–3
Language	10	8.75–11
Concentration	6	4.–8
Yesavage	3	0–7
Tinetti total index	19	12.50–24
Tinetti balance index	10	6–14
Tinetti gait index	9	6–10
Norton	18	15.50–19

term memory, and language functioning), we observed a significant and positive relationship between BDNF concentration and the concentration subscale ($r = 0.27$, $p = 0.01$; Fig. 2D), but no significant correlations were found with any of the other subscales (orientation: $r = 0.06$, $p = 0.57$, [Fig. 2B]; fixation: $r = 0.19$, $p = 0.09$, [Fig. 2C]; memory: $r = 0.08$, $p = 0.54$, [Fig. 2E]; language: $r = 0.06$, $p = 0.55$, [Fig. 4F]), using the Spearman test in all cases. All the significant correlations were also performed taking covariates due to potential variance i.e. gender, age, body mass index, and educational level into account. After this analysis the significant associations found between BDNF concentration in plasma and Barthel index and concentration subscale of MMSE were still significant ($p = 0.03$ and $p = 0.01$ respectively). No significant associations were found between BDNF concentration in plasma and the other measurements even controlling for covariates. In contrast, plasma BDNF levels were not significantly associated with other geriatric assessment scales: Tinetti total ($r = 0.89$, $p = 0.56$), Tinetti gait index ($r = 0.79$, $p = 0.59$), and Tinetti balance index ($r = 0.10$, $p = 0.50$) to determine the risk of falls, the Yesavage scale for geriatric depression ($r = 0.13$, $p = 0.35$), the Norton scale for pressure-ulcer assessment ($r = 0.15$, $p = 0.29$), or the Lawton index for the instrumental activities of daily living ($r = -0.48$, $p = 0.79$).

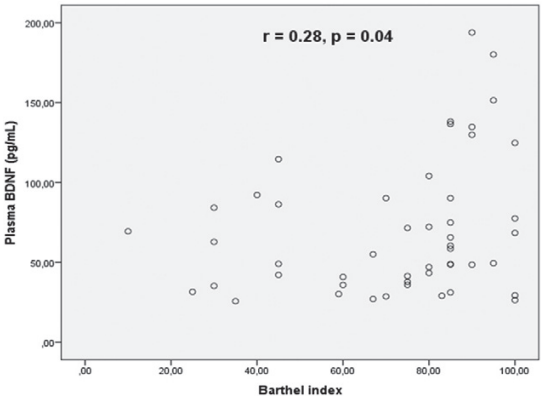

Fig. 1. Correlation between BDNF and the Barthel index to measure the instrumental activities of daily living. The Barthel index was implemented as described in the Materials and Methods section. Levels of BDNF (pg/mL) were measured in the plasma and they were plotted against the Barthel index for each participant enrolled in the study. The level of significance is indicated in the figure.

3.3. Evaluation of the relationship between plasma brain-derived neurotrophic factor levels and other analytical parameters

Other analytical parameters were also measured in blood samples in order to find any correlations with the plasma BDNF concentration. There were no significant correlations between plasma BDNF concentration and platelet count ($r = 0.08$, $p = 0.52$), leukocyte count ($r = 0.15$, $p = 0.26$; Fig. 3A), erythrocyte count ($r = -0.26$, $p = 0.85$), or hemoglobin concentration ($r = 0.03$, $p = 0.81$), using the Spearman test in all cases. The median values (interquartile range) for platelets, leukocytes, and erythrocytes were within the normal reference interval: platelet count: $205 \times 10^3/\mu L$, interquartile range: 176–248 (normal range: 130–450 $\times$ $10^3/\mu L$); leukocyte count: $6.35 \times 10^3/\mu L$, interquartile range: 5.20–8.47 (normal range: 3.8–10.8 $\times$ $10^3/\mu L$), and erythrocyte count: $3.88 \times 10^6/\mu L$, interquartile range: 3.57–4.24 (normal range: 3.7–5.2 $\times$ $10^6/\mu L$). In contrast, the median hemoglobin concentration was lower than average: 11.60 g/dL, interquartile range: 10.82–12.97 (normal range: 12–16 g/dL), although this is a common physiological finding in geriatric patients.

We compared the total leukocyte count, as well as their differentiated lineages: neutrophils, lymphocytes, monocytes, basophils, and eosinophils, with plasma BDNF concentrations to find a significant positive correlation with the eosinophil count (eosinophils: $r = 0.31$, $p = 0.01$, Spearman test [Fig. 3B]). There were no significant differences found for the other leukocyte subtypes using the Spearman test: neutrophils: $r = -0.10$, $p = 0.43$, (Fig. 3C); lymphocytes: $r = -0.001$, $p = 0.99$, (Fig. 3D); monocytes: $r = 0.07$, $p = 0.57$, (Fig. 3E); basophils: $r = -0.96$; $p = 0.48$, (Fig. 3F). All the significant correlations were also performed taking covariates due to potential variance i.e. gender, age, body mass index, and educational level into account and led to similar results (data not shown). The median values (interquartile range) for ach leukocyte subpopulation were within the normal range (neutrophil count: 60.25%, interquartile range: 52.60–66.17, normal range: 40–75%; lymphocyte count: 26.90%, interquartile range: 22.10–35.05, normal range: 20–45%; monocyte count: 8.25%, interquartile range: 7.10–10.15, normal range: 2–10%; basophil count: 0.50%, interquartile range: 0.30–0.67, normal range: 0–2%; eosinophil count: 2.95%, interquartile range: 1.80–4.67, normal range: 1–6%). No significant correlation was observed between plasma BDNF levels and typical parameters such as glucose, urea, urate, triglycerides, low-density lipoprotein cholesterol, creatinine, GOT and GPT, Na, K, Ca, Fe, Ferritin, B12, total proteins, or thyroid-stimulating hormone (glucose: $r = 0.05$, $p = 0.97$; urea: $r = 0.02$, $p = 0.91$; uric acid: $r = -0.08$, $p = 0.55$; creatinine: $r = 0.2$; $p = 0.85$; triglycerides: $r = -0.12$, $p = 0.36$, [Fig. 4B]; low-density lipoprotein cholesterol: $r = -0.27$, $p = 0.70$, [Fig. 4C]; GOT: $r = -0.18$, $p = 0.43$; GPT: $r = -0.22$, $p = 0.10$; Na: $r = -0.19$, $p = 0.26$; K: $r = -0.84$, $p = 0.54$; Ca: $r = -0.04$, $p = 0.97$; Fe: $r = -0.21$, $p = 0.14$; Ferritin: $r = 0.008$, $p = 0.95$; B12:$r = -0.10$, $p = 0.45$; total proteins: $r = 0.03$, $p = 0.82$; thyroid-stimulating hormone: $r = -0.20$, $p = 0.15$, all assessed using the Spearman test).

In contrast, a significant inverse correlation emerged between plasma BDNF levels and total cholesterol ($r = -0.26$, $p = 0.04$; Fig. 4A) and how-density lipoprotein cholesterol ($r = -0.31$, $p = 0.04$; Fig. 4D) using the Spearman test. The median values (interquartile range) of these biochemical parameters were also within the normal reference interval. Data from the analytical parameters are shown in Table 3.

A linear regression analysis, using BDNF plasma levels as a dependent variable and the factors that were significant in the previous analysis as independent variables, showed that gender ($r = 0.26$, $r^2 = 0.07$, $p = 0.02$), the concentration subscale of the MMSE test ($r = 0.30$, $r^2 = 0.09$, $p = 0.009$), and total cholesterol ($r = 0.32$, $r = 0.10$, $p = 0.01$) were independent factors associated with BDNF plasma levels. In contrast, the Barthel index ($r = 0.26$, $r^2 = 0.07$, $p = 0.06$), eosinophil count ($r = 0.15$, $r^2 = 0.02$, $p = 0.25$), and low-density lipoprotein cholesterol levels ($r = 0.29$, $r^2 = 0.08$, $p = 0.053$) were not significant.

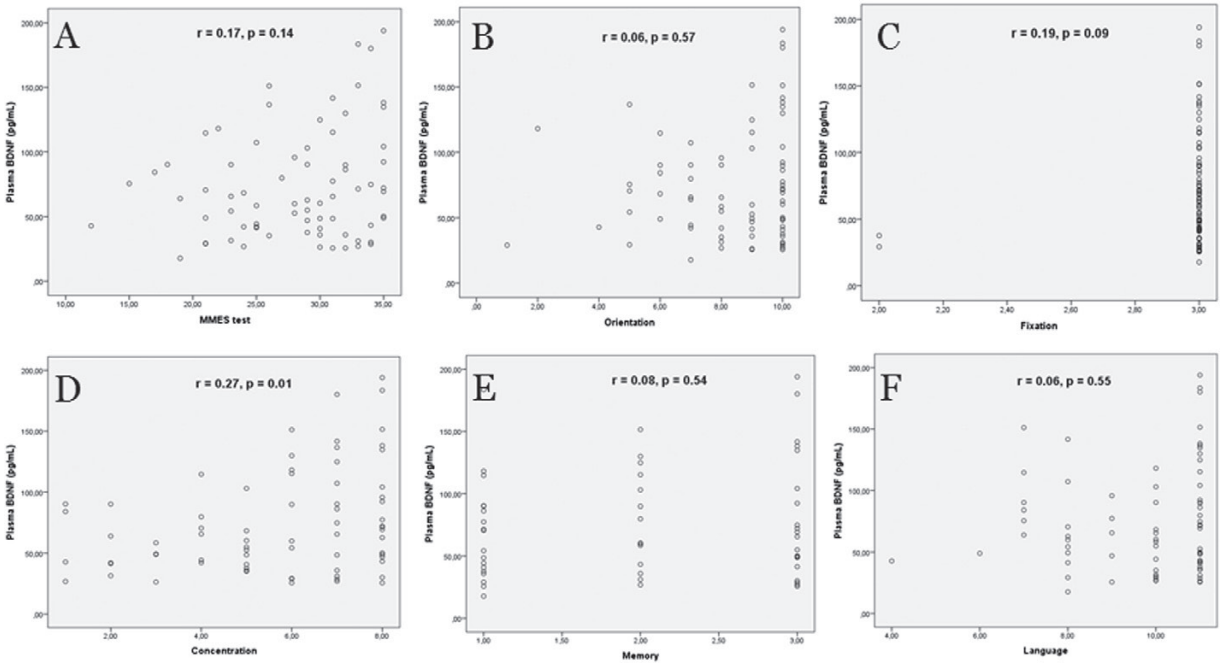

Fig. 2. Correlation between BDNF and the mini-mental score examination test (MMSE) and its subscales (Orientation, Fixation, Memory, Language, Concentration). The MMSE test and its subscales were measured as described in the Materials and Methods section. Levels of BDNF (pg/mL) were measured in the plasma and they were plotted against the MMSE test (A), and its subscale, results: Orientation (B), Fixation (C), Concentration (D), Memory (E), Language (F) for each participant enrolled in the study. The level of significance is indicated in the corresponding figure.

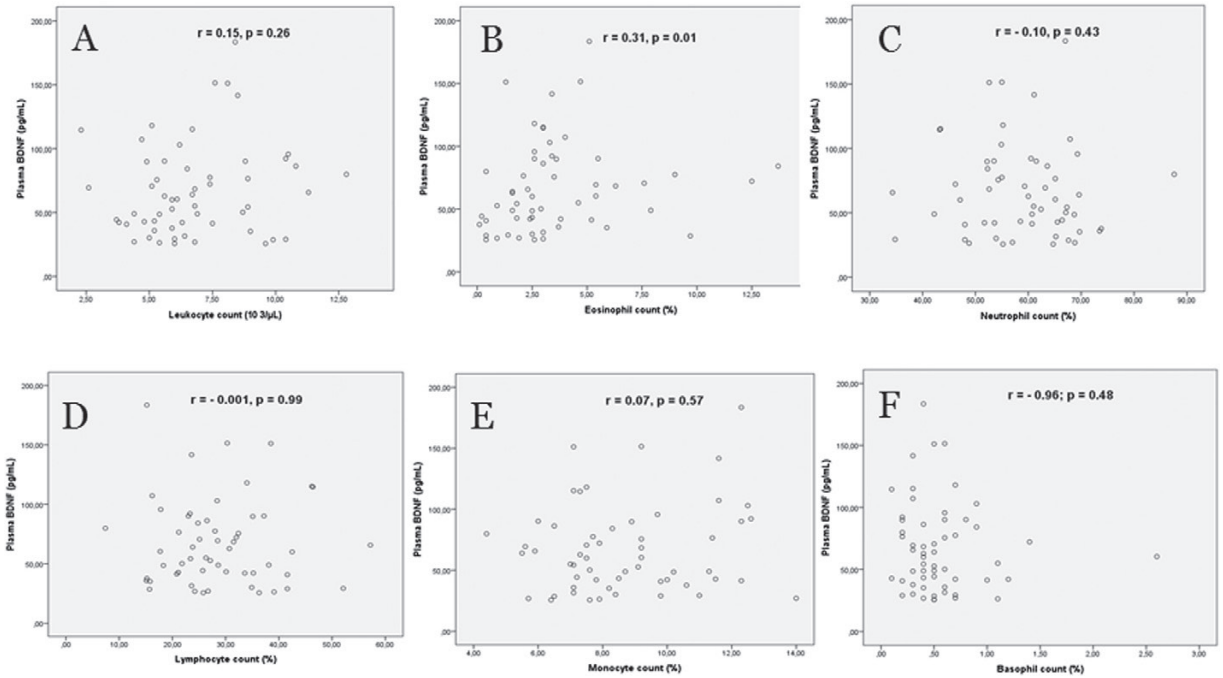

Fig. 3. Correlation between BDNF levels in plasma and the total-subtype leukocyte count. Levels of BDNF (pg/mL) were measured in the plasma as described in the Materials and Methods section. (A) leukocyte ($\times 10^3$/µL), (B) eosinophil (%), (C) neutrophil (%), (D) lymphocyte (%), (E) monocyte (%), and (F) basophil (%) counts were measured in the blood and were plotted against plasma levels of BDNF for each participant enrolled in the study. The level of significance is indicated in the corresponding figure.

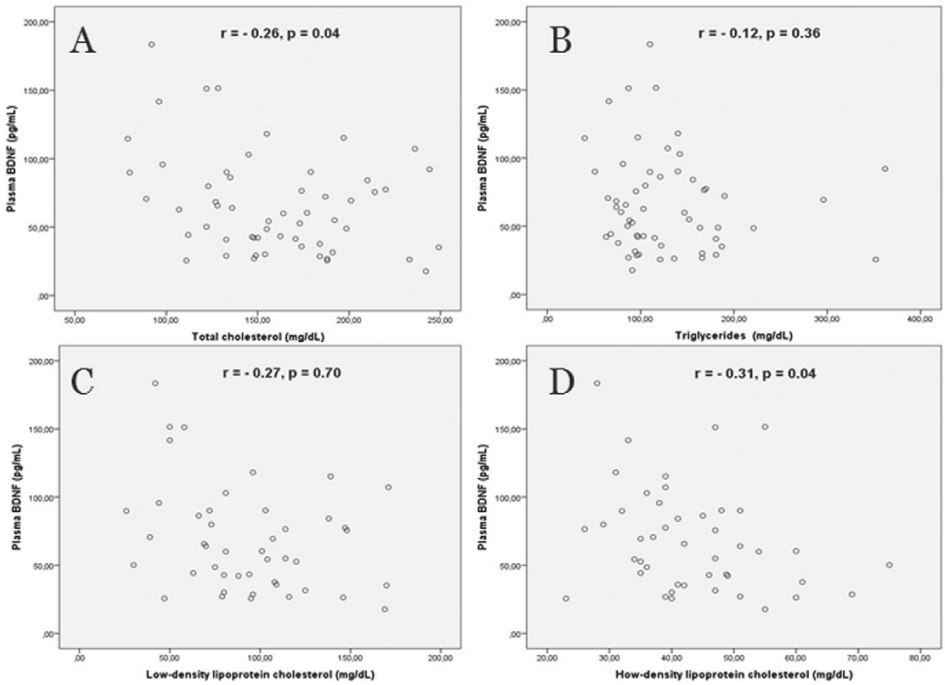

Fig. 4. Correlation between BDNF levels in plasma and the lipid profile. Levels of BDNF (pg/mL) were measured in the plasma as described in the Materials and Methods section. Total Cholesterol (mg/dL) (A), triglycerides (mg/dL) (B), low-density lipoprotein C (LDL-C; mg/dL) (C), and high-density lipoprotein C (HDL-C; mg/dL) (D) levels were measured in the blood and were plotted against plasma levels of BDNF for each participant enrolled in the study. The level of significance is indicated in the corresponding figures.

Table 3
Results of blood hematological and biochemical analytical analysis.
Values are reported as the median, interquartile range, and normal range. All of the biochemical and hematological parameter values were expressed in conventional units.

Analytical parameters	Median value	Interquartile range	Normal range
Platelet count (10^3/μL)	205	176–248	130–450
Erythrocyte count (10^6/μL)	3.88	3.57–4.24	3.7–5.2
Hemoglobin concentration (g/dL)	11.60	10.82–12.97	12–16
Leukocyte count (10^3/μL)	6.35	5.20–8.47	3.8–10.8
Neutrophil count (%)	60.25	52.60–66.17	40–75
Lymphocyte count (%)	26.90	22.10–35.05	20–45
Monocyte count (%)	8.25	7.10–10.15	2–10
Basophil count (%)	2.95	1.80–4.67	0–2
Eosinophil count (%)	0.50	0.30–0.67	1–6
Glucose (mg/dL)	82	73.50–99	74–106
Urea (mg/dL)	47.20	37.05–70.65	17–60
Urate (mg/dL)	5.40	4.20–6.10	2.6–6
Triglycerides (mg/dL)	110	87–159.80	50–150
Low-density lipoprotein cholesterol (mg/dL)	94	67.5–115	0–130
Creatinine (mg/dL)	0.94	0.73–1.10	0.51–0.95
GOT (U/L)	17.40	14–21.55	10–31
GPT (UL)	14	8–22.70	10–34
Na (mEq/L)	140.95	139.37–142.67	136–146
K (mEq/L)	4.50	4.20–4.77	3.5–5.1
Ca (mg/dL)	9.10	8.80–9.50	8.8–10.6
Fe (μg/dL)	54.50	42.25–67.75	60–180
Ferritin (μg/L)	126.20	54.90–193	10–120
B12 (pg/mL)	281.50	218–418.75	180–914
Total proteins (g/dL)	6.35	6.17–6.82	6.6–8.3
Thyroid-stimulating hormone (uUI/mL)	1.72	1.17–3.18	0.34–5.6
Total cholesterol (mg/dL)	155	128.07–188	100–200
How-density lipoprotein cholesterol (mg/dL)	41	35.50–50	40–200

4. Discussion

The most important new and significant finding in this report is that BDNF plasma levels correlate with functional and cognitive impairment in older individuals where no such relationship has been found with other psychological and geriatric measurements.

Several previous studies demonstrated that the BDNF concentration in serum decreases during aging (Ziegenhorn et al., 2007), and that it correlates with depressive symptoms and cognitive decline in depressed older patients (Bus et al., 2012b; Shimada et al., 2014). In addition, high serum BDNF concentrations seem to be a protective factor against Alzheimer disease, as shown in a powerful longitudinal study performed within the framework of the Framingham Heart Study (Weinstein et al., 2014). In our study, we choose to measure BDNF in plasma samples because this is a good reflection of the amount of BDNF stored in circulating platelets, whereas serum BDNF may be a better reflection of the steady-state situation and consequently the amount of bioactive BDNF protein (Fujimura et al., 2002; Radka et al., 1996). Thus, measuring BDNF in plasma is likely to be a better estimate of peripheral BDNF levels because it reduces the influence of the release of BDNF from platelets when measuring neurotrophin levels in serum.

It has been reported that plasma concentrations of BDNF significantly decrease with age (Krabbe et al., 2009; Lommatzsch et al., 2005; Tapia-Arancibia et al., 2008; Yasui-Furukori et al., 2013; Ziegenhorn et al., 2007), which contrasts with our findings which did not show this effect. This could be explained by the younger age of the subjects in these previous samples compared to the patients we included in our study with a median age of 83 years (interquartile range 78–89). In addition, plasma BDNF levels were higher in men compared to women in our study which also contrasts with results from other studies that show the opposite effect (Krabbe et al., 2009). This gender difference may be justified, in part, by hormonal changes experienced

by women with increasing age because estradiol may induce BDNF expression (Frye and Rhodes, 2005; Gibbs, 1999; Sohrabji and Lewis, 2006). The youngest woman in our sample was 62 years old; they were all postmenopausal and none were receiving any hormone replacement therapy. In agreement with our findings, Bus et al. (2012a) found a decrease in the systemic levels of BDNF with age, but only in women. Therefore, we should not rule out a gender difference in BDNF release into the blood circulation.

Another possible explanation for the gender difference could be the recently-proposed association between depression and decreased levels of BDNF (Molendijk et al., 2014; Pereira et al., 2013), which may even extend to a correlation between peripheral BDNF levels and the severity of depression (Gervasoni et al., 2005). Similarly, Lang et al. (2004) associated BDNF concentrations with personality traits related to depression; it is possible that this could be linked to the fact that postmenopausal women often display increased depressive behavior (MacQueen and Chokka, 2004).The results of our study showed that women had the highest scores on the Yesavage scale (an ad hoc geriatric depression scale) and thus showed a greater tendency toward depression than men (although this correlation was not significant), which may explain the lower BDNF levels found in women. This is probably because the sample was not specifically selected to study the role of BDNF in depression, meaning that several factors such as the heterogeneity of psychotropic medication, different lengths of antidepressant treatment schemes, and the presence of undiagnosed depressive symptoms and therefore the absence of suitable antidepressant treatment, can significantly affect statistical outcomes if not properly controlled for.

The mean plasma BDNF concentration (73.26 pg/ml) in this study was lower than the mean obtained in previous studies conducted in older adults without dementia or severe cognitive impairment (Krabbe et al., 2009; Pereira et al., 2013). These values may be due to the frailty of the subjects in our sample compared to younger individuals studied in the literature. Coelho et al. (2012) found lower circulating levels of BDNF in prefrail elderly women compared to non-frail women. So far no studies in the literature have associated BDNF levels in plasma with any frailty criteria or any particular frailty phenotype (frail, prefrail, or non-frail status), which may be because 80% of individuals were already frail (fulfilling 3 or 4 frailty criteria).

We also analyzed BDNF levels in the plasma in relation to other geriatric assessment scales and showed a correlation between BDNF plasma levels and the Barthel index for the basic activities of daily life and the concentration subscale on the MMSE test. This novel correlation between plasma BDNF levels and the Barthel index suggests that low levels of BDNF are associated with greater functional dependence in frail elderly patients. Perhaps linked to the aforementioned correlation with the concentration subscale, neurons are considered the main cellular source of BDNF (Kerschensteiner et al., 1999). However, there are other potential sources of BDNF in the circulation such as immune cells, vascular endothelial cells, and smooth muscle cells (Donovan et al., 1995; Lommatzsch et al., 1999; Nakahashi et al., 2000; Noga et al., 2003; Papathanassoglou et al., 2014), which may be connected to the fact that several studies have shown an increase in BDNF production during exercise (Kim et al., 2014).

Other studies have shown that BDNF can cross the blood–brain barrier in both directions, suggesting that measurements of blood BDNF levels reflect brain-tissue BDNF levels and peripheral BDNF could influence the brain (Klein et al., 2011; Pan et al., 1998; Sartorius et al., 2009), triggering greater neuroplasticity and contributing not only to an improvement in cognitive function, but also to an improvement in functional status. Several studies, positively correlated cognitive decline associated with age with various functional limitations, that is, decreased cognitive ability in elderly patients affects their ability to perform the activities of daily living, producing loss of independence, and the constant need for help (Giovannetti et al., 2008; Sitjas Molina et al., 2003). While there is still controversy regarding the relationship between cognitive impairment and functional deficit, it is possible that changes in brain plasticity processes cause this functional decline (Mahncke et al., 2006).

Therefore, given that BDNF is associated with neuronal plasticity and better cognitive functioning in old age (Calero and Navarro, 2007), and considering the interdependence between cognitive and functional aging, our findings suggest that BDNF is in some way linked with a higher level of independence and functional autonomy in old age. Consequently, BDNF plasma levels may be associated with functional risk, which is especially useful because functional impairment is predictive of poor outcome and mortality regardless of diagnosis (Trigás-Ferrín et al., 2011).

Several previous studies have shown that blood levels of BDNF are associated with cognitive impairment (Komulainen et al., 2008). In addition, many brain functions related to cognition are dependent on BDNF (Bird and Burgess, 2008) and there is evidence that it is an important molecular mediator of brain plasticity (Arancio and Chao, 2007; Schinder and Poo, 2000; Tanaka et al., 2008). In general, optimal cognitive functions are linked to efficient neuronal plasticity (Tapia-Arancibia et al., 2008), and although brain plasticity occurs throughout life, it decreases with aging (Tapia-Arancibia et al., 2008; Burke and Barnes, 2006).

Most non-demented older individuals generally show a gradual decline in cognitive functions, although there is wide inter-individual variability. In particular, brain functions associated with cortex or hippocampus alterations, two highly plastic regions which are crucial for learning and memory (Bird and Burgess, 2008; Tapia-Arancibia et al., 2008), are more susceptible to age-related brain function impairment (Lee et al., 2012: Lister and Barnes, 2009). In this context, we studied a possible association between BDNF and MMSE test scores. Our analysis showed a positive and significant relationship between plasma BDNF and the MMSE-test concentration subscale. Numerous studies have reported that depressive disorder in adults is associated with cognitive deficits (Talarowska et al., 2015), in particular with those related to the attention domain (Hasselbalch et al., 2012). In line with these results, our study shows a significant correlation between the Yesavage scale and the MMSE test concentration subscale, but not with the other subscales (data not shown). This means that subjects with concentration problems could have an underlying mood disorder, or at least some depressive symptoms, which are a widespread feature in the geriatric population.

Another relevant and unexpected new finding was the significant relationship between plasma BDNF and eosinophil count or other leucocyte-subtype counts. Notably, the association occurs within the physiological range of the eosinophil count. To date, only one study has reported BDNF production by eosinophils (Noga et al., 2003). Because eosinophils are the main cells that mediate allergic inflammation, high levels of BDNF have been observed in allergic diseases compared to levels observed in healthy controls (Bonini et al., 1996; Raap et al., 2005). However, we cannot rule out that other blood cells are likely to act as sources of peripheral BDNF (Noga et al., 2003). Therefore, our observation, at minimum, suggests that peripheral blood eosinophils might be a source of BDNF production.

