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SERUM 25-HYDROXYVITAMIN D, FRAILTY SYNDROME AND RISK OF DEMENTIA IN ELDERLY MEN AND WOMEN: Pro.V.A. STUDY

Direttore della Scuola: Ch.mo Prof. Gaetano Thiene
Coordinatore d'indirizzo: Ch.mo Prof. Fabrizio Fabris
Supervisore: Ch.mo Prof. Enzo Manzato

Dottorando: Dott.ssa Silvia Sarti

ABSTRACT

Background: Frailty is one of the most problematic expression of population ageing. It is a state of vulnerability and is associated with a greater risk for adverse outcomes such as falls, disability and death. Identifying early markers for the onset of this clinical syndrome is more than just a scientific challenge. One of the most important component of frailty is the sarcopenia, a progressive loss of skeletal muscle mass, strength, and power. Recent insight suggests that vitamin D may be important in preserving from muscle mass and, strength which are key components of frailty. Cognition and dementia have already been considered as components of frailty, but the role of frailty as a possible determinant of dementia has been poorly investigated. We examined the relationship between serum 25-hydroxyvitamin D (25OHD) levels and the presence of frailty syndrome, and the association between vitamin D deficiency and the onset of frailty in a sample of older male and female adults. We also estimated the predictive role of frailty syndrome on incident dementia in the same population.

Methods: This research is part of the Progetto Veneto Anziani (Pro.V.A), an Italian prospective population-based cohort study conduct in Italy between 1995 and 2001 with follow up assessment at 4.4 years. A total of 1577 subjects (978 women and 599 men) aged $\geq$ 65 years completed interviews, medical examinations and functional assessments, and provided blood samples. For the purpose of the second part of this study, 1181 partecipans (712 women and 469 men) were studied, excluding subjects with alterations of MMSE. Serum 25OHD

levels were measured at the baseline and categorized into clinical groups: 25OHD deficient (<50 nmol/L), insufficient (≥50 to <75 nmol/L) and sufficient (≥75 nmol/L). Frailty syndrome was defined using a modified measurement of Cardiovascular Health study criteria. Analyses were adjusted for relevant confounders including health status and physical performance.

Results: Frailty was present at baseline in 457 (29%) subjects. The prevalence of frail syndrome was significantly higher among 25OHD-deficient participants compared to 25OHD-sufficient ones (43.4% vs 20.7%, p<.0001). Compered to normal Vitamin D status, Vitamin D deficiency and vitamin D insufficient were independently associated to the likelihood of being frail, even adjusting for multiple potential confounders (OR: 1.657, 95% CI: 1.232-2.228; p<0.001 for the deficient group and OR: 1.392, 95% CI: 1.024-1.891; p<0.05). In a subsample of 1129 non-frail participants, the 27% became frail at 4.4 years; nevertheless after adjusting for health and functional confounders, Vitamin D deficiency was no longer associated to the risk of the onset of frailty. Participants who developed dementia in follow-up were about 19% (228) of the sample. The subjects with frailty status were more likely to be dementia (OR: 1.87, 95% CI 1.02-3.30). Frailty syndrome was also associated with a greater risk of developing dementia (RR: 1.94, 95% CI: 1.42-2.64).

Conclusion: Vitamin D deficiency is associated to frailty syndrome in elderly men and women, regardless of several confounding factors. By the way, vitamin D deficiency does not predict the onset of frailty, as well as other health condition. Frailty is a risk factor of dementia.

RIASSUNTO

Introduzione: la fragilità è una delle maggiori problematiche della popolazione anziana. E' uno stato di vulnerabilità ed è associata ad un aumento di diversi outcomes quali le cadute, la disabilità e la morte. Identificare precocemente marcatori dell'espressione clinica della sindrome è più di una sfida scientifica. Una delle più importanti componenti della fragilità è la sarcopenia, una progressiva perdita di massa, forza e potenza muscolare. Studi recenti suggeriscono che la vitamina D potrebbe avere un ruolo nel preservare massa e forza muscolari, componenti chiave della fragilità. Anche lo stato cognitivo e la demenza sono considerati componenti della fragilità, ma il ruolo della fragilità come possibile determinante della demenza è stato poco investigato. In questo studio è stata esaminata l'associazione tra la deficienza di vitamina D e la fragilità in un campione di uomini e donne anziani. Inoltre è stato esaminato il ruolo predittivo della fragilità sulla demenza nella stessa popolazione.

Metodi: Questa ricerca fa parte del Progetto Veneto Anziani (Pro.V.A.), studio prospettivo di coorte della popolazione italiana, condotto in Italia tra il 1995 e il 2001 con un follow-up a 4.4 anni. Un totale di 1577 soggetti (978 donne e 599 uomini) di età $\geq$ 65 anni ha completato le interviste, gli esami medici, le valutazioni funzionali e ha fornito un campione di sangue. Nella seconda parte dello studio, sono stati valutati 1181 partecipanti (712 donne e 469 uomini), escludendo i soggetti con MMSE < 24. I livelli di vitamina D sono stati misurati al basale e categorizzati in sottogruppi: livelli deficienti (<50 nmol/L),

insufficienti (≥50 e <70 nmol/L) e sufficienti (≥75 nmol/L). La fragilità è stata definita usando i criteri modificati del Cardiovascular Health study. Le analisi sono state aggiustate per fattori confondenti, tra cui lo stato di salute e la performance fisica.