The peripheral effects of circulating BDNF on non-neuronal cells still remain unclear (Fujimura et al., 2002). BDNF in skeletal muscle has been described to be involved in the development and differentiation of myoblasts and muscle fibers, as well as in regulating the survival of motor neurons, the presynaptic release of neurotransmitters, and maintenance of the postsynaptic region in skeletal myofibers (Raschke and Eckel, 2013; Sakuma et al., 2015). In addition, a recent report shows aerobic exercise induces an increase in peripheral BDNF and is associated with increases in muscle strength (Tsai et al., 2015) whereas frailty syndrome is characterized by a loss of strength and muscle mass (Evans et al., 2010). In this context, our results show a positive and significant correlation between eosinophil count and poor muscle strength, one of the five Fried frailty criteria, measured by evaluating hand grip strength (data not shown). It is possible that BDNF released by eosinophils may

be involved in muscle activity and thus a decrease in their count is related to the mechanisms involved in frailty. Therefore the influence of eosinophil counts on muscle strength in frail subjects might be an interesting topic for future research.

There is growing scientific interest in the role of BDNF in metabolic regulation. In this study plasma BDNF was positively related to total cholesterol and inversely related to low-density lipoprotein cholesterol levels. This finding supports a potential role for BDNF in lipid metabolism and is in line with the roles observed in other studies where circulating BDNF was positively correlated with low-density lipoprotein cholesterol, total cholesterol, and triglyceride levels (Golden et al., 2010; Jung et al., 2011). The role of BDNF in the pathogenesis of this metabolic disorder is still is not clear, however, this neurotrophin might be related to some pathophysiological aspect of cardiovascular disease.

In conclusion, using the Barthel index we found a specific association between the plasma concentration of BDNF and frailty in older individuals. Furthermore, our results indicate that a decrease in BDNF levels is related to a poor score in the MMSE-test concentration subdomain. These results provide further evidence that BDNF may play a role in the pathophysiology of functional impairment in frail older-adults. Because this is a cross-sectional study it was designed to evaluate the relationship between BDNF and functional and cognitive parameters in older individuals. However, it was not intended to adjust for all disease conditions, as suggested for other pathologies in which BDNF seems to play a role (Gass and Hellweg, 2010), because these conditions may be important intermediaries in creating any associations identified. In addition, this experimental design cannot determine causality, but ongoing follow-up studies are in progress to establish any such relationships.

Despite these limitations, evidence from this study supports a significant association between BDNF and some parameters related to functional and mental impairment in older individuals. These findings provide a basis for further investigation into the underlying mechanisms that contribute to some individuals undergoing a faster aging process so that potential interventional strategies can be developed in the future to increase BDNF with the aim of improving functional and cognitive abilities. We cannot be certain whether the reduction in BDNF concentration in blood is the cause of functional impairment and some forms of cognitive alterations; it may represent a downstream effect of these symptoms, and so ongoing follow-up studies are in progress to determine the prognostic value of reduced BDNF in functional and cognitive impairment over time. Longitudinal studies will be required to clearly understand the relationship between peripheral BDNF levels and functional and cognitive impairment, and to determine if plasma BDNF is a suitable marker for this characteristic.

Competing interests

The authors declare that they have no competing interests.

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ANEXO C: Artículo 3

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Original Article

Serum vitamin D and functional impairment in octogenarian women

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ABSTRACT

Purpose: Serum vitamin D deficiency has been associated with frailty in people aged 65 and over, however its relationship with functional impairment has not been investigated in octogenerian (aged 80–90 years) institutionalized women.
Methods: We assessed functional impairment in this latter group by measuring frailty syndrome and other geriatric and psychological assessment scales: the Tinetti gait and balance index to determine the risk for falls, the Barthel index to measure the basic activities of daily living, the Lawton index for instrumental activities, the mini-mental score examination test for cognitive impairment, the Yesavage scale for geriatric depression, and the Norton scale for the risk of ulceration.
Results: Frail individuals had significantly reduced serum vitamin D concentrations (measured as total 25-hydroxyvitamin D; 25(OH)D) compared to robust individuals, but reduced 25(OH)D concentration did not significantly correlate with frailty syndrome severity, and mean 25(OH)D concentrations were within the recommended levels in all groups. The 25(OH)D concentration did not correlate with any of the blood analytical parameters measured and with the geriatric assessment scales used, suggesting a selective relationship with frailty.
Conclusion: These results highlight the need to individualize treatment such as vitamin D supplementation in order to treat frailty syndrome.

1. Introduction

Frailty syndrome is characterized by decreased physical functioning and a higher risk for poor outcomes such as an increased incidence of falls, fractures, disability, and comorbidity, increased health care expenditure, and premature mortality (Fried et al., 2001; Fugate-Woods et al., 2005). The concept of frailty has grown in importance because its prevention or hindrance delays the onset of disabilities and dependence (Fried et al., 2001). Frailty syndrome is primarily defined using a standardized phenotype based on five physical criteria as first described by Fried et al. in their Cardiovascular Health Study (Fried et al., 2001), although several other scales for measuring frailty have also been proposed (Fernández-Garrido, Ruiz-Ros, Buigues, Navarro-Martinez, & Cauli, 2014). Frailty has been associated with changes in several physiologic systems, coagulation, hematologic, endocrine systems, micronutrients-vitamins and a low-chronic inflammatory state (Walston et al., 2002). Importantly, a decreased vitamin D concentration in blood has been repeatedly linked to frailty syndrome (Fernández-Garrido et al., 2014; Morley et al., 2013; Smit et al., 2012; Wilhelm-Leen, Hall, Deboer, & Chertow, 2010). An adequate concentration of this vitamin is required to maintain sufficient blood calcium and phosphorous levels to maintain strong bones, regulate cell growth and differentiation, modulate immune system activity, influence muscle metabolism, and regulate some cerebral processes (Adams & Hewison, 2010; Asamura et al., 2010). However, one recent clinical trials have found that vitamin D supplementation does not have any beneficial effects on frailty syndrome (Latham et al., 2003). Moreover daily administration of high vitamin D3 doses (50,000 IU) did not improve physical performance for patients with heart failure despite an increase in their serum 25(OH)D concentration (Boxer et al., 2013). These results suggest that under some circumstances the strength of the association between vitamin D and frailty can be weak or absent.

Most of the studies showing a link between blood vitamin D concentrations and frailty analyze the relationship in samples of community-dwelling individuals with a wide range of ages (generally aged 65 years or more) but exclude older institutionalized adults. In addition a large epidemiological study conducted in Italy demonstrated that vitamin D insufficiency was significantly associated with frailty only in men (Shardell et al., 2009). To our knowledge, the relationship between

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vitamin D, frailty, and other geriatric assessment measurements in old (aged 75 years or more) institutionalized women have not been investigated.

Our three main objectives in this work were:

(1) To evaluate the vitamin D concentration, measured as 25(OH)D, in blood in relation to the severity of frailty syndrome in older, non-osteoporotic, institutionalized women.
(2) To assess the relationship between vitamin D and geriatric assessment tool measurements (the Tinetti scale for gait and balance, the mini-mental score examination [MMSE] test for cognitive function, the Barthel index for the basic activities of daily life, the Norton scale for the risk of pressure ulcers, the Yesavage scale for geriatric depression, and the Lawton index for instrumental activities of daily life).
(3) To evaluate the relationship between vitamin D and other analytical parameters (erythrocyte and platelet count, hemoglobin, hematocrit, glucose, urea, uric acid, cholesterol, triglyceride, creatinine, glutamic oxaloacetic transaminase [GOT], and glutamic pyruvic transaminase [GPT] concentration).

2. Materials and methods

2.1. Study population

This was a clinical study with a cross-sectional design performed on institutionalized elderly women living in one of four nursing homes (*GeroResidencias La Saleta*, Valencia). The inclusion criteria were: institutionalized for at least 6 months, ability to rise from a chair and walk six meters, and aged 65 years or older. The exclusion criteria were: severe cognitive impairment (MMSE score less than 21), severe psychiatric disease or blindness, acute infections or known cancer, primary hyperparathyroidism, or vitamin D and/or calcium supplementation. The research was undertaken in compliance with the requirements of the Declaration of Helsinki and the entire study protocol was approved by the local ethical committee at the University of Valencia (reference number: H1384175284261). All participants gave written informed consent before being enrolled in the study.

2.2. Variables

The variables included socio-demographic characteristics (age, body mass index, and smoking), and measurement of the five frailty criteria (involuntary weight loss, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity) according to Fried et al. (2001) as described in the next section. Geriatric assessment was achieved by evaluating participants with the Tinetti scale (for gait and balance), the MMSE Test (for cognitive function), the Barthel index (for basic activities of daily life), the Norton scale (for the risk of pressure ulcers), the Yesavage scale for geriatric depression, and the Lawton index (for instrumental activities of daily life).

2.3. Measurement of frailty criteria

Frailty was measured by assessing the presence or absence of the five characteristics of the Fried criteria (Fried et al., 2001), which were defined and evaluated as follows:

1) Weight loss, defined as the unintentional loss of 4.5 kg or more in the past year.
2) The exhaustion criterion was considered present if the participant answered "Often" or "Most of the time" to the question "How often in the last week did you feel that everything you did was an effort?" included in the Center for Epidemiologic Studies-Depression scale (Asamura et al., 2010; Orme, Reis, & Herz, 1985; Radloff, 1977).
3) Physical inactivity: defined as participants who performed no physical activity, spent most of the time sitting, and rarely took a short walk or performed any other non-demanding physical activity. Low physical activity was quantified using the Spanish adaptation for women of the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ; Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Elosua, Marrugat, Molina, Pons, & Pujol, 1994; Elosua et al., 2000; Holick, 2004; Thomas et al., 1998; Ruiz-Comellas et al., 2012). The MLTPAQ is administered by a trained interviewer who is provided with detailed instructions and a list of very clearly defined physical activities (PAs). Total energy expenditure from leisure time PA (EEPAtotal) can be obtained with this questionnaire and was used to quantify PA (Jacobs, Ainsworth, & Hartman, 1993; Lips, 2001). Participants were given a list of suggested activities and asked to mark those performed in the last year. To avoid memory bias, as far as possible, the activities performed during the last week were collected first, followed by those performed the last month, the last quarter, and finally the last year, always including the former periods. For validation purposes, only information referring to the last year was used.
4) Slow walking speed, based on the 4,6 walk gait speed test, was defined when the participant corresponded to the worst quintile for the group, after adjustment for sex and height according to the standards of the Short Physical Performance Battery (Guralnik et al., 1994; Shardell et al., 2009).
5) To assess weakness hand grip strength was measured with a Jaymar hand-held hydraulic dynamometer as an approximation of general muscle strength, and assessed according to the standards of the Hispanic Established Populations for the Epidemiologic Studies of the Elderly (Dawson-Hughes et al., 2005; Ottenbacher et al., 2002).

2.4. Vitamin D measurement

25(OH)D (the sum of both 25(OH)D2 and 25(OH)D3, derived from ergocalciferol and cholecalciferol respectively) was measured in serum samples by gas chromatography. First a solid-phase extraction (SPE) was applied to serum samples according to a previously devised protocol (George & Szczesniewski, 2009). The elutes were evaporated and the residue was derivatized using a mixture of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA)/pyridine, then resuspended and dissolved in 60 μL of BSTFA with 1% trimethylchlorosilane (TMCS), and injected into the GC/MS instrument for analysis (Cauli, Mansouri, Agusti, & Felipo, 2009).

2.5. Measurement of hematological and biochemical markers

Fasting blood samples were collected in the morning. The hemoglobin concentration, and white blood cell, erythrocyte, and platelet counts were measured on automated instruments at local hematology laboratories near the nursing homes. Biochemical serum analyses included glucose, urea, urate, cholesterol, triglycerides, creatinine, GOT, and GPT. All samples were kept at 4–6 °C and processed within 2 hours of the blood collection. Blood serum (5 ml) was obtained by collecting blood in BD Vacutainer tubes and centrifuging them at 500 g for 10 min at room temperature.

2.6. Statistical analysis

Descriptive statistics, including a measurement of central tendency (mean), standard error of the mean (SEM), and range values were used to describe all the quantitative variables. The normal distribution of each variable was estimated with the Kolmogorov–Smirnov test. Given that none of the variables were normally distributed, correlation was analyzed using the Spearman correlation coefficient. Linear regression analysis was used to specify the association between changes in vitamin D (measured as 25(OH)D) and any variables that were significant in the previous analysis. The non-parametric Kruskal–Wallis test was performed to verify any possible differences between groups. Statistical

significance was set at $p < 0.05$ and the analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Frailty score and geriatric assessment

The sample comprised 104 very old (aged 80–90 years) institutionalized women; approximately 82% of the participants were widows. The severity of the frailty syndrome detected in the study population is represented in Fig. 1A. Forty-nine percent of the population met three of the Fried frailty criteria, followed by 20% that met four criteria. According to these criteria 21% of the population were robust (met 0 Fried criteria), 21% were prefrail (met 1 or 2 criteria), and 58% were frail (met more than 3 criteria; Fig. 1B), but none of the women fulfilled all 5 criteria for frailty syndrome. The most represented Fried criteria in the sample was *low physical activity* (93%) followed by *slow walking speed* (85%), *weakness* (71%), *exhaustion* (36%), and *weight loss* (9%). The results for age and the results from the different geriatric and psychological evaluations are summarized in Tables 1 and 2.

3.2. Vitamin D and calcium concentration

The serum vitamin D concentration (expressed as 25(OH)D) was 46 ± 6 ng/mL in robust women, 29 ± 4 ng/mL in prefrail women, and 28 ± 3 ng/mL in frail women (Fig. 2A). A Kruskal–Wallis test showed a significant difference between the groups ($p < 0.01$) and a post-hoc comparison test showed that both prefrail and frail women had significantly lower blood vitamin D concentrations compared to robust individuals ($p < 0.05$). No statistical difference was observed between the vitamin D blood concentration in prefrail and frail women. The serum calcium concentration was 7.9 ± 0.5 mg/dL in robust individuals, 8.2 ± 0.8 mg/dL in prefrail individuals, and 8.0 ± 0.4 mg/dL in frail individuals (Fig. 2B); no significant differences were observed in the calcium concentration between groups ($p = 0.9$, Kruskal–Wallis test). The concentration of parathormone and phosphate in all the blood samples were within the physiological ranges (data not shown), and similarly, the mean vitamin D concentration values in the three experimental groups were also within the physiologically-normal range.

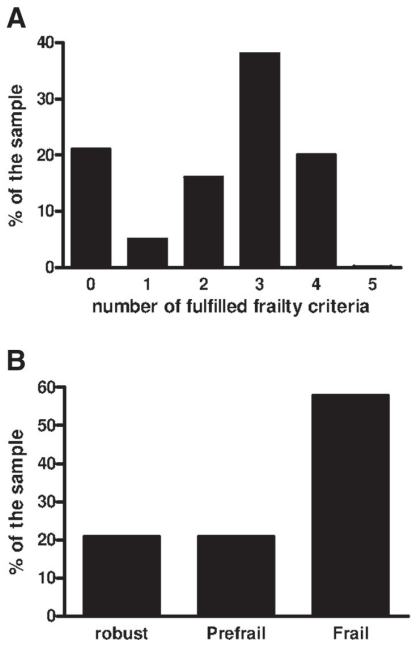

Fig. 1. Evaluation of frailty syndrome in the study sample. Frailty measurement methods are described in Materials and Methods. (A) Number of frailty criteria met in the sample, expressed as percentage of the entire population; (B) The severity of frailty, as reported by Fried et al. (2001), was expressed as follows: participants who met three or more criteria were classified as frail, those who met one or two were prefrail, and those who did not meet any of the criteria were non-frail (robust).

Table 1
Geriatric evaluation showing the age of participants enrolled in the study, and the results of the geriatric scale assessments with the mean and range for each value/scale.

	Mean value	Range
Age	84 years	75–99 years
Tinetti balance Index	9	0–14
Tinetti Gait Index	6	0–12
Norton	15	13–20
MMSE	24	21–30
Barthel Index	60	25–100
Lawton Index	4	2–7

The following validated scales were used: the Tinetti gait and balance index to determine the risk for falls, the Norton scale to assess the risk of pressure ulcers, the mini-mental score examination test (MMSE) for cognitive function, the Barthel index to measure the activities of daily living and mobility, and the Lawton index for Instrumental Activities of Daily Living.

3.3. Evaluation of the relationship between serum vitamin D status and geriatric assessment scale scores

No significant differences were observed in the scores obtained in a complete battery of geriatric assessment tests (as previously described) between robust, prefrail, and frail individuals. The only significant difference was the age of frail individuals which was higher ($p < 0.05$) than the age of robust women, although there was no significant age difference between prefrail and frail women. Statistical analyses which were adjusted for several variables such as age, sex, body mass index, smoking status, the Charlston index score for comorbidities, the total number of prescribed drugs as an index of polypharmacy, and calcium/vitamin D supplementation, showed no significant relationship between vitamin D and frailty ($p = 0.09$) suggesting that these parameters do contribute to the reduced vitamin D concentration found in frail institutionalized women.

Table 2
The relationship between frailty score and other biochemical blood parameters.

	p values for correlation analysis
Glucose	0.098
Urea	0.592
Uric acid	0.088
Urea	0.923
Creatinine	0.165
Cholesterol	0.366
Triglycerides	0.101
GOT	0.583
GPT	0.077
Hematocrit	0.800
Hemoglobin	0.082
Blood cell count	0.863
Barthel score	0.197
Tinetti	0.280
MMSE score	0.147
Yesavage	0.422
Norton	0.081

Correlation coefficients and significance after bivariate correlations between variables were evaluated using the Pearson (applied to data with a normal distribution) or Spearman (applied to data with non-normal distribution) correlation tests.

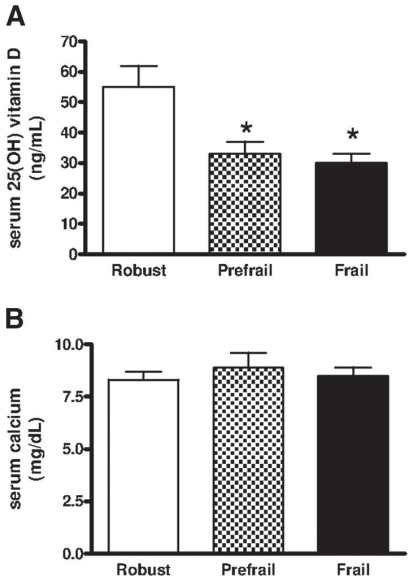

Fig. 2. Vitamin D (A) and calcium (B) concentration in the serum of frail individuals. Data are expressed as the mean ± the standard error mean (SEM) for each experimental group. *$p < 0.05$ compared to robust individuals, tested using Dunnet's post-hoc test.

3.4. Evaluation of the relationship between frailty score, vitamin D, and other analytical parameters using geriatric assessment tools

No significant correlation was observed between the serum vitamin D concentration and the severity of frailty syndrome in any of the three groups (robust, prefrail, and frail) as assessed using the aforementioned geriatric assessment tools and biochemical parameters including hemoglobin, glucose, urea, uric acid, cholesterol, triglycerides, creatinine, GOT, and GPT levels in blood.

4. Discussion

Before undertaking a full clinical trial to study the effects of vitamin D supplementation on frailty syndrome in very old institutionalized individuals, first we evaluated whether vitamin D correlates with the severity of frailty in these individuals. This work does not replicate similar previous studies because the features of our sample, i.e. institutionalized women with a mean age of 84 years, the type of diet (Mediterranean), and sunlight exposure levels (at a latitude of 39.47°) may modulate the relationship between vitamin D and frailty syndrome.

Here we report the following main results: 1) vitamin D concentration (measured as 25(OH)D) in serum is decreased in frail and prefrail very old institutionalized women; 2) the concentration of vitamin D was within the currently recommended levels for the older population in robust, prefrail, and frail women, i.e. all three groups had a serum 25(OH)D concentration of at least 20 ng/ml (50 nM) 3) blood vitamin D concentrations were similar in prefrail and frail women and so do not correlate with the severity of frailty syndrome. We discuss these findings in more detail below.

Several studies performed in old, male, community-dwelling individuals have found an inverse association between frailty syndrome and serum 25(OH)D concentration (Shardell et al., 2009; Smit et al., 2012), although this difference may be because basal 25(OH)D levels in serum are lower in women compared to men (Dawson-Hughes, Harris, & Dallal, 1997; Shardell et al., 2009). In addition, vitamin D intake has recently been estimated to be insufficient on a global scale (Bendik, Friedel, Roos, Weber, & Eggersdorfer, 2014). Similarly, in our sample significantly lower vitamin D concentrations were found in blood samples from both prefrail and frail women compared to robust women, thus demonstrating that a reduction in vitamin D concentration in blood is also associated with frailty syndrome in very old institutionalized women. Interestingly the association was specific for frailty syndrome and did not correlate with the participant's general health status, i.e. no significant relationships were observed between vitamin D and the other geriatric evaluation results from the Tinetti scale (gait and balance), MMSE test (cognitive function), Barthel index (basic activities of daily life), Norton scale (risk of pressure ulcers), the Yesavage scale (geriatric depression), and Lawton index (instrumental activities of daily life). This result suggests that vitamin D may be useful for treating frailty syndrome, even in very old institutionalized women. However vitamin D is liposoluble and its administration to individuals in supraphysiological amounts can lead to some major side effects.

Surprisingly, and in contrast with the literature in this field, in our study the concentration of vitamin D was reduced in frail women compared to non-frail women, but was still within the recommended range for all three groups (robust, prefrail, and frail individuals). However, we cannot generalize our results to include all institutionalized older individuals because our sample represents a segment of the population with good general health and excluded non-ambulating women (see the Barthel index values) and those with dementia (see the MMSE values).

Vitamin D deficiency, defined as serum 25(OH)D levels of less than 20 ng/ml (50 nM), has been reported in many older and middle-aged individuals living in U.S.A., Asia, and North Europe (Bouillon et al., 2005). However epidemiological data show that its prevalence ranges widely from 20 to 100% in the older population depending on the specific age, gender, and nationality (Bischoff-Ferrari et al., 2006; Thomas et al., 1998). The cause of widespread vitamin D deficiency is not fully understood and likely has many contributing factors including an age-related decrease in hydroxylation efficiency to form 25(OH)D and reduced sunlight exposure (Lips, 2001). Community-dwelling individuals may have decreased sunlight exposure due to economic difficulties, loneliness, participation in very few outdoor activities, or because of other more direct physical and mental effects of frailty. The individuals in our sample live in a geographic area with high levels of yearlong sunlight, and moreover, we collected the blood samples in spring when there is both an increased amount of sunlight and more incentives to go outside. Together these facts may have led our sample population to have had an increased amount of day-to-day sunlight exposure thus contributing to higher vitamin D serum concentrations compared to studies conducted in other countries.

Diet may also play a significant role in the regulation of vitamin D levels (Adams & Hewison, 2010; Bischoff-Ferrari et al., 2006). It is likely that institutional residents are better nourished than older people living in the general community because the meals they eat are carefully planned by nutritional and medical professionals to provide a balanced diet. This likely ensures adequate vitamin D intake (as in our study), whereas community-dwelling individuals may have serum vitamin D concentrations below the recommended level because they might not be able to properly plan, shop for ingredients, or prepare adequately-nutritional meals because of the effects of frailty. Similar to our findings, the National Health and Nutrition Examination Survey (NHANES), performed in a younger segment of the old-age population [(age 65 and older)], in US found that the mean serum 25(OH)D concentrations were within the recommended physiological range (60–72 nM) in frail, prefrail, and robust individuals (Smit et al., 2012). In addition, dietary intake of vitamin-D-fortified food products (e.g. yoghurt or milk) can vary among countries and individuals, giving rise to potential variation in blood vitamin D levels (Adams & Hewison, 2010; Rizzoli et al., 2013) which may skew the results. We did not attempt to scientifically account for this effect in this study, but it may be worth further investigation in future studies.

We showed that vitamin D levels were similar in prefrail and frail individuals, suggesting that it is not a biomarker for the severity of

frailty syndrome but rather it can be used to distinguish frail individuals from non-frail ones. No significant differences in serum calcium or parathormone concentration were observed between the groups, confirming previous findings that showed that the role of vitamin D in frailty syndrome is not related to the impairment of calcium metabolism, at least in terms of blood calcium availability (Bartali et al., 2006). These original findings provide the basis for further investigation into the underlying mechanisms that contribute to frailty in very old institutionalized individuals. Our results cannot be generalized to include all of the institutionalized older-population because we wanted to specifically study the association between vitamin D and frailty in very old women with good general health compared to the majority of individuals in this age range who are frail or prefrail (excluding those with dementia, the inability to walk, or without the ability to undertake the normal activities of daily life).

Conflict of interest statement

Authors have no conflicts of interest to declare.

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ANEXO D: Otras publicaciones relacionadas

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Review

Clinical features of prefrail older individuals and emerging peripheral biomarkers: A systematic review

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ABSTRACT

Frailty is a geriatric syndrome characterized by the clinical presentation of identifiable physical alterations such as loss of muscle mass and strength, energy and exercise tolerance, and decreased physiological reserve. Individuals with one or two of these alterations are defined as prefrail. The clinical features of prefrail older individuals have been investigated to a lesser extent compared to the frail population, even though this intermediate stage may provide insights into the mechanisms involved in the physical decline associated with aging and it is considered to be potentially reversible. We performed searches in the Medline, Embase, Scopus, Cinahl, and Cochrane databases from January 1995 to July 2013 for papers about the identification of prefrail people aged 65 and older published either in English or Spanish, and the reference lists of from the articles retrieved were pearled in order to identify any which may have been missed in the initial search. Two independent reviewers extracted descriptive information on frailty criteria and outcomes from the selected papers: of the 277 articles retrieved from the searches and 25 articles retrieved from pearling, 84 met the study inclusion criteria. The prevalence of prefrailty ranges between 35% and 50% in individuals aged over 60, is more common in women, and the age and the number of comorbidities in these individuals is similar to their frail counterparts. Weakness is the most prevalent symptom in prefrail individuals although there are some sex differences. Some serum biomarkers seem to discriminate prefrail from non-frail individuals but further research would be required to confirm this.

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1. Introduction

Frailty is a state of increased vulnerability to stressors (Morley et al., 2013), characterized by decreased physical functioning and an increased risk for poor outcomes, such as a higher incidence of falls, fractures, disabilities, comorbidities, health care expenditure, and premature mortality (Fried et al., 2001; Fugate Woods et al., 2005; Woo et al., 2012). Recently, the influence of genetic background has been explored in order to explain the variability of frailty phenotypes (Dato et al., 2012). The concept of frailty has grown in importance because of the need to better understand the health trajectory of older people, and to prevent, or at least to delay, the onset of late-life disabilities (Fried et al., 2001; Henly et al., 2011). Several models have been developed to assess frailty including the frailty index and frailty clinical scale (Hyde et al., 2010; Mitnitski, Mogilner, & Rockwood, 2001; Rockwood, 2005; Rockwood & Mitnitski, 2007) but the most used is that of Fried et al. (2001). The fragility index takes all the deficits that are present in an individual into account, including active diseases, ability to perform daily living activities, and physical signs from clinical and neurological examinations (from 20 to 70 different deficits). A third model, the FRAIL scale, integrates features from each of these models, combining physical symptoms, the inability to walk or climb a flight of stairs, weight loss, and exhaustion, with the presence of multiple illnesses. A fourth model, developed from the Study of Osteoporotic Fractures (SOF), leads to results similar to those obtained by evaluating frailty with Fried's criteria (Kiely, Cupples, & Lipsitz, 2009). At present there is no consensus on which measure should be used in the assessment of frailty, although difficulties in assessing frailty according to Fried's criteria in very old subjects (more than 85 years old) due to the high number of comorbidities in this population suggests that the frailty index or cumulative deficits index might be better used in these cases (Collerton et al., 2012; Kulminski et al., 2008).

Frailty syndrome is usually defined according to a well-established, standardized phenotype, based on five physical criteria as described by Fried et al. (2001) in the Cardiovascular Health Study (CHS): a clinical definition which has also been validated by other groups (Ahmed, Mandel, & Fain, 2007; Fugate Woods et al., 2005; Graham et al., 2009; Wilhelm-Leen et al., 2009). People meeting three or more criteria are classified as frail, those with one or two as prefrail (or intermediate-frail), and people without any as non-frail (Fried et al., 2001). By revising the literature, we found that individuals who meet one or two Fried criteria (intermediate-frail or prefrail) are sometimes not included in clinical studies, or that no statistical comparisons are made between prefrail, non-frail, and frail groups in terms of evaluating them with clinical scales or biomarkers. Logistic regression model results from analyzing the associations between frailty and risk-factor biomarkers in non-frail and prefrail subjects are often combined to focus on frailty and to create conservative models (Michelon et al., 2006; Semba et al., 2006; Szanton, Allen, Seplaki, Bandeen-Roche, & Fried, 2008). We believe that early identification of the prefrail population and characterization of its features is crucial in order to set therapeutic guidelines and nursing interventions aiming to prevent or minimize the conditions inherent to prefrailty, its transition to frailty, and the risk of acute clinical complications or disability and dependence (Fried, Ferrucci, Darer, Williamson, & Anderson, 2004; Walston et al., 2006). To our knowledge, to date no reviews have been published concerning the features of individuals in the prefrailty state, nor the presence of biomarkers related to prefrailty. In this work we specifically reviewed and discussed the following:

i. The features of prefrail individuals
ii. The progression of prefrailty
iii. The prevalence of Fried criterion in prefrail individuals
iv. Biomarkers for prefrailty

2. Materials and methods

The design of this study was developed according to PRISMA guidelines.

2.1. Literature search

A literature search using multiple electronic bibliographic databases was conducted. The Medline (OVID), Embase (OVID), Cinahl (OVID and EBSCO), Scopus, and Cochrane libraries were searched from January1995 to July 2013. Reference lists of all relevant articles were manually cross-referenced in order to identify additional articles. The primary search terms used were "*prefrail*" and "*prefrailty*". The search strategy used was *prefrail** with one of the following terms: *men, women, gender/sex differences, prevalence, physical activity, slowness, weakness, fatigue, weight loss, muscular strength, Fried criteria, age, aging, biomarker, white blood cells, leukocytes, inflammation, oxidative stress, testosterone, cortisol, vitamin, DHEAS (dehydroepiandrosterone)*, and *IL-6 (interleukin-6)*.

2.2. Inclusion/exclusion criteria

The following inclusion criteria were used: (1) acknowledged as an original article, (2) full-text published in either English or Spanish, (3) study participants were identified as "prefrail" or "pre-frail" in either the title, abstract, and/or text, (4) the frailty phenotype was assessed using the Fried criteria (Fried et al., 2001), (5) prefrail individuals were classified as those meeting one or two Fried criteria. Although most studies focused solely on analyzing and reporting on the frail group, the purpose of this systematic review was to focus exclusively on prefrailty.

2.3. Data collection and analysis

The database search results were uploaded into a web-based system which was used to manage the screening process, and duplicate citations were removed. To determine which studies would be included, four members of the review team independently screened the title and abstracts of the articles extracted from the literature search. The electronic full text was retrieved for studies on which the reviewers agreed, based on our inclusion/

exclusion criteria. For each of these articles, two reviewers independently extracted the following data: the country where the study was conducted, the number of participants in the non-frail and prefrail groups, the age of participants at the time of inclusion, the sex of participants, their living arrangements, and the definition and measurement method of frailty. Any disagreement between the two reviewers on the papers and data extracted from them was resolved by the corresponding author.

3. Results

Among the studies identified by our search strategy, 104 required further full-text screening, and 84 met the inclusion criteria. We separately summarized and discussed the results which emerged from the literature review in four sections: the features of prefrail individuals, the progression of prefrailty, the prevalence of each Fried criterion in prefrail individuals, and biomarkers of the prefrailty state.

3.1. The features of prefrail individuals

In contrast to the relatively low prevalence of frailty in the elderly (7–12% in population aged 65 years or more and around 25% in those over 85 years) the prevalence of prefrailty is much higher (Chen, Wu, Chen, & Lue, 2010; Danon-Hersch, Rodondi, Spagnoli, & Santos-Eggimann, 2012; Ensrud et al., 2007; Espinoza, Jung, & Hazuda, 2012; Fried et al., 2001; Masel, Graham, Reistetter, Markides, & Ottenbacher, 2009; Ottenbacher et al., 2009; Santos-Eggimann, Cuenoud, Spagnoli, & Junod, 2009; Semba et al., 2006; Smit, Winters-Stone, Loprinzi, Tang, & Crespo, 2012). Studies performed on large cohorts demonstrate that the prevalence of prefrailty ranges between 35% and 50% in the population aged over 65. Among the 5317 people evaluated in the CHS study (age range: 65–101) the prevalence of prefrailty was 47% (Fried et al., 2001), and similarly, among participants aged 60 years or older in the third national health and nutrition examination survey (NHANES III), 40.5% were prefrail (Smit et al., 2012). In the population-based cohort study performed in Italy (InCHIANTI; adults aged 65 years or more; $n = 1155$) the prevalence of prefrailty was 32% as assessed with a slightly modified Fried's criteria (Shardell et al., 2012). In the context of the Lausanne Cohort 65+ (Lc65+): a longitudinal, observational study performed at the University of Lausanne Hospital Center (Switzerland) on 1283 participants (aged 65–70 years), 71.1% were classified as non-frail, 26.4% as prefrail, and 2.5% as frail (Danon-Hersch et al., 2012; Santos-Eggimann et al., 2008). In this "young old" age group, only one-quarter of the population was classified as prefrail, which is lower compared to other studies. This difference was likely accounted for by the Lc65+ participants' younger age compared to other studies in which participants had a mean age 7–12 years older than those in the Lc65+ cohort.

A 2004 cross-sectional analysis of 16,600 community-dwelling individuals aged 50 years or older enrolled in the Survey of Health, Aging and Retirement in Europe (SHARE) in 10 European countries demonstrated that 37.4% of the middle-aged (50–65 years) population were prefrail (Santos-Eggimann et al., 2009). Significant differences in the prevalence of prefrailty (as well as frailty) were observed between countries: in both the middle-aged and the over 65 years (65+) age categories, prefrailty was the most prevalent in the middle-aged populations in Spain, Italy, and France (44.9%, 43.5%, and 40.3% respectively) and in the 65+ population in Spain, Italy, and Switzerland (50.9%, 45.6%, and 46.5% respectively). Similarly, some differences in the prevalence of prefrailty have also been reported among American older individuals in seven Latin American or Caribbean cities (Alvarado, Zunzunegui, Béland, & Bamvita, 2008). The reason for such differences may be due to the lower rates of institutionalization of older disabled people in these countries, although comparisons in the prevalence of prefrailty between studies should be conducted with caution because of potential differences in the definitions of frailty criteria, the distribution of confounding factors such as age, sex, or race (e.g. African American men and women show a higher prevalence of frailty), or exclusion criteria. For instance, the higher prevalence of prefrailty in the SHARE study can probably be explained by methodological differences: the SHARE study criteria were less specific than those defined in the CHS study with the exception of 'weakness' thus leading to a higher prevalence, particularly for the 'exhaustion' criterion (Santos-Eggimann et al., 2009).

Similar to frailty, prefrailty is also more common in women than in men, although this is not always reported (Garcia-Garcia et al., 2011). The prevalence of prefrailty in the 50–64 year-old group in the SHARE study was 42% in women and 33% in men, whereas this nearly equalized in the 65+ group, with prefrailty noted in 42.7% of women and 41.9% of men (Santos-Eggimann et al., 2009). Notably, the Lc65+ cohort identified 63% of prefrail individuals as women (Danon-Hersch et al., 2012). However some studies have not found such differences, for example Voznesensky, Walsh, Dauser, Brindisi, and Kenny (2009) reported that the prevalence of one Fried criterion was 42.5% in women and 48.2% in men, with the frequency of two criteria 13.4% and 18.2% in the female male populations respectively.

Prefrailty is associated with lower levels of education in elderly people living at home (Espinoza et al., 2012; Hoeck et al., 2012), lower acculturation (Masel, Howrey, & Peek, 2011), and a lower socio-economic burden (Fried et al., 2001). There is also a significantly higher level of comorbidity in prefrail individuals compared to non-frail individuals with the presence of two or more chronic diseases. Specific conditions that were more frequently reported in prefrail rather than non-frail subjects included chronic heart disease or other heart diseases, stroke, diabetes mellitus, hypertension, chronic respiratory diseases, osteoporosis, arthritis, balance issues, and depression (Blaum et al., 2009; Drey et al., 2010; Fried et al., 2001; Kang et al., 2009), however, at least in the case of hypertension, conflicting results have been reported (Bastos-Barbosa et al., 2012). The age or the number of comorbidities and the extent of cognitive decline in prefrail individuals is similar to that observed in frail individuals (Avila-Funes et al., 2009; Danon-Hersch et al., 2012; Hubbard, O'Mahony, Savva, Calver, & Woodhouse, 2009; Samper-Ternent, Al Snih, Raji, Markides, & Ottenbacher, 2008), suggesting that these underlying comorbidities do not explain the differences in frailty syndrome severities. Indeed, cognitive impairment, assessed by the mini-mental state examination (MMSE) score, is independently associated with an increased risk of both prefrailty and frailty over a 10-year period in older Mexican Americans (Raji, Al Snih, Ostir, Markides, & Ottenbacher, 2010).

Prefrail individuals have a reduced survival rate and a higher number of impairments to their daily living and instrumental activities than non-frail participants (Danon-Hersch et al., 2012; Faber et al., 2006; Fried et al., 2001; Snih Al et al., 2009; Wong et al., 2010). In the CHS cohort, the survival curves for each frailty group showed that after a 7-year interval, 43% of those who were frail had died, compared to 23% of those who were intermediate, and 12% of those who were robust at the baseline time point (Fried et al., 2001). Obesity is also more likely in prefrail than in non-frail or frail individuals (Blaum, Xue, Michelon, Semba, & Fried, 2005; Hubbard et al., 2009, 2010), although this may be explained by the unintentional loss of weight which is common in very frail subjects (Cawthon et al., 2007). Similarly, a cross-sectional analysis of baseline time point data from the US Women's Health and Aging Studies (WHAS) I (1992) and II (1994) revealed that prefrail individuals have a higher weight and prevalence of obesity (Blaum

et al., 2005). Moreover, the proportion of prefrail women increased with increasing body mass index (BMI), whereas, in contrast, the proportion of frail women was lowest for women with a BMI of 25–30 kg/m^2, and was highest for women with a BMI of 30 kg/m^2 or higher (Blaum et al., 2005). In line with the role of abdominal obesity, Hubbard, Lang, Llewellyn, and Rockwood (2010) recently demonstrated a significant positive association between BMI and frailty which showed a U-curve relationship.

It is not surprising that the "risk state" of frailty is associated with both BMI extremes: obesity (including sarcopenic obesity) and weight loss. Besides obesity, even excess weight recorded in mid-life has been associated with the development of prefrailty in a 26-year follow up of initially healthy men (Strandberg et al., 2012). The occurrence of diabetes also appears to be relevant to the transition to the prefrailty state (Barzilay et al., 2001, 2007; Smith & Aspray, 2011; Villareal, Banks, Siener, Sinacore, & Klein, 2004). Additionally, prefrail older adults show a significant reduction in lower-extremity muscular power compared to non-frail individuals evaluated in stair climb and sit-to-stand transfer tests for functional power (Zech, Steib, Sportwiss, Freiberger, & Pfeifer, 2011) suggesting that besides upper-extremity muscular strength (one of the five measurements of the physical phenotype of frailty syndrome) muscular strength in the lower-extremities is also decreased in the prefrailty state (see Section 4). However, despite these changes, the incidence of falls seems to be unaffected in prefrail individuals compared to robust individuals (Samper-Ternent et al., 2012) suggesting that physical alterations (gait, posture, etc.) other than decreases in muscular strength, must also account for an increased risk of falls.

3.2. The progression of prefrailty

Many studies have shown that prefrailty predicts frailty. Fried et al. demonstrated that in the CHS cohort, after adjusting for covariates, prefrail individuals are at more than twice the risk of becoming frail over three years, compared to non-frail individuals (Fried et al., 2001). In support of other previous findings (Walston et al., 2002), a long term follow up study of the cohort who were non-frail at the baseline time point in the original CHS showed that 26% did not develop prefrailty or frailty at either the 5-year or 9-year follow up; 66% remained prefrail or had developed prefrailty at years 5 and/or 9, and 8% became frail at years 5 or 9 (Barzilay et al., 2007). Of the individuals still alive at year 9, only 3.5% remained non-frail. In general, the same factors that predicted frailty also predicted prefrailty in many follow up studies. Those who became prefrail or frail were more likely to be older individuals, African American, and/or female, who at the baseline time point were heavier, had more central obesity, and/or had a lower self-assessed health status and MMSE scores than those who did not become frail (Barzilay et al., 2007). They had fewer years of education, a lower income, and were less likely to be married. Masel et al. (2011) demonstrated over the course of a 10 year study that increased acculturation protects older Mexican Americans against the transition to a prefrail or frail state, suggesting that language abilities with their consequences in the social and cultural domain are protective, at least in this huge subpopulation of Americans. Whether these factors played a causal role in the development of frailty or were a consequence of other unidentified factors that lead to frailty deserves further exploration. Prefrail individuals were about 50% more likely to have metabolic syndrome compared to non-frail individuals (Barzilay et al., 2007), and those who developed frailty were also more likely to have baseline depressive symptoms or arthritis and to use more medications (Fried et al., 2001). Blood pressure levels did not significantly differ between subjects who did or did not become frail, but those who became frail were also more likely to additionally develop diabetes mellitus and stroke. Hence these different studies provide support for the hypothesis that prefrailty and frailty represent different levels within the same progressive process (Barzilay et al., 2007; Drey, Pfeifer, Sieber, & Bauer, 2011).

The predictive association between the prefrailty stage and the incidence of falls, worsened mobility, impairment in the activities of daily living (ADL), the incidence of hospitalization, and death over a three or seven-year period, showed hazard ratios ranging from 1.28 to 2.10 for the prefrail group, and 1.82 to 4.46 in the frail group. The prefrail group also significantly predicted all outcomes after adjustment, but with lower strengths of association compared to the frail group (Fried et al., 2001). In contrast to frailty transition, hospitalization plays a minor or no role in the transition from prefrail or from non-frail to prefrail states, respectively (Gill, Gahbauer, Han, & Allore, 2011). It is possible that progression from the prefrail to frail status might have one set of etiological factors, whereas progression of the frailty to a more end-stage point might be associated with others; such factors might include declines in weight or low levels of albumin or cholesterol as a consequence of malnutrition or catabolism.

3.3. The prevalence of each Fried criterion in prefrail individuals

The five different components of frailty syndrome (Fried criteria) are generally evaluated and defined as follows:

(1) *Weight loss*. Defined as the unintentional loss of 4.5 kg in the past year.
(2) *Exhaustion*. If the participant answered "Often" or "Most of the time" to the question: "How often in the last week did you feel that everything you did was an effort?" included in the Center for Epidemiologic Studies-Depression scale (Radloff 1977); the exhaustion criterion was considered present.
(3) *Physical inactivity*. Participants who performed no physical activity, spent most of the time sitting, or rarely had a short walk (or undertook other non-demanding physical activities).
(4) *Slowness*. Measured as a slow walking speed, corresponding to the worst quintile for their sex and height group.
(5) *Weakness*. This is commonly assessed by measuring hand grip strength using a hand-held dynamometer.

It is relatively hard to establish which Fried criteria are more frequent in the prefrail population due to the fact that few articles collect detailed data on the prevalence of each individual criterion within this group. Studies dealing with the influence of sex, age, comorbidities, etc., with each Fried criterion in prefrail individuals are even more scant. Within the landmark Lc65+ study, the prevalence of single frailty criterion in prefrail individuals was 40.5% for weakness, 29.0% for weight loss, 17.1% for low physical activity, 11.5% for exhaustion, and 2.0% for slowness. People with weakness as the sole Fried frailty criterion differed significantly from non-frail participants: 66.7% were women, and they were significantly older than the other non-frail participants, and these individuals also needed help with daily (ADLs) and instrumental (IADLs) activities of daily life (Danon-Hersch et al., 2012). Interestingly, weakness was associated with a two to three times greater prevalence of heart diseases, diabetes mellitus, and arthritis (Danon-Hersch et al., 2012). Similarly, prefrail individuals displaying exhaustion as the single Fried frailty criterion also needed help with ADLs and IADLs significantly more often than non-frail participants, but in contrast they additionally reported depression more frequently (Drey et al., 2010).

In the WHAS II, a longitudinal study with a 7.5-year follow up period that included 420 non-frail women aged 70–79 at the baseline time point, weakness was the most common initial

manifestation in prefrail women and was moderately predictive of the onset of frailty (Xue, Bandeen-Roche, Varadhan, Zhou, & Fried, 2008). Low activity and slowness were the second and third criteria found in the prefrail group. The predictive value of weakness for the onset of frailty agrees with earlier reports that showed that loss of muscle strength begins in midlife (Lindle et al., 1997). Decline in strength has been attributed to the loss of muscle mass and muscle quality referred to as sarcopenia, resulting from anatomic and biochemical changes in aging muscle (Kamel, 2003).

With the landmark NHANES III study which assessed frailty with four Fried criteria (weight loss was excluded) in individuals 60 years and older Smit et al. (2012) found that the most common symptoms among prefrail individuals were muscle weakness (35%), and slow walking (36%) which are also the two features which were the most prevalent in the frail group. Of note, exhaustion was reported only by 4% of prefrail older adults, but in contrast it was reported by 63.4% of frail older adults suggesting that exhaustion and weight loss may be good indicators to discriminate prefrail and frail individuals. However, other studies have shown that exhaustion is more common in prefrail individuals (Drey et al., 2011; Santos-Eggimann et al., 2009) or in much older prefrail individuals (Dahlin-Ivanoff et al., 2010; Jürschik Giménez, Escobar Bravo, Nuin Orrio, & Botigué Satorra, 2011). Significant differences are found in the literature regarding exhaustion, and differences in the age of patients recruited across the different results only partially explains these discrepancies, thus making further study of this issue essential. One of the main reasons for different result in the prevalence of exhaustion could be due to differences in measuring this Fried criterion in, but not only, large epidemiological frailty studies (WHAS, CHS, InCHIANTI and MrOS).

However, despite the heterogeneous entry points into the frailty state, most transitions to frailty, especially in women, do involve adding exhaustion and/or weight loss (Xue et al., 2008), raising the possibility that decreased energy production or increased utilization (similar to wasting conditions), may be involved in the threshold transition toward frailty in a final common pathway. Weight loss and exhaustion rarely developed alone, but rather appeared concomitantly with other manifestations which would result in physical depletion or failure of compensatory mechanisms (Bortz, 2002; Drey et al., 2011; Gavrilov & Gavrilova, 2001; Kamel, 2003; Lindle et al., 1997). Drey et al. (2011) demonstrated that individuals with exhaustion have higher scores on the geriatric depression scale, suggesting a correlation between the two, and therefore might also explain why exhaustion is more common in frail or prefrail women (Xue et al., 2008). Weight loss is seldom observed in prefrail individuals and appears as a late manifestation of frailty and/or may reflect a disease end-stage strongly associated with mortality.

Similar to other geriatric diseases and syndromes, and as suggested by other studies, several gender differences have been observed in frailty (Carcaillon et al., 2012; Gale et al., 2013; Garrido, Serrano, Bartolomé, & Martínez-Vizcaíno, 2012; Walston & Fried, 1999). Sex-differences in the prevalence of Fried criteria among prefrail individuals have been reported; in the InCHIANTI cohort study Shardell et al. (2009) demonstrated that the most common Fried criteria in prefrail men were slowness (46.2%) and weakness (46.4%), whereas exhaustion (38%) and low energy expenditure (36.7%) were most prevalent among prefrail women. Further research is required to specifically delineate the types and prevalence of individual frailty criteria in pre-frail women and men. However, taking both genders into account, the most common Fried criteria observed in frail individuals are still slowness and weakness, concurring with previous findings. Similar to other geriatric diseases and syndromes, as suggested by other studies, several gender differences have been observed in frailty (Carcaillon et al., 2012; Gale et al., 2013; Garrido et al., 2012; Walston & Fried, 1999) and further research should address the type and the prevalence of individual frailty criteria in pre-frail women and men. Weight loss is seldom observed in prefrail individuals and appears as a late manifestation of frailty and/or may reflect an end-stage of disease strongly associated with mortality.

3.4. Biomarkers of prefrailty

The etiology of frailty is not well understood but it has been associated with changes in several physiological systems, including inflammation, coagulation, hematological, and endocrine systems, as well as changes to the balance of micronutrients and vitamins (Walston et al., 2002). Evidence suggests that these physiological changes are evident at a preclinical stage of frailty (prefrailty), and it is hypothesized that they are at the root of the characteristics used to classify the syndrome (Fried et al., 2001). The identification of blood markers that distinguish prefrail older adults would be useful for both prevention and etiological studies. We studied all the biomarkers that have been associated with prefrailty in order to identify any that might discriminate prefrail from non-frail subjects, although most of the studies did not statistically compare either non-frail vs. prefrail or prefrail vs. frail individuals and so final conclusions cannot be drawn from this data. We reviewed the main frailty biomarkers (inflammatory markers, hormones, vitamin D, and others) to check for their alteration in prefrail individuals.

3.4.1. Inflammatory markers

IL-6 is an important cytokine that modulates the immune system. Increasing evidence suggests that high IL-6 levels are associated with adverse outcomes, including disability and early mortality in older adults (Cohen, 1997; Ershler & Keller, 2000; Ferrucci et al., 1999; Harris et al., 1999). A number of studies have observed a significant association between elevated IL-6 blood concentrations and frailty in older adults (Leng et al., 2004; Leng, Xue, Tian, Walston, & Fried, 2007). Ronning et al. (2010) evaluated the relationship between IL-6 and frailty in a total of 187 post-operative patients (submitted for elective colon or rectum tumor resections) using Fried criteria and the comprehensive geriatric assessment scale (CGA): a multidimensional evaluation including comorbidity, polypharmacy, physical functioning, nutritional and cognitive status, depression, and social support (Balducci & Extermann, 2000). IL-6 and C-reactive protein (CRP) were significantly increased in prefrail subjects compared to robust subjects, however this was only true when prefrailty was assessed by CGA: other studies did not find significant changes in serum IL-6 concentration in prefrail subjects classified according to Fried criteria; indeed the percentage of subjects with detectable IL-6 were very similar at 41% and 44% in non-frail and prefrail cohorts respectively (Hubbard et al., 2009; Leng et al., 2007, 2011). Interestingly, a cross-sectional study of community-dwelling older women suggests that chronic CMV infection is associated with both prefrailty and frailty and has a significant multiplicative effect on IL-6 levels (Schmaltz et al., 2005) suggesting a relationship between a chronic asymptomatic infection and the prefrailty state.

Two studies found an association between TNF-alpha and the prevalence of frailty (Hubbard et al., 2009; Serviddio, Romano, Greco, & Rollo, 2009), but this association was not observed in the InCHIANTI study (Bandeen-Roche, Walston, Huang, Semba, & Ferrucci, 2009; Leng et al., 2004). Moreover, Hubbard et al. (2009) did not find any significant increase in TNF-alpha concentration in prefrail institutionalized patients when assessed with the frailty index. However, TNF-alpha concentration in serum is significantly

increased in prefrail oncology patients (Ronning et al., 2010) suggesting that TNF-alpha can trigger frailty in these patients.

The cross-sectional association study performed within the CHS revealed that the concentration of C-reactive protein levels in plasma was increased by 37% in prefrail individuals compared to non-frail individuals (mean 3.7 mg/L vs. 2.7 mg/L, respectively; Walston et al., 2002). However, Hubbard et al. (2009) and Wu, Shiesh, Kuo, and Lin (2009) failed to find any significant difference in the concentration of C-reactive protein levels in prefrail patients, although Hubbard et al. (2009) observed a tendency (3 mg/L and 4 mg/l in non-frail and prefrail patients respectively). White-blood cell counts were not significantly increased in prefrail individuals (Hubbard et al., 2009).

3.4.2. Testosterone

Low testosterone concentrations in blood have been related to frailty on many occasions, although with a certain variability of results among studies (O'Connell et al., 2011). The majority of testosterone in human serum is bound to either sex hormone-binding globulin (SHBG; 44%) or albumin (54%) with a higher affinity binding in the former and weaker in the latter. The remaining unbound fraction (2%) is referred to as 'free testosterone' and is available to all target tissues that express the androgen receptor; the albumin-bound fraction is considered to be bioavailable in some (but not all) target tissues. It is well established that male testosterone concentrations decline modestly with age at the rate of 0.4–2% a year (Harman et al., 2001) which contrasts with the dramatic decreases in hormone concentrations observed in menopause, a fact which may explain the increased prevalence of frailty in women (Walston & Fried, 1999). Many studies found that blood testosterone concentrations, and in particular the free testosterone concentrations, are significantly decreased in frail individuals (see Table 1). Although our careful qualitative analysis of published reports suggests that decreased testosterone blood concentrations may represent a useful prefrailty biomarker, further study, taking into account possible gender differences is required to definitively draw these conclusions. A careful qualitative analysis of published reports suggests that testosterone concentration in blood can be already decreased in prefrail subjects and this aspect deserves further studies and attention should be paid to possible gender differences (see Table 2).

3.4.3. Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are prominent adrenal steroid hormones in humans (Orentreich, Brind, Rizer, & Vogelman, 1984). DHEA/DHEAS influences peripheral tissues either indirectly via conversion to androgens, estrogens or both, or directly as a steroid hormone (Perrini, Laviola, Natalicchio, & Giorgino, 2005). DHEA shows a characteristic secretion pattern throughout life, with serum levels declining with increasing age. DHEA levels also change depending on gender, with higher levels in men (Orentreich et al., 1984), and lower DHEAS levels have been reported in the serum of frail subjects (Cappola, Xue, & Fried, 2009; Leng et al., 2004).