Risultati: La fragilità al basale era presente in 457 (29%) soggetti. La prevalenza della fragilità era significativamente più alta tra i soggetti con deficienza di vitamina D rispetto ai soggetti con sufficienza (43.4% vs 20.7%, p<.0001). Se paragonate a livelli sufficienti di vitamina D, la deficienza e l'insufficienza di vitamina D erano indipendentemente associate ad una maggiore probabilità di essere fragili, anche dopo correzione per multipli potenziali fattori confondenti (OR: 1.657, 95% CI: 1.232-2.228; p<0.001 per la deficienza, e OR: 1.392, 95% CI: 1.024-1.891; p<0.05 per l'insufficienza di vitamina D). Dei 1129 partecipanti non fragili, il 27% diventa fragile a 4.4 anni; tuttavia dopo correzione per fattori confondenti, la deficienza di vitamina D non risulta più associata al rischio di sviluppare fragilità. I soggetti che sviluppano demenza nel follow-up era circa il 19% (n=288) del campione. I soggetti fragili avevano maggiori probabilità di essere dementi (OR:1.87, 95% CI 1.02-3.30). Inoltre la fragilità risultava associata a un maggior rischio di sviluppare demenza (RR: 1.94, 95% CI 1.41-2.2.64), anche dopo correzione per fattori confondenti.

Conclusioni: la deficienza di vitamina D risulta associata alla fragilità in uomini e donne anziani, indipendentemente da fattori confondenti. Tuttavia, la deficienza di vitamina D non predice il rischio di sviluppare fragilità, quando corretta per lo stato di salute. La fragilità risulta un fattore di rischio nello sviluppo di demenza.

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INTRODUCTION

Frailty has been described by Fried as a biological syndrome of decrease reserved and resistance to stressors, resulting from cumulative declines across multiple physiologic systems and causing vulnerability to adverse outcomes (Fried LP et al., 2001). It is characterized by weight loss, exhaustion, physical inactivity, reduced muscle strength, and slowed motor function (Fried LP et al., 2001). The presence of one or two of these criteria denotes pre-frailty.

It independently predicts risks of adverse health outcomes including incident falls, fractures, disability and mortality. (Fried LP et al., 2001; Ensrud KE et al., 2007; Cawthon PM et al., 2007; Rockwood K et al. 2004).

Frailty is common in older adults; in a recent meta-analysis, the prevalence of frailty in community-dwelling adults aged ≥ 65 years was found to be 10.7% (Collard et al. 2012).

Identifying early markers for the onset of this clinical syndrome is more than just a scientific challenge. Preventing frailty may help slow progression of disablement process in older persons.

One of the most important component of frailty is the sarcopenia, a progressive loss of skeletal muscle mass, strength, and power, measured by gait speed and grip strength in presence of a low muscle mass (Morley JE et al., 2014; Cruz-Jentoft AJ et al., 2010). The phenotype of frailty including weakness and slowness (Fried LP et al., 2001).

Recent evidence supports a role of vitamin D (25(OH)D) in physical performance and strength through direct effects on muscular function (Dawson-Hughes B ,

2008; Ceglia L, 2008), as well as independently through its reported roles in cardiovascular diseases, diabetes mellitus, hypertension, pulmonary function, osteoarthritis (Holick MF , 2007) and cognitive disease (Toffanello ED et al., 2014), condition that frequently lead to declines in physical performance and strength (Fried LP , 1997). Poor physical performance and muscle weakness have been associated with low 25OHD levels among older in cross-sectional studies (Bischoff HA et al. 1999; Zamboni M et al., 2002; Bischoff-Ferrari HA et al., 2004; Houston DK et al., 2007; Annweiler C et al., 2010; Toffanello ED et al., 2002). Low 25-hydroxyvitamin D (25(OH)D) levels are common in older adults (Lip P, 2001; Visser M et al., 2003) and have been linked to falls (Bischoff-Ferrari HA et al, 2006; Snijder MB et al., 2006), fractures (Bischoff-Ferrari et al., 2006), pain (Lips P, 2001; Visser M, et al, 2003; Bischoff-Ferrari et al., 2006), sarcopenia (Visser M et al., 2003), poor physical function (Gloth FM et al., 1995; Verhaar et al., 2000). The importance of vitamin D in maintaining calcium homeostasis is well known. Cholecalciferol is synthesized in the skin in response to ultraviolet light or is ingested through food (eg, fatty fish, eggs, fortified dairy products) and hydroxylatated into 25(OH)D in the liver. Older persons often have low 25(OH)D levels owing to age-related decrease efficiency of hydroxylation and reduced sunlight exposure (Lips P, 2001; Lips P, 2006). When 25(OH)D levels are low, active metabolite 1,25-dihydroxyvitamin D (1,25-(OH)2D) and calcium absorption decrease. The reduced serum calcium causes parathyroid hormone (PTH) levels to rise to stimulate 1,25(OH)2D production, resulting in increased bone turnover and hip fracture risk (Lips P, 2001; Lips P, 2006). Emerging research suggests that vitamin D affects muscle strength and function (Visser M et

al., 2003; Bischoff-Ferrari HA et al., 2004), both directly and indirectly through PTH regulation and inflammation (Lips P, 2001; Visser M et al., 2003; Houston DK, et al. 2007; Thomas MK et al., 1998; Puts MT et al., 2005(b)).

There is evidence from several studies for a association between 25(OH)D levels and frailty (Puts MT et al., 2005(a); Shardall M et al., 2009; Ensrud KE et al., 2010; Ensrud KE et al., 2011; Wilhelm-Leen ER et al., 2010; Hirani V et al., 2013; Tajar A et al., 2013), nevertheless results from longitudinal studies on the association between 25(OH)D levels and incidents frailty are limited. Although low 25(OH)D levels were associated with incident frailty in some studies (Puts MT et al., 2005; Wong YY et al., 2013), no significant relationship was found in others (Ensrud KE et al., 2011; Shardell M et al., 2012; Schottker B et al., 2014).