A cross-sectional analysis of baseline time point information from three separate studies in healthy older subjects, including 898 participants aged 60 years or older (65.8% male), evaluated DHEAS serum concentrations and its relationship with frailty as assessed by Fried criteria (Voznesensky et al., 2009). In this study 48% of subjects in the highest DHEAS level quartile met no Fried criteria; 18% of non-frail individuals were included in the lowest DHEAS level quartile (15.0–27.3 μg/dL), which increased to 27% in intermediate-frailty individuals who met one Fried criteria and to 30% in intermediate-frailty individuals meeting two. In frail individuals who met 3–5 criteria the percentage reached 37% of this DHEAS subpopulation.

In the WHAS I and II the bottom quartile of serum DHEAS concentrations were more likely to be in the prefrail group than in the non-frail group (Cappola et al., 2009). However, there was little difference between prefrail and frail women in the magnitude of the association between individual deficiencies in DHEAS and frailty stage, suggesting that these relative deficiencies alone do not distinguish prefrail and frail women. In addition, the magnitude of association differed by very little hormonal burden in prefrail women. This nonlinearity suggests that association does not simply represent a dose-response effect, but rather the crossing of a threshold which results in multisystem deregulation and disrupted homeostasis.

3.4.4. Cortisol

Activation of the hypothalamic–pituitary–adrenal (HPA) axis to produce cortisol is a fundamental endocrine response to situations that threaten homeostasis. In addition to its well characterized roles of increasing glucose availability and inhibiting the inflammatory cascade, cortisol also mediates numerous other functions essential for stress responses. Elevated diurnal cortisol and an impaired HPA-axis response to stressors have been hypothesized to initiate or amplify alterations in many other important physiological systems.

Several studies demonstrated that higher levels and blunted diurnal variation of cortisol may be involved in the vulnerability and clinical presentation observed in frail older individuals (Johar et al., 2014; Varadhan et al., 2008). Within the framework of the WHAS II Varadhan et al. (2008) found a significant and positive association between frailty burden and 24-h mean cortisol levels (but not awakening levels) in elderly (80–90 years old) community-dwelling women, however, these changes in cortisol concentrations were not observed in prefrail women. Interestingly, in elderly residents in long-stay institutions, frailty is associated with an increase in the concentration of salivary cortisol, an association which also appears in prefrail individuals (de Almeida Holanda et al., 2012).

Table 1
Testosterone and frailty. Summary of the results obtained by measuring blood testosterone (total and free) and sex hormone binding globulin (SHBG) in frailty. S: significant; N.S.: not significant; N.D.: not determined; y: years; *n*: sample size. Arrows indicate 'significantly increased' or 'significantly decreased' compared to non-frail individuals.

Study	Gender	*n*	Age	Frailty assessment	Total testosterone		Free testosterone		SHBG
Mohr et al. (2007)	Men	646	50–86 y	Fried criteria	N.S.		N.S.		S:↑
Cawthon et al. (2009)	Men	1469	>65 y	Fried criteria	N.S.		S:↓		N.S.
Wu et al. (2010)	Men	54	=or >65 y	Fried criteria	Men	S:↓	S:↓		N.S.
	Women	54			Women	S:↓	S:↓		N:S
Travison et al. (2010)	Men	624	50–86 y	Fried criteria	N.S.		N.S.		N.S.
Travison et al. (2011)	Men	1645	>70 y	Fried criteria SOF index	S:↓		S:↓		S:↓
Eichholzer et al. (2012)	Men	461	>60 y	Fried criteria	N.S.		N.S.		N.S.
Carcaillon et al. (2012)	Men	552	>65 y	Fried criteria	Men	↓	Men	↓	N.D.
	Women	735			Women	N.S.	Women	N.S.	

Table 2
Testosterone and prefrailty. Values (*SD* or range) of mean testosterone concentration in blood in robust (non-frail) and prefrail individuals. Total testosterone concentration is expressed as nmol/L; free testosterone concentration (pmol/L); SHBG (sex hormone-binding globulin; nmol/L). N.D.: not determined; y: years; *n*: sample size.

Study	Gender	*n*	Age	Frailty assessment		Total testosterone	Free testosterone	SHBG
Mohr et al. (2007)	Men	646	50–86 y	Fried criteria	Robust, $n = 256$	15.2 ± 5.7 nmol/L	0.26 ± 0.10 pmol/L	50.6 ± 19.8 nmol/L
					Prefrail, $n = 295$	14.1 ± 5.5 nmol/L	0.25 ± 0.10 pmol/L	51.1 ± 20.1 nmol/L
Wu et al. (2010)	Men	54	= or >65 y	Fried criteria	Robust, $n = 9$	25.9 nmol/L (13.2–35.2)	0.30 pmol/L (0.267–0.40)	67.8 nmol/L (19.9–102.9)
					Prefrail, $n = 31$	19.4 nmol/L (7.2–39.9)	0.27 pmol/L (0.87–0.41)	59.3 nmol/L (26.5–145.8)
	Women	54			Robust, $n = 8$	0.45 nmol/L (0.36–1.25)	6.65 pmol/L (3.91–21.00)	58.0 nmol/L (33.1–77.8)
					Prefrail, $n = 36$	0.47 nmol/L (0.14–1.55)	4.66 pmol/L (1.57–15.10)	56.2 nmol/L (13.9–154.7)
Carcaillon et al. (2012)	Men	552	>65 y	Fried criteria	Robust, $n = 275$	4.43 ng/mL (3.56–5.98)	5.92 pg/mL (4.43–9.48)	N.D.
					Prefrail, $n = 241$	3.70 ng/mL (3.35–5.69)	5.00 pg/mL (3.63–9.20)	N.D.
	Women	735			Robust, $n = 363$	0.41 ng/mL (0.23–0.68)	0.37 pg/mL (0.13–0.93)	N.D.
					Prefrail, $n = 300$	0.40 ng/mL (0.25–0.67)	0.37 pg/mL (0.12–0.94)	N.D.

3.4.5. Vitamin D

Vitamin D is necessary to preserve adequate blood calcium and phosphorous levels, to maintain strong bones, to regulate cell growth and differentiation, to assist immune system activity, to influence muscle phosphate metabolism, and regulate some cerebral processes (Adams & Hewison, 2010); it is metabolized into 25-hydroxyvitamin D (25[OH]D) in the liver and extra-renal tissues and converted into the active form 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D$) in the kidneys. Concentrations of serum 25(OH)D are a good indicator of vitamin D status (Bouillon et al., 2008). Vitamin D deficiency, defined as serum (25(OH)D) levels of less than 20 ng/ml (50 nmol/L), is common in elderly U.S. and European adults, and the prevalence ranges widely from 20 to 100% in older populations (Bischoff Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Holick, 2004; Thomas et al., 1998). Several studies found an inverse association between frailty and blood 25(OH)D levels in both genders (Eichholzer et al., 2012; Smit et al., 2012; Wilhelm-Leen, Hall, DeBoer, & Chertow, 2010), whereas the Italian InCHIANTI study which included 561 women (55.8%) and 444 men (44.2%) demonstrated that Vitamin D insufficiency was significantly associated with frailty only in men (Shardell et al., 2009). Factors that contribute to this deficiency are an age-related decrease in hydroxylation efficiency to form 25(OH)D, and reduced sunlight exposure (Lips et al., 2006).

Among adult participants aged 60 years and older in the NHANES III study serum 25(OH)D concentrations were the lowest in frail participants (60.4 ± 2.3 nM, $n = 453$), were intermediate in prefrail participants (65.6 ± 1.1 nM, $n = 1915$), and the highest in participants who were not frail (71.9 ± 0.9 nM, $n = 2363$; (Smit et al., 2012). Even though basal serum levels of 25(OH)D are lower in women compared to men (Dawson-Hughes, Harris, Krall, & Dallal, 1997; Shardell et al., 2009) the percentage of non-frail individuals with a serum 25(OD)D deficiency was 40% whereas it was 63% in prefrail individuals. After adjusting for age, center, health, and lifestyle confounding factors, these lower levels were associated with prefrailty (confidence interval [CI]: 1.26–1.67) and frailty (CI: 1.30–2.76), but no significant differences were observed between non-frail and prefrail women (Shardell et al., 2009; Table 3). In addition, men with low 25(OH)D levels had an elevated risk of frailty and prefrailty compared with men with adequate 25(OH)D levels. These data suggest that the contribution of hypovitaminosis D in prefrailty (but not in frailty) is likely to be gender-dependent. Moreover there is statistical evidence suggesting that there are different associations between vitamin D insufficiency and single frailty criteria (low energy expenditure in both sexes, and slowness in men) when considering prefrail and frail subjects in a single cohort.

Similar to the findings by Wilhelm-Leen et al. (2010), the cross-sectional results from NAHNES III showed that participants with low serum 25(OH)D concentrations were 1.5 times more likely to be prefrail (and 1.7 times more likely to be frail). Because these data are cross-sectional, whether low serum 25(OH)D levels contribute to the development of prefrailty or vice versa cannot be resolved. Nonetheless, several mechanisms for the contribution of low vitamin D levels to prefrailty and/or frailty have been proposed: vitamin D can affect muscle metabolism by mediating gene transcription through allelic vitamin D receptor polymorphisms in muscles (Janssen, Samson, & Verhaar, 2002) and thus might play a role in the weakness and/or low muscle strength observed in prefrail individuals. Together these data suggest that vitamin D insufficiency is present in prefrail individuals and it is suitable as a possible prefrailty biomarker (Table 3).

When 25(OH)D levels are low, active metabolite 1,25-(OH) 2 D and calcium absorption decreases. This reduced serum calcium causes parathyroid hormone (PTH) levels to rise in order to stimulate 1,25-(OH)2D production, resulting in increased bone turnover and hip fracture risk (Lips et al., 2006). High serum PTH

Table 3
Vitamin D and prefrailty values (*SD* or range) of mean vitamin D concentration in serum in robust (non-frail) and prefrail individuals. D; y: years; *n*: sample size.

Study	Gender	*n*	Age	Frailty assessment	Serum 25(OH)D	
Shardell et al. (2009)	Men	151	≥ 65 y	Fried criteria	Robust, *n* = 242	61.5 nmol/L (41.7–78.4)
					Prefrail, *n* = 151	43.2 nmol/L (30.0–69.6)
	Women	237			Robust, *n* = 243	36.2 nmol/L (27.2–56.2)
					Prefrail, *n* = 237	34.2 nmol/L (21.7–47.7)
Smit et al. (2012)	Men + women	Robust: 46.5% male Prefrail: 36.8% male	≥60 y	Fried criteria	Robust, *n* = 2363	71.9 ± 0.9 nmol/L
					Prefrail, *n* = 1915	65.6 ± 1.1 nmol/L

levels (higher than 32.4 ng/L) were associated with prefrailty in men (Shardell et al., 2009): serum PTH concentrations were high in 12% of non-frail, 30% of prefrail, and 31% of frail men. Interestingly the hyper-PTH did not further increase in frail compared to prefrail men, and PTH concentrations were only slightly increased in prefrail women (but significantly increased in frail women; (Shardell et al., 2009). These data suggest that sex-differences may account for the different interplay between 25(OH)D and PTH in prefrail individuals. All five Fried criteria, except sarcopenia, are associated with 25(OH)D serum concentrations, while only weakness is associated with PTH serum concentrations (Tajar et al., 2013).

3.4.6. Other biomarkers

A decrease in serum albumin concentration is a well-established marker of aging (Crimmins, Hayward, Ueda, Saito, & Kim, 2008) and frailty (Walston et al., 2002; Wu et al., 2009; Wu, Lin, Liu, Tsai, & Shiesh, 2010), however, not all studies reported a significant decrease in this parameter in frail subjects (Eichholzer, Barbir, Basaria, & Dobs, 2012); serum albumin is decreased in prefrail subjects (Smit et al., 2012) although no direct comparison between prefrail and non-frail subjects was performed (Smit et al., 2012). Another report did not find any significant differences in serum albumin concentration between non-frail and prefrail subjects (Hubbard et al., 2009) or in men in the CHAMP and NHANES III cohorts (Eichholzer et al., 2012; Le Couteur et al., 2010), or the elderly Chinese population (Wu et al., 2009, 2010). A study performed in older women recruited in WHAS I and II showed a linear relationship between thyroglobulin antibody seropositivity and the prefrail to frail spectrum (Wang et al., 2010), but it was unclear whether these autoantibodies were pathogenic or were a feature of frailty syndrome.

Shamsi et al. (2012) found differences in the plasma glycoproteome of prefrail individuals before the onset of clinically recognizable frailty. Among these proteins the most encouraging biomarkers for future research were transferrin which was increased, and hemopexin, fibrinogen gamma chain, and apolipoprotein E which were decreased. In addition, the levels of reactive oxygen species, notably hydrogen peroxide, increase with age, and result in an accumulation of oxidative damage to DNA, lipids, and proteins. Guanine is the most easily oxidized nucleoside, producing 8-hydroxy-20-deoxyguanosine (8-OHdG) when oxidized in situ in the DNA carbon backbone. 8-OGdG serves as an oxidative stress marker; and results in mutations due to mismatching, thus compromising cells, as well as tissues and organ function; it accumulates with age and was increased in one prefrail Chinese population study after adjusting for inflammation and other metabolic markers (Wu et al., 2009), The significant association between 8-OHdG and frailty, after adjusting for inflammation and metabolic markers, pointed toward the possibility that oxidative stress is directly linked to prefrailty. Future studies must be conducted in order to confirm these findings and to determine if these and other biomarkers could identify older adults at risk for prefrailty and frailty and the poor outcomes associated with them.

The discovery of biomarkers associated with prefrailty may be useful to identify the target population most amenable to clinical and/or care interventions in order to delay the transition to the stage of frailty or disability.

3.4.7. Biomarkers of frailty unchanged in prefrail individuals

Data from InCHIANTI study showed that vitamin E concentration is not significantly altered in prefrail subjects but is significantly (Bartali et al., 2006; Ble et al., 2006; Michelon et al., 2006) or almost significantly (Smit et al., 2012) decreased in frail individuals. No alterations in the concentration of folate, vitamin A, total carotenoids (Sum of β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene), vitamin B12, vitamin C, or selenium in prefrail older American adult serum were found after proper adjustment for age, sex, race-ethnicity, smoking, education, income, BMI, and co-morbidity (Smit et al., 2012). Within the NHANES III study Eichholzer et al. (2012) found that free and total estradiol concentrations in blood were significantly reduced in frail but unchanged in prefrail men. The concentration of alanine transaminase and cholesterol are also significantly decreased in frail subjects but remain unchanged in prefrail subjects (Le Couteur et al., 2010) however reduced cholesterol in the serum in frail individuals has not been found in other studies (Eichholzer et al., 2012; Wu et al., 2009, 2010). The concentration of adiponectin, an important adipokine which has insulin sensitizing, anti-atherosclerotic, and anti-inflammatory properties, is increased in frail male individuals but remains unchanged in prefrail older individuals (Tsai et al., 2013).

4. Conclusions

A major public health goal for older individuals is to prevent disability and to enhance active life expectancy. Disease-oriented approaches do not completely define the complexity and variability of the functional health status of older adults. Frailty has been proposed as a new paradigm that shifts the focus away from disease-specific pathologies toward biological dysfunction and homeostatic impairments that can be analyzed using a multidisciplinary approach within the framework of health-trajectory research. In this review we outlined the features of prefrail older individuals, the progression of prefrailty, prevalence of each Fried criterion in prefrail individuals, and possible biomarkers for prefrailty. Detailed study of the features and biological alterations of prefrail individuals allows us to shed some light on the first alterations that can trigger the transition to frailty and thus, also to its poor outcomes.

Our literature review supports the notion that the features of prefrail and frail individuals are similar although in some cases qualitative differences ensue (i.e. the reduced vitamin D concentration in women, the changed distribution of some of the Fried criteria, or the biomarker changes discussed in Section 3.4.7). The existence of associations between prefrailty and single frailty criteria (most commonly weakness in prefrail individuals) suggests that specific physical alterations could result from

Table 4
Other prefrailty biomarker candidates. Summary of the biomarkers associated with prefrailty (except testosterone which is reported in Tables 1 and 2). Details of the cited studies are shown in the text.

Biomarker	Original research articles
DHEA/DHEAS	Cappola et al. (2009) and Voznesensky et al. (2009)
Cortisol	de Almeida Holanda et al. (2012)
Vitamin D	Shardell et al., 2009, 2012, Smit et al. (2012) and Wilhelm-Leen et al. (2010)
Transferrin, hemopexin, fibrinogen gamma chain, apolipoprotein E	Shamsi et al. (2012)
8-OHdG	Wu et al. (2009)

common pathophysiological changes. Prefrailty deserves further research because it is a continuous process that may be partly reversible, especially in its initial phases, and additional experimental work is required to identify the specific combination of clinical and laboratory biomarkers (see summary in Table 4) that can be used for the diagnosis of prefrailty. The identification of biomarkers associated with prefrailty state would be very helpful in pinpointing specific mechanisms responsible for the development of prefrailty, and may help us to tailor treatment or prevention strategies, or to promote suitable healthier life styles in older individuals.

Conflict of interest statement

None of the authors have any financial or personal relationships with conflicting individuals or organizations to disclose.

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The relationship between depression and frailty syndrome: a systematic review

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Objectives: Frailty is a geriatric syndrome characterised by the clinical presentation of identifiable physical alterations such as loss of muscle mass and strength, energy and exercise tolerance, and decreased physiological reserve. Frailty and depressive symptoms are common issues facing older adults and may be associated. It is not clear if the depression facilitates the appearance of frailty syndrome or vice versa or these two coexist independently in the same individuals.
Method: We performed searches in several databases (Embase, PubMed, CINAHL, Scopus, and PsycINFO) papers published between November 2003 to February 2014 about frailty syndrome and depression in people aged 65 and older published and the reference lists of from the articles retrieved were pearled in order to identify any which may have been missed in the initial search. Two independent reviewers extracted descriptive information on the prevalence and co-occurrence of frailty and depression in older individuals and of frailty criteria among depressed patients.
Results: Depression and frailty occur in a significant proportion of frail older individuals. Common pathophysiological alterations and biomarkers in the two syndromes have been recently described.
Conclusion: Studies on the causal relationship between the two syndromes are clearly necessary in the future.

Keywords: depression; mental health; biological markers

1. Introduction

Although chronological and biological age correlate, individuals with the same chronological age can vary widely in health and functional status (Mitnitski, Mogilner, MacKnight, & Rockwood, 2002). The concept of frailty tries to explain this heterogeneity in older adults and is thus an important concept for clinical practitioners and policy-makers. Frailty is a state of increased vulnerability to stressors (Fried, Ferrucci, Darer, Williamson, & Anderson, 2004; Morley et al., 2013), characterised by decreased physical functioning and an increased risk for poor outcomes, such as a higher incidence of falls, fractures, disabilities, comorbidities, health care expenditure, and premature mortality (Fried et al., 2001; Fugate Woods et al., 2005; Woo, Leung, & Morley, 2012). Recently, the influence of genetic background has started to be explored in order to explain the variability of frailty phenotypes (Dato et al., 2012). The concept of frailty has grown in importance because of the need to better understand the health trajectory of older people and to prevent, or at least to delay, the onset of late-life disabilities in worldwide growing aged population (Fried et al., 2001). The attributes of frailty in the elderly evidenced in literature are vulnerability to biopsychosocial and environmental stressors, walking changes, fatigue self-report, muscle weakness, and reduction of the handgrip strength in the dominant hand (Andrade, Fernandes, Nóbrega, Garcia, & Costa, (2012) for review).

The most used tool to evaluate frailty syndrome is that of Fried et al. (2001). Several other models have been developed to assess frailty including the frailty index and frailty clinical scale (Hyde et al., 2010; Mitnitski, Mogilner, & Rockwood, 2001; Rockwood & Mitnitski, 2007; Rockwood et al., 2005) which takes all the deficits that are present in an individual into account, including active diseases, ability to perform daily living activities, and physical signs from clinical and neurological examinations (20–70 different deficits). A third model, the FRAIL scale, integrates features from each of these models, combining physical symptoms, the inability to walk or climb a flight of stairs, weight loss, and exhaustion with the presence of multiple illnesses. Another model, developed from the Study of Osteoporotic Fractures (SOF), leads to results similar to those obtained using Fried's criteria (Kiely, Cupples, & Lipsitz, 2009). At present there is no consensus on which method of measurement should be used to assess frailty, although difficulties in its assessment according to Fried's criteria in very old subjects (more than 85 years old) due to the high number of comorbidities in this population, suggests that the frailty index or cumulative deficits index might be better used in these cases (Collerton et al., 2012; Kulminski et al., 2008).

Frailty syndrome is usually defined according to a well-established, standardised phenotype, based on the five physical criteria as described by Fried et al. (2001), namely unintentional weight loss, poor physical activity, slowness, reduced muscular strength, and exhaustion. These alterations can also be present in depressed patients and the overlapping of the frailty spectrum and depression is challenging. Depression is not a normal part of aging;

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while older adults may face widowhood or loss of function or independence, persistent bereavement or serious depression is abnormal and should be treated. Untreated depression presents a serious public health problem: it complicates chronic conditions such as heart disease, diabetes, and stroke, increases health care costs, and often accompanies functional impairment and disability (Frederick et al., 2007; Katon, 2003; Snowden, Dhingra, Keyes & Anderson 2010; Unützer et al., 1997). Depression and frailty are linked to higher health care costs (Fried et al., 2004; Unützer et al., 1997) and higher mortality (Frederick et al., 2007; Fried et al., 2001; Snowden, Dhingra, Keyes, & Anderson, 2010). To our knowledge, no systematic reviews have been published concerning the relationship between physical frailty syndrome and depression in older individuals. In this work, we specifically review and discuss the following:

(1) The prevalence and co-occurrence of frailty (defined using the Fried's frailty index) and depression in older individuals.
(2) The prevalence of frailty criteria among depressed patients.
(3) Causality between depression and frailty.
(4) Common biomarkers in frailty and depression.

2. Materials and methods

This systematic review was designed and developed according to the PRISMA guidelines.

2.1. Literature search

A literature search using multiple electronic bibliographic databases was conducted. Embase, PubMed, CINAHL, Scopus, and PsycINFO were searched for reports from November 2013 to February 2014. The primary search terms used were 'frail*' and 'depression' and results were limited to 'English and Spanish' for all the databases. Furthermore, PsycINFO and Scopus were also limited to 'Journal Article'.

2.2. Inclusion/exclusion criteria

The following inclusion criteria were used: (1) acknowledged as an original article, (2) full-text published in either English or Spanish, (3) the frailty phenotype was assessed using the Fried criteria (Fried et al., 2001), (4) study participants were identified as frail, prefrail, or non-frail (or robust) in the title, abstract, and/or main text, and (5) depressive symptoms or depression were measured in the study sample and mentioned in the title, abstract, and/or main text. Articles published between November 2003 and February 2014 were selected in order to elaborate this systematic review. Reports related to frailty measurement using Fried's criteria in a specific group of patients (e.g. HIV or oncological patients) were excluded.

2.3. Data collection and analysis

Citation lists of all articles were imported to an online citation manager (RefWorks) which was used to manage the screening process and to remove duplicate citations. To determine which studies would be included, two members of the review team independently screened the title and abstracts of the articles extracted from the literature search and when the reviewers agreed on inclusion/exclusion criteria the full electronic text was retrieved. For each of these articles, two reviewers independently extracted the following data: the country where the study was conducted, the number of participants, age at the time of inclusion, sex, living arrangements, and the definition and measurement method of frailty and depression. The reviewers were blind to each other. Any disagreement between the two reviewers on the papers or data extracted from them was resolved by the corresponding author and then accepted by all authors.

3. Results

Among the studies identified by our search strategy, 426 required further full-text screening, and 28 met the inclusion criteria (Figure 1). We summarised the results which emerged from the literature review into four sections: the prevalence of depression in frail individuals, the relationship between each Fried criterion and depression, causality between depression and frailty, and biomarkers observed in both frailty and depression.

3.1. Prevalence and the co-occurrence of frailty syndrome and depression

A recent systematic review evaluated the prevalence of frailty syndrome in community-dwelling adults aged 65 and older and found that the prevalence of frailty was 11% [21 studies; 61,500 participants (Collard, Boter, Schoevers, & Oude Voshaar, 2012)]. The weighted prevalence was 10% for physical frailty and 14% for the broad phenotype of frailty, however its prevalence in the community varies enormously (range 4%−59%) and depends on of the method used to assess it. Prevalence increases with age and is more frequent in females than age-matched males (Collard et al., 2012; Fried et al., 2004). Frailty syndrome occurs in more than 20% of community-dwelling adults aged 85 and over (Fried et al., 2004), and in institutionalised people the rate of frailty is even higher (Garcia-Garcia et al., 2011; González-Vaca et al., 2013). The prevalence of prefrailty (individuals who fulfil one or two Fried's criteria) in community-dwelling adults aged 65 and over is much higher (35%−53%) than for frailty (Collard et al., 2012; Fernández-Garrido et al., 2014).

The median global prevalence of serious depression in the elderly population is around 1%−5% (Barua, Kar, Ghosh, & Basilio, 2011; Comijs et al., 2011; Fiske, Wetherell, & Gatz, 2009), and if one considers all depressive disorders this increases to 10%−15% in the general population aged 65 and over (Barua et al., 2011; Blazer,

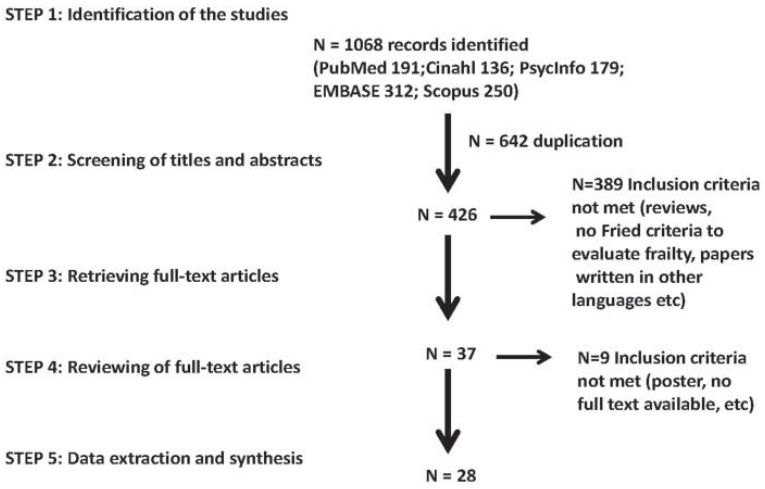

Figure 1. The PRISMA flowchart of the systematic literature review.

2003). Rates of depressive disorders are substantially higher among specific populations, ranging from 5%–10% in medical outpatients to 14%–42% in long-term care facility residents (Blazer, Hybels, & Fillenbaum, 2006; Comijs et al., 2011; Djernes, 2006), but few differences emerge by race or ethnicity in terms of prevalence of depression (Swenson, Baxter, Shetterly, Scarbro, & Hamman, 2000). Although in general women suffer from more depressive symptoms than men, widowhood, one of the life-events that is most associated with depression in the geriatric population, has a comparable depressive effect in both men and women (Lee & DeMaris, 2007; Schaan, 2013).

The co-occurrence of both frailty and depression among people aged 65 and over has only recently started to be investigated (Collard, Comijs, Naarding, & Voshaar, 2014; de Albuquerque Sousa, Dias, Maciel, & Guerra, 2012; Feng, Nyunt, Feng, Yap, & Ng, 2014; Garcia-Garcia et al., 2011; Samper-Ternent, Snih, Raji, Markides, & Ottenbacher, 2008; Wu, Shiesh, Kuo, & Lin, 2009); most studies found that around 4%–16% of frail individuals aged 60 and over had serious depression (Table 1). However, this percentage increases to 35% when an older (aged 75 and over) population is selected (Jürschik et al., 2012) or when considering frail men (Mohr et al., 2007). In addition, experimental data obtained in 620 women within the Women's Health and Aging Studies I and II (WHAS-I/II) (Chang, Weiss, Xue, & Fried, 2010) showed that the risk of frailty increases if depressive symptoms are also present, as defined by a geriatric depression score (GDS) of 9 or higher based on Yesavage's scale, a widespread used clinical scale to evaluate geriatric depression (Yesavage et al., 1982).

Interestingly, other diseases besides depression also increase the risk of frailty (e.g. pulmonary disease, cardiovascular disease, anaemia, and rheumatoid arthritis) and the co-occurrence of depressive symp-toms and anaemia synergistically interact to further increase the risk of frailty (Chang et al., 2010). A recent cross-sectional observational study performed in the framework of a cohort study, the Netherlands Study of Depression in Older persons (NESDO) determined the prevalence of physical frailty syndrome in depression in adults aged 60 years or more (evaluated by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [DSM-IV] (Collard et al., 2014). Logistic regression analyses in this study showed that the prevalence of frailty was three times higher in depressed than in non-depressed individuals (27.2% and 9.1%, respectively) after adjusting for confounding factors such as age, gender, and all baseline characteristics. Similarly, 22.7% of older frail Mexican individuals aged 60 and over also had clinically significant depressive symptoms (Sánchez-García et al., 2014). The prevalence of depression in frail individuals is as high as 46.5% as reported in older individuals in Spain (Garcia-Garcia et al., 2011) but some differences between countries appear. Higher age and severity of depression are independently associated with physical frailty in depressed older adults (Collard et al., 2014; Garcia-Garcia et al., 2011; González-Vaca et al., 2013) and interestingly, all measures of depressive symptoms are associated with frailty in the depressed individuals irrespective of the tool used to assess frailty syndrome (Collard et al., 2014; John, Tyas, & Montgomery, 2013; Jürschik et al., 2012; Lin et al., 2011; Ní Mhaoláin et al., 2012).

A report generated within the Study on Aging and Dementia in Mexico found that 23% of frail individuals had clinically significant depressive symptoms; moreover, they found a significant association between depression and either prefrailty or frailty (Sánchez-García et al., 2014). Two studies recently addressed the rate of frailty in depressed older people, each finding that the percentage of comorbid frailty reaches 46%–57% (Collard et al., 2014; Ní Mhaoláin et al., 2012); higher depression scores have also been reported in prefrail compared to robust individuals (Ní Mhaoláin et al., 2012). Once frailty

Table 1. Prevalence of depression or depressive symptoms in frail, prefrail, and non-frail (robust) individuals. Frailty is measured according to Fried's frailty criteria. Individuals who fulfilled zero criteria were robust, those who fulfilled one or two criteria were prefrail, and those who fulfilled more than or equal to three criteria were frail. MD = mildly depressed, D = depressed, CES-D = Center for Epidemiologic Studies Depression, GDS = Geriatric Depression Scale, BL = baseline, FU = follow-up, SD = standard deviation, OR = odds ratio, IC = confidence interval.

Reference	Sample	Depression and/or depressive symptoms evaluation	Robust	Prefrail	Frail
Brown et al. (2013)	$N = 1027$ Data obtained within the framework of the Nordic Research on Aging (NORA) between 1988−1991	CES-D (Center for Epidemiologic Studies Depression Scale) Non-depressed (ND) (CES-D ≤ 9), mildly depressed (MD) (CES-D between 10−15), depressed (D) (CES-D ≥ 16)	Not determined	Not determined	ND: 7% MD:10% D: 21%
De Albuquerque et al. (2012)	$N = 391$ Cross-sectional study	GDS-15	3.9%	18.2%	7.4%
Drey et al. (2011)	$N = 119$ Bavaria, Germany	GDS-4 Depressive symptoms (DS) GDS-4 ≥ 2	15%	8%	47%
Esrud et al. (2007)	$N = 6724$ (women) Data obtained within the framework of the Study of Osteoporotic Fractures (SOF). (September 1986−October 1988 at baseline. Follow-up between 1992−1994)	GDS-15 Depressive symptoms if GDS score ≥ 5	0.6 (±1.0) Mean ± SD of GDS Score	2.2 (±2.2) Mean ± SD of GDS Score	3.9 (±2.8) Mean ± SD of GDS Score
Feng et al. (2014)	$N = 1827$ Living in Singapore Data were collected from 2003 to 2005. Followed up twice: 2005−2007; 2007−2009.	GDS-15 Depressive symptoms if GDS score ≥ 5	Baseline 8.7% First follow-up 2.0% Second follow-up 1.3%	Baseline 15.6% First follow-up 6.2% Second follow-up 4.2%	Baseline 28.3% First follow-up 20.9% Second follow-up 8.0%
Garcia-Garcia et al. (2011)	$N = 2488$ Living in Toledo (Spain) Data were collected from 2006 to 2009.	GDS-15 Depressive symptoms if GDS score ≥ 5		41.8%	8.4%
Garrido et al. (2012)	$N = 281$ Living in Cuenca (Spain)	GDS-15 Depressive symptoms if GDS score ≥ 5	Men: 3.2 ±2.1 Women: 3.7 ± 2.8 Mean ± SD of GDS score	Not determined	Men: 5.8 ± 3.1 Women: 5.9 ± 3.4 Mean ± SD of GDS score
Gonzalez-Vaca et al. (2013)	$N = 331$ Living in Albacete (Spain)	GDS-15 Depressive symptoms if GDS score ≥ 5	3.5 ± 2.8 (robust and prefrail individuals were pooled together) Mean ± SD of GDS score	5.5 ± 3.2 Mean ± SD of GDS score	
Jürschik et al. (2012)	$N = 640$ Living in Lleida, Spain	CES-D 20-item version: score ≥ 16 indicates depression	25.5%	Not determined	32.5%
Lin et al. (2011)	$N = 933$ Living in Taiwan. Data were collected in June 2009	SF-36 questionnarie: includes mental health assessment (MH) (5 items)	1.67%	2.94%	8.8%

(*continued*)

Table 1. (*Continued*)

Reference	Sample	Depression and/or depressive symptoms evaluation	Robust	Prefrail	Frail
Mohr et al. (2007)	$N = 646$ (men) Data obtained within the framework of the Massachusetts Male Aging Study (MMAS)	CES-D 20-item version: score ≥ 16 indicates depression	2%	12%	33%
Ni Mhaolaein et al. (2012)	$N = 301$ Living in Dublin (Ireland). Data were collected between August 2007−July 20	CESD-8 Depressive disorder score ≥ 4	1.42 (CESD-8 score)	2.10 (CESD-8 score)	3.62 (CESD-8 score)
Ottenbacher et al. (2009)	Baseline $N = 2049$ Follow up $N = 777$ Living in Texas, New Mexico, Arizona, and California (USA) Data were collected at baseline 1993−1994 and follow-up conducted at 2−3 year intervals: wave 2: 1995−1996, wave 5: 2006	CES-D Subjects with high depressive symptoms those with a score ≥ 16	Baseline 4.17 (±5.39) Follow-up 4.38 (±5.22) mean ± SD Depressive symptoms	Baseline 7.84 (±8.51) Follow-up 8.14 (±7.09) mean ± SD Depressive symptoms	Baseline 13.18 (±10.64) Follow-up 15.16 (±10.67) mean ± SD Depressive symptoms
Samper-Ternet et al. (2008)	$N = 1370$ Living in Texas, New Mexico, Colorado, Arizona, and California; USA Data were collected from de Hispanic EPESE. The data obtained at the second (1995−1996), third (1998−1999), fourth (2000−2001), and fifth (2004−2005) wave (1995−2005).	CES-D: subjects with high depressive symptoms those with a score ≥ 16	3.8 (±5.1)	7.1 (±8.3)	10.0 (± 8.8)
Sánchez-Garcia et al. (2014)	$N = 1993$ Living in Mexico City (Mexico) Data were collected from the Study on Aging and Dementia in Mexico (SADEM) in September 2009−March 2010	Mexican version of the CES-D 20-items version). Cut-off points for identifying depressive symptoms were at > 16 points.	9.4% depressive symptoms	28.4% depressive symptoms	53.8% depressive symptoms
Vaz Fragoso et al. (2009)	$N = 374$ Living in New Haven Connecticut, USA	CES-D: subjects with high depressive symptoms those with a score ≥ 16	20.9%	Not determined	37.7%
Wu et al. (2009)	$N = 90$ In Southern Taiwan Cross-sectional study	CESD-10	0%	16.1%	23.3%

develops, there is a higher likelihood of clinically significant depression; thus, identification of frailty syndrome may be relevant in identifying older people at risk of deteriorating mental health.

The syndromes of frailty and depression are each associated with increased disability and death but their confluence has rarely been studied (Brown et al., 2013; Fried et al., 2001). Clinical depression is characterised by symptoms that interfere with the patient's ability to function normally for a prolonged period of time and can facilitate the progression of frailty syndrome. Depression often goes undiagnosed in elderly adults, especially in the context of multiple physical problems, and because of this it is under treated. A longitudinal study with a large cohort (around 2500 people) recently performed in Italy showed that both frailty and depressive symptoms (evaluated by the 30-question GDS) are associated with a greater risk of developing dementia (Solfrizzi et al., 2013).

3.2. *The prevalence of the Fried criterion in frail people with depression*

Very few studies have addressed the distribution of frailty criteria among depressed older individuals (Table 2), although some recent reports have shown a clear overlap between frailty syndrome and depression (Ní Mhaoláin et al., 2012): for instance, frail subjects that self-reported exhaustion as measured by the Fried criterion, which is very common in depressed individuals (Samper-Ternent et al., 2008), also had a clinical picture of depression. Sleep–wake disturbances, commonly observed in depressed patients, are also independently associated with frailty (Vaz Fragoso, Gahbauer, Van Ness, & Gill, 2009), which raises the question of whether depressed frail people might have increased depressive symptom scores (due to the shared characteristics of both syndromes). Collard et al. (2014) properly addressed this issue by deconstructing the concept of depression (by using a scoring system without items that might overlap with physical frailty) and by using different ways to measure frailty (e.g. weakness and slowness). However, there is still a significant association between the severity of all the different depressive symptoms and the frailty metrics used by these analyses (Collard et al., 2014), underlining that frailty and depression may not be distinct syndromes but rather represent a single construct at least in a subgroup of older individuals (Mezuk, Lohman, Dumenci, & Lapane, 2012). Thus, it is unlikely that the higher severity of depressive symptoms in frail compared to non-frail depressed individuals can be explained by these shared characteristics.

Data from the Nordic Research on Ageing Study (with a large number of participants aged 75 or over) showed that the individual frailty criterion (gait speed, physical activity level, grip strength, and levels of fatigue) differed from the depression status, and that the severity of each of these criteria was worse in the depressed rather than the non-depressed group. The combined effect of depression and the characteristics of frailty (specifically fatigue and impaired gait speed) on mortality are particularly deleterious for older women, and result in an increased risk of

Table 2. Prevalence of frailty criteria in depressed older individuals. Frailty is measured according to Fried's frailty criteria: unintentional weight loss, poor physical activity, slowing, reduced muscular Strength, exhaustion (Fried et al., 2001). MD = mildly depressed, D = depressed, ND = non depressed, M = men, W = women, F = frail, PF = prefrail, MH = mental health assessment, RE = emotional role, CES-D = Center for Epidemiologic Studies Depression, GDS = Geriatric Depression Scale, MMSE = Mini Mental State Examination, SD = standard deviation, OR = odds ratio, IC = confidence interval.

Reference	Sample	Scales used to evaluate depression and/or depressive symptoms.	Unintentional weight loss	Poor physical activity	Slowing	Reduced muscular strength	Exhaustion
Brown et al. (2013)	$N = 1027$ Data obtained within the framework of the Nordic Research on Aging (NORA) between 1988 and 1991	CES-D (Center for Epidemiologic Studies Depression Scale) Non-depressed (ND) (CES-D $\leq$ 9), Mildly depressed (MD) (CES-D between 10 and 15), Depressed (D) (CES-D $\geq$ 16).	Not determined	ND 23% MD 31% D 43%	ND 34% MD 34% D 49%	ND 21% MD 31% D 34%	ND 22% MD 20% D 43%
Collard et al. (2014)	$N = 511$ Data obtained within the framework of the The Netherlands Study of Depression in Older (NESDO).	The Composite International Diagnostic Interview (CIDI) used to determinate depression according to the criteria of DSM-IV and ICD-10. Severity of depression was measured by 30-item self-rating Inventory of Depressive Sympomatology (IDS) (0–84) → 0–13 normal, 14–25 mild depression, 26–38 moderate depression, 39–48 severe depression, 49–84 very severe depression.	D 35.4% ND 3.8%	D 42.3% ND 34.8%	D 26.5% ND 18.9	D 25.1% ND 19.7%	D 45.8% ND 3.8%

death; specifically, the presence of depression nearly doubled the effect of fatigue and slow gait speed on mortality in older women (Brown et al., 2013). A cross-sectional study by Yanagita et al. (2006) demonstrated that reduced handgrip strength (measured in a different way to Fried's criterion) was significantly lower in the depressed compared to the non-depressed group, the depressed group had a significantly lower physical activity index and total walking distance each day, and that in general participants with higher depression scores had poorer performance in all of the physical criteria measured. Moreover, the association of the time it took patients with depressive symptoms to walk 10 feet remained significant after adjusting for confounding factors. Thus, physical performance (especially gait speed) may be important in terms of its potential correlation with depression in community-dwelling older men (Yanagita et al., 2006). Hence, in order to improve health care of older depressed people it is important to study frailty further in this specific group with depression.

3.3. Causality between depression and frailty

It is critical to know whether frailty and the onset of depression are concurrent or whether one generally precedes the other. A cross-sectional epidemiological study performed in older individuals living in Brazil showed that depression (evaluated with the short version of GDS, GDS-15) was significantly associated with frailty (Sousa, Dias, Maciel, & Guerra 2012). No depressive symptoms were evaluated in this study but among those diagnosed with major depression 7% were frail, 18% were prefrail, and only 4% were non-frail (Lin et al., 2011; Sousa et al., 2012). When using a less sensitive scale such as GDS-4, frail people again scored positive for depression significantly more frequently than non-frail people (Drey, Pfeifer, Sieber, & Bauer, 2011); this increased prevalence of depressive symptoms in frail and prefrail older individuals is maintained after adjusting for sociodemographic factors, comorbidities, and functional disabilities (Feng et al., 2014; Lakey et al., 2012; Ní Mhaoláin et al., 2012; Wu et al., 2009).

While the association between frailty and depression has been repeatedly demonstrated in subgroups of frail individuals, few longitudinal studies have addressed the causality between the two syndromes. Data from Singapore longitudinal aging studies revealed that prefrail and frail individuals were more likely than non-frail individuals to show persistent and/or new depressive symptoms at follow-up (Feng et al., 2014). In addition, depressive symptoms were associated with an increased risk for becoming prefrail and frail in a large (N = ca. 28,000) US prospective cohort study, after adjusting for antidepressant use and other important covariates (Lakey et al., 2012). Interestingly, antidepressant users with depressive symptoms appeared to be at the highest risk of becoming frail (Lakey et al., 2012). Variables that were statistically significant predictors for frailty at follow-up included a (low) Mini Mental State Examination (MMSE) score and a negative affect score on the CES-D score subscale, but not the measures of basic or instrumental activity in the daily life or balance (Lakey et al., 2012), suggesting that it was not related to general body function impairment. Ottenbacher et al. (2009) found an association between negative affect scores on the CES-D subscale at baseline and a risk of frailty at 10-year follow-up within a longitudinal study carried out on Mexican−American older adults, although the complete CES-D score was not included in the regression models because two questions from the somatic and retarded activity subscale of the CES-D were used in the frailty index (Ottenbacher et al., 2009).

Other predictors of frailty at the 10-year follow-up include: age, marital status, arthritis, diabetes, smoking status, body mass index, MMSE score, the negative affect subscale score, and the number of comorbid conditions (Ottenbacher et al., 2009). Both frailty and depression have been associated with an increased risk of dementia and, in particular, once controlled for confounding factors, vascular dementia stands out (Solfrizzi et al., 2013). Longitudinal studies are required to shed light onto the causality between these factors, but unfortunately so far only one such study aimed at assessing whether frailty is a predictor of depression has been performed. This study, conducted in 1800 adults aged 55 or more, showed that prefrail and frail individuals were more likely than non-frail individuals to show persistent and new depressive symptoms at a (10-year) follow-up, and exhaustion, weakness, slowness, and low physical activity were physical frailty components that were individually predictive of the onset or persistence of depressive symptoms (Feng et al., 2014). Other studies have investigated the role of depression or depressive symptoms as a predictor of frailty, for instance, depressive symptoms were strong predictors of frailty and mortality in disabled women (Xue, Fried, Glass, Laffan, & Chaves, 2007). Taken together, these experimental data support a significant role for frailty syndrome as a predictor of comorbidity with depression in older adults and vice versa, although further large longitudinal studies should be performed to better define the relationship between these two factors.

3.4. Common biomarkers in frailty and depression

Indirect evidence points to several explanations for the association between physical frailty and depressive symptoms in older adults. First, it can be postulated that most severely depressed patients are more susceptible to developing frailty syndrome by lifestyle factors associated with depression such as reduced physical activity, inactivity, and medication non-compliance (in the case of somatic comorbidity). However, the two syndromes seem to have some common pathophysiological mechanisms; hormonal changes such as those activated by the hypothalamic−pituitary−adrenal (HPA) axis are common in the two syndromes (Fernández-Garrido et al., 2014; Maggio et al., 2005; Murri et al., 2014) and elevated diurnal cortisol and an impaired HPA-axis response to stressors have been hypothesised to initiate or amplify alterations in many other important physiological systems. Several

studies demonstrated that higher levels of cortisol and its blunted diurnal variation may be involved in the vulnerability and general clinical presentation observed in frail older individuals (Johar et al., 2014; Varadhan et al., 2008). Within the framework of the WHAS-II, Varadhan et al. (2008) found a significant positive association between frailty-burden and 24-h mean cortisol levels (but not awakening levels) in frail elderly (80−90 years old) community-dwelling women, changes which were not observed in prefrail women. Frailty is associated with an increase in the concentration of salivary cortisol in elderly residents in long-stay institutions, an association which appears at the prefrailty state (de Almeida Holanda et al., 2012); interestingly, both hypo- and hyper-cortisolemia have been reported in depression (Bremmer et al., 2007; Cubała & Landowski, 2014), and low salivary cortisol concentrations and a minimal difference between morning and evening cortisol concentrations are risk factors for depression (Grynderup et al., 2013).

Low-grade inflammation is generally considered to be one of the underlying mechanisms of both frailty (Fernández-Garrido et al., 2014; Leng, Xue, Tian, Walston, & Fried, 2007) and late-life depression (Bremmer et al., 2008; Milaneschi, Corsi, Penninx, & Bandinelli, 2009; Rudolf, Greggersen, Kahl, Hüppe, & Schweiger, 2014). Among the inflammatory factors, interleukin 6 (IL-6), an important cytokine that modulates the immune system, has been associated with frailty and prefrailty in older adults (Fernández-Garrido, Ruiz-Ros, Buigues, Navarro-Martinez, & Cauli, 2014; Leng et al., 2004, 2007). Further work is required to elaborate upon these results, but these data hold the promise of opening up new therapeutic avenues for the treatment frailty syndrome with or without co-morbid depression, and for monitoring the efficacy of pharmacological and non-pharmacological interventions.

4. Discussion

Frailty identifies groups of people in need of extra medical attention which can be useful when considering financial health care planning, especially given that frail older adults are among the most challenging in medical management. However, awareness of this syndrome and its risks can help us care for these patients more confidently and decrease their risk for adverse outcomes. Late-life depression with comorbid frailty syndrome has already been demonstrated, and a possible contribution to causality has also recently emerged from different studies. The results summarised in this systematic review support the hypothesis that frailty and depression are comorbid geriatric syndromes in a subgroup of older individuals; at the same time, frailty is also a risk factor for the development and persistence of depressive symptoms. The same common symptoms and risk factors may also explain why frailty could perpetuate depressive symptoms and predispose individuals to new symptoms of depression.

Caring for people with these two syndromes is challenging in clinical practice. Health promotion, proactively provided to frail older adults in nursing, increases the quality of life and reduces depressive symptoms in frail older people, and does not increase the overall cost of health care (Markle-Reid et al., 2006). In addition to psychosocial factors, multisystem physiological dysregulation in frailty is an important biological factor predisposing, precipitating, and perpetuating late-life depression. A possible explanation for the comorbidity of depression in a subgroup of frail individuals may be the presence of common underlying processes, i.e. low grade inflammation which is related to both frailty and depression, as discussed in Section 3.4. It is not known whether the proinflammatory state associated with specific diseases activates an etiological cascade which results in frailty, or whether this occurs through non-disease specific pathways. Identifying combinations of inflam-matory diseases that act synergistically to heighten the risk of frailty would provide information about the mechanistic pathways that induce a proinflammatory frail state, enhancing our ability to co-manage these diseases and potentially delaying frailty.

It is also worth pointing out that even though depression and frailty occur in a significant proportion of frail individuals, this is not a definitive general circumstance. In fact, older people with frailty can easily be misdiagnosed as suffering from a depressive disorder during periods of physiological low mood and therefore frequently receive antidepressant treatments inappropriately (Collard et al., 2014), with associated consequences and side-effects such as increased risk of fall, sedation, and eventual heart and/or gastroenteric complications.

Future research should investigate whether multimodal interventions targeting depression, mobility deficits, and fatigue can decrease morbidity and improve quality of life in older depressed individuals with concomitant frailty syndrome. By differentiating between frail and non-frail elderly individuals, it will be easier to treat them with appropriate, multidisciplinary interventions. Such treatment opportunities seem especially important in late-life depression with comorbid frailty, because lifestyle is thought to be the greatest contributor to the onset of frailty (Bortz, 2002) and late-life depression negatively affects lifestyle behaviour (Payne, Froggatt, Toye, & White, 2006; van Gool, 2003).

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Frailty syndrome and pre-operative risk evaluation: A systematic review

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ABSTRACT

Background: Frailty is a geriatric syndrome characterized by the clinical presentation of identifiable physical alterations and decreased physiological reserve. The assessment of frailty syndrome has been recently related with post-surgical outcomes and overall mortality in older individuals.
Design and data sources: We performed searches in Pubmed, Embase, Scopus, SCIELO and IME (Spanish medical index) databases from their start dates to February 2014 for original papers about the identification of the relationship between frailty and pre-operative risk evaluation in people aged 65 and over.
Review methods: We followed criteria of systematic PRISMA guidelines. Two independent reviewers extracted descriptive information on frailty criteria and outcomes from the selected papers: of the 77 articles retrieved from the searches, 32 met the study inclusion criteria.
Results: Severity of frailty syndrome significantly correlated with post-surgical mortality rates and with many although not all post-surgical complications. These relationships emerge in different type of surgical procedures and patients' features. The comparison of diagnostic tools to assess frailty in pre-operative risk evaluation are very few and to date, no recommendation can be made about the best scale to measure it.
Conclusion: Assessment of frailty syndrome should be added in the pre-operative risk assessment in older individuals.

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1. Introduction

Surgical procedures are a feature in every clinical setting and their frequency has greatly increased in modern medicine. It has been estimated that around 234 million surgical operations are performed every year worldwide (Weiser et al., 2008). Pre-operative risk evaluation is an essential tool for measuring the risk, performing cost-benefit analysis, and studying therapeutic trends. (Geissler et al., 2000) Within the next 20 years, the dramatic increase in the old-age population (75 years and over) will have a major social and health impact on the perioperative (intra- and postoperative) management of patients who require surgery. Epidemiological data showed that elderly people require surgical procedures four times more often than the rest of the population. (Poldermans et al., 2009). Advances in medicine and care allow people to live longer and with a better health status, thus the need for surgical management of elderly patients has become very common (Lee, Buth, Martin, Yip, & Hirsch, 2010). Chronological age should not be used as a proxy for clinical factors, because age itself seems to be partially responsible for an increase in post-operative complications. Greater risks are associated with the urgency of surgical procedure, as well as with the presence of significant cardiac, pulmonary, or renal disease (Fuertes and D'Urbano, 2002; Gallardo-Prieto et al., 2006; Poldermans et al., 2009; Sánchez Rosas, 2008). Indeed, in a prospective study of 364 surgical patients aged 60 years and over, Fuentes Valdés and Jiménez Paneque (2000) demonstrated that age had no influence on postoperative complications; similar results were found in another study with patients with different clinical features (Sundermann et al., 2011).

The risk of perioperative complications depends on the condition of the patient prior to surgery, the presence of co-morbidities, and the magnitude and duration of the surgical procedure (Poldermans et al., 2009). Adverse postoperative outcomes still remain common in older people when compared with their younger counterparts (Partridge, Harari, & Dhesi, 2012). Post-operative complication and mortality rates represent the factors helping clinicians/surgeons to decide whether older patients will ultimately benefit from surgery.

Although score systems used to estimate the risk of surgery were primarily designed to predict mortality, postoperative morbidity has been acknowledged as the major determinant of the ultimate cost and patient quality of life after surgery (Geissler et al., 2000; Kohl & Deutschman, 2006). Risk stratification for elderly patients undergoing major surgery is crucial for surgical planning; only in the light of a such an assessment can decisions about whether to perform surgery at all, the type of surgery, and the timing of the surgery, be made. Moreover, these stratification studies also identify patients who require a period of optimization before surgery. Risk stratification is generally performed by assessing signs and symptoms, comorbidities, and analyzing different biochemical and physiological markers (Tan et al., 2012).