Recent insight suggests that vitamin D may be also important in preserving cognitive function via different mechanisms. (Garcion E et al., 2002; Eyles DW et al., 2005; Annweiler C et al., 2011; Masoumi A et al., 2009; Annweiler C et al., 2010 (2)). Toffanello et al. (Toffanello ED et al., 2014) in the same population of this study showed that to be an independent association between low 25OHD levels and cognitive decline in elderly individuals. If low levels of vitamin D are associated with frailty and cognitive decline, probably also the frailty will be associated with cognitive decline.

In the ILSA, both lower cognition and greater depressive symptoms were cross-sectionally associated with physical frailty (Solfrizzi V et al., 2012). Moreover, frail demented patients were at higher risk of all-cause mortality, but not of disability, over 3- and 7-years follow-up periods. In a recent population-based study, frailty status in older Mexican Americans cognitively unimpaired at

baseline was an indipendet predictor of cognitive decline over a 10-years period (Samper-Ternent R et al., 2008) furthermore, several studies have reported that physical frailty is significantly associated with low cognitive performances, including incidente of Alzheimer's desease (AD) (Buchman AS et al., 2007), mild cognitive impairment (MCI) (Boyle PA et al., 2010) and AD pathology in older persons with and without dementia (Buchman AS et al., 2008).

Cognition and dementia have already been considered as components of frailty, but the role of frailty as a possible determinant of dementia has been poorly investigated. Certain studies consider cognition to be a component of frailty. Most recent studies examined the influence of physical frailty on cognitive function impairment, whereas several others assume cognition to be a phychological risk factor for frailty (Panza F et al., 2011). Recently, an International Consensus Group has defined a "cognitive frailty" condition. (Kelaiditi E et al., 2013). As mentioned, the associations between frailty and cognitive function have been examined in literature, but not confirmed.

Cross-sectional studies have found an association between frailty and poor cognitive function (Jacobs JM et al. 2011; Yassuda MS et al., 2012; Avila-Funes JA et al., 2011). Some studies even considered cognition to be a component of frailty (Sarkisian CA et al., 2008; Avila-Funes JA et al., 2008) which represents a clear risk factor for adverse health outcomes (e.g. physical disability or mortality) (Sakisian CA et al., 2008; Solfrizzi V et al., 2013). Longitudinal studies have found that physical frailty predicts future cognitive function decline and even the incidence of Alzheimer's disease (Solfrizzi v, et al. 2013; Buchman AS et al., 2007; Boyle PA et al., 2010; Mitnitski A et al., 2011). The rate of change in frailty

is also associated with cognitive decline (Buchman AS, et al, 2007). Frail older adults are less likely to show cognitive improvement or stabilization over time (Mitnitski A et al, 2011), as well as non-frail older people with poor cognition are more likely to became frail than those with good cognition (Raji MA et al., 2010). Frailty and cognitive function share related mechanisms. Risk factors commonly associated with frailty included gender (women), poor education, low socioeconomic status, marital status (never married, widow), disabling conditions, diabetes mellitus (especially if complicated), high comorbidity, depressive symptoms, fear of falling, social isolation, malnutrition, geriatric syndromes, retirement, and specific biomarkers (Woo J et al., 2005; Bischoff HA et al., 2006; Mhaolain AM et al., 2012). Factors related to poor cognitive function include age, gender (women), poor education, disabling conditions (especially in instrumental activities of daily living), diabetes, stroke, unhealthy behaviors, poor social support, and social isolation (Yen CH et al., 2010; Zunzunegui MV et al., 2003; Purser JL et al., 2005; Sabia S et al., 2009).

Based on available literature, a possible relationship between cognitive function and frailty can be describe in multiple ways. First, cognitive impairment is a component of frailty. Second, cognitive impairment and frailty are characterized by synergistic actions in the development of negative health outcomes (e.g. mortality or disability). Third, cognitive impairment determines frailty. Fourth, frailty determines cognitive impairment.

AIM OF THE STUDY

The objectives of this study were to examine, at baseline, the relationship between serum 25-hydroxyvitamin D levels and the presence of frailty syndrome, and in the follow-up the association between vitamin D deficiency and the onset of frailty.

In the subjects without alterations of MMSE, the objective was examine the possible role of frailty syndrome on incident dementia in a sample of older adults.

METHODS

Study population

Data for this analysis were drawn from the Progetto Veneto Anziani (Pro.V.A.), an observational cohort study on an Italian population of older men and women (aged ≥ 65 years) living in two areas in the north-east of Italy.

The baseline assessment started in 1995, finished in 1997, and was followed by 2 in-person follow-up visits (at 5 and 7 years), with ongoing morbidity and mortality surveillance. All participants provided informed written consent to study participation and to morbidity surveillance. Participants were interviewed at their homes and were subsequently examined by physicians and nurses at the 2 study clinics using an extensive battery of clinical, instrumental, biochemical, and physical performance tests. The physician who performed the physical examination determined the disease status, integrating information from the interview, examination, use of medications, and medical records review.

Briefly, the baseline Pro.V.A study population consisted of 3099 age- and sex-stratified Caucasian community-dwelling participants (1854 women and 1245 men) randomly selected between 1995-97 using a multistage stratification method designed to keep the male-to-female ratio at 2:3 and to oversample the oldest age group. No exclusion criteria were used. Follow-up assessments were conducted after 4 years (mean [SD] follow-up, 4.4 [1.1] years). For the purposes of the present study, individuals without details of baseline serum 25(OH)D levels (n=272) or without one of the criteria to define frailty at baseline (n=826), were excluded, as were as subjects with severe renal failure (n=11) (glomerular filtration rate [GFR] <30), with primitive hyperparathyroidism (n=17), and

participants with leg and/or arm amputations (n=23), unable to walk and/or in wheelchairs (n=89). Of the remaining 1861 participants who have all baseline data, 181 subjects died during the follow-up, whereas 103 were alive but did not come back for the second visit. The final sample consisted of 1577 participants: 599 men (mean [SD] age: 72.8 [7.0]) and 978 women (mean [SD] age: 74.6[6.7]). Compared with the sample as a whole, the excluded subjects (n=284) had the same distribution in male and female (63.0% vs 62.0% female and 37.0% vs 38.0% male; Chi-Square test, p=0.7462). They were older (mean [SD] age: 79.4 [7.3] vs 73.4 [6.4] years), and had lower serum vitamin D levels (mean [SD], 67.0[20.2] vs 88.6 [53.8] nmol/L) (one-way ANOVA, $p<.0001$ for all comparisons). They were more likely than our study partecipants to have a diagnosis of depression (57.2% vs 29.2%; chi-square test, p<0.0001), dementia (61.6% vs 26.9%, chi-square test, p<0.0001), cardiovascular disease (36.6% vs 15.2%, chi-square test, p<0.0001), neurologic disease (12.0% vs 1.8%, chi-square test, p<0.0001), diabetes (14.4% vs 7.9%, chi-square test, p=0.0003), and COPD (14.1% vs 7.3%, chi-square test, p=0.0001). Compared to participants, the excluded subjects were also more likely to be dependent in ADL (33.8% vs 10.0%, chi-square, p<0.0001), and to be frailty (76.8% vs 29.0%, chi-square test, p<0.0001).

For the purpose of the second part of this study, 1181 (469 men and 712 women) participants were studied, excluding subjects with MMSE $\geq$ 24 in baseline. Compared with the sample as a whole, the excluded subjects (n=396) had a same distribution in male and female (60.3% vs 59.1% female, and 39.7% vs 38.0%

male; Chi-Square test, p=0.7431) and they were older (mean[SD], 78.3[8.1] vs 72.0 [5.6] years).

Ethics approval

The local Ethical Committees of Padua University and the Veneto Region's Local Health Units (ULSS) n.15 and n.18 approved the study protocol, and participants gave their written informed consent.

Clinical and laboratory data

Participants were examined at city hospitals by trained physicians and nurses both at the baseline and at the follow-up. Information on formal education, physical activity and smoking habits was collected during an in-person interview. Smoking habits were classified as "never/former" (for at least a year in the past) versus "current" smokers. Physical activities were grouped into two categories, i.e. outdoor activities (brisk walking, cycling, gardening, and fishing), and indoor activities (dancing, exercising at the gym). Participants were asked to report how many hours a week they had spent on each of the above-mentioned pastimes in the previous month. An activity was considered regular if it had been practiced for more than 1 hour a week during the previous month. Any diseases existing at the baseline were ascertained by board-certified physicians who examined all the clinical details, including medical history, self-reported symptoms, medical and hospital records, blood tests, and physical examination. Prior major diseases included any of the following: cardiovascular diseases (CVD: hypertension, congestive heart failure, angina and myocardial infarction, stroke, and peripheral

artery disease), diabetes, chronic obstructive pulmonary diseases (COPD), cancer, osteoarthritic diseases (including hand/knee/hip osteoarthritis, and hip fracture). Presence of depressive symptoms was assessed using the Geriatric Depression Scale, GDS (scores ≥11 were indicative of mood disorders) (Parmelee PA, Lawton MP, Katz IR. Psychological Assessment: A Journal of Consulting and Clinical Psychology 1989; Vol 1(4): 331-338). Disability was defined as the inability or need for assistance to perform one or more activities of daily living (ADL): bathing, dressing, eating, using the toilet, or transferring.

Venous blood samples were obtained after an overnight fast, centrifuged and stored at -80°C. Routine biochemical tests were performed at city hospitals, and PTH and 25OHD tests at the Padua University laboratory. Serum 25OHD levels were measured by radioimmunoassay (RIA kit; DiaSorin). The intra- and inter-assay coefficients of variation for 25OHD were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin), with intra- and inter-assay coefficients of variation for PTH of 3.0% and 5.5%, respectively. Serum creatinine was measured using a standard creatinine Jaffé method (Roche Diagnostics, Germany) and GFR was calculated with the MDRD formula (Levey AS et al. 2009).

Frailty status

Frailty status was defined using the following criteria, similar to those proposed by Fried and colleagues using data collected in the Cardiovascular Health Study (Fried LP, et al., 2001):

- Weight loss: identified by weight loss > 5 kg in the past year;

- Exhaustion: identified by an answer of "no" to the question "Do you feel full of energy?" and GDS score ≥ 10;

- Weakness: identified by inability to stand from a chair unaided, or without using the arms;

- Slowness: defined as a mean gait speed < 0.8 m/s during 4 meters walks;

- Low physical activity: identified by asking "Do you practice regular physical activity?", defining physical activity as ≥ 4 hours/week in the previous month of the least moderate physical activity.

Subjects with 3 or more components were considered to be frail.

Cognitive function assessment

Cognitive function was assessed at baseline and follow-up by administering the 30-item Mini-Mental State Examination (MMSE) (Folstein MF et al., 1983; **Figure 1**), a validated neuropsychological tool for measuring global cognitive function with orientation, concentration, language, praxis and memory components designed to screen for cognitive impairment. When used repeatedly, the MMSE is also able to measure changes in cognitive status (Chea E et al., 2011). MMSE scores range from 0 to 30; crude MMSE scores obtained in our sample were adjusted for age and formal education using coefficients proposed for the Italian population (Magni E et al., 1996). Adjusted scores lower than 24 indicated a cognitive impairment (Malloy PF et al., 1997). A decline of 3 or more points at follow-up was define as a substantial cognitive decline (Bracco L et al, 1998; Zhao Q et al., 2014).