Evaluation of frailty syndrome has recently emerged as an important variable for the estimation of perioperative risk in these patients (Makary et al., 2010; Partridge et al., 2012; Robinson et al., 2013). The need of measuring frailty lies in its utility both as a tool for pre-operative risk stratification, and as a method for identifying potentially modifiable factors that can be optimized in order to increase the chance of a better outcome after surgery.

In this systematic review we assessed the following issues:

1. The relationship between frailty syndrome and postoperative mortality.
2. The relationship between frailty syndrome and postoperative complications.
3. Comparison of different tools for measuring frailty syndrome in relation to postoperative outcomes.

2. Materials and methods

The design of this study was developed according to PRISMA guidelines (Liberati et al., 2009) (Fig. 1).

2.1. Literature search

A literature was conducted search using multiple electronic bibliographic databases i.e. PubMed, Embase, Scopus, SCIELO, and IME (a Spanish medical index). Reference lists of all the relevant articles were uploaded in the Refworks software in order to

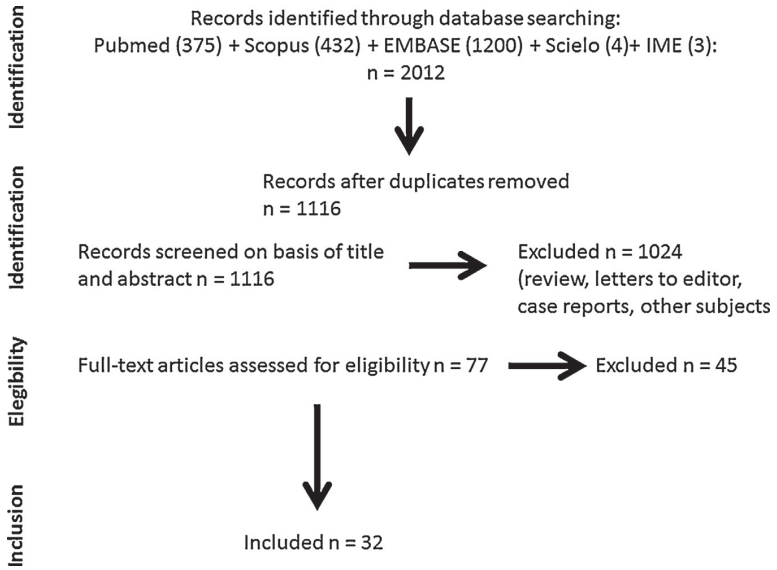

Fig. 1. The PRISMA flowchart of the systematic literature review.

identify additional articles that were potentially useful for the purposes of this study. The primary search terms used were "frail* and "surgery". The search strategy used was "frailty and surgery". The secondary search was "pre-operative (or pre-operative) and frail*". Both strategies were used for all for all five databases from beginning to February 2014.

2.2. Inclusion/exclusion criteria

The following inclusion criteria were used: (1) acknowledged as an original article, (2) full-text published in either English or Spanish, (3) study participants were identified as "not frail" or "frail" in the title, abstract, and/or text. In some databases such as PubMed, some limits were established, for example "species: humans". All the articles retrieved were published between 2009 and 2014. Exclusion criteria were: (1) papers that measured only postoperative frailty and (2) articles that evaluated only one frailty criterion (e.g. sarcopenia).

2.3. Data collection and analysis

The database searches were uploaded into a web-based system, which was used to manage the screening process (RefWorks), and duplicate citations were removed. To determine which studies should be included, we screened the title and abstracts of the articles extracted from the databases. The electronic full text was retrieved for those studies that fulfilled the inclusion criteria. For each article, we extracted the following data: the country where the study was conducted, the number of participants in the non-frail, prefrail, and frail groups, the age of participants at the time of inclusion, their gender, and the frailty definition and its measurement method. Furthermore, the principal outcomes following surgery and the conclusions of each study were carefully analysed. For each of the retrieved articles, two reviewers independently extracted the data. Any disagreement between the two reviewers on the papers, or data extracted from them, was resolved by the corresponding author and accepted by all the authors.

3. Results

Among the studies identified by our search strategy, 77 required further full-text screening, and 32 met the inclusion criteria. We separately summarized and discussed the results that emerged from the literature review in the following three sections: (1) the relationship between frailty syndrome and postoperative mortality; (2) the relationship between frailty syndrome and postoperative complications; (3) Comparison of different tools for measuring frailty syndrome in relation to postoperative outcomes.

3.1. The relationship between frailty syndrome and postoperative mortality

Mortality, i.e. the survival rate, after major surgery is the most direct clinical outcome which can be used to evaluate the efficacy of the surgical procedure or to assess postsurgical complications over time. Mortality rates not only depend on perioperative issues (e.g. intraoperative complications, the type of surgery performed, underlying comorbid diseases, etc.) but also on the preoperative health status of patients, hence its relationship with frailty syndrome in older individuals (Table 1). A large cohort (1940 individuals) prospective study performed in open lobectomy (a surgical procedure that removes one of the lobes of the lungs) patients in the US demonstrated that the severity of preoperative frailty is significantly and directly associated with postoperative mortality. Robust (non-frail) individuals have a mortality rate of around 1%, whereas those with the highest frailty score have a risk of mortality which is increased by almost 6-fold (Tsiouris et al., 2013).

Velanovich et al. (2013) found that although the mortality risk increased according to the severity of pre-operative frailty, it was largely dependent on the type of surgical procedure that these patients underwent. For instance, the odds ratio (OR) for mortality increased from 1.24 in patients undergoing general low-complexity thoracic surgery to 3.36 for low-complexity orthopedic surgery (Velanovich et al., 2013). A large population study (the National Surgical Quality Improvement Program; NSQIP) demonstrated that pre-operative frailty state is a strong predictor of cardiac arrest and death, and showed that it's a better indicator of post-operative mortality compared to the scoring system of the American Society of Anesthesiologists' (ASA) (Larsen & Rubinfeld, 2012). Frailty, assessed using a modified frailty index (mFI), was also associated with a higher mortality-risk in patients who underwent otolaryngology surgery: robust individuals had a mortality rate of around 0.2% whereas those with a high frailty score had a much higher value at around 12% (Adams et al., 2013)

Among the different physical frailty components included in the Fried's scale (Fried et al., 2001), slow gait speed was an independent predictor of mortality among patients scheduled for cardiac surgery (Afilalo et al., 2010). In another study, frailty was a factor associated with poorer 90-day and 1-year overall survival (OR = 10.4 and 8.4 respectively) in the oldest elderly-patient group (aged 80 and over) who underwent elective surgery for colon cancer (Neuman et al., 2013). However, other factors, besides frailty syndrome, were also strongly associated with poorer survival, including older age (OR = 1.1), male gender (OR = 1.6), a high number of hospitalizations in the prior year (OR = 1.2), and dementia (OR = 4.5). In general, specific comorbidities were not associated with 90-day or 1-year survival, except for some neurological disorder and electrolyte abnormalities (Neuman et al., 2013). The newest Groningen Frailty Indicator scale (Tegels, de Maat, Hulsewe, Hoofwijk, & Stoot, 2014) also showed a statistically significant association between increasing frailty and in-hospital mortality in gastric cancer patients, (OR = 1.35) which was independent of age, ASA-classification, neoadjuvant chemotherapy, type of surgery, and tumour stage. However another study performed in oncological patients with different types of tumours and cancer stages found no relationship between preoperative frailty and postsurgical mortality (Saxton & Velanovich, 2011), suggesting that, in the case of malignancies, factors other than frailty (tumour location and the presence of metastases) likely play a major role.

3.2. The relationship between frailty syndrome and postoperative complications

The type and severity of complications are important gauges of the successful surgical procedures (Clavien, Sanabria, & Strasberg, 1992). The National Veterans Affairs Surgical Risk Study prospectively collected data on major surgical operations at 44 North American Veterans Affairs hospitals (Khuri et al., 1995). Based on these data, the authors developed risk-adjusted models for 30-day morbidity and mortality for a number of surgical subspecialties (Daley et al., 1997; Khuri et al., 1997). Following this study, the Veterans Affairs NSQIP was set up and led to a 45% reduction in morbidity and a 27% decrease in mortality (Khuri et al., 1998). However, accurate estimation for surgical complication rates is difficult to obtain because of the lack of consensus of what constitutes a postoperative complication (Shah & Hamilton, 2013). Clavien et al. (1992) proposed a model for classifying surgical complications (grades I–V as well as suffix "d" for disability), and to establish differences between complications and other negative

Table 1
Frailty syndrome and mortality rate after surgery.

Reference	Population	Type of surgery	Assessment of frailty	Results on mortality rate after surgery
Tsiouris et al. (2013)	*N* = 1940 Mean age (±S.E.M.): 66 ± 11 years (51% women) Study design: cohort prospective study	Open lobectomy patients (surgeries performed between 2005 and 2010)	Modified frailty index (mFI) according to Mitnitski, Mogilner, and Rockwood (2001)	An mFI score of 0 was associated with a mortality rate of 1%, compared with 5.6% for mFI score of 0.27.
Velanovich et al. (2013)	*N* = 971,434 Mean age (±S.E.M.): 55 ± 17 years 57.4% women Study design: prospective longitudinal cohort study.	Cardiac, surgery (0.5%), General surgery (74.84%), Gynecologic (2,42%), Neurosurgery (1.21%), Orthopedic (4.85%), Otolaryngologic (1.02%), Plastic (0.9%), General Thoracic (0,47%), Urologic (1.65%), and Vascular (12.05%) (surgeries performed between 2005 and 2009)	Modified frailty index (mFI) according to Mitnitski et al. (2001).	For each increase in mFI, the OR for mortality increased from 1.24 (cardiac surgery at the moderate complexity level) to 46.33 (general surgery at the low complexity level).
Farhat et al. (2012)	*N* = 35,334 patients. Age >60 years 54.4% female 77.1% white 8.9% black Study design; retrospective observational study	Patients older than 60 years undergoing emergency general surgery (surgeries performed between 2005 and 2009)	Canadian Study of Health and Aging frailty-index (mFI) according to Rockwood et al. (2005)	mFI was the strongest predictor of death among all variables with an odds ratio of 11.70.
Afilalo et al. (2012)	*N* = 152 Mean age (±S.E.M.): 76 ± 4 years 34% women. Study design: prospective, multicenter cohort study	Coronary artery bypass and/or valve surgery or repair via a standard sternotomy approach (surgeries performed between 2008 and 2010)	Four different frailty scales: Fried criteria according to Fried et al. (2001) 7-item expanded CHS accord Rothman et al. (2008) 4-item MSSA4-item MacArthur Study of Successful Aging frailty scale subdimensions according to Sarkisian et al. (2008) Gait speed ≥6 s to walk 5 m	Slow gait speed was associated with an increase in mortality or major morbidity OR 2.63; CHS scale OR 1.36; modified CHS scale OR 1.26; MSSA subdimensions OR 1.24.
Adams et al. (2013)	*N* = 6727 mean (range) age of 54.7 (range 16–90 years) 49.7% women 10.2% African American Study design: retrospective review of medical records.	Inpatients who underwent surgical procedures in otolaryngology ward. (surgeries performed between 2005 and 2010)	mFI according to Saxton and Velanovich Saxton & Velanovich, (2011) (obtained mapping 11 variables present in the CSHA-FI according to Rockwood, (2005) plus 15 variables in the NSQIP data set.	Multivariate logistic regression was used to compare mFI with age, ASA, and wound classification. As the mFI increased from 0(no frailty-associated variables) to 0.45 (5 of 11) or higher, mortality risk increased from 0.2% to 11.9%. The mFI was the dominant significant predictor, with a relative OR of 11.
Sundermann et al.(2011)	*N* = 400 patients Mean age (±S.E.M.): 80.1 ± 4 years. 51% women Type of study not specified	Patients undergoing elective cardiac surgery (surgeries performed between 2008 and 2010)	Comprehensive Assessment of Frailty (CAF, according to Sundermann et al. (2011) and Fried criteria (according to Fried et al. (2001). Selected laboratory tests: serum albumine, creatinine and brain natriuretic peptide.	One-year mortality was related to the CAF-score (OR 1.11). The largest effect was found for the test item 'Chair rise' followed by the items 'Weak', 'Stair', 'Creatinine'.
Obeid et al. (2012)	*N* = 58,448 58.7% were older than 60 years, with 14.5% older than 80 years. 52.1% women Study design: retrospective study	Patients undergoing laparoscopic and open colectomies. (surgeries performed between 2005 and 2009)	mFI according to Mitnitski et al. (2001)	As the frailty index increased from 0 to 0.64, mortality increased from 0.7% to 43.2%.
Green et al. (2012)	*N* = 159 Mean age (±S.E.M.: 86 ± 8 years. 50% women Study design: prospective cohort study	Patients undergoing transcatheter aortic valve replacement (TAVR) for symptomatic aortic stenosis. (time of surgeries not specified)	Markers of frailty were chosen to loosely parallel those by Fried et al. (2001). Activities in daily living was assessed by the Katz survey (Katz, Downs, Cash, & Grotz, 1970). Serum albumin was measured used as a marker of malnutrition and wasting. These components were summed to derive a frailty score for each subject (possible range 0 to 12).	Frailty score >5 was associated with increased mortality after TAVR (adjusted OR: 3.51)

Table 2
Classification of surgical complications.

Clavien–Dindo clasification of surgical complications	
Grades	Definitions
Grade I	• Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and • Radiological interventions. • Acceptable therapeutic regimens are antiemetics, antipyretics, analgesics, and diuretics drugs and electrolytes administration and physiotherapy. • This grade also includes wound infections opened at the bedside.
Grade II	• Requiring pharmacological treatment with drugs other than such allowed for grade I complications. • Blood transfusions and total parenteral nutrition are also included.
Grade III	Requiring surgical, endoscopic or radiological intervention
Grade III-a	Intervention not under general anesthesia
Grade III-b	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)‡ requiring IC/ICU-management
Grade IV-a	Single organ dysfunction (including dialysis)
Grade IV-b	Multi-organ dysfunction
Grade V	Death of a patient
Suffix 'd	If the patient suffers from a complication at the time of discharge the suffix "d" (for 'disability') is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication

outcomes as the result of surgical procedures, such as sequelae and surgical therapy failures (Table 2).

This model was updated in 2004, using a cohort of 6336 patients who underwent elective general surgery, to allow more efficient grading of postoperative complications (Dindo, Demartines, & Clavien, 2004) and these new Clavien–Dindo classifications were evaluated through an international survey with two questionnaires sent to 10 surgical centres. Results from physicians in the second survey indicated that these classifications include simple (92% of the respondents), reproducible (91%), logical (92%), useful (90%), and comprehensive (89%). Therefore, the authors suggest that subjective, inaccurate, or confusing terms such as "minor" or "major" should be removed from the surgical literature (Clavien et al., 2009). Dindo and Clavien also tried to standardize the concept of 'surgical complication' by proposing a definition for it as "any deviation from the ideal postoperative course that is not inherent in the procedure and does not comprise a failure to cure" (Dindo & Clavien, 2008).

In order to properly record postoperative complications and calculate surgery risk, it is important to evaluate the patient's physiological status explaining why evaluation of frailty has emerged as a crucial variable in the assessment of risk stratification (Table 3). In colorectal cancer patients who were frail preoperatively (as assessed by the comprehensive geriatric assessment [CGA] scale) the most common postoperative complications were related to pulmonary (24% cases), cardiac (23%) system, and in 13% of individuals, delirium (Kristjansson et al., 2012). Moreover, frail individuals were more likely to suffer a severe postoperative complication or die (62%) in comparison to robust (33%) or intermediate/prefrail (36%) individuals (Kristjansson et al., 2012). A growing body of literature supports the concept that accumulated frailty deficits are related to adverse postoperative outcomes in older adults (Table 3). There are six studies published that have been analysed in details in the next section dividing them in prospective, retrospective studies and those performed in a specific population.

3.2.1. Prospective studies considering the relationship between frailty syndrome and postoperative complications

Dasgupta et al. (2009) prospectively evaluated the relationship between frailty and postoperative complications in 125 patients (70 years and older) who underwent elective surgery (mostly orthopaedic). The Edmonton Frail Scale (which comprehensively evaluates several criteria such as functional ability, cognition, nutrition, geriatric syndromes, and health status) was used to assess frailty. Frail individuals were more likely to have postoperative complications (OR = 5.2) and had a significantly reduced chance of being discharged. Makary et al., (2010) also conducted a prospective study in patients (594 patients aged 65 years and over) who underwent an elective surgical procedure. This study is the only one that specifically evaluate the relationship between "phenotypic frailty" and postsurgical outcomes (Makary et al., 2010). Phenotypic frailty was evaluated by the Fried criteria (Fried et al., 2001) i.e. weight loss (shrinking), decreased grip strength, exhaustion, low activity, and slow walking speed. Makary and colleagues (2010) demonstrated that the severity of frailty is significantly correlated with an increased risk for postoperative complications, longer length of hospital stay, and an increased likelihood of postoperative transfer into an institutional care facility.

3.2.2. Retrospective studies considering the relationship between frailty syndrome and postoperative complications

Lee et al. (2010) retrospectively studied frailty in 3826 patients (of all ages) who underwent cardiac surgery; they defined frailty as the presence of one or more of three frailty deficits: dependence in one or more daily living activity, use of a walking aid, and/or a documented history of dementia. Their results showed that frailty independently predicted hospital mortality, institutional discharge time, and mid-term mortality (up to three years). Similarly, Saxton and Velanovich (2011) performed a retrospective study on 226 patients who had been admitted for general surgical procedures. Frailty was evaluated by using the 70-item Canadian Study of Health and Aging Frailty Index, which includes the criteria of functionality, cognition, geriatric syndromes, co-morbidity burden, nutrition, and mood. Their results demonstrated that patients with postoperative complications had a significantly higher median frailty-index score in the preoperative evaluation than those without complications.

3.2.3. Preoperative frailty syndrome studies in specific subgroups and their relationship with postoperative complications

Erekson et al. (2011) demonstrated that a preoperative dependent functional-status and unintentional weight loss (one of most frequently measured criterion on several frailty scales)

Table 3
Frailty syndrome and post-operative complications.

Reference	Population	Type of surgery	Assessment of frailty	Classification of post-operative complications	Main results
Kristjansson et al. (2012)	*N* = 176 70–94 years (mean age 80 years). (57% women) Study design: cohort prospective longitudinal study	Stage I to IV colorectal cancer. (November 2006- June 2008)	CGA (Comprehensive geriatric assessment). according to (Jones et al., 2004Jones, Song, & Rockwood, 2004). Modified physical phenotype of frailty (PF). According to Fried et al. (2001).	Clavien classification according to Clavien et al. (1992)	CGA significantly predicted any post-operative complication. PF was neither a significant predictor of any complication nor of severe complications. The most common severe complications in the CGA-frail group were pulmonary complications in 18 patients (24%), cardiac complications in 17 patients (23%), and delirium in 10 patients (13%). Increasing frailty identified from the modified PF was neither a significant predictor of any complication nor of severe complications.
Tsiouris et al. (2013)	*N* = 1940 Mean age (±S.E.M.): 66 ± 11 years (51% women) Study design: cohort prospective study	Open lobectomy patients (surgeries performed between 2005 and 2010)	Modified frailty index (mFI) according to (Mitnitski et al., 2001)	Clavien-Dindo Clasification according to (Clavien et al., 2009)	Failure to wean from the ventilator, reintubation, surgical site infections, pneumonia, and Clavien 4 and above complications occurred in 1.8%, 2.6%, 2.2%, 5.4%, and 4.2%, respectively, in patients with an mFI of 0, compared with 7.4%, 7%, 3.2%, 10.9%, and 14.4%, respectively, in patients with mFI of 0.27. Multivariate logistic regression also demonstrated that mFi >0.27 (OR 4.8), wound class 3 (OR 4.8), ASA score 4 (OR 6.8), and dependent functional status (OR 4.7), were predictors of Clavien 4 complications.
Velanovich et al. (2013)	*N* = 971,434 Mean age (±S.E.M.): 55 ± 17 years 57.4% women Study design: prospective longitudinal cohort study.	Cardiac, surgery (0.5%), General surgery (74.84%), Gynecologic (2,42%), Neurosurgery (1.21%), Orthopedic(4.85%), Otolaryngologic (1.02%), Plastic (0.9%), General Thoracic (0,47%), Urologic (1.65%), and Vascular (12.05%) (surgeries performed between 2005 and 2009)	Modified frailty index (mFI) according to (Mitnitski et al., 2001)	According to the criteria proposed by American College of Surgeons National Surgical Quality Improvement Program (NSQIP)	The trend of odds was statistically significantly increased with each increase in FI, from 1.24 (general thoracic surgery at the low complexity level) to 3.36 (orthopedic surgery at the low complexity level).
Farhat et al. (2012)	*N* = 35,334 patients. Age >60 years 54.4% female 77.1% white 8.9% black Study design; retrospective observational study	Patients older than 60 years undergoing emergency general surgery. Most common operations: partial colon resection (16.7%), small bowel resection (10.1%), and laparoscoic appendectomy (9.5%). (surgeries performed between 2005-2009)	Canadian Study of Health and Aging frailty-index (mFI) (Rockwood, 2005)	The American College of Surgeons National Surgical Quality Improvement Program (NSQIP),	The category of any infection rate increased from 15.7% in patients with an mFI of 0 to 39.9% in those with an mFI of 0.64 and then decreased to 33.3% in patients with an mFI of 0.73 and greater. Patients with mFI of 0 had a wound infection rate of 9.0%, which increased to 12.1% in patients with an mFI score of 0.27 and then decreased to 9.8% in patients with an mFI score of 0.73. Wound occurrence rate was 9.6% with an mFI of 0, increasing to peak at 14.0% withan mFI of 0.27 and then decreasing to 9.8% in those with an mFI score <0.73. The rate of any occurrence increased from 21.0% in patients with an mFI score of 0 to 54.9% in patients with an mFI of 0.73 and greater.
Tan et al. (2012)	*N* = 83 Mean age: 81.5 years (range 75–93) Gender distribution was not specified Study design: prospective cohort study at 2 centers from	Colorectal resection. (surgeries performed between February 2008 and April 2010).	Fried criteria according to (Fried et al., 2001)	Clavien-Dindo classification of type II according to (Clavien et al., 2009)	Frail patients syndrome have a significant 4 times higher risk of developing major complications (OR = 4.1).
Larsen & Rubinfeld (2012)	*N* = 971,455 Study design: study population. Age ≥40 Gender distribution was not specified.	Patients of all type of surgery. (surgeries performed between 2005 and 2009)	Frailty index (CSH-FI) (Rockwood, 2005)	Cardiac arrest requiring cardiopulmonary and resuscitation, myocardial infarction (MI), and death	Frailty and ASA class were the strongest predictors of cardiac arrest; the OR for frailty was 26.4, and the ORs for ASA Classes 3, 4, and 5 were 1.2, 3.5, and 7.5, respectively. For perioperative MI, frailty and ASA class also were the most powerful predictors, with the OR for frailty being 41.8, and the ORs for ASA Classes 3, 4, and 5 being 6.9, 12.3, and 14.9, respectively.

Kristjansson et al. (2010)	*N* = 178 Age range: 70–94 years, and 10 patients were 90 years or older. 57% women Study design: prospective observational cohort study	Colorectal cancer. (surgeries performed between November 2006 and June 2008)	CGA (comprehensive geriatric assessment) (Jones et al., 2004)	Clavien classification (Clavien et al., 1992)	The rate of complications for patients operated laparoscopically was significantly lower than for patients operated by open or converted surgery. Being frail compared with non-frail increased the relative risks of experiencing any complication and severe complications by 1.59 and 1.75, respectively. Increasing age and ASA classification were not associated with complications.
Garonzik-Wang et al. (2012)	*N* = 183 Age: 53 ± 14 36% women, 40% African American. Study design: prospective study single center study	Patients with end-stage renal disease (ESRD) for kidney transplant. (surgeries performed between December 2008 and April 2010)	Fried criteria (Fried et al., 2001)	The primary outcome was delayed graft function (DGF), defined as the need for dialysis in the first week after a kidney transplant.	The rate of DGF was 30% in frail patients and 15% in non frail kidney transplant recipients. After adjusting for multiple donor and recipient factors, frailty was independently associated with twice the risk of DGF (relative risk [RR], 1.94.
Adams et al. (2013)	*N* = 6727 mean (range) age of 54.7 (range 16–90 years) 49.7% women 10.2% African American Study design: retrospective review of medical records.	Inpatients who underwent surgical procedures in otolaryngology ward. (surgeries performed between 2005 and 2010)	mFI according to Saxton and Velanovich (Saxton & Velanovich, 2011), 2011 (obtained mapping 11 variables present in the CSHA-FI (Rockwood, 2005) plus 15 variables in the NSQIP data set.	Clavien-Dindo grade IV complications (Clavien et al., 2009)	The risk of Clavien-Dindo grade IV complications increased from 1.2% (non frail) to 26.2% (frail). The risk of all complications increased from 9.5% (non frail) to 40.5% (frail). mFI was the dominant significant predictor, with a relative OR of 11. Functional status was the second dominant predictor, with a relative OR of 5. Age had a relative OR of 1. When the model was run with functional status included within the mFI, them FI became far more powerful, with a relative OR of 109. In this model, ASA classes 1 through 4 were significant.
Makary et al. (2010)	*N* = 594 Age was 71.3 (range 65–94 years). 67.6% women Caucasian 84 % Study design: prospective longitudinal study	Patients selected for elective surgery (between July 2005 and July 2006)	Fried criteria (Fried et al., 2001)	Surgical complication was defined using the American College of Surgeons National Surgical Quality Improvement Program (NSQIP) (Khuri et al., 1998)	Pre-operative frailty was associated with an increased risk for postoperative complications (intermediately frail: OR [OR] 2.06; 95% CI 1.18–3.60; frail: OR 2.54; 95% CI 1.12–5.77), length of stay (intermediately frail: incidence rate ratio 1.49; 95% CI 1.24–1.80; frail: Frailty improved predictive power ($p < 0.01$) of each risk index (i.e., American Society of Anesthesiologists, Lee, and Eagle scores).
Dasgupta et al. (2009)	*N* = 125 Mean age was 77.4 (range 70–92 years) Study design: exploratory study	Older adults with medical illness undergoing non-cardiac major elective surgery. (surgeries performed between June 2002 and April 2003)	Edmonton Frail Scale (EFS)(Rolfson, Majumdar, Tsuyuki, Tahir, & Rockwood, 2006)	Wound infection or excessive bleeding into the surgical site. The primary outcome were cardiac or pulmonary complications, delirium, stroke, gastrointestinal bleeding.	Increasing frailty was significantly associated with postoperative complications, increased length of hospitalization and inability to be discharged home, independent of age. EFS scores of 3 or less were associated with a lower risk of having a complication (age-adjusted OR 0.27) and a higher chance (80%) of being discharged home. EFS scores exceeding 7 were associated with increased complications (OR 5.02) and a lower chance of being discharged home (40%).
Lasithiotakis et al. (2013)	*N* = 57 Median age 73 years (8.8) 50.9% women Study design: prospective cohort study	Elective laparoscopic cholecystectomy (surgeries performed between October 2008 and December 2011)	Comprehensive Geriatric assessment (CGA) (Pal, Katheria, & Hurria, 2010)	Dindo classification (Dindo et al., 2004)	The overall incidence of postoperative complications was 23.7%, most of which were grade I and II (18.8%). Frail patients experienced a significantly higher incidence of postoperative complications compared to their fit counterparts (84.6 vs. 15.4%,) OR (1.0 vs 6.0). Frail patients experienced a significantly higher frequency of prolonged (more than 2 days) postoperative hospital stay compared with their fit counterparts (65 vs 35%), OR (1.0 vs 4.2). No statistically significant association was between age, gender, and the incidence of postoperative complications.
Courtney-Brooks et al. (2012)	*N* = 37 women Mean age: 73 years (range: 65–95) Women Study design: prospective Cohort study, pilot study	Gynecologic oncology patients (surgery performed between March and December 2011)	Fried criteria (Fried et al., 2001)	Surgical complications defined by the American College of Surgeons National Surgical Quality Improvement Program (NSQIP)	The rate of 30-day surgical complications increased with frailty score and was 24%, versus 67% for women who were not frail as compared to the frail.