Figure 1: Mini-mental state examination questionnaire

Mini-Mental State Examination (MMSE)

Maximum Score	Score	
		ORIENTATION
5	( )	What is the (year), (season), (date), (day), (month)
5	( )	Where are we (state), (county), (town or city), (hospital), (floor)
		REGISTRATION
3	( )	Name 3 common objects, (e.g. 'apple', 'table', 'penny'). Take 1 second to say each. Then ask the patient to repeat all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. Count trials and record.
		Trials:
		ATTENTION AND CALCULATION
5	( )	Spell 'world' backwards. The score is the number of letters in the correct order (D__ L__ R__ O__ W__)
		RECALL
3	( )	Ask for the 3 objects repeated above. Give 1 point for each correct answer. [Note: recall cannot be tested if all 3 objects were not remembered during registration.]
		LANGUAGE
2	( )	Name a 'pencil' and 'watch' (2 points)
1	( )	Repeat the following "No, ifs, ands, or buts" (1 point)
3	( )	Follow a 3-stage command: 'Take a paper in your right hand, Fold it in half, and Put it on the floor' (3 points)
		Read and obey the following:
1	( )	Close your eyes (1 point)
1	( )	Write a sentence (1 point)
1	( )	Copy the following design (1 point)

Score Ranges

24 – 30	Normal
18 – 23	Mild dementia
10 – 17	Moderate dementia
<10	Severe Dementia
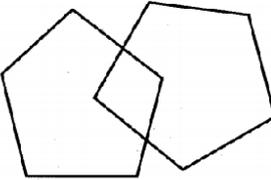
Total Score _____

Statistical analysis

Participants' baseline characteristics were summarized using means (± SD) for continuous variables and counts and percentage for categorical variables. Means and proportions were calculated in the sample as a whole, and by clinical group for serum 25OHD leveles, i.e. 25OHD deficient (<50 nmol/L); 25OHD insufficient (≥50 to <75 nmol/L); and 25OHD sufficient (≥75 nmol/L). (Holick MF, 2007).

Differences in baseline characteristics by 25OHD levels were compared using chi-square tests for categorical variables, analysis of variance for continuous variables with normal distributions, and the Kruskal-Wallis test for variables with skewed distributions.

Multivariable logistic regression models were used to examine the association between baseline 25OHD levels and the odds of frailty in all participants.

In secondary analyses, excluding participants with a baseline diagnosis of frailty, logistic regression models were applied to identify any association between 25OHD levels and the onset of a frail condition at the follow-up.

For all analyses, 25OHD status was coded as an indicator variable, considering the subjects with the highest serum vitamin levels (>75 nmol/L) as the reference group.

In a basic adjusted model we checked for age in years, sex, body mass index (BMI; calculated as the weight in kg/height in meters squared), season of blood draw (autumn, winter, spring and summer) and smoking habits. In a second model, variables identified as potential confounders in studies on frailty or

25OHD levels were also included, i.e. depression, cardiovascular diseases, osteoarthrosis, neurological diseases, diabetes and COPD. PTH concentrations were also initially considered for inclusion in the analysis because they might act as intermediate factors of altered 25OHD levels, but they were subsequently removed from the models due to a high collinearity, as quantified by the variance inflation factor (VIF).

In secondary analyses, excluding both partecipants with a baseline diagnosis of dementia and MMSE scores < 24, logistic regression models were applied to identify any association between frailty status and the onset of cognitive impairment (follow-up MMSE score < 24). In a basic adjusted model, we checked for age in years and sex. In a second model, variables identified as potential confounders were also included, i.e. smoking habit, MMSE, depression, cardiovascular diseases, diabetes, chronic obstructive pulmonary diseases, and osteoarthritis.

All analyses were performed using the SAS rel. 9.13 (cary NC: SAS Institute). All statistical tests were two-tailed and statistical significance was assumed for p-value <0.05.

RESULTS

The sample consisted of 1577 community-dwelling elderly individuals with a mean [SD] age of 73.4 [6.4] years. The mean [SD] baseline 25OHD serum level was 88.6 [56.0] nmol/L. Vitamin D deficiency (25OHD < 50 nmol/L) was identified in about 25% of the sample. At baseline about 29% of the participants was frail according to the revised criteria of the CHS, and about 43% of these frail subjects had a vitamin D deficiency.

Table 1 shows the baseline characteristics of the sample, as a whole and by baseline vitamin D status. Participants who were 25OHD deficient were older than those with higher serum 25OHD levels, and they were more likely to be female. Subjects with lower 25OHD levels were more likely to be frail. Even controlling for age and sex, differences in baseline characteristics across 25OHD levels remain, for the exception of MMSE scores, dementia, and osteoarthrosys.

In logistic regression models (**Table 2**), both vitamin D deficiency and insufficiency were independently associated to the likelihood of being frail. Even adjusting for multiple potential confounders, vitamin D deficient and insufficient partipants were at higher risk of being frail individuals compared to the sufficient subjects (OR 1.898, 95% CI 1.449-2.485 for the deficient group and OR 1.392, 95% CI 1.024-1.891 for the insufficient, compared to the reference group).

A total of 1129 subjects out of the 1577 participants were classified as non-frail at the baseline evaluation, and they represented the sub-sample for secondary longitudinal analyses. Of these non-frail individuals a total of 304 (27%) match the frail criteria at the 4.4-year follow-up examinations, and low baseline vitamin

D levels (serum 25OHD <75 nmol/L) were observed in less than a health of them (47%). On logistic regression modeling of the relative risk of the onset of frail condition at follow-up, in individuals who did not match the frail criteria at the baseline evaluation (n= 1129) both vitamin D deficiency and insufficiency were not associated with a higher 4.4-year risk of becoming frail (**Table 3**).