Table 3 (*Continued*)

Reference	Population	Type of surgery	Assessment of frailty	Classification of post-operative complications	Main results
Dale et al. (2014)	$N=76$ Mean age 67 years (nearly 80% were older than 60 years) Study deisgn: prospective cohort study	Patients undergoing a pancreatico-duodonectomy (PD) for pancreatic tumors. (surgeries performed between October 2007 and July 2011)	4 (out of 5) Fried criteria (reduced physical activity was not measured in the study) (Fried et al., 2001)	Clavien-Dindo classification (Dindo et al., 2004)	"Exhaustion" predicted major complications [odds ratio (OR) = 4.06; $p=0.01$], longer hospital stays ($\beta=0.27$; $p=0.02$), and surgical intensive care unit admissions (OR = 4.30; $p=0.01$). No other measures were statistically associated with the outcomes.
Sundermann et al. (2011)	$N=400$ Mean age was 80.1 ± 4 years. 51% women	$N=400$ patients Mean age (±S.E.M.): 80.1 ± 4 years. 51% women Type of study not specified	Patients undergoing elective cardiac surgery (surgeries performed between 2008 and 2010, Germany)	Comprehensive Assessment of Frailty (Sundermann et al., 2011) and Fried criteria (Fried et al., 2001) Selected laboratory tests: serum albumine, creatinine and brain natriuretic peptide.	CAF score was positively correlated with the length of ventilator assistance. No correlation of the CAF-score to other secondary endpoints could be detected. Age as single factor was not a significant predictor of one-year mortality.
Erekson et al. (2011)	$N=22.214$ 497 women two age categories (<80 years, and ≥80 years old) aged ≥80 years (2.23%), and 97.7% younger. 61.8% white. Study design: longitudinal observational study	Major gynecologic surgery. (surgeries performed between 2005 and 2009)	Markers of frailty: decreased cognition, poor nutrition (measure by lower serum albumin and hematocrit levels), recent unexplained falls, and dependent functional status for activities of daily living, according to Robinson et al. (2013)	The primary outcome was composite 30 day major postoperative complications (Heisler, Melton, Weaver, & Gebhart, 2009)	Age ≥80 years (adjusted OR = 1.8), dependent functional status (adjusted OR = 2.37), and unintentional weight loss (adjusted OR = 2.49) were significantly associated with postoperative morbidity after adjusting for confounding factors (diabetes mellitus, known bleeding disorder, morbid obesity, ascites, preoperative systemic infection, procedures for gynecologic cancer, disseminated cancer, emergency procedures, operative time and wound class).
Obeid et al. (2012)	$N=58,448$ 58.7% were older than 60 years, with 14.5% older than 80 years. 52.1% women Study design: retrospective study	Patients undergoing laparoscopic and open colectomies. (surgeries performed between 2005 and 2009)	mFI according to (Mitnitski et al., 2001)	Clavien-Dindo classification (Clavien et al., 2009)	After adjusting for known risk factors and relevant patient variables, frailty index remained an independent predictor of Clavien class IV and V complications with the highest OR of 14.4, as did open colectomy (vs. laparoscopic) and ASA score ≥4, although with smaller ORs. In patients who underwent emergency operations, as the mFI increased from 0 to 0.64, the percent of patients who experienced Clavien IV and V complications increased from 9.1% to 77.1%.
Saxton & Velanovich (2011)	$N=226$ Age 61 ± 13 years, 53% women 63% white 25% African American Study design: retrospective observational study.	56% cancer patients (31% superficial/ laparoscopic, 17% open, intra-abdominal for benign disease, and 52% open/laparoscopic for malignant disease) (time of surgeries not specified)	mFI according to Mitnitski (Mitnitski et al., 2001)	Clavien classification (Dindo et al., 2004)	Increasing the FI scores were associated with increasing numbers of postoperative complications. There was a statistically significant increased risk for the development of any complication (OR 1.48), but not for the most severe complications or death. In the multivariate analysis for the development of any postoperative complications, an FI >0.12 increased the risk (OR 2.71).
Leung et al. (2011)	$N=63$ Age 73 ± 6.0 50% women Studydesign: prospective longitudinal cohort study, pilot study	Surgical type: general arthroplasty spine thoracic (surgeries performed in 2007)	Fried criteria (Fried et al., 2001)	Postoperative delirium defined using the Confusion Assessment Method (Inouye et al., 1990)	Higher frailty score was significantly associated with higher risk for delirium. The incidence of delirium increased steadily from 0% for those with frailty scores of 0 to 57% for those with frailty scores of 4 or more.

Green et al. (2012)	$N = 159$ Mean age (±S.E.M.): 86 ± 8 years. 50% women Study design: prospective cohort study	Patients undergoing transcatheter aortic valve replacement (TAVR) for symptomatic aortic stenosis. (time of surgeries not specified)	Markers of frailty were chosen to loosely parallel those operationalized by (Fried et al., 2001). Activities in daily living was assessed by the Katz ADL survey (Katz et al., 1970) Serum albumin. These components were summed to derive a frailty score for each subject.	Life-threatening or major bleeding, major vascular complications, and major stroke were assessed according to the Valve Academic Research Consortium criteria (Leon et al., 2011)	After adjustment for several factors frailty score of >5 was associated with in-hospital life-threatening or major bleeding evens (OR 2.2). There was a higher frequency of blood transfusions after TAVR in the group with a frailty score >5 (24 (32.9%) versus 15 (18.1%). There was no significant interaction between access route and frailty score category for the life-threatening or major bleeding endpoint.
Revenig et al. (2013)	$N = 189$ patients. Mean age 62 years, 40.2% women 71.4% Caucasian. Study design: prospective study	Surgical type: 117 from urology, 52 from surgical oncology, and 20 from general surgery clinics. (time of surgeries not specified)	Hopkins Frailty Score, (shrinking weakness, exhaustion, low activity, and slowed walking speed) (Makary et al., 2010)	Clavien–Dindo classification (Dindo et al., 2004)	The composite of the 5 measures of the Hopkins Frailty Score, preoperative shrinking or weight loss, and a patient's preoperative hemoglobin were all statistically significant predictors of a postoperative complication of any grade. An analysis of major complications (Grade III or greater) revealed no statistically significant relationship with frailty status. On multivariate analysis, only a patient's composite frailty score (OR 2.07) was predictive of a postoperative complication, and higher hemoglobin (OR 0.84) was protective for an adverse postoperative outcome.
Tegels et al. (2014)	$N = 180$ patients 41.1% women. Mean age at time of surgery (range) 69.8 (range 37–88 years). Study design: Retrospective study.	Patients with gastric cancer. (surgeries performed between 2005 and 2012)	Frailty was assessed using the Groningen Frailty Indicator (GFI) (Schuurmans, Steverink, Lindenberg, Frieswijk, & Slaets, 2004)	Outcome measurements were serious adverse events (complications at Clavien–Dindo grade ≥3a) (Clavien et al., 2009;Dindo et al., 2004) length of stay.	GFI ≥3 was associated with increased risk of serious adverse events (Clavien–Dindo grade ≥3a) at 21.6% in the lower group versus 50% in the higher group. This was independent of age, type of surgery, tumor stage, ASA classification, and neoadjuvant chemotherapy in multivariate analysis (OR 3.62).

were both significantly associated with postoperative morbidity in older women who underwent major gynaecological surgery. Interestingly, these associations remained significant even after adjustment for confounding factors including diabetes mellitus, known bleeding disorders, morbid obesity, ascites, preoperative systemic infection, history of gynaecological/disseminated cancer or emergency procedures, surgery time, and wound class (Afilalo et al., 2012).

Robinson et al. (2013) evaluated preoperative frailty in colorectal cancer and cardiac patients with a different evaluation tool including the simple criterion "timed up-and go" i.e., the time required to stand up from a chair, walk 10 feet, return to the chair, and sit back down in it (an abnormal score was defined as 15 seconds or more). In the colorectal surgery population, an increasing frailty burden was associated with advancing age, but other co-morbidities were similar when the three frailty colorectal groups (robust, intermediate-frail, and frail) were compared. In the cardiac surgery population, age and other co-morbidities were similar between these three frailty groups, except the incidence of stroke in the medical history (prior to surgery), which was a higher in the frail group. These findings support the notion that frailty is not strictly related to biological age and that the number of comorbidities does not fully correlate with their severity (Afilalo et al., 2012; Farhat et al., 2012; Fernández-Garrido et al., 2014a; Fried et al., 2004; Partridge et al., 2012; Robinson et al., 2013) As the frailty burden increases, the occurrence of one or more complications are more likely. Robinson et al. (2013) reported that infection was the most common complication in both surgical groups, and perhaps related to this, a decrease in immune function has also been described in frail individuals (Furlan et al., 2013; Heffernan et al., 2012; Leng et al., 2009; Reddan et al., 2003). Length of hospital stay increased as the frailty burden accumulated, and thus these patients also accrued higher economic and health-professional costs during this postsurgical period (Cohen et al., 2012; Lasithiotakis et al., 2013; Lee et al., 2010; Makary et al., 2010; Robinson et al., 2013).

By using the Clavien–Dindo classification system for surgical complications (see Table 2), Obeid et al. (2012) showed that the frailty index is directly and significantly associated with an increased risk of mortality or class IV or V complications in older patients who underwent laparoscopic and open colectomies. After adjusting for known risk factors and relevant patient variables, the frailty index remained an independent predictor of Clavien-Dindo class IV and V complications (with a maximum OR of 14.4). Similarly, an ASA score of 4 or more for open (vs. laparoscopic) colectomy was also predictive of class IV and V complications, although the ORs were less (Obeid et al., 2012). These results were confirmed by Adams et al. (2013) that showed that the risk of grade IV complications increased from 1.2% to 26.2% for open or laparoscopic colectomy, and the overall risk of any complication increased from 9.5% to 40.5%.

Delirium is a severe postoperative complication that has rarely been investigated in relation to preoperative frailty. Leung, McArdle, and Wong (2011) demonstrated that higher frailty scores (measured using the Fried criteria) are associated with a higher risk of postoperative delirium: The incidence of delirium increased steadily from 0% for those with frailty scores of 0, to 57% for those with frailty scores of 4 or more (Leung et al., 2011). Notably, not all postoperative complications, for instance the incidence of wound infection are related to the preoperative frailty status (Farhat et al., 2012). This suggests that frailty is not a general risk factor for all postoperative complications and that not all physiopathological alterations observed in frail individuals play a significant role in the occurrence of postsurgical complications (Farhat et al., 2012).

3.3. Comparison of different tools for measuring frailty syndrome in relation to postoperative outcomes

Only two studies have compared the ability of different frailty scales to predict postoperative complications and mortality (Afilalo et al., 2012; Kristjansson et al., 2012). Kristjansson et al. (2012) compared the CGA scale to a modified version of the physical phenotype of frailty (PF; slightly modified from the original Fried criteria) in a cohort of older adults with colorectal cancer. They found that a multi-domain frailty measurement based on the CGA was more useful than frailty identified by the modified PF criteria in predicting postoperative complications, although both frailty scales were predictive of mortality rate. Thus, even though the physical phenotype of frailty is less time-consuming than the CGA assessment it does not necessarily identify remediable conditions such as malnutrition and depression (Buigues et al., 2014), that can be optimized preoperatively (although both these factors are linked to frailty in a subpopulation of frail older individuals). Afilalo et al. (2012) compared the ability of four frailty scales to predict postoperative complications and mortality in patients undergoing coronary artery bypass and/or valve surgery. The scales analysed in that report were the 5-item Cardiovascular Health Study (CHS) frailty scale (gait speed, handgrip strength, inactivity, exhaustion, and weight loss) (Fried et al., 2001), the 7-item expanded CHS frailty scale (CHS scale criteria plus cognitive impairment and depressed mood) (Rothman, Leo-Summers, & Gill, 2008), the 4-item MacArthur Study of Successful Aging frailty scale (gait speed, handgrip strength, inactivity, and cognitive impairment) (Sarkisian, Gruenewald, Boscardin, & Seeman, 2008); and gait speed alone, with frailty defined as the individual requiring 6 s or more to walk 5 m. The proportion of patients categorized as frail varied depending on the scale used, ranging from 20% using the CHS frailty scale to 46% using the single measurement of 5-m gait speed. In this study only the 5-m gait speed was significantly associated with an increase in mortality or major morbidity (OR = 2.63) and demonstrated a superior predictive-ability compared with other frailty scales. This finding is consistent with previous studies which showed that gait speed is the best to predict 6-month mortality compared to more complex measurements including the CHS and Rockwood frailty scales, or to other simple measurements such as handgrip strength, and balance (Purser et al., 2006). One reason for this finding may be the simple and reliable nature of the measurement from both the patient's and observer's perspective, the possibility to use it in patients with cognitive impairment thus eliminating those subjective questions included in several frailty scales. It is difficult to suggest the optimal frailty tool for preoperative risk evaluation. It seems that long and exhaustive scales such as the CGA have the best predictive power for postsurgical complications and mortality in oncological patients, whereas in patients undergoing coronary artery bypass and/or valve surgery, a simple test such as 5-m gait speed alone (as defined above) displayed the best sensitivity to postsurgical outcomes. This apparent discrepancy may be due to the different population of older individuals in these studies: oncological patients versus patients requiring surgery for coronary artery bypass and/or valvulopathy treatment. In order to shed some lights on this crucial issue it's still advisable to assess frailty syndrome by a validated scale as those reported in Table 4 and

Table 4
Frailty scales used in the studies analyzed in the review.

Diagnostic tool to assess frailty	Total number of patients screened	Number of studies	References
CGA (comprehensive geriatric assessment) according to Jones et al. (2004)	233	2	Kristjansson et al. (2012) Lasithiotakis et al. (2013)
Phenotype of frailty according to Fried et al. (2001)	1923	10	Kristjansson et al. (2012) Afilalo et al. (2012) Sundermann et al. (2011) Green et al. (2012) Tan et al. (2012) Garonzik-Wang et al. (2012) Makary et al. (2010) Courtney-Brooks et al. (2012) Dale et al. (2014) Leung et al., (2011)
Modified frailty index (mFI) according to Mitnitski et al. (2001)	1.032.048	4	Tsiouris et al. (2013) Velanovich et al.(2013) Obeid et al. (2012) Saxton & Velanovich, (2011)
Canadian Study of Health and Aging frailty-index (mFI) according to Rockwood et al. (2005)	1.006.789	2	Farhat et al. (2012) Larsen & Rubinfeld, (2012)
mFI according Saxton & Velanovich (2011) (obtained mapping 11 variables present in the CSHAFI (according to Rockwood (2005) plus 15 variables in the NSQIP data set.	6727	1	Adams et al. (2013)
Comprehensive Assessment of Frailty (CAF, according to Sündermann et al. (2011)).	400	1	Sundermann et al. (2011)
Groningen Frailty Indicator (GFI) according to Schuurmans et al. (2004)	180	1	Tegels et al. (2014)
Edmonton Frail Scale (EFS) according to Rolfson et al. (2006)	125	1	Dasgupta et al. (2009)
Hopkins Frailty Score: shrinking,weakness, exhaustion, low activity, and slowed walking speed according to Makary et al. (2010)	189	1	Revenig et al. (2013)
Other scales: 7-item expanded CHS according to Rothman et al. (2008). 4-item MSSA: MacArthur Study of Successful Aging frailty scale subdimensions according Sarkisian et al. (2008). Gait speed ≥6 s to walk 5 m Others markers of frailty according to Robinson et al. (2013)	22366	2	Afilalo et al. (2012) Erekson et al. (2011)

Table 5
Frailty and post-operative mortality in oncologic patients.

Reference	Population	Type of surgery	Assessment of frailty	Results on mortality rate after surgery
Kristjansson et al. (2012)	$N = 176$ 70–94 years (mean age 80 years) (57% women) Study design: cohort prospective longitudinal study	Patients with colorectal cancer (Stage I–IV). (surgery performed between November 2006–June 2008)	CGA (Comprehensive geriatric assessment). According to Jones et al. (2004) Modified physical phenotype of frailty (PF). According to Fried et al. (2001)	Both CGA and PF significantly ($p < 0.01$) predicts survival.
Neuman et al. (2013)	$N = 12,979$ oldest-old patients among Medicare beneficiaries age ≥80 years	Elective colectomy for stage I–III colon cancer from the Surveillance, Epidemiology and End Results (SEER)-Medicare database	Defined by the John Hopkins' Adjusted Clinical Groups case-mix system. Frailty assessed by the presence of 11 conditions (e.g., difficulty walking, weight loss, frequent falls, malnutrition, impaired vision, decubitus ulcer, incontinence, etc.)	Frailty strongly associates with decreased survival (90-day survival: odds ratio 10.4 [95% confidence interval 7.6–14.2], $p < 0.001$; 1-year survival: odds ratio 8.4 [95% confidence interval 6.4–11.1], $p < 0.001$
Tegels et al. (2014)	$N = 180$ patients 41.1% women. Mean age at time of surgery (range) 69.8 (range 37–88 years). Study design: retrospective study.	Patients with gastric cancer. (surgeries performed between 2005–2012)	Frailty was assessed using the Groningen Frailty Indicator (GFI) according to Schuurmans et al. (2004)	Multivariate analysis showed a significant association between increasing GFI and in-hospital mortality, OR 1.35 independent for age, ASA-classification, neoadjuvant chemotherapy, type of surgery, and tumor stage.

compared the results with individual less time-consuming measurements such as gait speed and handgrip strength.

4. Discussion

In order to evaluate the risks and benefits of surgery in older patients it is important to differentiate between chronological age and the physiological age for making-decision purposes. Several studies showed that increasing age was neither a predictor of postoperative morbidity nor of overall survival, suggesting that older individuals can well tolerate surgical procedures (Kristjansson et al., 2012).

The greatest risks are associated with other factors such as the level of urgency and concomitant cardiac, pulmonary, or/and renal diseases. Frailty syndrome arises from the need to identify those individual with decreased functional capacity, and therefore to identify patients with an increased risk of negative consequences such as falls, institutionalization, hospitalization, or even an increased risk of mortality (Fried et al., 2001). Lee et al. (2010) analysed a cohort of patients undergoing cardiac surgery in a single institution and found that frailty markedly increased the risk of these postoperative outcomes, and furthermore, the effect of frailty was independent of age.

OR risk scores do not contain all the relevant factors associated with increased risk in the elderly population, in particular, frailty and disability are not assessed (Afilalo et al., 2012). Based on the best evidence currently available many studies suggest that frailty is an independent predictor of adverse outcomes following cardiac surgery (Bagnall, Faiz, Darzi, & Athanasiou, 2013). The importance of assessing frailty before surgery has also been demonstrated in other surgical patients. In older oncologic patients, where treatment modalities such as surgery and chemotherapy may pose a high risk of complications and toxicity, identifying frailty represents a strong predictor for postoperative mortality (Table 5). Higher scores on the mFI have been more closely associated with increased morbidity and mortality rates than wound-class classification, ASA scores, or age, in patients requiring otolaryngology surgery or urgent surgery in an emergency hospital unit (Adams et al., 2013; Farhat et al., 2012). Moreover, the mFI can help surgeons to better assess complications and mortality rates after open lobectomy (Tsiouris et al., 2013). Frailty may also be useful as a tool to risk-stratify candidate patients for kidney transplant in the preoperative setting and may provide further insights into the mechanism of early graft dysfunction (Garonzik-Wang et al., 2012).

Many reports have demonstrated that frailty is more common in women, (Bartali et al., 2006; Cappola et al., 2009; Fernández-Garrido et al., 2014b; Kamel, 2003) indeed specific pre-operative frailty assessments are well-accepted by gynecological cancer patients, and are a feasible tool in a clinical setting (Courtney-Brooks et al., 2012). Dale et al. (2014) demonstrated that previous knowledge about the exhaustion or physical performance deficits in patients with pancreatic tumour considered suitable for pancreaticoduodenectomy can help to predict clinically relevant outcomes. Even though several reports demonstrated that frailty is an independent factor useful for measuring risk stratification, the question about the best clinical tool for assessing frailty remains unanswered. There are various risk-scoring systems described in the literature, and their use to assess preoperative risk has provided a useful and necessary tool for measuring patients' risk and to make informed decisions regarding surgery (Geissler et al., 2000).

Our systematic review supports that frailty should be routinely measured as part of the preoperative patient risk assessment in older individuals. Based on the analysis of the current literature we cannot recommend the best clinical tool to evaluate frailty in preoperative settings. Therefore, various scoring systems have been used (see Table 4). Although each of these scales efficiently identifies individuals with a high risk of adverse outcomes, they have analysed different subpopulations and differ in their ability to predict the prognostic outcomes (Amrock & Deiner, 2014).

Therefore, further investigation is required to choose the best combination of criteria for evaluating preoperative surgical risk. Most surgical risk scores largely ignore frailty syndrome, even though it has a strong predictive value for mortality and adverse outcomes, as discussed in this review. Furthermore, it is important to point out that assessment of frailty should be also performed for predicting postoperative complications, and to program the hospital stay for improving health condition of the patient.

Conflicts of interest

The authors have no conflicts of interest.

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Effect of a Prebiotic Formulation on Frailty Syndrome: A Randomized, Double-Blind Clinical Trial

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Abstract: Aging can result in major changes in the composition and metabolic activities of bacterial populations in the gastrointestinal system and result in impaired function of the immune system. We assessed the efficacy of prebiotic Darmocare Pre® (Bonusan Besloten Vennootschap (BV), Numansdorp, The Netherlands) to evaluate whether the regular intake of this product can improve frailty criteria, functional status and response of the immune system in elderly people affected by the frailty syndrome. The study was a placebo-controlled, randomized, double blind design in sixty older participants aged 65 and over. The prebiotic product was composed of a mixture of inulin plus fructooligosaccharides and was compared with placebo (maltodextrin). Participants were randomized to a parallel group intervention of 13 weeks' duration with a daily intake of Darmocare Pre® or placebo. Either prebiotic or placebo were administered after breakfast (between 9–10 a.m.) dissolved in a glass of water carefully stirred just before drinking. The primary outcome was to study the effect on frailty syndrome. The secondary outcomes were effect on functional and cognitive behavior and sleep quality. Moreover, we evaluated whether prebiotic administration alters blood parameters (haemogram and biochemical analysis). The overall rate of frailty was not significantly modified by Darmocare Pre® administration. Nevertheless, prebiotic administration compared with placebo significantly improved two frailty criteria, e.g., exhaustion and handgrip strength ($p < 0.01$ and $p < 0.05$, respectively). No significant effects were observed in functional and cognitive behavior or sleep quality. The use of novel therapeutic approaches influencing the gut microbiota–muscle–brain axis could be considered for treatment of the frailty syndrome.

Keywords: fatigue; biomarker; leucocytes; inflammation; aging

1. Introduction

Health care for older people in western societies, with high rates of chronic diseases and mental and physical impairment, has become a challenge for every health professional. Frailty is a geriatric syndrome describing physical and functional decline that occurs as a consequence of certain diseases (e.g., cancer, chronic infection, *etc.*) but also even without disease. This syndrome is characterized by an increased risk for poor outcomes related with accidental falls, fractures, disability, comorbidity,

health care expenditure and premature mortality [1]. In a recent publication, our group has shown [2] that an increase in neutrophil count and a decrease in lymphocyte count are significantly associated with less hand grip strength and low physical activity, two criteria of frailty syndrome [3]. We wish to test with a confident perspective that any measure that alters the number of leukocytes (decrease in neutrophil count and increase in lymphocyte count) can prevent the progression of frailty syndrome since it has been consistently demonstrated that proper immune function in aging is associated with the absence of other signs and symptoms [4]. The gut microbiota and their metabolites play a central role in modulating gut health and disease [4,5] in all age groups, especially in elderly people [6]. In the elderly, there seems to be a decline in microbiota diversity [7] with lower numbers of bifidobacteria, an increase in Enterobacteriaceae [8] and certain Proteobacteria. Moreover, these changes are suspected to play a role in the causation of bowel disease [9]. Age-related changes in gastro-intestinal physiology and function, such as greater permeability of mucosal membrane, reduced transit times, and secretion of acids by the gastric mucosa, can result in a significant change in the composition of the intestinal microbiota, marked by a decline in bifidobacterial numbers and an increase in putatively detrimental populations such as clostridia and enterobacteria [10–12]. This altered composition of the gut microbiota can affect the immune system causing profound and multifaceted changes in the elderly [13]. Prebiotics are food ingredients that, when selectively fermented, may alter the composition and/or activity of the intestinal microbiota and bring possible benefits to the individual's health. Oral administration of formulas containing prebiotics and fermented milk products may improve intestinal microbiota in this population and may contribute to the regulation of the gastro-intestinal functions and activity of the immune–gut system [14]. The potential use of prebiotics as a modulator of the immune function in older individuals has been suggested [15]. The rotational of our clinical trial was based on previous *in vitro* studies that demonstrated the ability of prebiotics to regulate the immune response through lymphocyte regulation and subsequently the inflammatory response [16–18]. Recently, studies performed in healthy humans found that administration of prebiotics increase the percentages of some lymphocyte subtypes [19,20].

Considering that alterations in neutrophil and lymphocyte counts are associated with frailty syndrome and, particularly with two frailty criteria, poor muscular strength and low physical activity [2], the primary goal of our study was to test whether the administration of the prebiotic formulation, Darmocare Pre® (Bonusan Besloten Vennootschap (BV), Numansdorp, The Netherlands), improves frailty syndrome in older individuals by altering the number of neutrophil and lymphocyte counts in the blood. In addition, as secondary outcomes, we evaluated whether Darmocare Pre® administration improves functional capacity, and some aspects usually present in frail older people, e.g., poor quality of sleep, disabilities in daily functioning, and cognitive function.