Table 4 shows the baseline characteristics of cognitively intact sample, as whole and by dementia at follow-up and not dementia at follow-up. Over a 4-4-years follow-up, 228 (about 19%) of 1181 older subject developed dementia. There were older than those did not developmend dementia and they were more likely to be female. At baseline, those subjects who developed dementia during the study period (n = 228), after age and sex adjusted, had lower level of education, lower MMSE score, more serious comorbidity, as well as frailty status, COPD, to be more depressed. The subjects with frailty status were more likely to be cognitively impaired, compared with the subjects without frailty (OR: 1.87; 95% CI: 1.02-3.30). The incidence of dementia was higher in individuals with frailty syndrome than no-frail subjects (35.92% vs 18.02%).

On logist regression modelling of the relative risk of the onset of dementia condition at follow-up, in individuals cognitively intact at the baseline evaluation (n = 1181), frailty syndrome was associated with a greater risk of developing dementia (RR: 1.94; 95% CI: 1.42-2.64) (**Table 5**).

Table 1: Baseline characteristics of the sample as a whole and by serum vitamin D levels.

	Serum 25OHD levels, nmol/L					
Characteristics	ALL (1577: M:599 F:978)	<50 (n=389) (24.7%)	≥50 and <75 (n=359) (22.7%)	≥75 (n=829) (52.6%)	p Value	Age and sex adjusted p values
Age (y)	73.4±6.4	75.6±6.9	73.4±6.4	72.3±5.8	<0.0001	-
Sex female, %	62.0 72.54	80.74	72.54 47.01	47.01	<0.0001	-
BMI (m^2/kg)	27.8±4.4	28.2±5.2	28.1±4.4	27.4±4.0	0.03	0.04
Season of blood collection: Winter	26.3	20.8	23.7	28.7		
Autumn	30.9	25.6	36.6	30.8		
Spring	25.7	33.9	20.3	24.9		
Summer	17.1	19.7	19.3	15.5	<0.0001	<0.0001
Dependency ADL, %	10.0	18.0	11.1	5.8	<0.0001	0.0001
Regular physical activity, %	79.4	67.3	77.2	86.0	<0.0001	<0.0001
Smokers, %	12.7	9.5	13.1	14.0	0.01	0.06
Speed, m/s	0.70±0.20	0.64±0.21	0.71±0.17	0.77±0.17	<0.0001	<0.0001
6-minWT, m	349.1±97.5	299.4±100.6	342.1±90.5	375.5±89.1	<0.0001	<0.0001
GDS (score)	8.2±6.0	9.9±6.5	8.5±5.9	7.3±5.7	<0.0001	<0.0001
Depression, %	29.2	39.8	28.9	24.4	<0.0001	0.001
MMSE (score)	25.2±3.7	24.8±4.0	24.9±3.7	25.6±3.5	<0.0001	0.3
Dementia, %	26.9	33.2	29.2	22.9	<0.0001	0.3
CVD, %	15.2	18.0	12.3	15.2	0.37	0.34
Neurological disease, %	1.8	3.6	1.96	0.97	<0.0001	0.03
Diabetes, %	7.9	8.5	7.2	7.8	0.20	0,3
COPD, %	7.3	8.0	6.7	7.2	0.17	0.002
OA	33.3	37.5	36.9	29.3	0.01	0.82
Frailty, %	29.0	43.4	32.6	20.7	<0.0001	<0.0001
Biochemical						
25 OHD, nmol/L	88.6±56.0	33.5±11.2	61.5±7.2	126.1±52.2	-	-
Calcium, mg/dL	9.6±4.2	9.6±2.2	9.5±0.4	9.7±5.6	0.38	0.45
PTH,	37.7±22.2	43.9±22.0	40.3±19.1	33.5±22.6	<0.0001	<0.0001
Albumina, g/L	4.4±1.4	4.4±0.4	4.4±0.3	4.5±1.8	0.17	0.41
GFR, mg/mL	70.4±18.0	67.9±18.7	69.0±17.1	73.5±17.1	<0.0001	0.53

Abbreviations: BMI= body mass index; ADL=activities of daily living; 6-min WT= 6 minutes walking test; GDS= geriatric depression scale; MMSE= mini-mental state examination; CVD=cardiovascular diseases; COPD=chronic obstructive pulmonary disease; OA= osteoarthrosis; 25 OHD: hydroxyvitamin D3, PTH: parathyroid hormone; GFR: glomerular filtration rate.

Table 2: Cross-sectional association between 25-OHD3 levels and odds of greater frailty status.

Odds ratio (95% CI) by serum 25OHD levels			
	25OHD <50 nmol/L	25OHD 50-74	25OHD ≥75
Model 1[a]	1.898 (1.449-2.485) p<0.0001	1.436 (1.086-1.90) p= 0.01	1.00 (referent)
Model 2[b]	1.657 (1.232-2.228) p<0.0008	1.392 (1.024-1.891) p=0.03	1.00 (referent)

a: age, gender, BMI, season of blood draw, smoking.
b: age, gender, BMI, season of blood draw, smoking, depression, cardiovascular diseases, osteoarthrosis, neurologic diseases, diabetes, COPD.

Table 3: Logistic regression model for the relative risk of frailty at 4.4 years in no frail participants, according to 25OHD serum levels.

Relative Risk (95% CI) by serum 25OHD levels			
	25OHD <50 nmol/L	25OHD 50-74	25OHD ≥75
Model 1[a]	1.057 (0.737-1.517) p=0.76	0.921 (0.650-1.3) p=0.64	1.00 (referent)
Model 2[b]	1.044 (0.716-1.522) p=0.82	0.886 (0.613-1.28) p=0.51	1.00 (referent)

a: age, gender, BMI, season of blood draw, smoking.
b: age, gender, BMI, season of blood draw, smoking, depression, cardiovascular diseases, osteoarthrosis, neurologic diseases, diabetes, COPD.

Table 4: Baseline characteristics of the cognitively intact sample as a whole and by dementia at follow-up and not dementia at follow-up.

Characteristics	ALL (n:1181; M: 469 F: 712)	Subjects who did not developed dementia (n=953) (80.7%)	Subjects who developed dementia (n=228) (19.3%)	p Value	Age and sex adjusted p values
Age (y)	72.12±5.6	71.2±5.1	75.9±6.1	<0.0001	
Sex female, %	60.3	58.3	68.4	0.005	
BMI (m²/kg)	27.8±4.3	27.9±4.3	27.7±4.5		0.82
Education (years)	6.22	6.38	4.01		0.015
Independency in ADL, %	92.2	93.5	86.8		0.22
Regular physical activity, %	82.1	82.8	79.4		0.78
Smokers, %	14.6	15.5	11.0		0.80
GDS (score)	7.6±5.8	7.2±5.8	9.1±5.9		0.0002
Frail status (%)	25.1	22.5	36.0		0.05
MMSE (score)	27.1±1.9	27.4±1.7	25.7±2.1		<0.0001
CVD, %	14.25	12.7	20.7		0.1
OA	30.9	28.9	39.1		0.41
Diabetes, %	7.79	7.35	9.65		0.11
COPD, %	7.8	6.41	13.6		0.0005
Albumina, g/L	4.4±1.5	4.5±1.7	4.4±0.3		0.98

Abbreviations: BMI= body mass index; ADL=activities of daily living; GDS= geriatric depression scale; MMSE= mini mental state examination; CVD=cardiovascular diseases; COPD=chronic obstructive pulmonary disease.

Table 5: Logistic regression model for the relative risk of dementia at 4.4 years in frail participants.

Model	Model 1[a]	Model 2[b]	Model 3[c]
RR (95% CI)	1.94 (CI 1.42-2.64) P<0.0001	1.36 (1.12-1.91) P=0.05	1.27 (1.11-1.80) P=0.05

a: unadjusted model
b: age, gender.
c: age, gender, smoking, MMSE, depression, CVD, diabetes, COPD, AO.

DISCUSSION

Our study identified an association between vitamin D deficiency and frailty syndrome, regardless of several confounding factors.

Previous cross-sectional studies including or limited to older women have inconsistently observed an independent association between lower 25(OH)D levels and frailty. The Longitudinal Aging Study Amsterdam (LASA) of 1321 adults ≥65 years old (Puts MT et al., 2005) measured serum 25(OH)D using a competitive binding protein assay, defined frailty as the presence of three out of nine frailty indicators, and reported that the odds of being classified as frail (*vs.* non-frail) was 1.7-fold higher among those with levels between 10 and 20 ng/ml and 2.6-fold higher among those with levels <10 ng/ml, compared with that among the referent group (>20 ng/ml). The Women's Heath and Aging Studies (Michelon E et al., 2006) measured serum 25(OH)D using a competitive binding protein assay, defined frailty using similar criteria to those used in the Cardiovascular heaith study index, and found an age-adjusted 1.7-fold higher odds of being classified as frail (*vs.* non-frail) among women with levels in the lowest quartile compared with that among women with levels in the upper three quartiles, but the association did not persist after further adjustment. Finally, the InCHIANTI study of older Italian men and women (Shardell M et al., 2009) measured serum 25(OH)D levels using a RIA, defined frailty with a modified CHS index, and reported that lower levels (<20 ng/ml) were associated with frailty in men, but not in women. While the InCHIANTI study found an independent association between lower 25(OH)D levels (<20 ng/ml) among women and the presence of only one of five individual frailty components (low

activity level), the current study using a similar frailty definition observed independent associations between lower 25(OH)D levels (<15 ng/ml or in the case of weakness <20 ng/ml) and the presence of each of five individual frailty dimensions (weakness, shrinking/sarcopenia, exhaustion, slowness, low activity level).

Vitamin D deficiency may be associated with frailty through several biological pathways, including effects on bone, muscle, and immunity.

Effects of vitamin D on bone health are well known (Lips P, 2006). Low 25(OH)D levels are associated, both directly and mediated through elevated PTH, with osteoporotic fractures and osteomalacia, a condition whereby new bone matrix is not properly mineralized (Lips P, 2001). These conditions, along with poor balance, (Zamboni M et al. 2002) may result in bodily pain (Mascarenhas R et al., 2004) (eg, via progression of osteoarthritis) (McAlindo TE et al., 1996; Zhang Y et al., 2000) and fear of falling, which may contribute to sedentariness (Lips P, 2001; Lips P, 2006; Tinetti ME et al., 1993).

Additionally, low 25(OH)D may affect frailty via effects on muscle strength. Vitamin D receptors (VDRs) are located in skeletal muscle cells (Simpson RU et al., 1985), and low 25(OH)D may result in decreased muscle strength from both decreased muscle synthesis and altered contractile properties of muscle (Wassner SJ et al., 1983). Muscle protein synthesis is initiated by binding $1,25\text{-}(OH)_2D$ to its nuclear receptor. The influence of $1,25\text{-}(OH)_2D$ on calcium homeostasis is believed to influence contractile properties of muscle cells via both a VDR-mediated genomic pathway and a nongenomic rapid mechanism (Boland R et al., 1995). Thus, the association between low 25(OH)D and frailty may be explained

by associations of insufficient 25(OH)D with sarcopenia and muscle weakness because both are central to the frailty syndrome (Fried LP et al., 2001). Previous studies found associations of low 25(OH)D with low muscle strength (Visser M et al., 2003), poor balance (Zamboni M et al., 2002; Bischoff HA et al., 1999), and falls (Snijer MB et al., 2006).

A third pathway through which low 25(OH)D may affect frailty stems from hypothesized anti-inflammatory properties of vitamin D (Lips P, 2006). The VDR has been identified on most immune system cells (Holick MF, 2005; van Etten et al., 2005).