2. Results

2.1. Design and Study Population

The clinical trial was conducted in 60 volunteers. The participants lived in nursing homes and were non-demented and able to walk (see inclusion and exclusion criteria in the Experimental Section). They were randomized to a parallel group intervention of 13 weeks' duration with a daily intake of Darmocare Pre® or placebo (Figure 1). Allocation of the participants was blinded. The intervention group used Darmocare Pre®. The composition of Darmocare Pre® was Inulin min. 3375 mg fructooligosaccharides (FOS) min. 3488 mg of per level measuring spoon (of 7.5 grams): Excipients: none guaranteed to contain no genetically modified organisms, maize, soy, yeast, gluten, lactose, added saccharose, gelatine, animal substances, preservatives, artificial coloring, flavoring and aromatic substances. The placebo group used an indistinguishable placebo product, which consisted of a corresponding dose of maltodextrin. The mean energy intake in Darmocare Pre®-group was 1720 $\pm$ 184 kcal/day, and in the placebo group was 1658 $\pm$ 220 kcal/day. We did not observe any statistically significant difference ($p = 0.83$) between the two experimental groups regarding the

energy intake. Power calculation for the study was estimated as a function of the differences in the measure between the groups that should be detected, and the standard deviation of these values, the *p*-value (α value), and sample size. The probability is 90 percent that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.000 units. This is based on the assumption that the standard deviation of the difference in the response variables is two. The standard deviation for frailty criteria that we considered for the intervention was two (based on trial aimed to reduce frailty syndrome as a primary outcome). Thus, to achieve 90% power at a two-sided 5% significance level, and to detect a minimum difference of one piece of frailty criteria between placebo and Darmocare Pre®-groups, it was calculated that a sample size of a total of 44 patients will enter this two-treatment crossover study [21]. Therefore, to allow for dropouts of people that could not complete the investigation, it was planned to recruit 60 volunteers.

Figure 1. Design of the clinical study. N: number of individuals.

Both prebiotic formulation and placebo were administered after breakfast (between 9–10 a.m) dissolved in a glass of water, which was carefully stirred just before consumption. The primary outcome measured was the effect of treatment on several frailty criteria: weight loss, self-reported exhaustion, low physical activity (PA) level, slowed motor performance and weakness according to Fried *et al.* [3] and as explained below. The secondary outcome was the effect on functional activity assessed by the Barthel Index, cognitive impairment by the Mini-Mental examination score and subjective quality of sleep by the Athens Scale. Moreover, we also analyzed if treatment influenced the values of common blood analytical parameters and the haemogram. Enrollment of participants and evaluations at baseline (week 0, see Figure 1) was performed during the first four months of 2015. Randomization was carried out once the enrollment and baseline evaluations were completed. The intervention took place between June–September in 2015. The participants were selected based on inclusion and exclusion criteria (see study population paragraph). Baseline evaluation was performed 1–2 months before randomization. Within one month after completion of the study, all participants were re-evaluated (frailty syndrome, functional, cognitive and sleep impairment), blood was drawn and faecal samples were collected. The present analyses included the administration of Darmocare Pre® or placebo during 13 week daily administration as described below.

2.2. Sample and Dropouts

A total of fifty participants completed the study (83.3%) (15 men and 35 women). The mean age of participants was 73.8 ± 1.6 standard error of the mean (SEM) (range 66–90 years). No significant difference ($p = 0.74$) was observed between the mean age of individuals in the Darmocare Pre® (74.2 ± 1.6) compared to the placebo group (73.4 ± 1.8). No significant difference was found regarding the distribution of sexes in the experimental groups (nine men and 19 women in the Darmocare Pre® and six men and 16 women in the placebo group). Dropout rates were significantly ($p < 0.05$) different

between groups (eight participants in the Darmocare Pre® group stopped the treatment and two participants from the placebo group). Four participants from the Darmocare Pre® dropout for cramps, two participants for episodes of diarrhea and two for unknown reasons. One participant that dropped out from the placebo groups suffered from cramps, and the other one stopped for unknown reasons. Among participants that concluded the trial, nine of 22 in the Darmocare Pre® group referred to the appearance of gas, which lasted between 3–8 days. In the placebo group (maltodextrin), none of the participants that concluded the trial referred to any side effects. The two experimental groups (Darmocare Pre® or placebo) were not significantly different at baseline (see Table 1).

Table 1. Frailty criteria and geriatric evaluation at baseline and post-treatment with Darmocare Pre® or placebo. * $p < 0.05$; ** $p < 0.01$.

Variable	Baseline		Significance	Post-Treatment		Significance
	Placebo Group (n = 22)	Dermocare Pre® (n = 28)	p Value	Placebo Group (n = 22)	Dermocare Pre® (n = 28)	p Value
Exhaustion (score 0–3: 0 "never"; 1 "A few times" (1–2 days per week); 2 "Often" (3–4 days per week); or 3 "Most of the time" (almost each day))	1.1 ± 1.7	1.4 ± 1.7	0.74	1.7 ± 1.2	0.8 ± 1.4 **	0.002
Slow walk (s) (time needed to walk 4.6 m)	8.6 ± 9.0	8.4 ± 6.0	0.91	8.7 ± 4.2	7.9 ± 4.5	0.48
Grip strength (right hand, kg)	11.5 ± 5.7	10.6 ± 8.2	0.61	10.2 ± 4.1	12.4 ± 3.2 *	0.04
Grip strength (left hand, kg)	10.2 ± 5.8	10.1 ± 7.6	0.92	9.1 ± 3.7	9.8 ± 3.5	0.50
Self health-perception (score 0–10, being 0 the worst and 10 the best)	7.1 ± 2.3	7.1 ± 2.1	0.96	6.8 ± 2.4	6.8 ± 2.0	0.96
Body mass index	26.1 ± 4.1	25.8 ± 4.2	0.97	26.0 ± 3.8	25.9 ± 4.1	0.96
Athens insomnia scale	3.4 ± 3.0	4.1 ± 4.7	0.77	4.5 ± 5.3	4.0 ± 4.3	0.68
Barthel index	76.2 ± 13.0	74.6 ± 17.7	0.69	78.3 ± 13.9	77.1 ± 29.9	0.87
Mini–Mental state examination	26.1 ± 2.2	26.5 ± 3.1	0.89	25.9 ± 2.1	26.4 ± 2.2	0.85

2.3. Effect of Darmocare Pre® Administration on Frailty Criteria

At baseline and at the end of treatment, participants fulfilled 1–4 frailty criteria according to Fried *et al.* [3]. The overall rate of frailty was not significantly modified by Darmocare Pre® administration. Mean frailty score was 2.8 ± 1.0 at baseline and 2.5 ± 0.8 after intervention in the Darmocare Pre® group and 2.9 ± 0.7 at baseline and 2.8 ± 0.8 after intervention in the placebo group. However, when we analyzed the effect on each of the frailty criteria separately, we observed a significant effect of Darmocare Pre® administration. There was a significant ($p < 0.05$, Mann–Whitney test) improvement (Table 1) in exhaustion and muscle strength in the group of patients who received Darmocare Pre® compared to the placebo group. Exhaustion and muscle strength were not significantly different between groups at baseline (before intervention, see Table 1). The other criteria of frailty syndrome *i.e.*, weight loss, slow walking speed and reduced physical activity were not significantly different between groups neither at baseline nor after the intervention (Table 1). The group of patients who took the prebiotics showed a reduction in fatigue score from 1.4 ± 1.7 at baseline to 0.8 ± 1.4 after intervention ($p < 0.05$) and, in turn, an increase in muscle strength was also observed (measured with hand strength) from 10.6 ± 8.2 at baseline to 12.4 ± 3.2 ($p < 0.05$) after intervention. This strength increase occurred in fact in the dominant hand in 100% of the participants in the Darmocare Pre® group.

We observed an improvement in some other parameters of the frailty syndrome among the group of patients taking prebiotics when comparing values at baseline and after the intervention. We measured an improvement in walking speed in the intervention group (from 8.4 ± 6.0 s at baseline to 7.9 ± 4.5 s. after intervention) *versus* an almost equal result in the placebo group before and after intervention (from 8.6 ± 9.0 at baseline to 8.7 ± 4.2 after). However, this improvement did not reach statistical significance. No significant differences were observed in the Athens scale for subjective sleep quality after Darmocare Pre® administration. The score in Athens scale was 4.18 ± 4.73 at

baseline and 4.00 ± 4.38 after intervention in the Darmocare Pre® group; 3.4 ± 3.0 at baseline and 4.5 ± 5.3 after intervention in placebo groups (Table 1). No significant differences were observed in the evaluation of functional activity measured with the Barthel Scale: 74.6 ± 17.7 at baseline and 77.1 ± 29.9 after intervention, in the Darmocare Pre® group, and 76.2 ± 13.0 at baseline and 78.3 ± 13.9 after intervention in the placebo group. No significant differences were found for the mini mental state examination (MMSE), 26.5 ± 3.1 at baseline and 26.4 ± 2.2 after intervention in the Darmocare Pre® group; and 26.1 ± 2.2 at baseline and 25.9 ± 2.1 after intervention in the placebo group.

2.4. Effect of Darmocare Pre® on Blood Analytical Parameters

We analyzed several parameters in blood: leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, erythrocytes, haemoglobin, glucose, glutamic oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), high-density lipoproteins (HDL) cholesterol, low-density lipoproteins (LDL) cholesterol, triglycerides, total proteins, creatinine calcium, sodium and potassium. As shown in Table 2, no significant differences were observed between Darmocare Pre® and placebo group (significance at $p < 0.05$). At baseline, no significant differences were observed in these analytical parameters between the two groups (data not shown).

Table 2. Blood analysis and haemogram after treatment with Darmocare Pre® or the placebo. GOT: glutamic oxaloacetic transaminase; GPT: glutamic-pyruvate transaminase; HDL: high-density lipoproteins; LDL: low-density lipoproteins; TNF: tumor necrosis factor α.

Variable	Placebo Group (n = 22)	Darmocare Pre® (n = 28)	p Value
Leukocytes ($\times 10^3/\mu L$)	7.6 ± 0.5	7.7 ± 0.8	0.92
Neutrophils ($\times 10^3/\mu L$)	4.5 ± 0.2	4.6 ± 0.3	0.79
Lymphocytes ($\times 10^3/\mu L$)	2.2 ± 0.2	2.3 ± 0.1	0.64
Monocytes ($\times 10^3/\mu L$)	0.61 ± 0.04	0.55 ± 0.03	0.23
Eosinophils ($\times 10^3/\mu L$)	0.22 ± 0.04	0.22 ± 0.05	0.88
Basophils ($\times 10^3/\mu L$)	0.03 ± 0.01	0.03 ± 0.01	1.00
Platelets ($\times 10^3/\mu L$)	220 ± 28	225 ± 36	0.92
Erythrocytes ($\times 10^6/\mu L$)	5.0 ± 0.6	4.8 ± 0.7	0.83
Haemoglobin (g/dL)	12.8 ± 1.3	12.9 ± 1.1	0.95
Glucose (mg/dL)	95 ± 11	92 ± 10	0.84
Urea (mg/dL)	44 ± 5	40 ± 3	0.48
GOT (U/L)	28 ± 3	27 ± 4	0.85
GPT (U/L)	25 ± 2	24 ± 4	0.84
HDL cholesterol (mg/dL)	46 ± 6	44 ± 7	0.83
LDL cholesterol (mg/dL)	120 ± 9	126 ± 11	0.69
Triglycerides (mg/dL)	131 ± 21	122 ± 16	0.73
Total proteins (g/dL)	7.5 ± 0.4	7.4 ± 0.5	0.88
Creatinine (mg/dL)	0.71 ± 0.10	0.74 ± 0.15	0.88
Calcium (mg/dL)	8.6 ± 1.0	8.9 ± 0.8	0.81
Sodium (mEq/L)	140 ± 3	141 ± 3	0.82
Potassium (mEq/L)	4.7 ± 0.8	4.8 ± 0.5	0.91
C-reactive protein (mg/L)	4.8 ± 1.5	4.9 ± 1.8	0.97
TNF-α (pg/mL)	1.8 ± 0.2	2.0 ± 0.3	0.60

3. Discussion

Improving the function of the immune–gut axis in the elderly has been proposed as an important target for improving health through reduced disease risk and reducing the progression of frailty syndrome that represents the previous steps towards disability, dependence, morbidities and mortality in adults [18]. One of the interventions to improve gut functions are the administration of prebiotics, natural available or synthetic oligosaccharides such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Several studies found that administration of prebiotics provide beneficial effects on health [18,22,23]. Based on this finding, we designed a randomized,

placebo-controlled study in order to assess the efficacy in frailty syndromes in older individuals, as these individuals often display gastrointestinal problems and frailties that both are characterized by immune dysfunctions among other physiopathological alterations [2–4,18,24]. Our results show the following two main results: (1) prebiotic administration as Darmocare Pre®, consisting of a mixture of inulin and fructooligosaccharides administered orally for 13 weeks, has a significant and beneficial effect on two criteria of frailty syndrome in older people age 65 and over, namely, hand grip and exhaustion; and (2) prebiotic administration is a safe therapeutic treatment with few and tolerable side effects.

Frailty is considered a syndrome characterized by poor muscle strength that is considered highly prevalent in old age and represents a high risk factor for falls, disability, hospitalization, and mortality [3,24]. In our study, we measured frailty criteria in each participant [3] using muscle weakness, measured by grip strength, as one of the criteria that identify frailty and sarcopenia [25–27]. The molecular mechanisms leading to poor muscular strength in frail individuals have not been addressed but inflammation and inappropriate nutritional status likely play a role [28]. Our study results showed a beneficial effect on muscular hand strength after Darmocare Pre® administration induced a beneficial effect on muscular hand strength compared to the placebo group, suggesting that prebiotics may influence the muscular system. Interestingly, a recent multicentric observational study carried out in institutionalized older adults (age > 70) in Spain found that oral nutritional supplement with prebiotic fiber (30% inulin, 70% fructooligosaccharides) for 12 weeks also improved handgrip strength [29]. However, in this latter study, the administration of prebiotics was combined with supplementation of vitamin D and calcium, and thus no conclusion could be made about which factor was responsible for this improvement. The improvement in muscular strength afforded by the prebiotic preparation may also explain the reduced feeling of exhaustion since the two features may likely be closely related. Several mechanisms could account for the improved muscular strength and reduced fatigue found in the subjects treated with the prebiotics. For instance, the reduction in cytokine production [23] might account for these effects [30,31]. Schiffrin *et al.* [32] found, in a prospective, randomized, double-blind, controlled study performed in community dwelling elderly, that oral nutritional supplementation with oligosaccharides (fructooligosaccaride 1.3 g/250 mL) attenuates the expression of mRNA for pro-inflammatory cytokines tumor necrosis factor α (TNF-α) and IL-6 mRNA in blood leucocytes, whereas no significant effects were detected in the fecal gut flora or in the nutritional parameters [32]. Prebiotics may have several effects upon immune function, including the growth of beneficial bacteria leading to inhibition of pathogenic growth, thereby reducing the stimulation of other proinflammatory cytokines (IL-6, IL-1) [22] or reducing activation of macrophages [33,34]. In order to shed some light on the mechanisms by which prebiotic administration exerts its beneficial effects in frailty syndrome, we measured the concentration of C-reactive protein whose levels generally rise in response to acute inflammation, and we did not find any significant differences between groups. We also evaluated the concentration of the pro-inflammatory cytokine TNF-α, but we did not find any significant difference in the concentration of TNF-α between prebiotic and placebo groups. Other cytokines may be involved in fatigue in frail individuals, and future studies should investigate any possible biomarker of this effect. We and other groups have previously published that frailty syndrome is associated with a change in leukocyte counts, and, in particular, in neutrophil and lymphocyte counts [2,35,36], suggesting that interventions that modify neutrophil and/or lymphocyte counts may represent new therapeutic strategies in frailty syndrome. Supporting this goal, it has been demonstrated, at least in healthy humans, that the administration of the prebiotic β2-1 fructan increased the percentages of lymphocyte counts in blood (CD3+CD8+ and CD8+ subtypes) compared to the placebo group [19]. Administration of a formulation containing three prebiotics including galacto-oligosaccharides (also present in Darmocare Pre®) for 28 weeks increases the white blood cell counts in blood [20]. For the reasons above, we postulated that prebiotic administration could enhance, among other mechanisms, the number of lymphocytes in the blood of frail older individuals, and, in turn, improve frailty syndrome. However, we were not able to find any significant

changes in leukocyte counts in those individuals treated with Darmocare Pre®, suggesting that the positive effect obtained by prebiotic administration in frailty was not related to a modulation of leukopoyesis but likely to influence levels of inflammatory mediators or metabolism. Gut microbiota may directly influence muscle cells by several molecular pathways that could potentially mediate the gut microbiota–muscle axis such as energy sparing from the diet, and in the regulation of host immunity and metabolism [23,37]. Thus, this needs to be evaluated in future. Scientific evidence from these reports and our results suggest that prebiotic administration can be an attractive tool to improve health in older individuals, probably through their effects on the modulation of the immune–gut system [27]. The second main result of the clinical trial refers to the safety of Darmocare Pre® administration in frail older individuals, which enables its use with little medical surveillance. Prebiotic administration did not significantly alter cognitive function (assessed by the MMSE), functional impairment (Barthel index), and participants did not refer to any sleep change (Athens scale) during the course of the treatment. However, it should keep in mind that even MMSE is extensively used in clinical practice, including in the primary care setting, as a screening test for dementia [38,39]. Thus, it could not be a sensitive tool to detect small changes in cognition after prebiotic administration. We cannot rule out that other tests more sensitive to detect cognitive alterations in non-demented people would have produced some effects considering the proposed role of immune–gut system of brain functions. During the course of intervention with Darmocare Pre®, only modest side effects such as flatulence and cramping occurred in about 30% of individuals, and these symptoms disappeared during treatment, thus suggesting that administration of prebiotics could be well- tolerated and likely usable in those cases of persistence with administration of drugs for the relief of abdominal discomfort and flatulence. In around 4% of cases, the administration of Darmocare Pre® induced diarrhea, although a direct causality was not demonstrated. The total drop out number of the intervention group was eight out of 36 and was due to digestive tract symptoms such as cramps/diarrhea, whereas nearly 50% of the prebiotic group experienced mild flatulence. The majority of symptoms disappeared after a very short period of eight days, but eight participants still dropped out before symptoms vanished. No major adverse effects of the use of prebiotics are known next to the symptoms our participants suffered [40], and these effects only seem serious with an intake of more than 30 gr/day [41]. The symptoms of bloating and gaseousness are probably adaptive to chronic consumption [42], which was also made evident in our study where adverse symptoms vanished after an average of eight days. Our study has several limitations. We did not analyze several immune parameters such as IL-6 and IL-10 with which we could have researched the pro- or anti-inflammatory effects of prebiotics in the elderly, and the same holds for the absence of other immunological measurements such as subpopulations of T and B lymphocytes. The small number of participants also limited the outcomes and the development of hard conclusions. Nevertheless, our results are still promising and bigger studies using clinical hard endpoints and more biochemical and immunological parameters are warranted. Another test that we could not include was total micro biome in stool; this limitation was due to financial burden. However, the improvement of several frailty parameters through the use of prebiotics is important, and when confirmed by bigger studies, could be used not only as a cure but perhaps even as a preventive measurement.

4. Experimental Section

4.1. Study Population

Participants were recruited from individuals living in nursing homes located in Valencia province (Spain). The inclusion criteria were the ability to get up from a chair and walk 6 m, and an age of 65 years or older. The exclusion criteria were dementia, major psychiatric disease (schizophrenia, bipolar disorders, *etc.*) or blindness, acute infections, or known cancers. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures

olving human participants were approved by the Ethics Committee of the University of Valencia lencia, Spain) (H1424156718665). Written informed consent was obtained from all participants.

Intervention

The prebiotic Darmocare Pre® and the placebo (maltodextrin) were physically indistinguishable. products were always stored at room temperature. Nurses or the participants themselves dissolved spoon of Darmocare Pre® or placebo in a glass of water, and it was administered in the morning rtly after breakfast (9–10 a.m.). The intervention and placebo products were comparable in visual pearance and taste. The participants were instructed to communicate any secondary effects of trointestinal alteration including changes in stool consistency or defecation frequency and to intain habitual dietary habits. The participants were interviewed about all types of potential erse effects including the most common side effects *i.e.*, gas and cramping; in addition, they were ed specifically for changes in stool characteristics (consistency and frequency). All participants in clinical trial were recruited from individuals living in nursing homes (as stated in the Experimental tion), in which diet was constantly supervised by a team of nutritionists/dietitians. In addition, in er to compare with the results on diet intervention, we estimated the energy intake in the study nple by assessment with a three-day food diary (one weekend and two weekdays) before the inning of the study and twice during the intervention period. A nutritional program (using a macro cedure of a spreadsheet) including a food database for Spanish population was used.

Measurement of Frailty Criteria

Frailty level was measured according to the five Fried criteria [3] as previously reported by our up [2]. Briefly, the criteria were assessed as follows: (1) unintentional body weight loss (5 percent 4.5 kg or more in the last year); (2) self-reported chronic fatigue: participants met the criteria if they wered "A few times", "Often", or "Most of the time" to the question "How often in the last week you feel that everything you did was an effort?", included in the Center for Epidemiologic Studies pression scale [43]; (3) low physical activity level was measured using the Spanish adaptation of the nnesota leisure time physical activity questionnaire [44–46]; (4) according to the standards of the rt physical performance battery [47], participants who walked 4.6 m in a longer time than the worst ntile of the sex and height-adjusted sample fulfilled the reduced walking speed criterion; our values re: men taller than 173 cm: ⩾6 s, height < 173 cm: ⩾7 s; women taller than 159 cm: ⩾6 s, height < cm: ⩾7 s; and (5) to measure muscle weakness, grip strength (Kg) was measured three times in h hand alternately with a hydraulic dynamometer (Jaymar, J.A. Preston, Corp., Jackson, MS, USA) ording to the standards of the Hispanic established populations for epidemiologic studies of the erly [48]. Participants were considered frail if they met at least three criteria and prefrail if they met or two. All measurements were performed by trained members of the Department of Nursing at University of Valencia, using a questionnaire with detailed instructions.

Geriatric Assessment

Functional status was evaluated using the Barthel index that defines the ability to perform the basic vities of daily living [49] and measures the level of independence related to the following 10 items: ding, bathing, dressing, grooming, defecating, urinating, toilet use, transfers (e.g., from armchair to), walking, and climbing stairs. The index has a score range 0–100, where 0 is total dependence and corresponds to total independence. The MMSE test was used to detect cognitive impairment. It luates different items grouped into five sections: orientation, immediate memory, attention and culation, delayed recall, and language and construction with a score range of 0–30, the highest res indicating better performance [38]. Another important issue evaluated was sleep quality using Athens Insomnia Scale (AIS) [50]. It consists of eight items related to sleep induction, awakenings ing the night, final awakening, total sleep duration, sleep quality at night, well-being, functioning acity, and sleepiness during the day. Each item of the AIS can be rated 0–3, (with 0 corresponding

to "no problem at all" and 3 "very serious problem"). The scale has a score range 0–24, where 0 denotes absence of any sleep-related problem and 24 means the most severe degree of insomnia.

4.5. Blood Analytical Parameters

Blood samples were obtained from each subject between 7:30 a.m and 10 a.m under at least 8 h in fasting conditions. Blood was collected by collecting 10 mL blood each into two Becton Dickinson (BD) Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid sodium salt (EDTA). After extraction, the blood samples were allowed to stand for 15 min and were centrifuged at 1500 rpm for 10 min at room temperature. Subsequently the plasma supernatants were aliquoted and stored at −20 °C until analysis. After thawing, the samples were centrifuged at 1500 rpm for 10 min at room temperature to completely remove all cells. For all other analytical determinations, residential center control blood extractions were used. Hematological parameters (white blood cells, hemoglobin, erythrocytes, and platelets) and biochemical parameters (glucose, urea, urate, cholesterol, triglycerides, creatinine, glutamic oxaloacetic transaminase (GOT), and serum glutamic pyruvic transaminase (GPT), and C-reactive protein) were measured in clinical laboratories belonging to local public health centers. Hematologic analysis included total red blood cell count (RBC) and white blood cell count (WBC) counts obtained by hemocytometer methodology (Autocrit Ultra3 Centrifuge, Becton Dickinson). Serum analytic values were determined on a laboratory chemistry analyzer (Dimension Xpand Plus Integrated Chemistry System, Siemens, Erlangen, Germany). Serum concentration of TNF-α was measured using a commercial enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (TNF-α (ab100654), Human ELISA Kit, Abcam®, Cambridge, UK). To minimize assay variance, all the measurements were conducted in duplicates and on the same day.

4.6. Statistical Analysis

Descriptive statistics, including measurements of central tendency (mean), median, and range values, were used to describe all the quantitative variables. The normal distribution of each variable was estimated with the Kolmogorov–Smirnov test. The non-parametric Mann–Whitney U test was performed to verify any possible differences between the two experimental groups. Statistical significance was set at $p < 0.05$ and statistical analysis was performed with the software SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

5. Conclusions

The use of prebiotics such as Darmocare Pre® in older individuals influences certain frailty symptoms and improves the levels of fatigue and muscle strength. Prebiotics could therefore be included in the total treatment protocol of people suffering especially from frailty, and as a preventive intervention in general. Nevertheless, it is necessary to identify those individuals who would benefit the most from the use of prebiotics through selected randomized trials.

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Author Contributions: Mary Martínez-Martínez, Yolanda Verdejo, Mari Carmen Mascarós and Carlos Peris administered the prebiotic or placebo and evaluated cognitive function and functional impairment. Omar Cauli analyzed the data; Cristina Buigues, Julio Fernández-Garrido, Rut Navarro-Martínez and Omar Cauli evaluated frailty, sleep quality, and drafted the manuscript; Omar Cauli, Leo Pruimboom and Aldert J. Hoogland revised the manuscript; Omar Cauli designed and conceived the study and was responsible for obtaining project grant funding.

Conflicts of Interest: Aldert J. Hoogland is a product developer in the company (Bonusan) that provided the Darmocare Pre® and the placebo for the study, and he performed the blinded randomization. Leo Pruimboom is a regular teacher in courses and seminars organized by Bonusan. Aldert J. Hoogland and Leo Pruimboom never saw any of the participants or saw any of the results in this study. Cristina Buigues and Julio Fernández-Garrido wrote the manuscript. Omar Cauli and Leo Pruimboom reviewed and corrected the manuscript. The remaining

authors (including Omar Cauli) declare that they have no competing interests, and they were blind to the drug treatment until the completion of the study.

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