As well as the association between vitamin D deficiency and frailty, we evaluate the relationship of vitamin D status and risk of developing frailty. Previous large population-based cohort studies investigating the association between 25OHD levels and the risk to be frail reported conflicting results.

Ensrud et al. among women classified as nonfrail (robust or intermediate) at baseline, founds some evidence of an independent association between lower 25(OH)D levels (<20 ng/ml) at baseline and a higher odds of incident frailty or death at follow-up 4.5 years later (Ensrud KE et al., 2010). The LASA study (Puts MT et al., 2005) reported that among 885 nonfrail men and women at baseline, those with 25(OH)D levels <10 ng/ml, but not those with levels between 10–20 ng/ml, had increased odds of being classified as frail (*vs.* nonfrail) at the 3-yr follow-up exam, compared with that among the referent group (>20 ng/ml). In contrast, an analysis of 463 women in the Women's Health and Aging Study I (Semba RD et al., 2006) classified as nonfrail at baseline reported that the odds of becoming frail at follow-up did not differ between women in the lowest quartile of

25(OH)D as compared with that among women in the top three quartiles. In addition, among a sample of 1267 nonfrail men enrolled in the baseline examination of Osteoporotic Fractures in Men (MrOS) study, there was no association between lower baseline 25(OH)D level (<20 ng/ml) and odds of greater frailty status at a 4.6-year follow-up examination (Ensrud KE et al., 2011). In our study vitamin D deficiency does not predict the onset of frailty.

Since there was no evidence to support an association between lower 25(OH)D levels and greater frailty status at follow-up, the observed cross-sectional association in this study may be due to the presence of residual confounding.

Analyses were adjusted for multiple factors, but the possibility of residual confounding cannot be eliminated.

Moreover, we had limited power to detect a longitudinal association because of smaller sample size.

Another hypothesis is that in patients with hypovitamisis D, low levels of vitamin D could lead, over the years, conditions that become more important to involve frail syndrome, such as osteoporosis, fractures, sarcopenia and disability (Bischoff-Ferrari HA et al., 2006; Visser M et al., 2003).

In the second part of the study, in our large cohort of Italian older individuals free of cognitive impairment at baseline, we found that frailty syndrome at baseline was a risk factor of developing dementia.

Previous studies have reported that frailty was associated with the level of cognition and dementia (Fried LP et al., 2001; Solfrizzi V et al., 2012; Panza F et al., 2011; Samper-Ternent R et al., 2008; Solfrizzi V et al., 2013). Two longitudinal populatio-based studies indicated that frailty syndrome was a

predictor of cognitive impairment in a 10-year follow-up (Samper-Ternent R et al., 2008), and that it was associated with the rate of cognitive decline in a 3-year follow-up period (Buchman AS et al., 2007). The Rush Memory and Aging Project also found that physical frailty increased the risk for MCI (Boyle PA et al., 2010), altrough there is still controversy whether MCI is a separate syndrome or, indeed, a sign of early dementia (Boyle PA et al., 2010; Buchman AS et al., 2008). In the ILSA sample (Solfrizzi V et al., 2013), frailty syndrome was a predictor of overall dementia. Several of the individual components used to construct the measure of frailty in this study, including altered gait, slower movement, weight loss, and muscle weakness, have been associated with the development of dementia (Cronin-Stubbs D et al., 1997; Scarmeas N et al., 2005; Wilson RS et al., 2003; Boyle PA et al., 2009). Furthemore, increased muscle strenght was associated with a decrease in the risk of incident Alzheimer's disease and incident MCI and with a slower rate of decline in global cognitive function during a mean follow-up of 3.6 (boyle PA et al., 2009).

Our finding suggested that factors associated with the development of frailty and its components were also associated with the development of dementia. For example, risk factors for cardiovascular disease (e.g. diabetes) have been related to both frailty (Afilalo J et al., 2009) and dementia (Bowler JV, 2005). In fact, several studies showed that comorbidities such as congestive heart failure, myocardial infarction, peripheral vascular disease, diabetes, and hypertention increase the risk for frailty (Afilalo J et al., 2009).

Beyond the possible role of vascular risk factors and vascular-related diseases, there are several potential path-ways by which frailty could contribute to

cognitive decline; however, at present, the mechanisms underlying this suggested association remained unclear. One of these underlying pathogenetic factors may be inflammation. Increased markers of inflammation, such as C-reactive protein or proinflammatory interleukins, are common and have been implicated in frailty (Puts MT et al., 2005) (as already mentioned), cognitive impairment (Weaver JD et al., 2002), and dementia (Ma SL, 2005).

In some forms of dementia, particularly AD, primary and supplementary motor cortices, the substantia nigra, and the striatum are often altered (Wolf DS et al., 1999). Studies have shown that alterations in these areas of the brain are associated with modifications in the components of frailty such as weight loss and slow gait (Schneider JA et al., 2006, Buchman AS et al., 2006), suggesting the possibility that changes in neural systems that control motor function, metabolism, and fatigue may be present in frailty.

One limitation of this part of our study is the small number of new cases of dementia. We also used the MMSE to diagnose dementia. Several studies report limitations of the MMSE in screening for dementia and cognitive impairment, specially in subcortical infarctions and small vessel disease, where it would not differentiate between focal and diffuse lesions. Furthermore, it would be insensitive to right-sided lesions (O'Sullivan M et al., 2005; Fure B et al., 2006).

CONCLUSION

In conclusion, vitamin D deficiency at the baseline is independently associated with greater evidence to frailty syndrome in elderly; by the way, vitamin D deficiency were not predict the onset of frailty at 4.4 years, as well as other health condition. Future research is waranted to address the directionality of this association.

Furthermore, frailty syndrome at baseline is associated with a greater risk of developing dementia.

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